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# Disturbance events in arid ecosystems : comparisons of enzyme activity profiles across multiple soil microbial communities

Daniel Warnock

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Daniel Warnock

*Candidate*

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Biology

*Department*

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This dissertation is approved, and it is acceptable in quality and form for publication:

*Approved by the Dissertation Committee:*

Dr Robert Sinsabaugh , Chairperson

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Dr Marcy Litvak

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Dr Cristina Takacs-Vesbach

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Dr Catherine Gehring

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**Disturbance events in arid ecosystems: comparisons of enzyme activity profiles  
across multiple soil microbial communities**

by

**Daniel Douglas Warnock**

B.S. Biology, University of Oregon, 2004

M.S. Integrative Microbiology and Biochemistry, University of Montana, 2009

DISSERTATION

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Requirements for the Degree of

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**ABSTRACT**

Arid ecosystems are home to approximately 35% of earth's human population, and approximately 40% of earth's terrestrial carbon. These systems are especially prone to releasing the stored carbon when under global climate change (GCC) related pressures. My goal for these studies was to expand on our already growing knowledge base of how the soil microbial communities in disturbed, arid ecosystems undergo shifts in functional patterns, as they respond to a rapidly changing environment.

In chapter two, I examined the overall functional activity levels, and functional behaviors exhibited by both pinon and juniper supported RAM communities in the context of widespread, disproportionate pinon mortality. More specifically, I examined the rhizosphere level effects of various types of nearest neighbor competition, and tree physiological status on RAM functional behaviors and activity rates, after all pinon trees >7cm diameter at breast height, had been girdled. From our girdled site results, 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. Additionally, the ordination plots for intact PJ woodlands show 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.

For chapter three, I expanded analyses of the data presented in chapter two by performing three different multivariate statistical methods in an effort to explain how widespread pinon mortality events affect the interactions between numerous soil parameters, and multiple aspects of RAM soil activity behaviors. I also used data from an additional study conducted at a nearby juniper savannah to serve as a reference for the directions the RAM activity trends at mortality affected pinon juniper woodland sites could take, as the dead pinons give way, and junipers take over. In general, results from these two chapters 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.

For chapter four, I sought to address questions regarding the effects of other types of GCC related disturbances, e.g., shrub encroachment, and fire, on the RAM communities on three other types of arid biomes, grasslands, shrub-lands and shrub-grass ecotone. With this study, the results also show the ability of different types of disturbance events to disrupt the previously established relationships between soil parameters and RAM activity patterns. The results from this chapter suggest that observed changes in EEA profiles may be related to the dominant plant functional type and the mechanism of disturbance that each biome type has encountered.

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## Chapter 1

### **The multifaceted responses of root associated microbial communities to various flavors of global climate change related disturbances**

#### *Context for the study of widespread tree mortality in pinon-juniper woodlands*

Arid ecosystems cover approximately 40% of earth's surface (Anderson-Teixeira et al., 2011), support a large portion of earth's population, i.e., more than 2 billion people (Safriel et al., 2005), and store approximately twice as much carbon as all temperate forests (Anderson-Teixeira et al., 2011). Further previous research suggests that these systems are highly sensitive to global climate change related disturbances (Diffenbaugh et al. 2008; IPCC 2007; Liancourt et al., 2012; Smith et al., 2005), which would likely lead to massive quantities of carbon released into earth's atmosphere. However, despite this knowledge regarding the importance of arid ecosystems, and the growing understanding of how some of above ground responses of some of these systems to disturbance events, such as drought induced tree mortality (Guardiola-Claramonte et al., 2011; Krofcheck et al., 2014; Royer et al., 2011), comparatively less is known about how different types of disturbance events will affect below ground ecosystem features, such as soil nutrient cycles, and soil microbial community composition (Berryman et al., 2013; Dean et al., 2015). Fortunately, from a small number of studies, conducted independently from one another, present results regarding how below a few different ground processes, e.g., soil respiration, or EEA rates, respond to various disturbance events such as N deposition (Ladwig et al., 2012; Dean et al., 2013), shrub encroachment (Thomey et al., 2014), drought (Thomey et al., 2011, 2014; Petrie et al., 2015), and tree mortality (Berryman et al., 2013; Krofcheck et al., 2014). Thus, within the context of these

previous studies, I conducted this dissertation work, with the intent of answering multiple questions regarding the pervasive effects of various types of disturbance, both gradual, e.g., shrub encroachment, and punctuated, e.g., fire, on multiple aspects of RAM community dynamics within four different arid ecosystems. Further, wherever possible used the same study designs, and analytical methods, to study the various effects of disturbance within the four different arid ecosystems.

***Elucidation of the influences of widespread piñon mortality on soil microbial community dynamics***

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 US (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). 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. Finally, even though 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., 2015).

Thus, to assess the aggregate impacts of piñon mortality on soil microbial community activity, I collected samples from an experimental PJ woodland study system in which all piñon trees  $\geq 7.0$ cm diameter at breast height were girdled in 2009, and a

paired control site where no trees were girdled (Krofcheck et al., 2014). I 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. The hypotheses for chapter two 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.

Overall, the results of this first piñon-juniper woodland study supported the 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), and by allowing for a competitive release effect experience by both juvenile piñons and adult junipers at the girdled PJ woodland field site. Lastly the results from chapter two 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. Lastly, 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.

### ***The study of site-level effects of piñon mortality***

As previously shown, piñon mortality is capable of altering whole ecosystem processes such as, respiration ( $R_{es}$ ), net ecosystem exchange (NEE), and ecosystem carbon sink strength (Krofcheck et al., 2014). Beyond these documented stand level effects, piñon mortality also affects soil microbial communities, and the belowground

processes they mediate, such as soil organic matter (SOM) turnover, soil nutrient cycling and ultimately soil carbon sequestration (Berryman et al., 2013; Dean et al. 2015; Drake et al., 2013; Krofcheck et al., 2014; Warnock et al., in press). Recent studies that have evaluated the effects of piñon mortality on soil microbial communities, and their overall functional behaviors, have generally focused on the responses of individual rhizospheres (Berryman et al., 2013; Dean et al., in revision Warnock et al., in press), which still leaves questions regarding how soil processes will respond, e.g., SOM turnover, at the ecosystem level, as a function of piñon survivorship. Thus, to better resolve the causal relationships between piñon mortality, soil physicochemical attributes, and microbial function, we applied a combination of linear models (LM), structural equation models (SEM) (Bowker et al., 2013; Gaitan et al., 2014; Hallett et al., 2014; Hill et al., 2012; 2014), and principal components analyses (PCA) to data from two piñon-juniper woodland sites, and a juniper savannah site, which are all monitored by the Ameriflux eddy covariance network. We added the data from the juniper savannah based on the assumption that these biomes may be regional endpoints for climate driven succession in PJ woodlands (Swaty et al., 2004; Sankey and Germino 2008). Overall, the multivariate analyses from chapter three produced results showing the capacity for variations across field sites, e.g., PJ girdle vs. juniper savannah, with respect to both soil microbial community properties, and edaphic characteristics, to resist changes in below ground processes, despite multiple convergences in analogous above ground ecosystem processes (Litvak et al., unpublished data; Krofcheck et al., 2014, Petrie et al., 2015; Thomey et al., 2011; 2014).

***The differential effects of multiple disturbance types on soil ecosystem processes***

Multiple global climate change (GCC) related phenomena, such as drought, and land cover change, are significantly altering critically important ecosystem processes throughout the western USA (Anderson-Teixeira et al., 2011; Berryman et al., 2013; Krofcheck et al., 2014; Petrie et al., 2013; Thomey et al., 2011; Warnock et al., in press). Several significant ecosystem processes have already been affected, including net primary productivity (NPP), and ecosystem respiration ( $R_{ES}$ ) (Anderson-Teixeira et al. 2011; Berryman et al., 2013; Krofcheck et al., 2014). If these trends continue, ecosystem carbon balance may shift, resulting in greater greenhouse gas emissions (Krofcheck et al., 2013), from multiple GCC affected biomes (Anderson-Teixeira et al. 2011; Berryman et al., 2013; Krofcheck et al., 2014; Petrie et al., 2013; ).

Thus, for chapter four of this dissertation, I sought to assess how GCC related disturbances such as drought, fire and shrub encroachment are altering the coupling between soil microbial community activity patterns and size to ecosystem GPP and ecosystem respiration in multiple lower elevation biomes, we sampled from four different field sites, over two successive growing seasons. The sites I sampled from include burned grassland, and unburned grassland, a desert shrub land, and a shrub-grass ecotone. From the samples collected at these sites, I was able to demonstrate how the influence of seasonal rainfall patterns and disturbances on soil processes affect soil microbial activity patterns at the scale of individual plant rhizospheres, as well as across whole plots that feature a variety of plant functional types, and different kinds of ecosystem disturbances.

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## Chapter 2

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

Daniel D. Warnock<sup>1\*</sup>, Marcy E. Litvak<sup>1</sup>, Laura Morillas<sup>1</sup> Robert L. Sinsabaugh<sup>1</sup>

<sup>1</sup>UNM Biology Department, University of New Mexico, Albuquerque, NM, 87131, USA

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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

#### Research Highlights

- 1) Widespread piñon mortality alters soil microbial activity at ecosystem scale
- 2) Soil enzyme activity varies with host tree and nearest neighbor identity
- 3) Piñon associated soil communities respond rapidly to increased host tree activity

## 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 nmol g<sup>-1</sup> h<sup>-1</sup>.

### *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<sub>4</sub><sup>3-</sup> and cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>) 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<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup> 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  $\text{W} / \text{m}^2$ ).

#### *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), 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. 4a). 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. 4b). 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. 4b). 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. 4b). 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. 5a). 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. 5a). For BG, activity under juniper (LJ/LJ) was 143% and 175% greater, respectively, than activity under piñon in LP/DP gradients (Tables s4, Fig. 7a). There were no significant differences between sampling locations in July as the growing season began (Table 2, Fig. 5b).

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. 6a). 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. 6b). 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. 6b). Further,  $\beta$ -Gluc activity beneath juniper canopies (LJ/LJ) was 170% greater than under piñon canopies in LP/LP (Table 2, Fig. 6b).  $\beta$ -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. 6b). AAP activity under piñon (LP/LP) was also 210% and 175% greater, respectively, than activity from LP/DP (Table 2, Fig. 6b). 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. 4a). 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. 6b). 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 3<sup>rd</sup> 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 28<sup>th</sup>, 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 15<sup>th</sup>. 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 3<sup>rd</sup> 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  $\text{NO}_3^-$  (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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**Table 2.1.** 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.

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

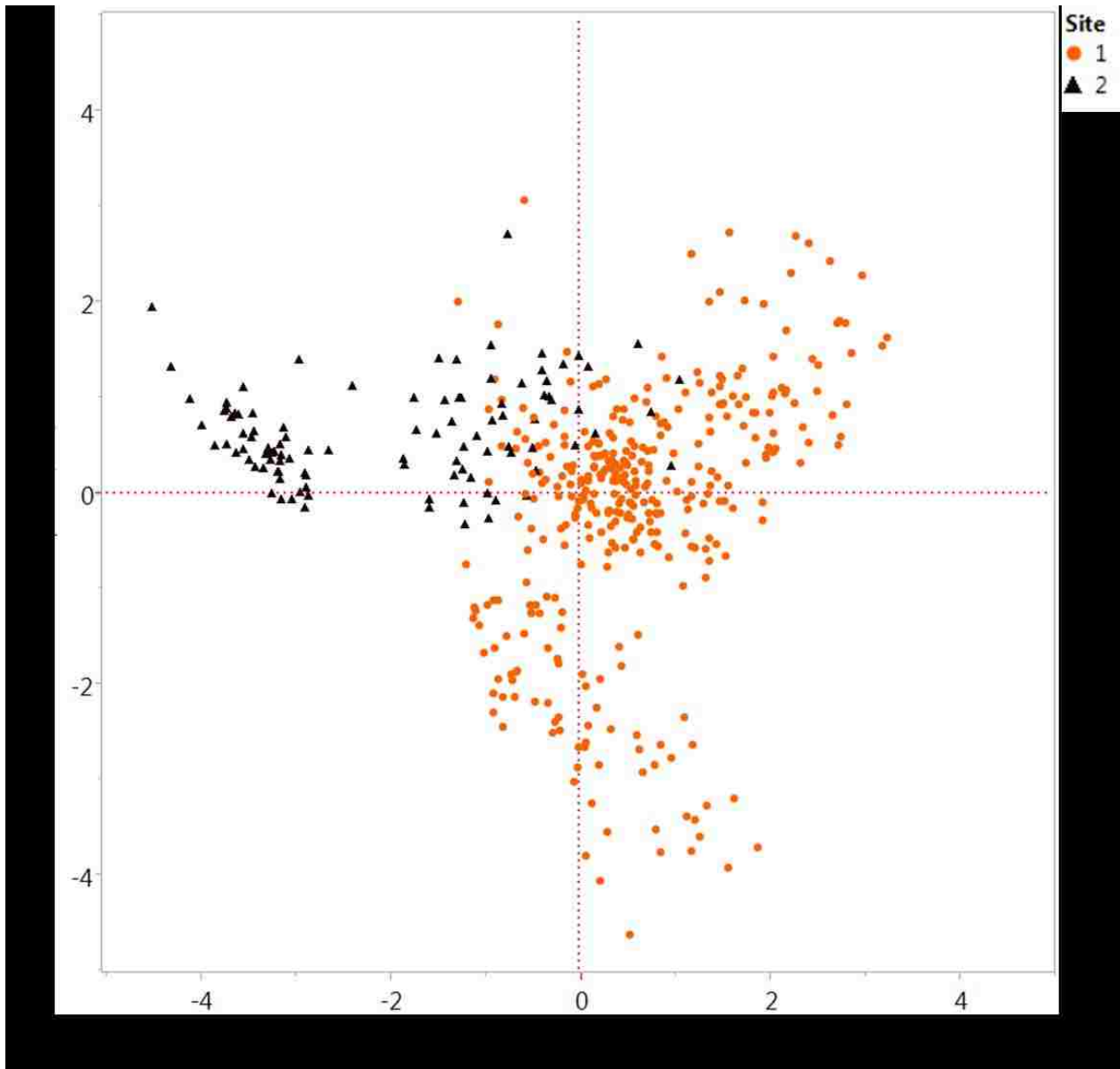
**Table 2.2.** One-way ANOVA statistics from the EEA results from all individual sampling locations, from both sites, and for all sampling dates.

Site	Sampling period	<sup>1</sup> AlaAP (nmol/h/g OM)	<sup>1</sup> Alk P (nmol/h/g OM)	$\beta$ -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

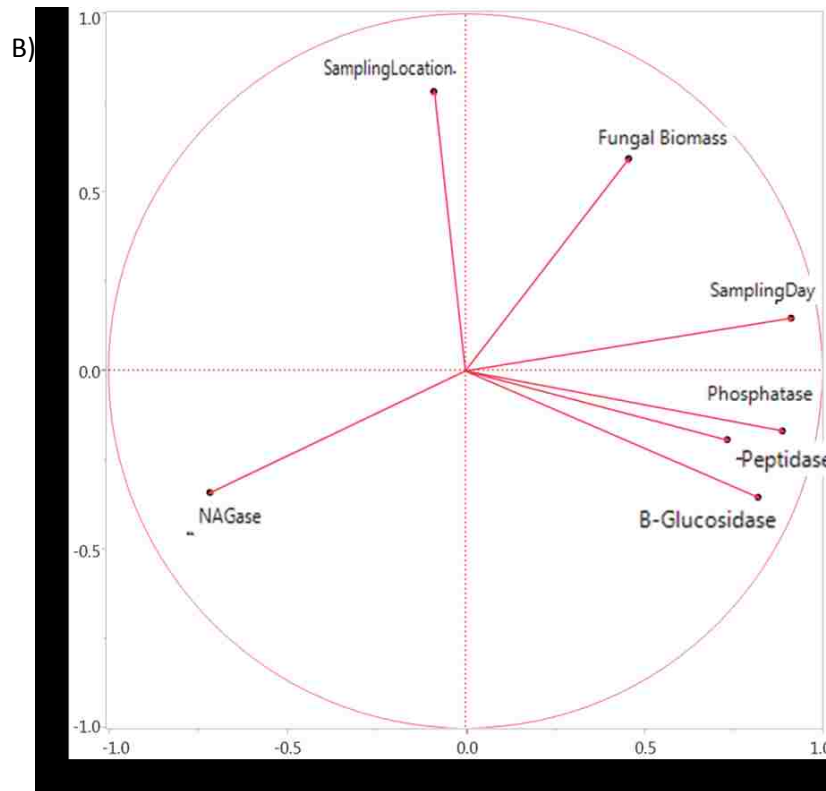
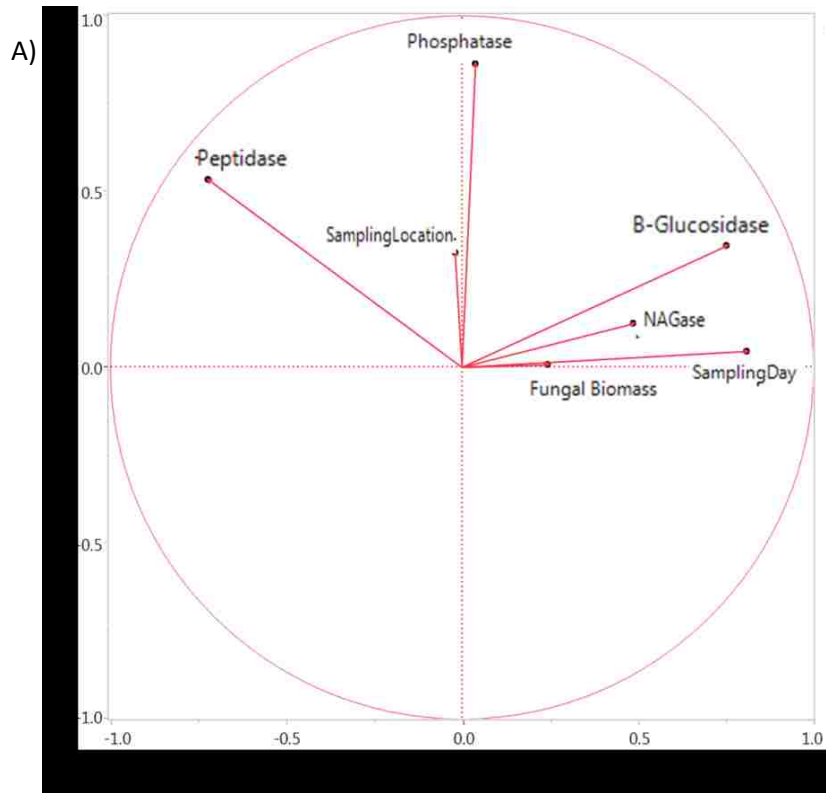


**Table 2.3.** Soil water and soil organic matter contents for both sites, from all sampling dates. Values in table correspond to values included in calculations for soil EEA's shown in Figures 3-5.

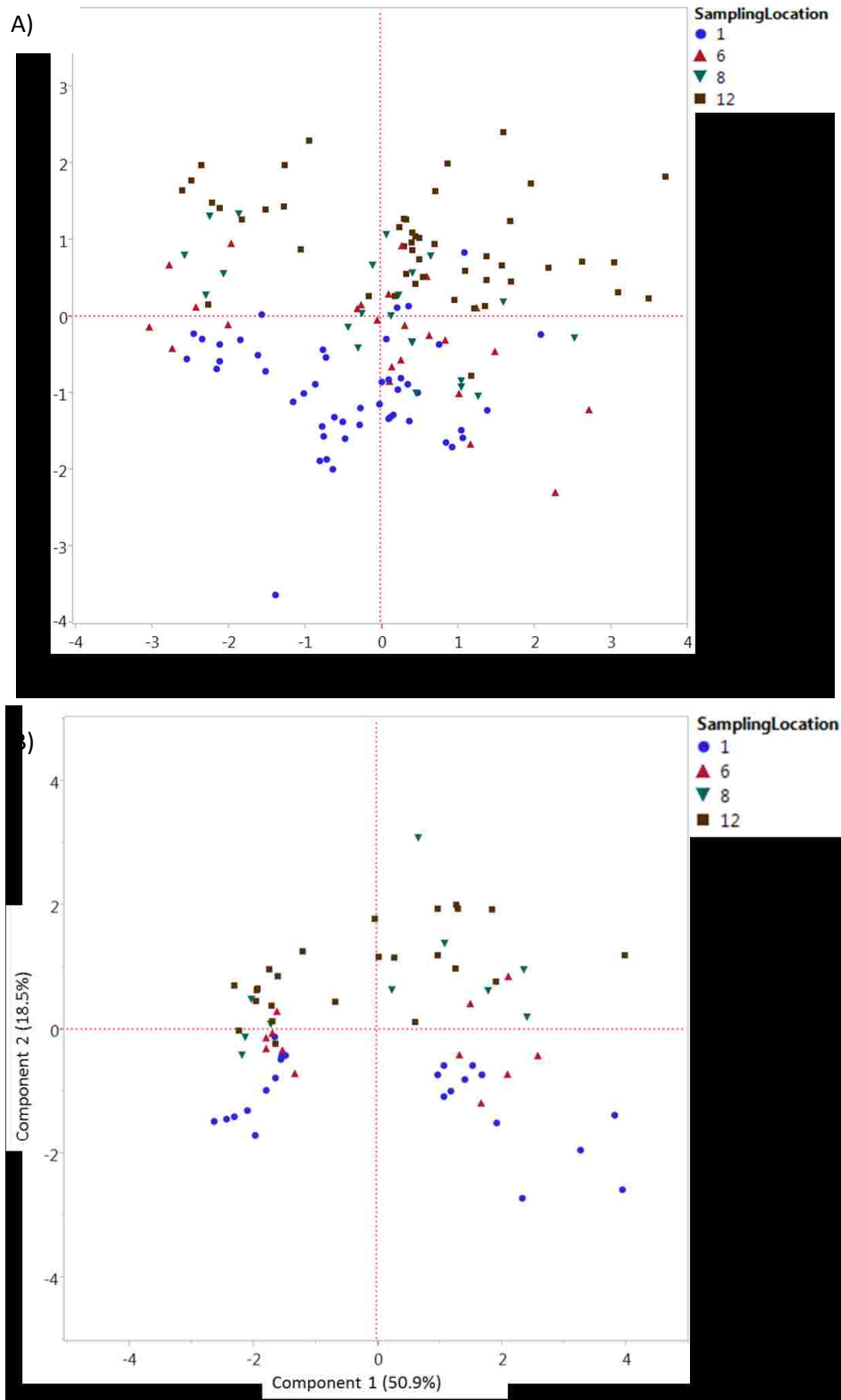
Site	Sampling Gradient	Sampling Location	June 2011		August 2011		September 2011	
			SWC ± (1 SD)	gOM/ g Soil	SWC ± (1 SD)	gOM/ g Soil	SWC ± (1 SD)	gOM/ g Soil
control	All averaged together	Site-wide means	0.034 (0.01)	0.089 (0.027)	No Data	No Data	0.11 (0.02)	0.12 (0.044)
girdled	(LJ/LJ)	live juniper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



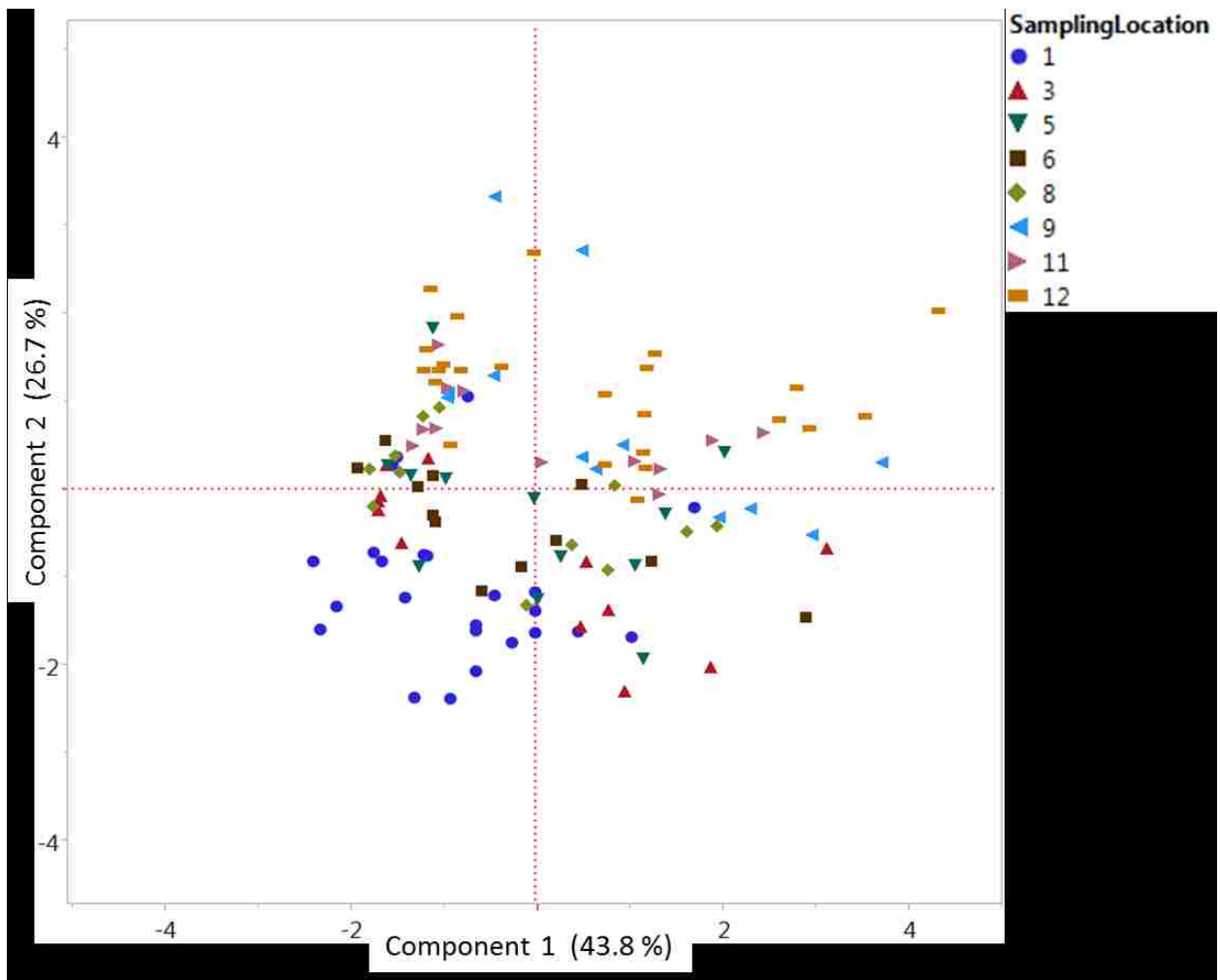
**Figure 2.1.** The ordination plot from the PCA analyses of all data considered together.



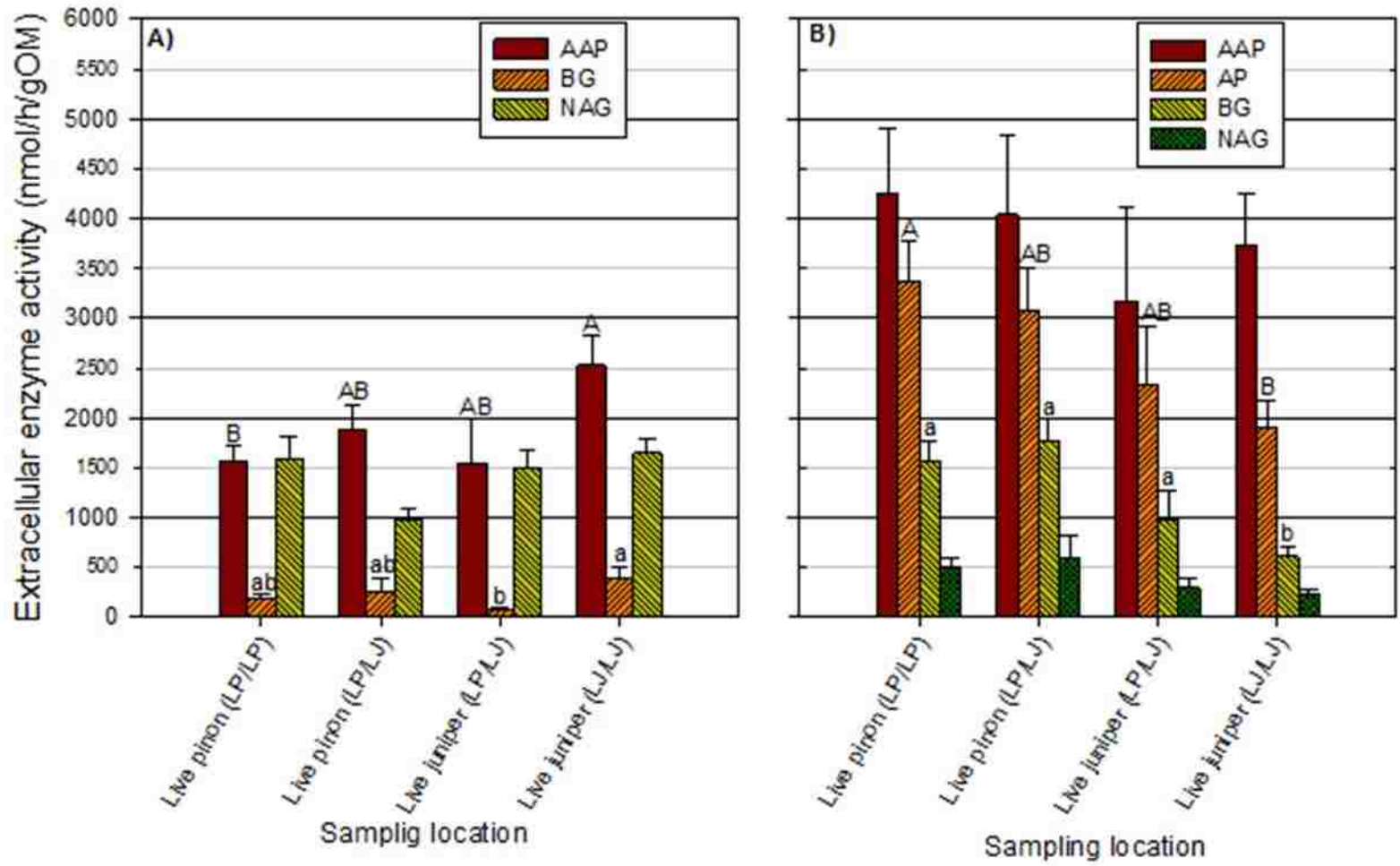
**Figure 2.2.** Graphical results from PCA analyses of A) all data from the control site, and B) all data from the girdled site.



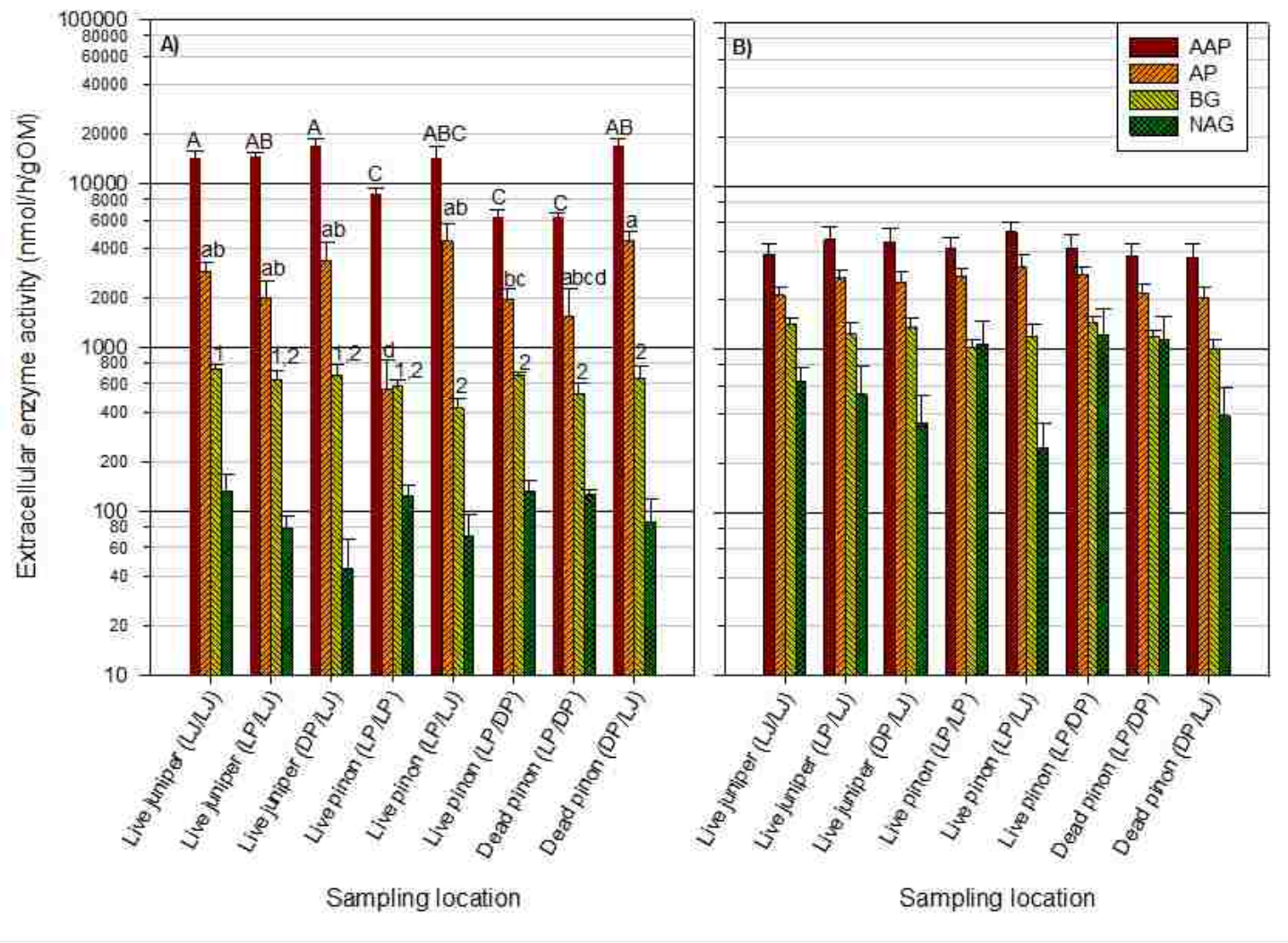
**Figure 2.3.** PCA ordination plots from the LP/LP, LJ/LJ, and LP/LJ gradients from the a) control site data, and b) the girdled site data. For both plots, the sampling location number corresponds to the following beneath tree canopy locations, one (LP) in LP/LP, six (LP) in LP/LJ, eight (LJ) in LP/LJ, and twelve (LJ) in LJ/LJ.



**Figure 2.4.** A PCA ordination plot of the August and September FBM,  $\beta$ -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.

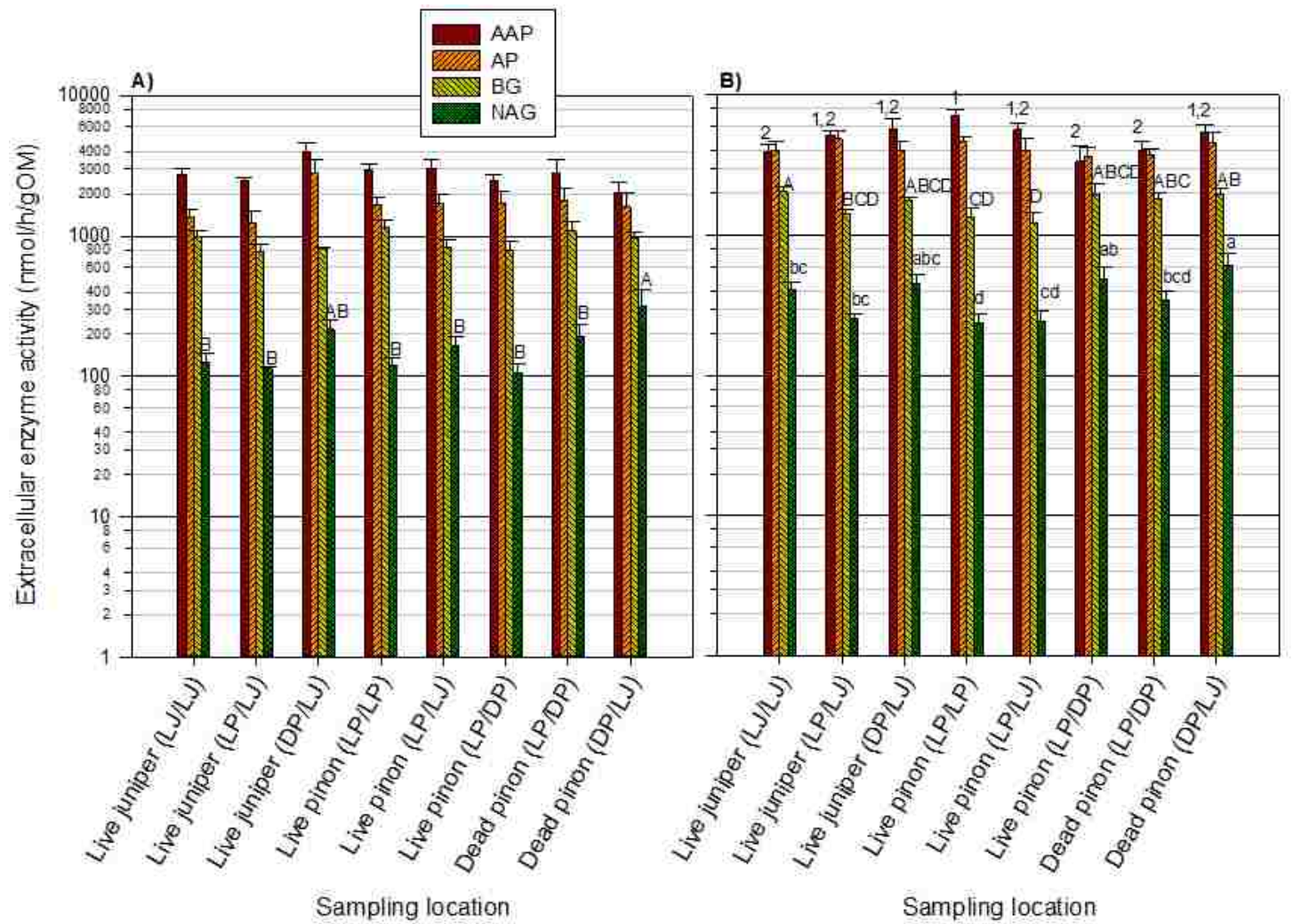


**Figure 2.5** Mean soil enzyme activity rates from the control site, for both A) dry period 2011 and B) wet period 2011. Y axis scale is the same, for both panels.



**Figure 2.6.** 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 2.7.** 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.S1.** Soil hydrolytic exoenzyme activities along with soil physicochemical properties, for samples collected from the Control PJ woodland on June 28th, 2011

Location	Gradient	<sup>1</sup> AlaAP (nmol/h/gOM)	β-Gluc (nmol/h/gOM)	NAGase (nmol/h/gOM)	Fungal Biomass (mg fungi/ g soil)	SWC (%) ± (1 SD)	gOM/ g Soil
Live Pines	LP/LP	1461.22 (409.98) <sup>C</sup>	188.086 (129.584) <sup>BC</sup>	1590.82 (732.432)	0.989 (0.538)	3.36 (1.26)	0.084 (0.027)
Interspace	LP/LP	1037.65 (511.92) <sup>C</sup>	131.495 (76.428) <sup>BC</sup>	1581.33 (514.056)	0.890 (0.440)	3.72 (1.48)	0.068 (0.029)
Live Junipers	LJ/LJ	2489.37 (603.55) <sup>AB</sup>	283.203 (190.762) <sup>B</sup>	1648.22 (527.861)	1.060 (0.470)	3.14 (0.71)	0.11 (0.040)
Interspace	LJ/LJ	3014.28 (1556.45) <sup>A</sup>	474.123 (368.931) <sup>A</sup>	1213.38 (530.384)	0.596 (0.327)	3.18 (0.85)	0.12 (0.080)
Live Pines	LP/LJ	1874.02 (639.95) <sup>BC</sup>	136.342 (129.165) <sup>BC</sup>	1240.42 (315.98)	0.744 (0.252)	3.20 (1.18)	0.098 (0.032)
Interspace	LP/LJ	1395.12 (483.2) <sup>C</sup>	147.437 (76.595) <sup>BC</sup>	1498.31 (396.892)	1.009 (0.327)	2.66 (0.67)	0.083 (0.027)
Live Juniper	LP/LJ	1539.67 (1118.92) <sup>C</sup>	68.887 (87.725) <sup>C</sup>	1648.22 (527.861)	0.897 (0.202)	3.93 (1.46)	0.11 (0.040)
		<b>K = 18.7</b> <b>P = 0.005</b>	<b>F=3.72</b> <b>P = 0.004</b>	F = 1.75 P = 0.18	F=0.993 P=0.442	F=0.72 P=0.64	F=1.36 P=0.25

<sup>1</sup>Data were non-parametric.

**Table 1.S2.** Soil hydrolytic enzyme activities and physicochemical properties for samples collected from the control PJ woodland on September 13, 2011.

Location	Gradient	<sup>1</sup> AlaAP (nmol/h/g OM)	<sup>1</sup> Alk P (nmol/h/g OM)	<sup>2</sup> β-Gluc (nmol/h/g OM)	NAG (nmol/h/g OM)	Fungal Biomass (mg fungi/ g soil)	SWC (%)	gOM/ g Soil
live piñons	LP/LP	3821.0 (1658.4)	3369.2 (1332.5) <sup>A</sup>	1749.8 (809.1) <sup>A</sup>	240.2 (100.9)	1.159 (0.461) <sup>BC</sup>	10.4 (1.8)	0.098 (0.026)
interspace	LP/LP	3288.2 (1600.7)	2772.8 (380.4) <sup>ABC</sup>	1200.2 (641.2) <sup>AB</sup>	431.1 (299.2)	0.950 (0.726) <sup>BC</sup>	10.6 (2.1)	0.095 (0.052)
live junipers	LJ/LJ	3732.2 (1610.4)	1909.3 (909.9) <sup>C</sup>	607.6 (307.1) <sup>B</sup>	496.5 (336.5)	1.667 (0.911) <sup>AB</sup>	11.8 (0.80)	0.13 (0.045)
interspace	LJ/LJ	5104.4 (1262.4)	2749.1 (1095.0) <sup>ABC</sup>	1781.3 (1530.1) <sup>AB</sup>	502.1 (414.0)	0.812 (0.376) <sup>C</sup>	12.8 (4.1)	0.12 (0.079)
live piñons	LP/LJ	3301.0 (818.6)	3082.7 (1054.5) <sup>AB</sup>	1777.2 (486.3) <sup>A</sup>	590.9 (500.5)	1.569 (0.900) <sup>ABC</sup>	12.0 (2.1)	0.12 (0.038)
Interspace	LP/LJ	4278.7 (2746.7)	1768.8 (327.3) <sup>BC</sup>	1468.6 (1194.6) <sup>AB</sup>	367.4 (284.7)	1.402 (0.857) <sup>BC</sup>	11.3 (2.1)	0.12 (0.055)
live juniper	LP/LJ	3177.8 (2271.4)	2333.2 (1448.9) <sup>ABC</sup>	974.5 (742.8) <sup>AB</sup>	294.3 (228.8)	2.334 (0.960) <sup>A</sup>	11.4 (3.1)	0.15 (0.046)
		F=0.78 P=0.57	<b>F=2.42</b> <b>P=0.041</b>	<b>K=14.5</b> <b>P=0.025</b>	F=2.16 P=0.63	<b>F=2.80</b> <b>P=0.021</b>	F=0.88 P=0.52	F=1.20 P=0.32

<sup>1</sup>Data were log transformed prior to performing ANOVA statistics. <sup>2</sup>Data were non-parametric.

**Table 1.S3** Soil hydrolytic extracellular activity for samples collected from the Girdled Piñon Juniper woodland in June, 2011. Numbers in parentheses are equal to 1 standard deviation of the mean.

Trees	Gradient	Location	<sup>1</sup> AlaAP (nmol/h/g OM)	<sup>2</sup> AlkP (nmol/h/g OM)	<sup>1</sup> β-Gluc (nmol/h/g OM)	<sup>2</sup> NAG (nmol/h/g OM)	Fungal biomass (mg/g soil)	Soil Water Content (%)	<sup>2</sup> gOM/ g Soil
junipers	LJ/LJ	juniper	14300.0 (5408.5) <sup>AC</sup>	2928.1 (1169.2) <sup>A</sup>	743.9 (153.1) <sup>A</sup>	133.5 (119.2) <sup>AB</sup>	1.129 (0.302)	5.10 (0.007) <sup>BCD</sup>	0.11 (0.033) <sup>A</sup>
	LP/LJ	juniper	14514.9 (2210.8) <sup>AC</sup>	1999.6 (1229.8) <sup>ABC</sup>	637.3 (187.1) <sup>ABC</sup>	77.9 (39.6) <sup>ABC</sup>	0.863 (0.432)	3.90 (0.008) <sup>DE</sup>	0.093 (0.017) <sup>ABC</sup>
	DP/LJ	juniper	16926.3 (4510.7) <sup>AC</sup>	3354.3 (2540.6) <sup>D</sup>	680.1 (247.9) <sup>AB</sup>	44.2 (52.0) <sup>BCD</sup>	1.186 (0.56)	4.60 (0.016) <sup>CDE</sup>	0.096 (0.030) <sup>AB</sup>
piñons	LP/LP	live piñons	8644.2 (2336.7) <sup>B</sup>	550.0 (978.0) <sup>ABC</sup>	575.6 (183.9) <sup>BCD</sup>	124.3 (61.7) <sup>AB</sup>	0.541 (0.183)	5.70 (0.005) <sup>BC</sup>	0.080 (0.014) <sup>BC</sup>
	LP/DP	live piñons	6282.1 (1549.4) <sup>D</sup>	2619.1 (1567.9) <sup>ABCD</sup>	675.8 (67.1) <sup>AB</sup>	133.1 (48.8) <sup>AB</sup>	0.818 (0.415)	7.30 (0.017) <sup>A</sup>	0.083 (0.016) <sup>ABC</sup>
	LP/DP	dead piñons	6286.9 (652.2) <sup>D</sup>	1537.9 (1493.1) <sup>ABC</sup>	518.5 (197.9) <sup>BCD</sup>	127.6 (14.6) <sup>AB</sup>	0.558 (0.118)	6.00 (0.019) <sup>ABC</sup>	0.096 (0.022) <sup>AB</sup>
	LP/LJ	live piñons	14356.3 (6426.3) <sup>A-E</sup>	4442.3 (3197.1) <sup>B</sup>	426.2 (134.7) <sup>CD</sup>	71.0 (60.8) <sup>ABCD</sup>	0.987 (0.411)	4.3 (0.011) <sup>DE</sup>	0.11 (0.035) <sup>A</sup>
	DP/LJ	dead piñons	22394.6 (8846.1) <sup>A</sup>	4469.1 (1457.6) <sup>B</sup>	603.2 (221.7) <sup>ABC</sup>	85.2 (83.2) <sup>ABCD</sup>	0.573 (0.338)	3.80 (0.010) <sup>E</sup>	0.096 (0.032) <sup>AB</sup>
interspaces	LP/LP	interspace	7376.4 (4976.1) <sup>C</sup>	0 (0) <sup>E</sup>	438.7 (116.2) <sup>CD</sup>	125.0 (21.2) <sup>AB</sup>	0.701 (0.464)	6.20 (0.012) <sup>AB</sup>	0.055 (0.028) <sup>C</sup>
	LP/DP	interspace	6111.9 (3187.1) <sup>E</sup>	1334.4 (1343.7) <sup>C</sup>	467.6 (180.2) <sup>BCD</sup>	99.2 (16.7) <sup>AB</sup>	1.012 (0.224)	5.30 (0.009) <sup>BCD</sup>	0.055 (0.028) <sup>ABC</sup>
	LP/LJ	interspace	16655.0 (4690.8) <sup>A</sup>	2712.4 (1501.5) <sup>ABC</sup>	552.7 (124.8) <sup>ABCD</sup>	13.3 (18.3) <sup>D</sup>	0.601 (0.306)	4.10 (0.006) <sup>DE</sup>	0.093 (0.039) <sup>ABC</sup>
	DP/LJ	interspace	16901.7 (3788.4) <sup>A</sup>	5204.5 (2476.3) <sup>B</sup>	644.0 (315.9) <sup>ABCD</sup>	21.5 (36.2) <sup>C</sup>	1.195 (0.548)	4.60 (0.016) <sup>CDE</sup>	0.072 (0.001) <sup>BC</sup>
	LJ/LJ	interspace	14520.6 (7068.2) <sup>ABCD</sup>	2269.5 (562.5) <sup>ABCD</sup>	393.3 (148.4) <sup>D</sup>	79.5 (55.0) <sup>ABC</sup>	1.072 (0.636)	4.90 (0.014) <sup>BCDE</sup>	0.088 (0.022) <sup>A</sup>
			<b>K=45.4</b> <b>P&lt;0.0001</b>	<b>K=38.8</b> <b>P&lt;0.0001</b>	<b>F=3.04</b> <b>P = 0.0016</b>	<b>K=27.2</b> <b>P=0.0072</b>	K = 14.5 P = 0.27	<b>F = 4.93</b> <b>P &lt; 0.0001</b>	<b>F = 2.54</b> <b>P = 0.028</b>

<sup>1</sup>Data were Log10 transformed prior to performing ANOVA. <sup>2</sup>Data are non-parametric.

**Table 1.S4.** Soil hydrolytic extracellular activity for samples collected from the Girdled Piñon Juniper woodland in July, 2011. Numbers in parentheses are equal to 1 standard deviation of the mean.

Trees	Gradient	Location	<sup>1</sup> AlaAP (nmol/h/g OM)	<sup>1</sup> AlkP (nmol/h/g OM)	<sup>1</sup> β-Gluc (nmol/h/g OM)	<sup>2</sup> NAG (nmol/h/g OM)	Fungal biomass (mg/g soil)	Soil Water Content (%)	<sup>2</sup> gOM/ g Soil
junipers	LJ/LJ	juniper	3770.6 (2174.0)	2298.7 (820.7)	1376.7 (299.0)	544.3 (408.9)	0.810 (0.351)	3.35 (0.1)	0.12 (0.020)
	LP/LJ	juniper	3849.8 (1285.9)	2455.1 (252.9)	1415.6 (252.5)	527.4 (626.1)	1.595 (1.686)	2.83 (0.62)	0.10 (0.030)
	DP/LJ	juniper	4554.6 (2311.7)	2581.0 (948.7)	1349.1 (468.6)	213.0 (259.9)	0.775 (0.158)	3.98 (1.13)	0.11 (0.051)
piñons	LP/LP	live piñon	5041.7 (2102.9)	3286.2 (1295.6)	1214.2 (453.2)	424.8 (434.8)	0.865 (0.495)	3.37 (0.67)	0.084 (0.023)
	LP/DP	live piñon	3718.0 (1703.5)	2211.3 (681.7)	1188.9 (289.5)	724.0 (440.8)	1.182 (0.346)	3.57 (0.56)	0.11 (0.035)
	LP/DP	dead piñon	3635.5 (2014.6)	2081.4 (717.6)	1008.3 (340.1)	218.3 (224.3)	1.027 (0.372)	4.27 (0.75)	0.12 (0.036)
	LP/LJ	live piñon	4148.1 (2132.6)	2880.3 (714.8)	1315.1 (216.6)	1211.8 (1377.8)	1.176 (0.629)	3.60 (1.00)	0.010 (0.027)
	DP/LJ	dead piñon	3585.8 (1509.2)	2423.3 (218.1)	965.9 (107.5)	737.5 (666.8)	0.792 (0.160)	0.036 (0.44)	0.12 (0.029)
interspaces	LP/LP	interspace	4514.5 (1263.4)	2249.7 (713.6)	1084.5 (161.6)	84.1 (137.1)	0.522 (0.227)	4.0 (0.50)	0.093 (0.023)
	LP/DP	interspace	4339.9 (2232.9)	2592.2 (863.9)	1098.1 (327.6)	149.3 (177.6)	0.866 (0.637)	3.55 (0.62)	0.095 (0.027)
	LP/LJ	interspace	3812.2 (1180.8)	2836.4 (665.7)	1217.9 (158.0)	628.8 (721.9)	0.680 (0.266)	3.98 (0.44)	0.087 (0.029)
	DP/LJ	interspace	3583.8 (1492.1)	2296.6 (348.9)	1124.1 (182.0)	257.6 (294.0)	0.800 (0.420)	3.46 (0.72)	0.10 (0.019)
	LJ/LJ	interspace	2654.8 (593.2)	2084.1 (148.6)	1169.4 (284.3)	336.1 (449.6)	0.880 (0.490)	4.0 (0.91)	0.12 (0.037)
			K=2.64 P=0.099	F=0.51 P=0.724	K=15.1 P=0.24	K=14.8 P=0.25	K = 8.72 P = 0.73	F = 1.47 P = 0.15	F=1.42 P=0.18

<sup>1</sup>Data were Log10 transformed prior to performing ANOVA. <sup>2</sup>Data are non-parametric.

**Table 1.S5.** Soil hydrolytic extracellular activity for samples collected from the Girdled Piñon Juniper woodland in August 2011. Numbers in parentheses are equal to 1 standard deviation of the mean.

Trees	Gradient	Location	<sup>1</sup> AlaAP (nmol/h/g OM)	<sup>1</sup> AlkP (nmol/h/g OM)	<sup>1</sup> β-Gluc (nmol/h/g OM)	<sup>2</sup> NAG (nmol/h/g OM)	Fungal biomass (mg/g soil)	Soil Water Content (%)	<sup>2</sup> gOM/ g Soil
junipers	LJ/LJ	juniper	2724.9 (846.4)	1257.8 (534.4)	829.8 (198.8)	126.1 <sup>CD</sup> (61.7)	1.283 (0.389) <sup>AB</sup>	16.7 (1.48) <sup>AB</sup>	0.10 (0.024) <sup>CD</sup>
	LP/LJ	juniper	2832.0 (1649.1)	1245.6 (614.9)	776.1 (234.6)	113.1 (8.1) <sup>CD</sup>	0.643 (0.437) <sup>CDE</sup>	15.1 (2.33) <sup>ABC</sup>	0.13 (0.039) <sup>ABC</sup>
	DP/LJ	juniper	2463.6 (234.8)	2120.7 (660.3)	810.7 (68.8)	216.0 (90.6) <sup>BC</sup>	1.013 (0.363) <sup>BCD</sup>	14.2 (1.09) <sup>D</sup>	0.12 (0.029) <sup>ABCD</sup>
piñons	LP/LP	live piñon	2724.4 (1214.8)	1674.9 (782.2)	987.0 (298.4)	118.7 (64.2) <sup>CD</sup>	0.857 (0.531) <sup>CDE</sup>	17.3 (1.79) <sup>A</sup>	0.13 (0.042) <sup>ABCD</sup>
	LP/DP	live piñon	2421.0 (749.4)	1708.7 (867.6)	689.7 (119.1)	106.5 (37.9) <sup>CD</sup>	1.179 (0.370) <sup>ABC</sup>	14.6 (1.14) <sup>BCD</sup>	0.15 (0.014) <sup>AB</sup>
	LP/DP	dead piñon	3049.9 (948.8)	1785.7 (1010.1)	929.3 (226.1)	192.3 (92.4) <sup>CD</sup>	0.927 (0.385) <sup>BCDE</sup>	14.1 (1.30) <sup>CD</sup>	0.12 (0.029) <sup>A</sup>
	LP/LJ	live piñon	2467.4 (606.1)	1694.9 (714.6)	828.6 (284.8)	167.0 (67.6) <sup>CD</sup>	0.888 (0.319) <sup>BCDE</sup>	18.0 (1.74) <sup>A</sup>	0.12 (0.023) <sup>ABCD</sup>
interspaces	DP/LJ	dead piñon	2592.6 (374.6)	1265.4 (676.5)	972.5 (204.2)	315.0 (239.1) <sup>AB</sup>	1.54 (0.681) <sup>A</sup>	14.4 (2.66) <sup>BCD</sup>	0.11 (0.021) <sup>A</sup>
	LP/LP	interspace	2752.4 (545.1)	1927.4 (656.0)	804.2 (229.3)	59.1 (30.8) <sup>D</sup>	0.500 (0.151) <sup>E</sup>	15.9 (3.64) <sup>ABCD</sup>	0.11 (0.023) <sup>ABCD</sup>
	LP/DP	interspace	2996.5 (738.8)	1678.4 (843.5)	878.7 (205.6)	140.0 (76.9) <sup>CD</sup>	0.753 (0.285) <sup>CDE</sup>	13.6 (1.24) <sup>D</sup>	0.11 (0.027) <sup>BCDE</sup>
	LP/LJ	interspace	2277.5 (450.7)	1523.1 (740.1)	919.1 (370.0)	107.3 (69.5) <sup>CD</sup>	0.654 (0.226) <sup>CDE</sup>	16.7 (1.59) <sup>ABCD</sup>	0.12 (0.037) <sup>ABCD</sup>
	DP/LJ	interspace	3239.6 (1372.2)	1836.4 (569.1)	1094.3 (444.3)	142.7 (94.2) <sup>CD</sup>	0.714 (0.327) <sup>CDE</sup>	13.3 (2.11) <sup>D</sup>	0.08 (0.025) <sup>E</sup>
	LJ/LJ	interspace	3622.8 (1965.8)	1347.4 (517.5)	938.2 (247.8)	382.5 (158.9) <sup>A</sup>	1.031 (0.365) <sup>BCD</sup>	14.9 (3.53) <sup>D</sup>	0.10 (0.022) <sup>DE</sup>
			K=5.71 P=0.93	F=0.863 P=0.586	F=0.9249 P=0.528	<b>K=22.6</b> <b>P=0.0309</b>	<b>F = 3.50</b> <b>P = 0.0005</b>	<b>F=3.6</b> <b>P=0.0003</b>	<b>F=2.47</b> <b>P=0.01</b>

<sup>1</sup>Data were Log10 transformed prior to performing ANOVA. <sup>2</sup>Data are non-parametric.

**Table 1.S6.** Soil hydrolytic extracellular activity for samples collected from the Girdled Piñon Juniper woodland in September 2011. Numbers in parentheses are equal to 1 standard deviation of the mean.

Trees	Gradient	Location	<sup>1</sup> AlaAP (nmol/h/g OM)	<sup>1</sup> AlkP (nmol/h/g OM)	<sup>1</sup> β-Gluc (nmol/h/g OM)	<sup>2</sup> NAG (nmol/h/g OM)	Fungal biomass (mg/g soil)	Soil Water Content (%)	gOM/ g Soil
junipers	LJ/LJ	juniper	3898.5 (1831.8) <sup>BC</sup>	4018.4 (2160.5)	2041.3 (615.9) <sup>A</sup>	365.6 (122.0) <sup>BCD</sup>	1.454 (0.370) <sup>A</sup>	8.90 (1.74) <sup>A</sup>	0.14 (0.033)
	LP/LJ	juniper	5145.4 (945.6) <sup>AB</sup>	4844.7 (1755.5)	1399.2 (318.0) <sup>ABC</sup>	252.7 (46.6) <sup>CD</sup>	0.766 (0.439) <sup>BC</sup>	7.50 (1.50) <sup>BC</sup>	0.10 (0.028)
	DP/LJ	juniper	5712.8 (2023.7) <sup>AB</sup>	4041.2 (1472.1)	1785.1 (146.1) <sup>ABC</sup>	449.2 (176.1) <sup>ABC</sup>	1.268 (0.202) <sup>AB</sup>	6.75 (0.52) <sup>C</sup>	0.13 (0.050)
piñons	LP/LP	live piñon	7088.3 (2572.7) <sup>A</sup>	4672.3 (1290.4)	1197.6 (608.9) <sup>C</sup>	237.4 (117.8) <sup>D</sup>	0.632 (0.405) <sup>C</sup>	6.74 (0.81) <sup>C</sup>	0.09 (0.017)
	LP/DP	live piñon	3369.4 (2228.4) <sup>BC</sup>	3662.7 (1264.0)	1955.9 (829.3) <sup>AB</sup>	490.0 (256.1) <sup>AB</sup>	0.724 (0.568) <sup>BC</sup>	6.90 (2.19) <sup>BC</sup>	0.12 (0.034)
	LP/DP	dead piñon	4038.7 (1424.7) <sup>BC</sup>	3326.7 (1040.9)	1808.6 (522.7) <sup>ABC</sup>	343.1 (134.0) <sup>BCD</sup>	1.042 (0.706) <sup>ABC</sup>	6.14 (0.72) <sup>C</sup>	0.11 (0.018)
	LP/LJ	live piñon	5605.2 (1488.8) <sup>AB</sup>	4043.3 (2068.9)	1229.3 (526.8) <sup>BC</sup>	242.2 (110.9) <sup>D</sup>	0.723 (0.250) <sup>BC</sup>	7.26 (0.92) <sup>BC</sup>	0.09 (0.022)
interspaces	DP/LJ	dead piñon	5432.4 (1539.4) <sup>AB</sup>	4589.0 (2124.1)	1953.1 (442.7) <sup>AB</sup>	607.5 (314.0) <sup>A</sup>	1.22 (0.367) <sup>AB</sup>	6.74 (0.64) <sup>C</sup>	0.12 (0.041)
	LP/LP	interspace	5425.5 (2052.7) <sup>AB</sup>	4145.1 (1896.7)	1129.8 (449.4) <sup>C</sup>	196.5 (59.5) <sup>D</sup>	0.838 (0.536) <sup>BC</sup>	7.04 (1.47) <sup>BC</sup>	0.10 (0.035)
	LP/DP	interspace	2239.7 (1648.2) <sup>C</sup>	2419.7 (1351.5)	1714.5 (934.2) <sup>ABC</sup>	317.9 (112.4) <sup>AB</sup>	1.068 (0.389) <sup>ABC</sup>	7.10 (1.45) <sup>BC</sup>	0.12 (0.027)
	LP/LJ	interspace	5208.4 (2962.7) <sup>AB</sup>	4202.7 (1515.9)	2041.3 (615.9) <sup>BC</sup>	331.7 (131.5) <sup>BCD</sup>	1.502 (0.718) <sup>A</sup>	7.45 (0.75) <sup>BC</sup>	0.10 (0.032)
	DP/LJ	interspace	5098.3 (2783.2) <sup>AB</sup>	4064.6 (1165.8)	1686.5 (736.3) <sup>ABC</sup>	314.4 (136.0) <sup>BCD</sup>	1.17 (0.3303) <sup>AB</sup>	7.20 (0.64) <sup>BC</sup>	0.10 (0.037)
	LJ/LJ	interspace	3718.6 (2020.8) <sup>BC</sup>	2816.0 (797.7)	1286.8 (547.7) <sup>BC</sup>	235.7 (111.8) <sup>D</sup>	1.25 (0.789) <sup>AB</sup>	8.42 (2.01) <sup>AB</sup>	0.13 (0.052)
			<b>K=45.4</b> <b>P&lt;0.0001</b>	K=16.7 P=0.392	<b>F=3.04</b> <b>P = 0.0016</b>	<b>K=27.2</b> <b>P=0.0072</b>	<b>F=2.32</b> <b>P=0.016</b>	<b>K=21.8</b> <b>P=0.04</b>	<b>K=15.2</b> <b>P=0.23</b>

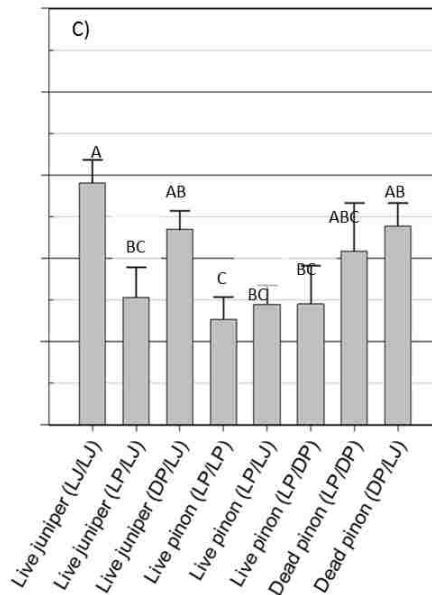
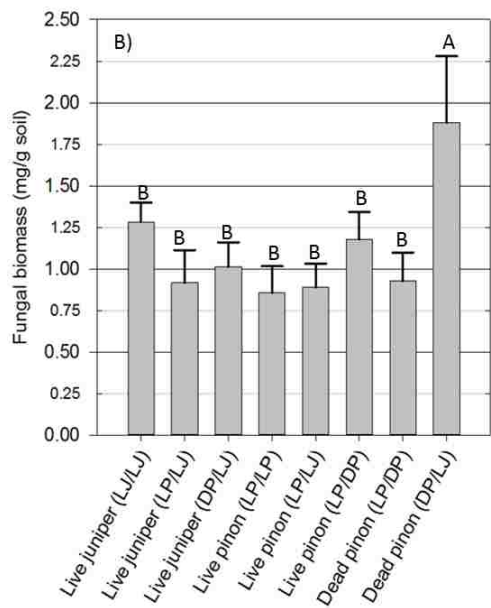
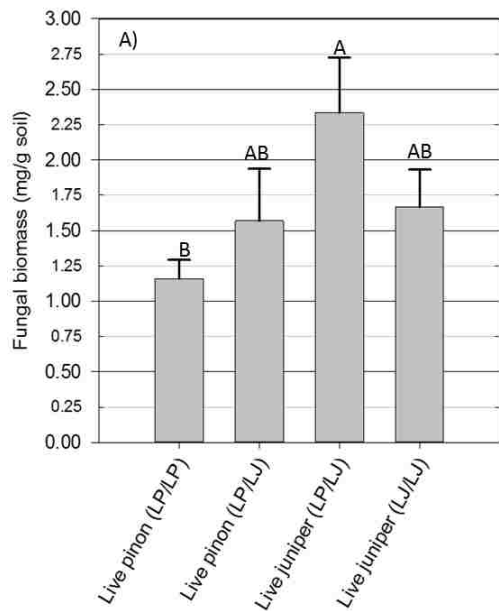
<sup>1</sup>Data were Log10 transformed prior to performing ANOVA. <sup>2</sup>Data are non-parametric.

**Table 1.S7.** Soil physicochemical properties for samples collected from the control PJ woodland in June and September of 2011. Numbers in parentheses are equivalent to 1SD of the mean.

Sampling period	Sampling Site	Sampling location	<sup>1</sup> CEC ± eq/100g	<sup>2</sup> pH	NH <sub>4</sub> <sup>+</sup> (ppm)	<sup>1</sup> NO <sub>3</sub> <sup>-</sup> (ppm)	PO <sub>4</sub> <sup>3-</sup> (ppm)
July	girdled	Site wide means	37.5 (8.80)	7.47 (0.22)	No Data	No Data	27.4 (11.9)
September		Site wide means	40.5 (8.63)	7.68 (0.20)	7.89 (5.48)	7.11 (1.10)	18.9 (7.73)
June	control	Site wide means	36.0 (10.0)	7.34 (0.28)	No Data	No Data	27.4 (11.9)
September		piñons (LP/LP)	29.7 (4.81) <sup>B</sup>	7.31 (0.31) <sup>B</sup>	7.68 (2.32)	7.03 (2.85) <sup>B</sup>	31.7 (20.2)
September	control	junipers (LJ/LJ)	38.1 (7.04) <sup>A</sup>	7.69 (0.065) <sup>A</sup>	8.13 (0.69)	6.49 (3.32) <sup>B</sup>	20.5 (7.82)
September		piñons (LP/LJ)	31.8 (3.37) <sup>B</sup>	7.38 (0.19) <sup>B</sup>	8.32 (0.97)	9.28 (6.23) <sup>AB</sup>	29 (24.0)
September	control	junipers (LP/LJ)	39.8 (14.8) <sup>AB</sup>	7.43 (0.38) <sup>B</sup>	9.54 (0.97)	15.1 (9.06) <sup>A</sup>	27.3 (15.8)
			$\chi^2=15.2$ P=0.002	$\chi^2=12.2$ P=0.007	F=0.954 P=0.428	F=3.85 P=0.02	$\chi^2=2.85$ P=0.41

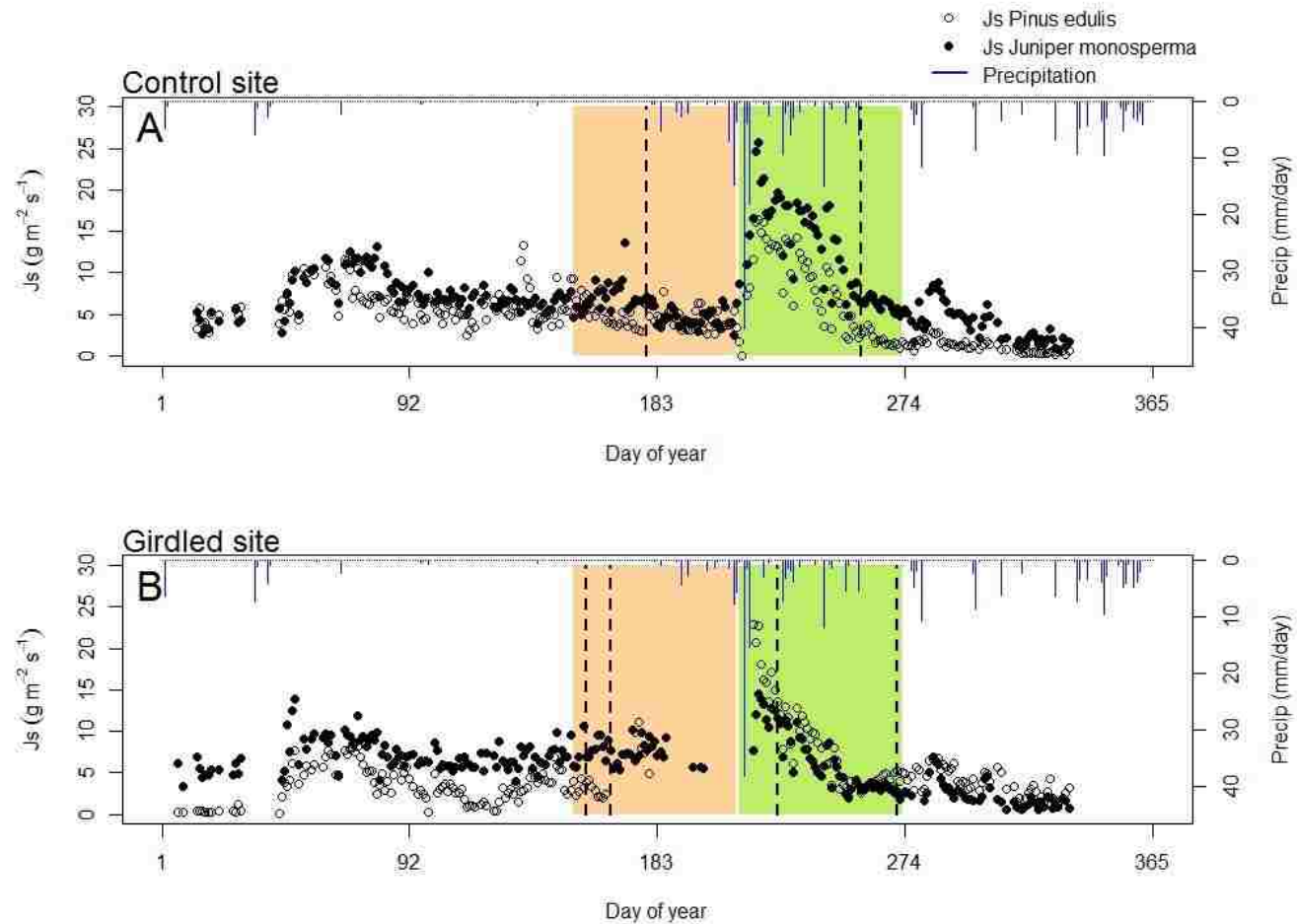
<sup>1</sup>Data were log transformed prior to performing ANOVA statistics.

<sup>2</sup>Data were non-parametric.



**Figure 1.S1.** Mean soil fungal biomass from samples collected during the wet period at both the A) control site and the girdled site during B) August 2011 and C) September 2011. The x-axis label is the location of sample collection, for all three panels Error bars represent one standard error of the mean.





**Figure 1.S2.** Piñon and juniper daily means of sap flow density ( $J_s$ ) for year 2011. Dry (June-July) and wet (August-September) periods considered in this work have been highlighted in brown and green respectively. Dashed line is showing soil-sampling dates at both sites. There were no sap flow data collected on July 19<sup>th</sup> from the girdled site, which is day number 200 on the y axis.

## Chapter 3

### **Disturbance events in arid ecosystems alter relationships among hydrolytic enzyme activities**

Daniel D Warnock<sup>1</sup>, Marcy E. Litvak<sup>1</sup>, Robert L Sinsabaugh<sup>1</sup>

<sup>1</sup>UNM Biology Department, University of New Mexico, Albuquerque, NM, 87131, USA

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#### **Abstract:**

Piñon (*Pinus edulus*) - Juniper (*Juniperus monosperma*) (PJ) woodlands cover 17+ million hectares in the western US. However, these numbers are changing, due to multiple, prolonged, drought induced die-offs among the piñon trees. Piñon die-offs may influence the functional activities of resident soil microbial communities, perhaps facilitating the transition of PJ woodlands into juniper savannahs. To assess the aggregate influences of piñon mortality on soil microbial community functionality, we collected samples from beneath tree canopies at two piñon-juniper woodland sites. One site had many dead piñons killed by girdling as part of an ecosystem scale manipulation. The other site was not experimentally disturbed but has experienced progressive piñon mortality. We also collected samples from a nearby juniper savannah site, as a reference for ecosystem transition. We analyzed multiple soil physicochemical properties, soil fungal biomass, and the activities of four hydrolytic enzymes, alanine aminopeptidase (AAP), alkaline phosphatase (AP),  $\beta$ -D-glucosidase (BG), and  $\beta$ -N-acetyl glucosaminidase (NAG). Step-wise regressions and structural equation models (SEM) indicated that widespread piñon mortality significantly altered the interactions among both soil physicochemical properties and hydrolase activities. In general, our SEM, and

step-wise ANOVA results suggest that piñon mortality has increased the number of significant interactions between soil parameters and EEA rates, and the number of functional dimensions needed to describe enzymatic C, N and P acquisition, though the specific responses we observed were unique for each site.

## **1. Introduction:**

Persistent drought events are affecting multiple ecosystem processes within the approximately 40 million ha of piñon (*Pinus edulis*) juniper (*Juniperus spp.*) (PJ) woodlands in the western USA (Floyd et al., 2009; 2015). These effects are driven by die-offs of piñons, which have claimed 32% to 65% of all piñons, with even greater rates among the sub-population of older, reproductive piñon trees, across Colorado, New Mexico and Arizona (Floyd et al., 2009; Krofcheck et al., 2013). These die-offs have caused major shifts in plant community composition and significant changes in ecosystem structure and function (Berryman et al., 2013; Krofcheck et al., 2014; Limousin et al., 2013; McDowell et al., 2008; Plaut et al., 2012). In addition, these mortality events may be the initial step in a state transition that converts piñon-juniper (PJ) woodlands into juniper savannas, or juniper dominated woodlands (Dean et al., 2015; Swaty et al., 2004; Sankey and Germino 2008).

At the stand level, widespread piñon mortality significantly alters both ecosystem respiration and gross primary productivity (GPP), which likely affects carbon sequestration (Berryman et al. 2013; Krofcheck et al., 2014). Further, piñon death also affects soil microbial communities, and the belowground processes they mediate, such as soil organic matter (SOM) turnover, soil respiration ( $R_s$ ), and nutrient cycling (Berryman

et al., 2013; Dean et al., 2015; Drake et al., 2013; Krofcheck et al., 2014; Warnock et al., in press). At the rhizosphere scale, piñon mortality modifies microbial community function and community structures, (Dean et al., 2015; Warnock et al. 2015). However, the implications of these responses on whole ecosystem processes such as, SOM turnover,  $R_s$ , and GPP, as functions of piñon survivorship are unknown (Berryman et al., 2013; Krofcheck et al., 2014).

Recent studies conducted within regional grassland and shrubland ecosystem sites located within the Sevilleta National Wildlife refuge in New Mexico, USA (Ladwig et al., 2012, 2014, 2015; Petrie et al., 2015; Thomey et al., 2011; 2014; Warnock et al., in prep), provide comparative data for the effects of climate driven disturbances such as fire, drought and woody plant encroachment, on soil microbial community function at the level of individual rhizospheres (Ladwig et al., 2012, 2015; Thomey et al., 2011, 2014; Warnock et al., in prep), as well as the ecosystem scale (Petrie et al., 2015; Thomey et al., 2011; Warnock et al., in prep). Specifically, these disturbance events altered the relationships among the activities of soil enzymes responsible for generating C, N, and P for microbial growth (Sinsabaugh et al., (2008; 2011), as well as the relationships between these activities, and soil properties (Thomey et al., 2011, 2014; Warnock et al., in prep –b). These responses ultimately underlie ecosystem processes such as NEE and  $R_{ES}$  (Krofcheck et al., 2014; Petrie et al., 2015; Thomey et al., 2011).

To resolve the relationships between piñon mortality, soil physicochemical attributes, and microbial function, e.g., respiration, we applied a combination of step-wise regression models, structural equation models (SEM) (Bowker et al., 2013; Gaitan et al., 2014; Hallett et al., 2014; Hill et al., 2012; 2014), and principal components analyses

(PCA) to data from two piñon-juniper woodland sites monitored by the Ameriflux eddy covariance network. Data from a monitored juniper savanna site were included in the analyses on the assumption that these biomes may be regional endpoints for climate driven succession in PJ woodlands (Swaty et al., 2004; Sankey and Germino 2008). We hypothesized that site wide mortality among mature piñons disrupts soil microbial community dynamics, such that the suite of significant indicator variables for soil enzyme activity becomes more complex and less predictable. We further hypothesized that responses to disturbance, vary by site in relation to edaphic characteristics.

We focused specifically on hydrolytic enzymes because in many systems the activities of the most commonly measured enzymes are highly correlated (Sinsabaugh et al., 2008, 20011, 2014, 2015, Sinsabaugh and Shah 2011). Large scale meta-analyses have shown that the activity of  $\beta$ -glucosidase, an enzyme involved in the degradation of cellulose and other beta-linked glucans, scales linearly with the activities of  $\beta$ -N-acetylglucosaminidase and leucine aminopeptidase, which contribute to the acquisition of N (and C) from amino polysaccharides and proteins, and with alkaline (acid) phosphatase, which hydrolyzes phosphate from phosphosaccharides and phospholipids (Sinsabaugh et al., 2008; 2011). However, disturbances, including droughts, and conifer mortality, can disrupt these patterns (Burns et al, 2013; Grandy et al., 2011; Sinsabaugh and Shah 2011; Warnock et al., 2015). Extrapolated to an ecosystem scale, these disruptions may underlie observed changes in NEE,  $R_{ES}$ , and SOM dynamics (Berryman et al., 2013; Burns et al., 2013 Krofcheck et al., 2014).

## 2. Materials and Methods:

PJ woodland datasets were based on a total of 468 soil samples collected from two different PJ woodland field sites, between June 2011, and September 2011, described previously in Warnock et al. (in press). The two PJ woodland sites were separated by 3 km, and are located near Mountainair, New Mexico, USA (Berryman et al., 2013; Krofcheck et al., 2014). Both sites are part of the New Mexico Elevation Gradient (NMEG; Anderson-Teixera et al. 2011), and the Ameriflux network. The long-term annual precipitation at the study area is 372 mm (Berryman et al., 2013; Krofcheck et al., 2014). Both sites have soils characterized as lithic mollic Calciorthid, and flat terrain with less than 1% slope (Berryman et al., 2013).

At the girdled PJ woodland site (34.45N, 106.21W), 1632 adult piñon trees (>7 cm diameter at breast height, DBH) were killed by girdling in September 2009 (Krofcheck et al., 2014). Six replicate plots, with five nearest neighbor gradients within each plot were established in May, 2011. 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). All samples were collected in 2011. Six similar plots were established at the untreated reference site (34.44N, 106.24W), however, at this site there were only 3 nearest neighbor gradients, per plot: LP/LP, LP/LJ, and LJ/LJ. Soil samples were collected 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.

Juniper savanna samples were collected from a site located at (34.425°N, 105.861°W), where *Juniperus monosperma* is the only woody species present. The soils are described as Witt loam, which have been classified as mesic Ustic calciargids (NRCS soil survey). The juniper savanna site received 130.0 mm of rainfall between 1 January 2012 and 1 May 2012, and 488.4 mm rainfall between 1 June 2012 and 10 October 2012. In 2013, the rainfall totals were 32.5 mm between 1 January 2013 and 14 June 2013, and 210.1 mm from 15 June through 13 September 2013.

### *2.1 Soil sample collection*

Piñon juniper samples were collected 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 the 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 4C until analyzed (within 72h).

Juniper savanna soil samples were collected in June 2012, July 2013 and August 2013. For each sampling date, three subsamples, 0 to 10cm depth, were taken from beneath eight different juniper canopies, and seven different bare soil locations, and were

subsequently combined to generate a composite sample from each individual sampling location. After collection, all samples were placed on ice and stored in a cooler during transport to the lab. Once at the lab, samples were refrigerated at 4C until analyzed (within 72h).

## *2.2 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).

## *2.3 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  $\text{nmol g}^{-1} \text{h}^{-1}$ .

## *2.4 Soil physical and chemical analyses*

Gravimetric soil water content (SWC) and soil organic matter content (SOM) were determined for all samples by oven drying at 60C for 24 h and combusting at 550C for 3 h. Juniper savanna soil samples collected in June 2012 and August 2013 were selected for further physicochemical analyses. For these samples, bulk soil pH was measured 1:1 in deionized water. Soluble  $\text{PO}_4^{3-}$ , and  $\text{K}^+$ , were extracted from 2 g of air-dried soils using 20 mL Mehlich-3 solution (Mehlich, 1984) and analyzed using a Spectro



CirOs ICP Spectrometer. Soil mineral N availability was determined via extracting 5 g of air-dried soils with 25 mL 1M KCl. Concentrations of  $\text{NO}_3^-$ , and  $\text{NH}_4^+$  were measured with a La Chat Quick Chem 8000 flow injection analyzer. All analyses were performed by the Oklahoma State University Soil, Water and Forage Analytical Laboratory (Stillwater, Oklahoma, USA ).

### *2.5 Statistical analyses*

Data from the three sites were analyzed separately. Further, data from the juniper savanna were analyzed using both one-way and two way ANOVA techniques, in order to determine the effects of sample collection time and location on soil nutrient physicochemical properties, soil fungal biomass, and soil microbial community functioning (Table s7), as described in Warnock et al., in (prep –b). For all three sites, we performed PCA analyses using JMP version 11 (SAS Institute Inc., Carey, North Carolina, USA), and all Structural Equation Models (SEMs) were constructed using the SPSS AMOS software package (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Lastly, all stepwise linear regression models were constructed using SPSS version 22.0. For all tests, statistical significance was accepted at an alpha of 0.05.

## **3.Results:**

### *3.1 General trends from all three sites*

Each site-level PCA procedure yielded a unique plot, as well as unique loading and correlation matrices (Fig 1-3, Tables s1-s6). Stepwise linear regressions and SEM procedures also produced site-specific results (Figs 4-6, Table 1). In general, these multivariate analyses show that both the number and the strength of the significant interactions among soil physicochemical variables, e.g.  $\text{PO}_4^{3-}$ , or  $\text{NH}_4^+$ , and individual hydrolase activities are influenced by sampling location, and collection date, (e.g., Figs. 1-6, Tables s1-s6). In addition, stoichiometric relationships such as the ratios of BG:AAP and BG:AP varied by site (Table 2).

### *3.2. Results from PJ girdle:*

The PCA for the girdled PJ site explained 46.8% of the total variation with two factors. Factor 1 (27.7%,) was positively loaded by sampling date (0.86), SWC (0.50), soil pH (0.38), BG (0.63) and NAG (0.33) (Table s1) and negatively loaded by AAP activity (-0.76). Factor two (19.1%) was positively loaded by sampling location (0.25), and all four enzyme activities (AAP (0.23), AP (0.65), BG (0.65), and NAG (0.55) (Table s1). The PCA correlation matrix showed both sampling date and SWC shared correlations ranging from  $-0.52 \leq r \leq 0.51$  with all four enzymes (Table s4). Lastly, correlations among enzyme activities were -0.19 for both AAP: BG and AAP: NAG, 0.26 for AAP:AP, 0.31 for BG:NAG, and 0.17 for BG:AP (Fig 1b, Table s4).

The stepwise regression model for predicting AAP activity included six factors, the most significant being BG activity (Table 1). The model for AP activity included only AAP activity (Table 1). The models for predicting BG activity and NAG activity

included six significant components, the most significant component in each model being AAP (Table 1).

The SEMs from the girdled site included the greatest number of significant interactions linked to enzyme activities among the three field sites (Fig 1). The most prominent factors were sampling date, and the respective activities of the other hydrolases (Fig 1). Overall, the models explained between 51% (BG) and 65% (AP) of the variation (Fig 1).

### *3.3 Results from PJ control site*

The PCA for the control PJ site explained 52.2% of total variation with two factors. Factor one (39.0%) was positively loaded by sampling day (0.95), SWC (0.88), FBM (0.45), as well as AAP (0.70) and BG (0.85) (Table s2) and negatively loaded by NAG (-0.72). Factor 2 (13.2%) was most heavily loaded by sampling location (0.55), SOM (0.80);  $\text{PO}_4^{3-}$ , AAP and BG, which were negatively associated with PC2, ranging from -0.33, to -0.37 (Table s2). The PCA correlation matrix presented correlations among EEA rates for AP, AAP and BG that all ranged between 0.62 and 0.69, while all three shared negative correlations with NAG, which were  $r = -0.28$  (AAP),  $r = -0.31$  (BG), and  $r = -0.57$  (AP) (Table s5).

The stepwise linear regressions models for predicting individual EEA rates were all significant ( $P < 0.0001$ ), with  $R^2$  values ranging from 0.13 to 0.70 (Table s2). The AAP model included four factors ( $R^2 = 0.57$ ), the most significant being BG ( $R^2 = 0.48$ , Table1). The AP model included three factors ( $R^2 = 0.69$ ), with the most significant factor was sampling day (Table 1). The BG model included three factors, the most

significant factor was AAP (Table 1). Lastly, the NAG model included two factors, the strongest predictor was sampling day.

The SEMs from PJ control site also showed multiple soil and experimental factors influencing EEA (Fig 5). The NAG model only accounted for 16% of the variation in the activity, and none of the factors shown to be significant drivers of EEA rates in the other three SEMs, were significant predictors of NAG activity.

### *3.4 Juniper savanna results*

The PCA for the juniper savanna site data (Table s7), explained 47.1% of the total variation with two factors (Fig 3). Factor 1 explained 29.4% of the variation, and Factor 2 explained 17.7% (Fig 3). The model loadings for both axes were complex, and included more than 10 factors with loading values exceeding  $\pm 0.15$ , for each axis (Table s3). The PCA correlation matrix presented correlations between the soil factors SWC, SOM, soil pH, FBM, and the individual EEA rates that were frequently  $r \geq 0.1$  (Table s6). Lastly, there were strong correlations between individual enzymes, AAP: AP ( $r = 0.74$ ), AAP: NAG ( $r = 0.44$ ), and BG: NAG ( $r = 0.22$ ) (Table s6).

The stepwise linear models for predicting individual EEA rates at the juniper savanna site all included multiple significant factors (Table 1). The AAP model featured two factors, the strongest was AP (Table 1). The AP model included five factors, the strongest predictor was AAP activity (Table 1). The BG model had four factors, and was the only model from any of the three study sites that featured a soil nutrient,  $\text{NH}_4^+$ , as the first factor in the step-wise model (Table 1). Lastly, the model for predicting NAG activity included only two factors, the strongest predictor was AP activity (Table 1).

The SEMs from this site explained between 29% (NAG), and 80% (AP) of the variation in EEA (Fig. 6). As with other sites, the NAG model showed the lowest number of significant correlations between variables, while the model for AP activity showed the largest (Fig 6).

#### **4. Discussion:**

The disturbances of drought and piñon mortality significantly altered EEA, as well as the relationships among enzymes, across the three juniper dominated, or co-dominated, field sites. Significant shifts in BG: AAP, and BG: AP relationships brought about by ecosystem wide disturbance (Table 2) were accompanied by large changes in the number of significant factor(s) influencing EEA relationships as shown by three different statistical approaches, PCA, step-wise linear regression models (step-wise), and structural equation models (SEM) (Figs. 1-6, Tables 1-2, and s1-s6).

##### *4.1 Widespread piñon mortality alters soil microbial community functional behavior*

The results support our first hypothesis that widespread piñon mortality disrupts soil microbial community dynamics at the plot scale. The SEM models for PJ-G with the exception of NAG, show an increase in the number of significant relationships compared to the PJ control site (Figs. 2-3), and a decrease in the strength of each model (Figs 4 and 5). Additionally, the interaction between NAG and BG activity, a measure of the coupling of C and N acquisition, is significant in PJ-G models, but not in the PJ-C models (Fig 4c,d and 5c,d). Similar differences across models are also evident in the relationships between SWC and BG, as well as between soil  $\text{PO}_4^{3-}$  and BG (Figs 2 - 4). Further, the more complex models from PJ-G also have lower  $R^2$  values than the models

from PJ-C, with the exception of NAG (Figs 2 -3). Thus, piñon mortality has generally caused the interactions that regulate EEA rates, with the exception of NAG (Figs 1c, and 2c) to become more diffuse.

The results from the PCA and stepwise analyses support those from the SEMs (Tables, s2-s3, figs 1-2). First, the PCA plots from the PJ woodland sites are distinct with AP, NAG and BG clustering for the PJ-G site, and AP, AAP and BG clustering for the PJ-C site. Second, the correlations among variables within the PJ-C correlation matrix are generally stronger than those in the PJ-G site matrix (Tables s4, s5). The stepwise models from the girdled site include more variables than the corresponding models for the control site (Table 1), and in three of the four stepwise models, both  $R^2$  values, are lower in the girdled site models (Table 2).

Overall, this comparison suggests piñon mortality attenuates the relationships between microbial activity, soil nutrients and soil properties. Similar trends have been observed for other arid ecosystems subjected to climate driven disturbance (Warnock et al., in prep –b; Ladwig et al., 2012, 2015; Petrie et al., 2014, 2015; Thomey et al., 2011; 2014). Ultimately shifts in EEA profiles and therefore microbial community functional behaviors, may be significant enough to significantly alter ecosystem GPP rates through changes in nutrient availability and biotic interaction (Krofcheck et al., 2013; Petrie et al., 2015; Thomey et al., 2011; 2014). Such changes are already causing multiple, arid ecosystems to become less stable, i.e., more year to year variation in GPP rates (D'Odorico et al., 2014; Ruppert et al., 2014; Schlesinger et al., 1990), and less productive (Krofcheck et al., 2014; Ruppert et al., 2014; Pangle et al., 2015; Petrie et al., 2015). These reductions in primary productivity could potentially cause multiple arid,

dry-land ecosystem types, which include PJ woodland ecosystems, to transition from carbon sinks, into carbon sources (Ahlstrom et al., 2015; Anderson-Teixeira et al., 2011; Serrano-Ortiz et al., 2014).

#### *4.2 soil microbial community functional behaviors varies across all three field sites*

The results also support our second hypothesis that each site has a unique functional profile (Figs. 1-6). The foundation begins with distinct soil types, e.g., a lithic mollic Calciorthid at PJ (Berryman et al., 2013), and a mesic Ustic calciargid at juniper savanna (NRCS soil survey). Second, the juniper savanna receives more annual precipitation, than either PJ site (Anderson-Teixeira et al., 2011). Third, the PJ sites and juniper savanna differ with respect to both soil phosphate availability and SOM content, with higher values at juniper savanna (Table s7). Lastly both fungal community composition and total richness differ between PJ sites and juniper savanna (Dean et al., 2015).

Based on regional climate trends, we originally predicted that belowground processes at the girdled PJ woodland site could represent the early stages of a successional transition from a woodland to a savanna landscape (Dean et al., 2015; Swaty et al., 2004). However, numerous mechanistic differences, as shown by the statistical models (Figs 1-6, Tables 1, and s1-s6), are still evident. Edaphic factors likely lag plant responses to climate change, perhaps limiting the potential for ecosystem succession. (Berryman et al., 2013; Krofcheck et al., 2014; Warnock et al., in press). Similar lags have been shown for various soil chronosequences, especially those established after

recent (< 150 years) disturbance events (Dawoe et al., 2014; Gros et al., 2004; Liao and Boutton 2008; Souza-Alonso et al., 2015; Welc et al., 2012). In such sequences, multiple below ground processes, e.g., respiration, and N<sub>2</sub> fixation, show significantly different process rates over time periods ranging from less than 10 years (Dawoe et al., 2014; Gros et al., 2004; Souza-Alonso et al., 2015 ) to more than 100 years (Gros et al., 2004; Liao and Boutton 2008; Souza-Alonso et al., 2015). Collectively, all of these results suggesting that a new kind of post-mortality juniper savanna may be emerging at the PJ-G site, which may be distinct from native juniper savannas biomes for quite some time.

#### *4.3 Concluding remarks:*

Overall, our multivariate analyses produced results that supported hypotheses one and two, and partially supported hypothesis three. These results also illustrate the capacity for differences in both soil microbial community properties, and their functional behaviors to be preserved, despite multiple convergences in above ground ecosystem processes (Litvak et al., unpublished data; Krofecheck et al., 2014, Petrie et al., 2015; Thomey et al., 2011; 2014). Thus, a greater number of piñon mortality affected sites need to be investigated to achieve a more realistic perspective on the potential for disturbance driven transition of PJ woodlands to juniper savannas.

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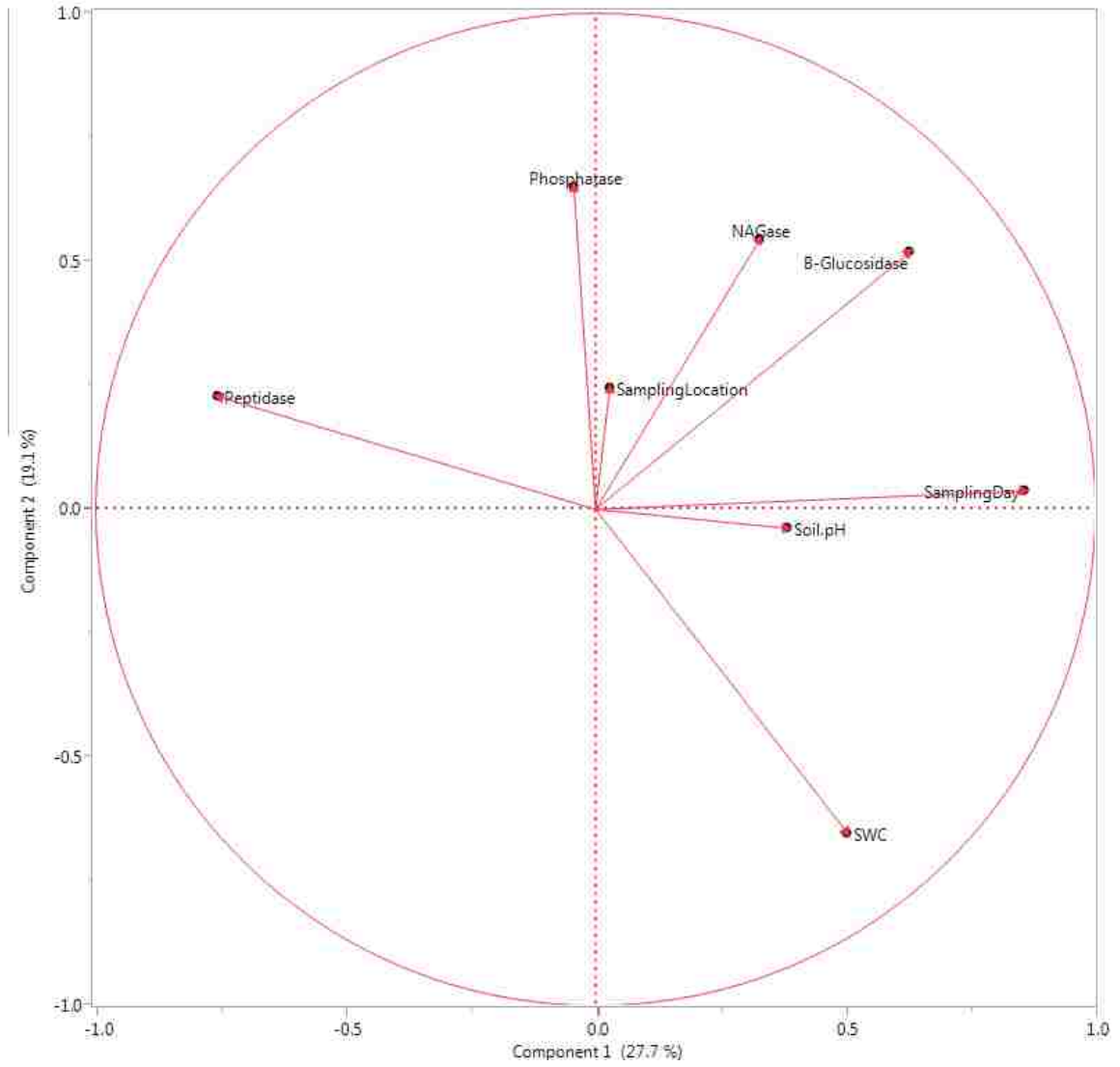
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**Table 3.1.** Stepwise ANOVA results from soil property and EEA analyses of soil samples collected at both PJ woodland field sites, as well as the juniper savannah field site.

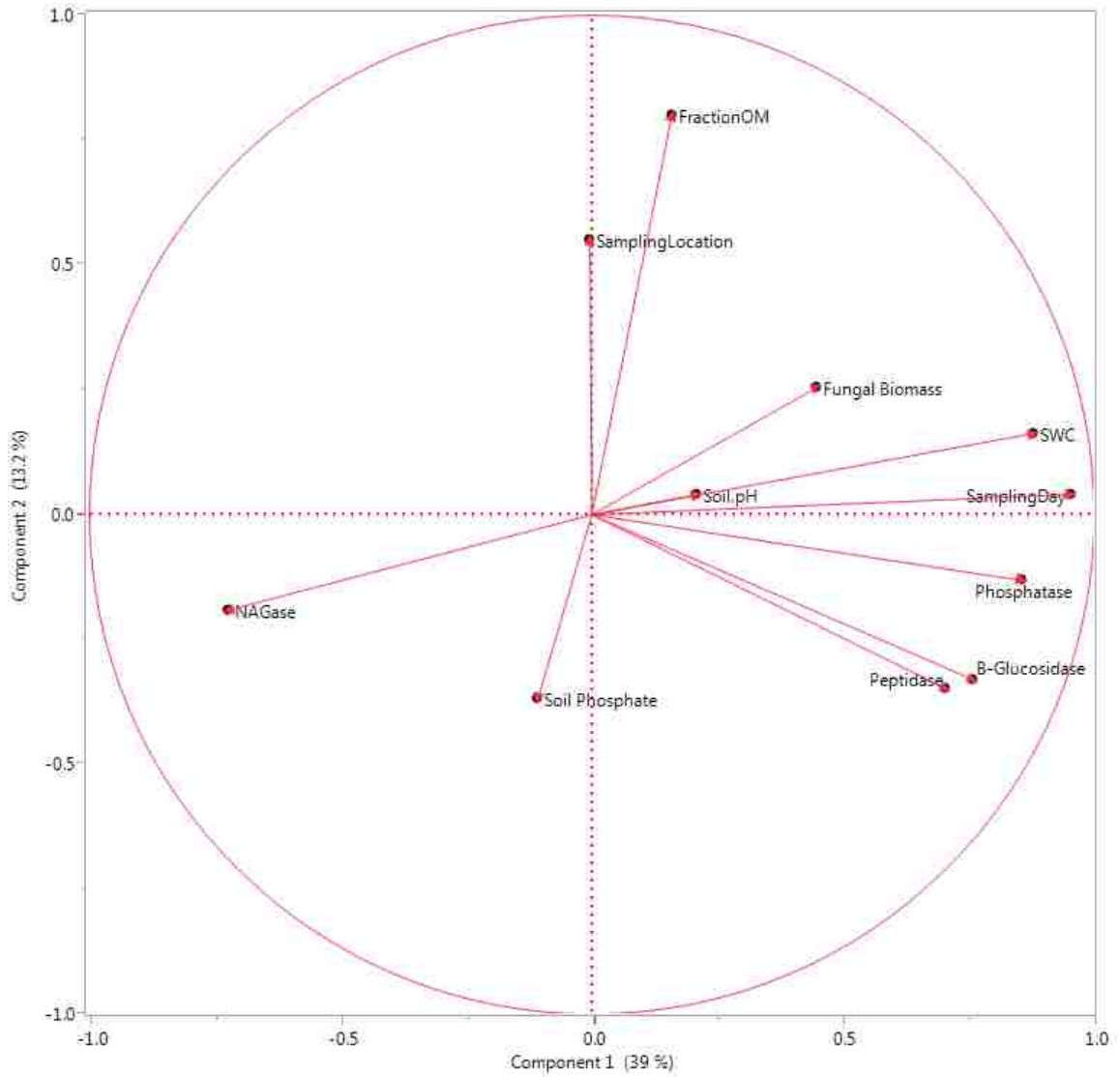
Field Site	Full step-wise model			Best single predictor		
	PJ Girdle	PJ Control	Juniper savannah	PJ Girdle	PJ Control	Juniper savannah
<b>Enzyme</b>						
<b>AAP</b>	BG, SD, NAG, AP, PO <sub>4</sub> <sup>3-</sup> , SOM: R= 0.70, R <sup>2</sup> = 0.49	BG, AP, Soil pH, SOM: R= 0.76, R <sup>2</sup> = 0.55	AP, SD: R= 0.82, R <sup>2</sup> = 0.67	BG : R= 0.40, R <sup>2</sup> = 0.16	BG : R= 0.69, R <sup>2</sup> = 0.48	AP : R= 0.73, R <sup>2</sup> = 0.54
<b>AP</b>	AP: R= 0.24, R <sup>2</sup> = 0.06	Day, AAP, FBM: R= 0.829, R <sup>2</sup> = 0.688	AAP, NAG, SWC, SOM, soil K: R= 0.901, R <sup>2</sup> = 0.812	Day: R= 0.80, R <sup>2</sup> = 0.70	Day: R= 0.79, R <sup>2</sup> = 0.69	AAP: R= 0.73, R <sup>2</sup> = 0.54
<b>BG</b>	AAP, Day, NAG, PO <sub>4</sub> <sup>3-</sup> , SWC, SOM: R= 0.72, R <sup>2</sup> = 0.52	AAP, Day, Sampling location: R= 0.77, R <sup>2</sup> = 0.59	NH <sub>4</sub> <sup>+</sup> , SD, SOM R= 0.69, R <sup>2</sup> = 0.48	AAP: R= 0.40, R <sup>2</sup> = 0.16	AAP: R= 0.69, R <sup>2</sup> = 0.48	NH <sub>4</sub> <sup>+</sup> : R= 0.33, R <sup>2</sup> = 0.11
<b>NAG</b>	BG , AAP, SWC, SOM, Location, NH <sub>4</sub> <sup>+</sup> : R= 0.46, R <sup>2</sup> = 0.21	Day, NH <sub>4</sub> <sup>+</sup> : R= 0.252, R <sup>2</sup> = 0.125	AP, AAP: R= 0.54, R <sup>2</sup> = 0.29	BG: R= 0.26, R <sup>2</sup> = 0.07	Day: R= 0.29, R <sup>2</sup> = 0.084	AP R= 0.44, R <sup>2</sup> = 0.19

**Table 3.2.** The slopes for the correlation rates of BG activity to AAP activity and of BG activity to AP activity.

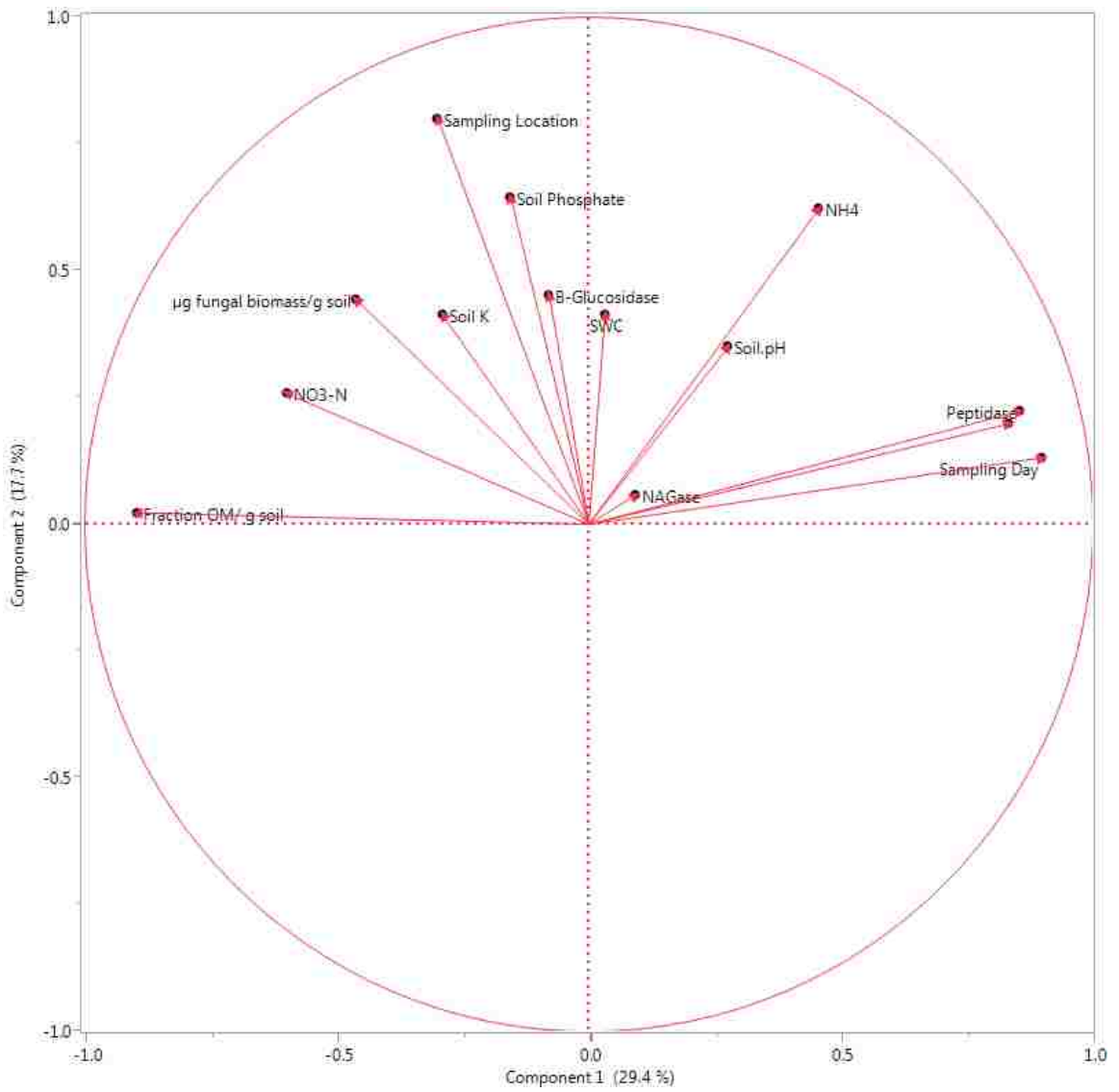
<b>Study Site:</b>	<b>BG: AAP</b>			<b>BG: AP</b>		
	<b>Slope</b>	<b>F=</b>	<b>P=</b>	<b>Slope</b>	<b>F=</b>	<b>P=</b>
<b>PJ-Control</b>	1.45x	91.6	0.0001	1.28x	65.5	0.0001
<b>PJ-Girdle</b>	-2.18x	32.2	0.0001	0.85x	39.3	0.0001
<b>J-Sav</b>	0.45x	0.36	0.55	0.38x	0.37	0.17



**Figure 3.1. PCA analyses from data collected at the girdled PJ woodland site**

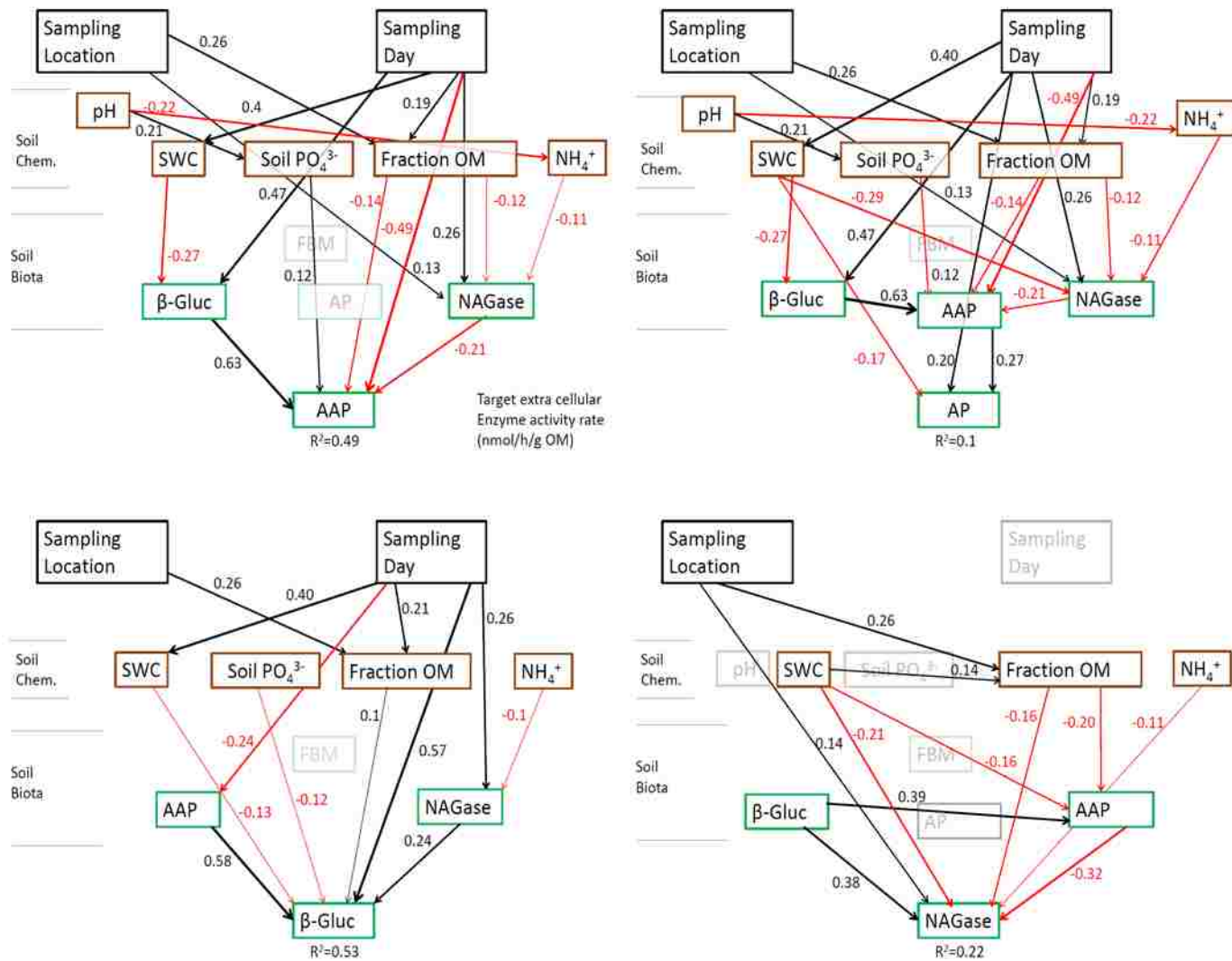


**Figure 3.2. PCA analyses from data collected at the control PJ woodland site**

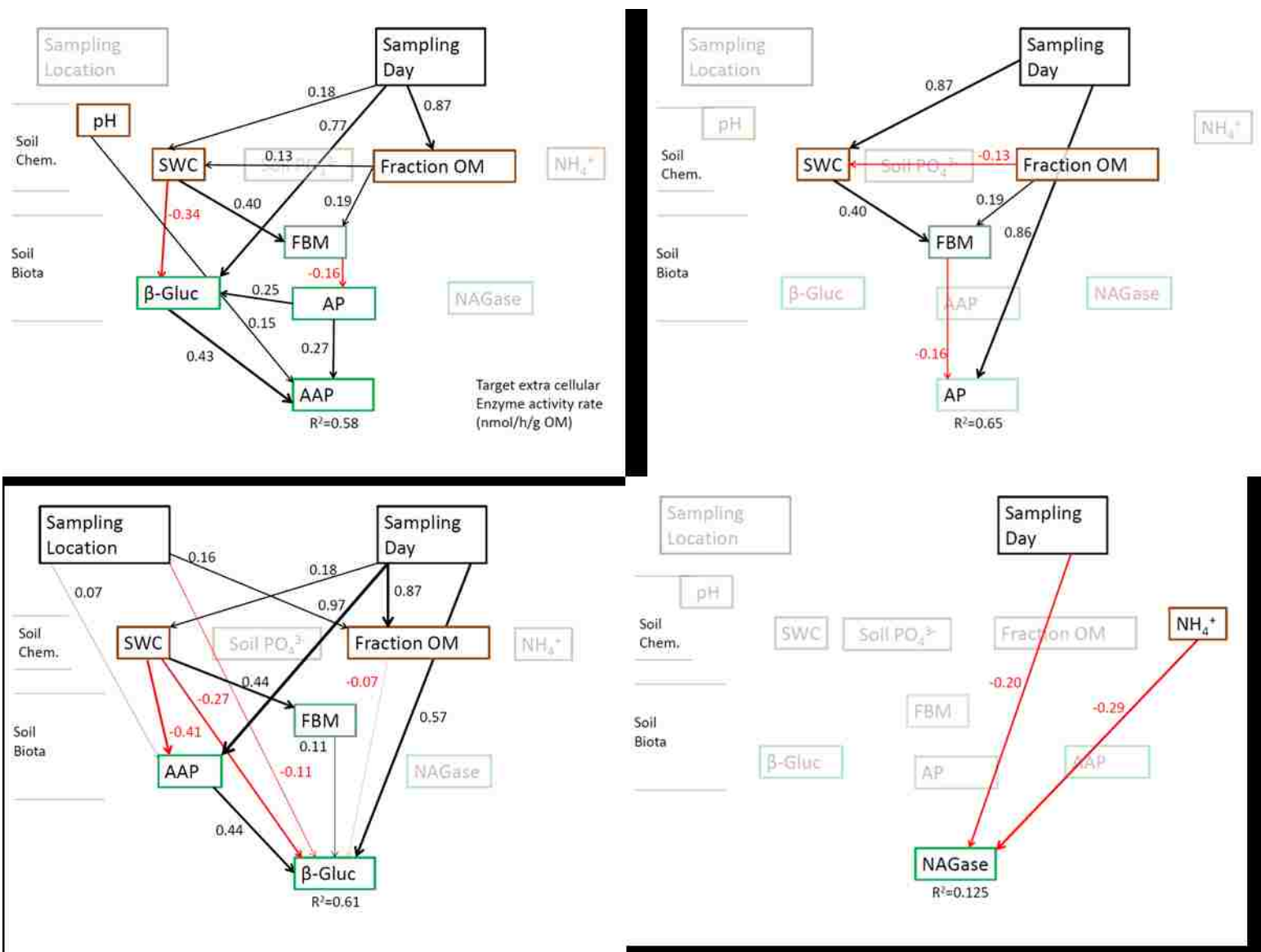


**Figure 3.3: PCA analyses from data collected at the juniper savanna site.**

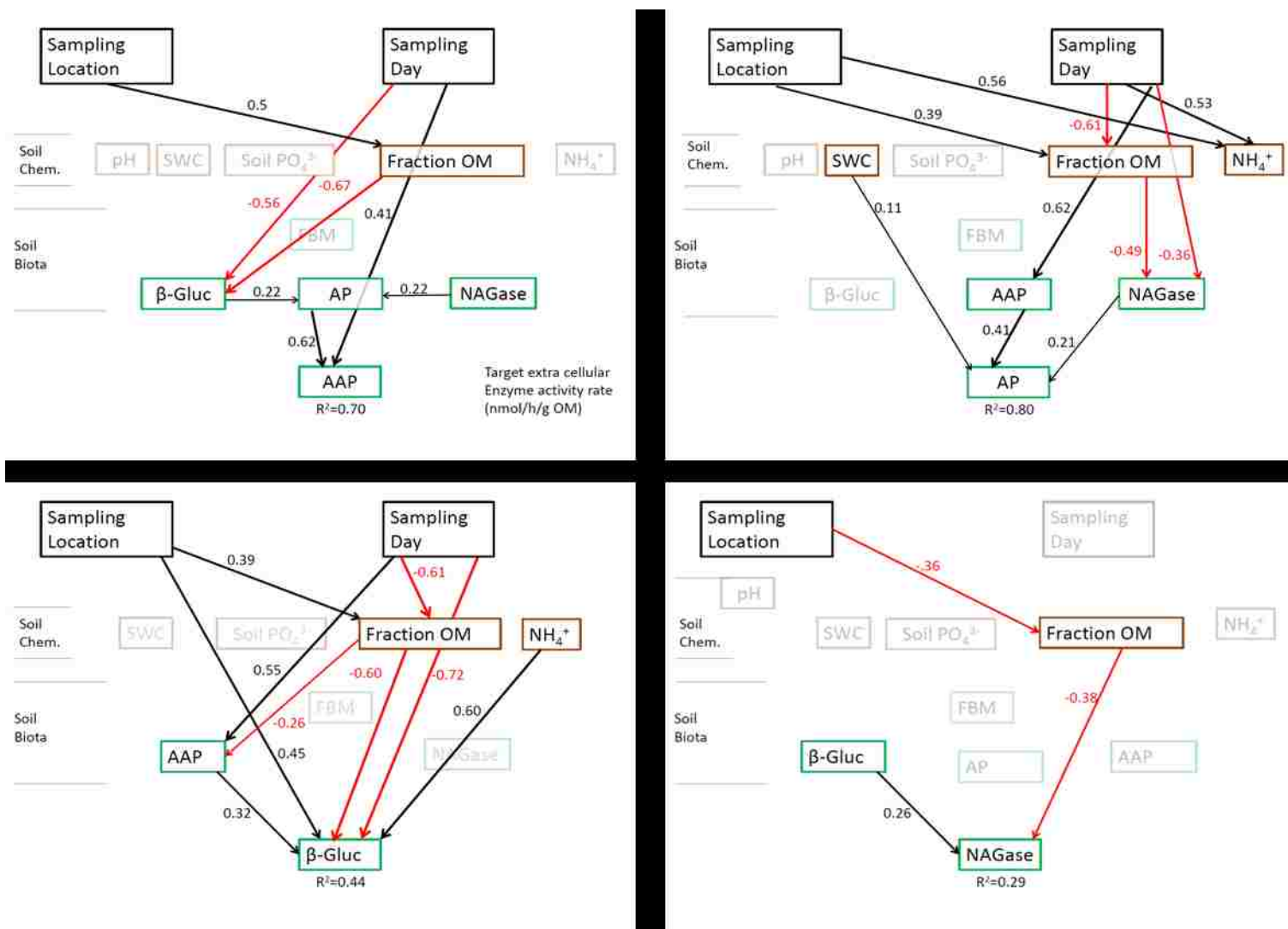




**Figure 3.4.** Structural equation models for all four hydrolytic extra cellular enzyme activity rates assessed from soil samples collected at the girdled PJ woodland site in 2011.



**Figure 3.5.** Structural equation models for all four hydrolytic extra cellular enzyme activity rates assessed from soil samples collected at the control PJ woodland site in 2011.



**Figure 3.6.** Structural equation models for all four hydrolytic extra cellular enzyme activity rates assessed from soil samples collected at the juniper savannah site in the summers of 2012 and 2013.

**Table 3.S1.** PCA loading matrix from soil property and EEA data generated soil samples collected at the girdled PJ woodland field site during the summer growing season of 2011.

<b>Variable</b>	<b>Prin1:</b>	<b>Prin2:</b>
<b>Eigenvalues</b>	2.33	1.65
<b>SamplingLocation</b>	0.0283	0.246
<b>SamplingDay</b>	0.859	0.0369
<b>SWC</b>	0.504	-0.653
<b>Soil.pH</b>	0.383	-0.0380
<b>Peptidase</b>	-0.755	0.227
<b>Phosphatase</b>	-0.0435	0.651
<b>B-Glucosidase</b>	0.629	0.518
<b>NAGase</b>	0.329	0.545

**Table 3.S2.** PCA loading matrix from soil property and EEA data generated soil samples collected at the control PJ woodland site during the summer growing season of 2011.

<b>Variable</b>	<b>Prin1:</b>	<b>Prin2:</b>
<b>Eigenvalues</b>	4.23	1.52
<b>SamplingLocation</b>	-0.00406	0.54954
<b>SamplingDay</b>	0.95304	0.0407
<b>SWC</b>	0.87774	0.16277
<b>Soil.pH</b>	0.21004	0.04079
<b>FractionOM</b>	0.15913	0.7999
<b>Fungal Biomass</b>	0.44788	0.25499
<b>Soil Phosphate</b>	-0.10694	-0.36684
<b>Peptidase</b>	0.70399	-0.34761
<b>Phosphatase</b>	0.85634	-0.13175
<b>B-Glucosidase</b>	0.75685	-0.32945
<b>NAGase</b>	-0.72332	-0.19101

**Table 3.S3.** PCA loading matrix from soil property and EEA data generated soil samples collected at the juniper savannah field site during the summer growing season of 2011.

<b>Variable:</b>	<b>Prin1:</b>	<b>Prin2:</b>
<b>Eigenvalues</b>	4.39	2.97
<b>Sampling Location</b>	-0.29984	0.80022
<b>Sampling Day</b>	0.90022	0.13176
<b>SWC</b>	0.03187	0.41322
<b>Soil.pH</b>	0.276	0.34961
<b>Soil Phosphate</b>	-0.15448	0.64351
<b>Soil K</b>	-0.29048	0.4133
<b>NH4</b>	0.45612	0.62265
<b>NO3-N</b>	-0.59961	0.25848
<b>Fungal Biomass</b>	-0.46157	0.44284
<b>Fraction OM/ g soil</b>	-0.89606	0.02269
<b>Peptidase</b>	0.85652	0.22343
<b>Phosphatase</b>	0.83321	0.19831
<b>B-Glucosidase</b>	-0.07818	0.45001
<b>NAGase</b>	0.09308	0.0583

**Table 3.S4.** PCA correlation matrix from soil property and EEA data generated soil samples collected at the girdled PJ woodland site during the summer growing

	<b>Location</b>	<b>Day</b>	<b>SWC</b>	<b>pH</b>	<b>AAP</b>	<b>AP</b>	<b>BG</b>	<b>NAGase</b>
<b>Location</b>	1.00	-0.01	-0.00	0.11	0.08	0.05	0.04	0.11
<b>Day</b>	-0.01	1.00	0.41	0.23	-0.52	0.13	0.51	0.13
<b>SWC</b>	-0.00	0.41	1.00	0.21	-0.35	-0.22	-0.05	-0.20
<b>pH</b>	0.11	0.23	0.21	1.00	-0.08	0.01	0.11	0.07
<b>AA</b>	0.08	-0.52	-0.35	-0.08	1.00	0.26	-0.29	-0.24
<b>AP</b>	0.05	0.13	-0.22	0.01	0.26	1.00	0.19	0.05
<b>BG</b>	0.04	0.51	-0.05	0.11	-0.29	0.19	1.00	0.29
<b>NAGase</b>	0.11	0.13	-0.20	0.07	-0.24	0.05	0.29	1.00

**Table 3.S5.** PCA correlation matrix from soil property and EEA data generated soil samples collected at the control PJ woodland site during the summer growing season of 2011.

	<b>Sampling Location</b>	<b>Sampling Day</b>	<b>SWC</b>	<b>Soil pH</b>	<b>SOM</b>	<b>FBM</b>	<b>Soil PO<sub>4</sub><sup>3-</sup></b>	<b>AAP</b>	<b>AP</b>	<b>BG</b>	<b>NAGase</b>
Sampling Location	1.00	0.00	0.00	0.01	0.16	-0.07	-0.28	0.05	-0.02	-0.11	-0.03
Sampling Day	0.00	1.00	0.90	0.11	0.16	0.38	-0.08	0.58	0.80	0.65	-0.73
SWC	0.00	0.90	1.00	0.09	0.26	0.36	-0.02	0.44	0.69	0.51	-0.71
Soil.pH	0.01	0.11	0.09	1.00	0.10	0.15	0.05	0.23	0.11	0.11	-0.13
Fraction OM	0.16	0.16	0.26	0.10	1.00	0.27	-0.11	-0.14	0.04	-0.09	-0.18
Fungal Biomass	-0.07	0.38	0.36	0.15	0.27	1.00	-0.05	0.19	0.19	0.34	-0.21
Soil Phosphate	-0.28	-0.08	-0.02	0.05	-0.11	-0.05	1.00	-0.10	-0.11	-0.12	-0.03
Peptidase	0.05	0.58	0.44	0.23	-0.14	0.19	-0.10	1.00	0.62	0.69	-0.28
Phosphatase	-0.02	0.80	0.69	0.11	0.04	0.19	-0.11	0.62	1.00	0.63	-0.57
B-Glucosidase	-0.11	0.65	0.51	0.11	-0.09	0.34	-0.12	0.69	0.63	1.00	-0.31
NAGase	-0.03	-0.73	-0.71	-0.13	-0.18	-0.21	-0.03	-0.28	-0.57	-0.31	1.00



**Table 3.S6.** PCA correlation matrix from soil property and EEA data generated soil samples collected at the juniper savannah field site during the summer growing season of 2011.

	<b>Location</b>	<b>Day</b>	<b>SWC</b>	<b>pH</b>	<b>PO<sub>4</sub><sup>3-</sup></b>	<b>K<sup>+</sup></b>	<b>NH<sub>4</sub><sup>+</sup></b>	<b>NO<sub>3</sub><sup>-</sup></b>	<b>FBM</b>	<b>SOM</b>	<b>AAP</b>	<b>AP</b>	<b>BG</b>	<b>NAGase</b>
<b>Location</b>	1.00	-0.14	0.26	0.35	0.39	0.17	0.42	0.32	0.46	0.36	-0.12	-0.15	0.35	-0.15
<b>Day</b>	-0.14	1.00	0.04	0.41	0.00	-0.15	0.53	-0.43	-0.44	-0.76	0.77	0.67	-0.22	-0.07
<b>SWC</b>	0.26	0.04	1.00	0.13	0.18	0.03	0.13	0.22	0.20	0.00	0.21	0.16	-0.17	-0.09
<b>pH</b>	0.35	0.41	0.13	1.00	-0.03	0.16	0.10	0.16	-0.06	-0.26	0.33	0.17	-0.18	-0.21
<b>PO<sub>4</sub><sup>3-</sup></b>	0.39	0.00	0.18	-0.03	1.00	0.44	0.19	0.18	0.32	0.03	-0.03	-0.00	0.19	0.08
<b>K<sup>+</sup></b>	0.17	-0.15	0.03	0.16	0.44	1.00	-0.01	0.41	0.07	0.17	-0.08	-0.23	0.14	0.16
<b>NH<sub>4</sub><sup>+</sup></b>	0.42	0.53	0.13	0.10	0.19	-0.01	1.00	-0.22	0.03	-0.31	0.43	0.40	0.41	0.02
<b>NO<sub>3</sub><sup>-</sup></b>	0.32	-0.43	0.22	0.16	0.18	0.41	-0.22	1.00	0.09	0.61	-0.32	-0.38	-0.03	-0.12
<b>FBM</b>	0.46	-0.44	0.20	-0.06	0.32	0.07	0.03	0.09	1.00	0.41	-0.30	-0.18	0.21	0.11
<b>SOM</b>	0.36	-0.76	0.00	-0.26	0.03	0.17	-0.31	0.61	0.41	1.00	-0.67	-0.70	0.01	-0.22
<b>AA</b>	-0.12	0.77	0.21	0.33	-0.03	-0.08	0.43	-0.32	-0.30	-0.67	1.00	0.81	-0.04	0.09
<b>AP</b>	-0.15	0.67	0.16	0.17	-0.00	-0.23	0.40	-0.38	-0.18	-0.70	0.81	1.00	0.11	0.18
<b>BG</b>	0.35	-0.22	-0.17	-0.18	0.19	0.14	0.41	-0.03	0.21	0.01	-0.04	0.11	1.00	0.29
<b>NAGase</b>	-0.15	-0.07	-0.09	-0.21	0.08	0.16	0.02	-0.12	0.11	-0.22	0.09	0.18	0.29	1.00

**Table 3.S7.** Soil extracellular enzyme activity rates, and physicochemical properties from the juniper savannah site for all four sampling dates. Numbers in parentheses are equal to +/- 1 SD of the mean.

	<sup>2</sup> Peptidase		<sup>1</sup> Phosphatase		β-Glucosidase		<sup>1</sup> NAGase		<sup>2</sup> Oxidase		<sup>2</sup> Peroxidase	
Sampling Date	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy
June 2012	2706.4 <sup>B</sup>	2811.7 <sup>B</sup>	2152.2 <sup>B</sup>	1740.6 <sup>B</sup>	2151.0 <sup>A</sup>	1672.5 <sup>B</sup>	333.0	222.5	45.1 <sup>B</sup>	45.3 <sup>B</sup>	2949.6 <sup>A</sup>	1940.4 <sup>A</sup>
	(905.2)	556.5	587.1	427.1	532.2	477.5	169.4	115.1	81.4	49.8	691.8	570.8
July 2013	7391.3 <sup>A</sup>	6242.5 <sup>A</sup>	4316.0 <sup>A</sup>	3426.1 <sup>A</sup>	1640.6 <sup>A</sup>	1672.7 <sup>A</sup>	399.9	219.3	311.2 <sup>A</sup>	231.9 <sup>A</sup>	1353.5 <sup>B</sup>	758.1 <sup>B</sup>
	2288.8	2748.8	1349.0	1314.4	482.6	<sub>B</sub> 849.8	269.5	72.0	268.7	101.5	766.0	300.3
August 2013	9191.3 <sup>A</sup>	8758.3 <sup>A</sup>	3390.2 <sup>A</sup>	3534.8 <sup>A</sup>	682.4 <sup>B</sup>	2230.1 <sup>A</sup>	222.4	259.5	23.1 <sup>B</sup>	43.4 <sup>B</sup>	64.5 <sup>C</sup>	60.5 <sup>C</sup>
	4913.9	3078.5	1435.9	1137.2	375.0	620.0	95.4	69.5	21.9	30.8	29.0	40.5
Effect tests			F=	P=	F=	P=	F=	P=				
Location Day #	χ=29.2, P<0.0001		2.00	0.17	2.7199	0.1062	2.05	0.16	χ=18.4, P<0.0024		χ=37.3, P<0.0001	
	18.0		<0.0001		0.6125	0.5465	0.43	0.65				
	Soil moisture content		Soil organic matter content		Soil pH		Soil NO <sub>3</sub> <sup>-</sup>		Soil NH <sub>4</sub> <sup>+</sup>		Soil PO <sub>4</sub> <sup>3-</sup>	
	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Bare	Bare	Canopy	Bare	Canopy	Bare	Canopy
June 2012	0.077	0.090	0.044	0.059	7.77	8.16	7.50	16.5	2.93	2.76	73.50	89.75
	0.010	0.013	0.014	0.014	0.35	0.12	(7.12)	(10.8)A	(1.18)	(0.51)B	27.42	42.49
September 2012	0.11	0.13	0.025	0.048	No	No	No	No	No	No	No	No
	0.018	0.028	0.0062	0.019	Data	Data	Data	Data	Data	Data	Data	Data
August 2013	0.086	0.087	0.025	0.028	8.23	8.20	6.32	6.16	2.55	7.88	62.56	103.89
	0.013	0.012	0.0062	0.0039	0.035	0.0	(0.94)	(0.92)B	0.61	(1.73)A	14.23	31.79
Effect tests	F=	P=	F=	P=	F=	P=	F=	P=	F=	P=	F=	P=
Location Day #	4.445	0.0407	14.0967	0.0005	2.91	0.0076	-1.66	0.11	-3.96	0.0004	-1.67	0.1074
	23.6892	<.0001	17.1951	<0.0001	2.46	0.0214	-2.47	0.02	-4.71	<0.0001	-0.23	0.8236

## Chapter 4

### **Disturbance events differentially affect interactions among individual soil extra cellular enzymes in multiple arid and semi-arid biomes.**

Daniel D Warnock<sup>1\*</sup> Marcy E. Litvak<sup>1</sup> Robert L Sinsabaugh<sup>1</sup>

<sup>1</sup>UNM Biology Department, University of New Mexico, Albuquerque, NM, 87131, USA

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#### **Abstract**

Global climate change (GCC) and ecosystem conversions are altering key processes in multiple arid ecosystems. These processes likely include, soil organic matter turnover, soil nutrient cycling and soil carbon storage. We assessed the aggregate influences of two GCC linked disturbances, woody plant encroachment and fire, on soil microbial activities rates at contiguous semiarid shrub and grassland sites in central New Mexico. Soil samples from beneath plant canopies, and adjacent bare soils were collected from both burned and unburned grassland sites, a shrub site, and a shrub/grass ecotone site, during the dry and wet seasons of 2012 and 2013. We analyzed eleven soil physicochemical properties along with fungal biomass, and the activities of alanine aminopeptidase (AAP), alkaline phosphatase (AP),  $\beta$ -D-glucosidase (BG), and  $\beta$ -N-acetyl glucosaminidase (NAG), phenol oxidase (POX) and peroxidase (PER). Enzyme activities varied with plant functional type, e.g. grass vs. shrub, sampling location, disturbance, and sampling time. Our univariate and multivariate analyses procedures demonstrated the capacity of environmental perturbations to significantly disrupt pre-

established relationships such that each disturbed site displayed unique relationships between soil enzymes and soil variables, in comparison to the intact sites. Results from multivariate analyses suggested that disturbances caused increased integration among hydrolytic enzyme activities, while weakening relationships with most soil variables. Ultimately disturbances alter microbial community organization and behaviors in ways that are unique for each site.

## **1. Introduction:**

Global climate change (GCC) related phenomena, such as drought, and land cover change, are significantly altering critically important ecosystem processes throughout the western USA (Anderson-Teixeira et al., 2011; Berryman et al., 2013; Krofcheck et al., 2014; Petrie et al., 2015; Thomey et al., 2011; Warnock et al., 2015), including net primary productivity (NPP), and ecosystem respiration ( $R_{ES}$ ) (Ahlstrom et al., 2015; Anderson-Teixeira et al., 2011; Berryman et al., 2013; Krofcheck et al., 2014). If these trends continue, multiple arid ecosystems are expected to shift from net carbon sinks to net carbon sources (Ahlstrom et al., 2015; Anderson-Teixeira et al., 2011; Berryman et al., 2013; Krofcheck et al., 2014; Petrie et al., 2015).

Even though the number of studies focusing on land-cover changes resulting from fire and drought is increasing, the effects of these disturbances on soil microbial communities, and their associated functionalities, remains understudied (Berryman et al., 2013). Recent work suggests that arid, and semiarid systems are especially susceptible to such processes (Ahlstrom et al., 2015; Anderson-Teixeira et al., 2011; Petrie et al., 2015; Thomey et al., 2011; 2014). Because these systems cover approximately 40% of earth's

terrestrial surfaces (Lal 2004; Shen et al., 2008) the conversion of sinks to sources could accelerate the rate of atmospheric GHG accumulation (Anderson Teixeira et al., 2011; Krofcheck et al., 2014; LaQuere et al., 2009; Petrie et al., 2015).

Recent studies conducted within the Sevilleta National Wildlife refuge in New Mexico, USA (Ladwig et al., 2012, 2014, 2015; Petrie et al., 2015; Thomey et al., 2011; 2014; Vargas et al., 2012), illustrate the potential for multiple GCC induced events, e.g. persistent droughts, tree mortality and woody plant encroachment to cause large portions of arid and semiarid ecosystems to transition from net C sinks, to net C sources (Ahlstrom et al., 2015; Anderson Teixeira et al., 2011; Berryman et al. 2013; Breshears et al., 2009; Drake et al., 2013; Floyd et al. 2009; 2015; Petrie et al., 2015, Krofcheck et al., 2014). Disturbance events have affected above ground ecosystem processes such as  $R_{ES}$ , and GPP, as well as key soil ecosystem processes such as soil organic matter (SOM) turnover, soil nutrient cycling, and soil respiration ( $R_S$ ) (Collins et al., 2014; Petrie et al., 2015; 2015; Thomey et al., 2011; 2014). What are less well understood however, are the drivers responsible for converting C sequestered in stable SOM pools, into more labile and volatile forms, which ultimately contribute to increased GHG emissions, (Ahlstrom et al., 2015; Berryman et al., 2013; Collins et al., 2014; Petrie et al., 2015; Pointing and Belknap 2012).

To assess how GCC related disturbances such as drought, fire and shrub encroachment are altering the coupling between soil microbial community activity and ecosystem GPP and ecosystem respiration, we sampled four sites, burned and unburned grassland, desert shrubland, and a shrub-grass ecotone over two successive growing seasons. Samples were collected from beneath the canopies of the dominant plants and

from the interspaces, often covered with biocrust, between plants. The field sites are part of the New Mexico elevation gradient / Ameriflux network of eddy flux covariance tower sites previously described in Anderson Teixeira et al. (2011), Petrie et al., (2015), and Thomey et al. (2011; 2014), as well as two additional sites disturbed by fire and desert shrub encroachment (Thomey et al., 2014; Vargas et al., 2012).

We hypothesized that individual hydrolytic and oxidative enzyme activities would be greatest beneath plant canopy, with the exception of alanine aminopeptidase (AAP), which is high in biocrust soils because such soils are enriched with photosynthetic organisms (Green et al., 2008; Heinze et al., 2006; Pointing and Belknap 2012; Stursova et al., 2006; Sinsabaugh et al., 2015). More generally, we hypothesized that enzyme activities would be more closely correlated with fungal biomass, soil water content and nutrient concentrations in less disturbed sites, and less closely integrated in sites where disturbances are altering steady state relationships and driving ecosystem transitions. Within this context, there should be characteristic biome-specific patterns.

Prior meta-analyses have shown that the activity of  $\beta$ -glucosidase (BG), an enzyme involved in the degradation of cellulose and other beta-linked glucans, scales linearly with the activities of  $\beta$ -N-acetylglucosaminidase (NAG), and leucine aminopeptidase (AAP), which contribute to the acquisition of N (and C) from amino polysaccharides and proteins, and with alkaline (acid) phosphatase, which hydrolyzes phosphate from phosphosaccharides and phospholipids (Sinsabaugh et al., 2008, 20011, 2014, 2015, Sinsabaugh and Shah 2011). However, local trends may differ from this global pattern. We included phenol oxidase and peroxidase activities because of the role these enzymes play with respect to the mining of humic materials to access chemically

protected C, N and P and thereby moving protected carbon into more volatile pools (Grandy et al., 2008; Sinsabaugh and Shah 2011; Sinsabaugh et al., 2012).

## **2. Materials and Methods:**

This study was conducted within the fetch of four eddy covariance tower sites, New Mexico, USA, which are part of the Ameriflux network (Anderson-Teixeira et al., 2011). We established our plots in June 2012 within the four following sites, a desert shrub site dominated by creosote (*Larrea tridentata*) (34.335° N, 106.744° W), a shrub grass ecotone (34.337°N, 106.732°W), an unburned grassland, dominated by black grama (*Bouteloua eriopoda*) (34.355° N, 106.675° W), and a second black grama dominated grassland that had burned in 2009 (34.362° N, 106.702° W).

The soils at both grassland sites and the ecotone sites are described as Turney loams, and are formally classified as thermic Typic Haplocalcids (NRCS soil survey). The soils at the desert shrub site are described as Nickel-Caliza, which are gravely, loamy soils (NRCS soil survey) that are formally classified as thermic typic Calciorhtids (NRCS soil survey). All sites field sites are located within the boundaries of the Sevilleta National Wildlife Refuge (SNWR).

### *2.1 Soil sampling*

For each of the our four field sites, soil samples were collected from sampling locations that were either beneath the plant canopy, or the adjacent bare soils. We collected samples from all four sites in June 2012, September 2012, July 2013 and August 2013. For each sampling date, three subsamples, 0 to 10 cm depth, were taken from each beneath each plant canopy location, and each bare soil location, and subsequently combined to generate a composite sample from each individual sampling

location. Eight beneath canopy and seven bare soil samples were collected from both grassland sites, and the desert shrub site. A total of six samples were collected from all three locations, i.e., beneath shrub canopy, grass canopy, and from bare soil, at the shrub-grass ecotone site. All composite samples were analyzed separately. After collection, all samples were then placed on ice and stored in a cooler during transport to the lab. Once at the lab, samples were refrigerated at 4C until analyzed (within 72h).

### *2.2 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 5.0  $\mu\text{g}$  ergosterol per mg fungal biomass (Antibus and Sinsabaugh 1993, Gessner and Newell 2002).

### *2.3 Extracellular enzyme assays*

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

### *2.4 Soil physical and chemical analyses*

Gravimetric soil water content (SWC) ( $\text{mg H}_2\text{O}/ \text{g soil}$ ) and soil organic matter content (SOM) ( $\text{mg OM}/ \text{g soil}$ ) were determined for all samples by oven drying at 60C for 24 h and combusting at 500C for 3 h. Samples collected in June 2012, as well as July and August from 2013 were selected for further physicochemical analyses. For these



samples, bulk soil pH was measured 1:1 in deionized water. Soluble  $\text{PO}_4^{3-}$ ,  $\text{K}^+$ , and were extracted from 2 g of air-dried soils using 20 mL Mehlich-3 solution (Mehlich, 1984) and analyzed using a Spectro CirOs ICP Spectrometer. Soil mineral N availability was determined via extracting 5 g of air-dried soils with 25 mL 1M KCl. Concentrations of  $\text{NO}_3^-$ , and  $\text{NH}_4^+$  were subsequently measured with a La Chat Quick Chem 8000 flow injection analyzer. All available nutrient ion concentrations were measured in parts per million (ppm). All analyses were performed by the Oklahoma State University Soil, Water and Forage Analytical Laboratory (Stillwater, Oklahoma, USA ).

### *2.5 Statistical analyses*

Principal components analyses (PCA) were used to compare correlation patterns within and across sites. Two-way ANOVAs were performed to test for interactions between sampling location, and sampling date.. When the data fulfilled assumptions of normality, one-way ANOVAs were also used to evaluate both site and seasonal effects, for individual enzymes. When normality assumptions were not met, a Kruskal- Wallis non-parametric, one-way ANOVA, along with a Wilcoxon one-way test for multiple comparisons analyses, were performed instead.

For all tests, statistical significance was accepted at an alpha of 0.05. All ANOVA, and PCA analyses were carried out using JMP version 11.2 (SAS Institute Inc., Carey, North Carolina, USA). All step-wise ANOVA procedures were performed using IBM SPSS v22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

### 3. Results:

#### *3.1 Multivariate analyses of pooled data*

ANOVA and *post hoc* comparisons showed that fungal biomass, soil physicochemical properties, and EEA significantly varied by sampling date and site (Table s1-s6). The step-wise regression models provided evidence that the principal variables associated with any specific EEA, was the potential activity of the five other extracellular enzymes (Table 1).

For the data set as a whole, PCA Factor 1 explained 24.2% of the variability with Factor 2 accounting for 17.0%. Factor one was positively loaded by all soil physicochemical variables, with the lone exception being fungal biomass (FBM) and negatively loaded by all six enzyme activities, and (Table 2). Factor two was positively loaded by all six EEA rates, and a subset of edaphic variables that excluded SWC and SOM (Table 2). The PCA correlation matrix indicated that rates of AP, BG and NAG shared strong mutual correlations ( $68.0\% \leq r \leq 86.8$ ), while AAP was only strongly correlated with PER,  $r = 55.4\%$  (Table 2). However within this general pattern, each site presented a unique pattern of functional relationships (Figs 1-6, Tables s6-s13).

#### *3.2 Results from the grassland site*

Results from both 2012 and 2013 show soil fungal biomass under grass canopies was consistently higher than fungal biomass in bare soils (Table s1). Potential AAP activity was also higher in bare soil samples, in both June 2012, and in August 2013 (Table S2). In contrast to AAP, BG and NAG activities from June 2012 and July 2013 were higher under canopies, with the NAG results from September 2012, showing the

same trend (Table s2). Lastly, three soil physicochemical parameters showed significant relationships with either sampling time, soil pH, or sampling day,  $\text{NO}_3^-$ , and SWC (Table S2).

All six stepwise linear regression models yielded significant results (Table 1). In the models constructed to predict the activity rates of AP, BG, and NAG, the single strongest predictor, was the rate of another hydrolytic EE (Table 1). In contrast, the single strongest predictor for the potential activity rates of both POX, and PER, was sampling day (SD) (Table 1). The most complex models were generated for predicting AP, BG and phenol oxidase activity, with 3 factors each, while the simplest model, consisting of one factor, was generated for predicting NAG activity (Table 1).

The PCA analyses of the grassland site data set explained 45.5% of total variation (Fig. 1). Factor 1 explained 22.3% of the variability with Factor 2 accounting for 20.2%. Factor one was positively loaded by all variables, except soil K,  $\text{NO}_3^-$ , and FBM. Factor two was most positively loaded by  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , AP, BG, NAG and PER, and most negatively by  $\text{PO}_4^{3-}$ , SOM, AAP and oxidase (Table 1). The rates of AP, BG and NAG all showed strong correlations with each other,  $45.0\% \leq r \leq 86.1\%$ , while AAP only showed one strong correlation, which was with AP, where  $r = 61.8\%$  (Table s2). Correlations between EEA rates and soil variables showed values of  $-49.6\% \leq r \leq 58.7\%$  (Table s2).

### *3.2 Results from burned grassland site*

All hydrolase activities were significantly greater in samples collected beneath grass canopies. All four enzymes showed this trend especially well during September

2012 (Table s3). For the oxidative enzymes, the highest rates were measured in soils collected in July 2013, with no significant variation across sampling locations (Table s3). Lastly, all soil physicochemical parameter values, except SWC, and  $\text{PO}_4^{3-}$ , showed significant interactions with sampling location or sampling day (Table S3).

All six stepwise regression models yielded significant results, with the single strongest factor for all observed enzyme activity rates being the activity rate of another individual enzyme activity (Table 2). Three of the four models constructed to predict hydrolytic EEA included three factors. The models generated for predicting the activity rates of the oxidative enzymes, included the activity rate of the other oxidative enzyme (Table 1).

The PCA Factor 1 explained 33.5% of the variance for the burned grassland data and factor 2 accounted for 15.1%. Factor one was positively loaded by soil K,  $\text{NH}_4^+$ , FBM, SOM, AAP, AP and BG, and negatively loaded with cover type, pH,  $\text{PO}_4^{3-}$ , FBM, NAG, POX and PER (Table 2). Factor two was positively loaded with sampling day, SWC,  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$ , and FBM, and strongly negatively loaded by  $\text{NO}_3^-$ , NAG, POX and PER (Table 2). All six EEAs were strongly correlated with soil mineral N availabilities,  $-38.3\% \leq r \leq 68.0\%$  (Fig 3, table s?) Additionally, the PCA plot showed two tight groupings of EEA rates (Fig. 3). All four hydrolytic EEAs grouped together along axis 1, and share correlations exceeding,  $r = 74.5\%$ , while both oxidative EEAs grouped together in quadrant three and shared an r value of  $r = 66.0\%$ .

### *3.3 Shrub-grass ecotone*

Significant interactions were present between sampling date and soil water content, soil organic matter content, and soil pH, while soil  $\text{PO}_4^{3-}$  showed a significant interaction with sampling location (Table s3). AAP activities, samples beneath bare soils exceeded those from beneath either plant canopy type in June 2012, and again in August 2013 (Table S4). In contrast, NAG and POX, activities were greater beneath plant canopies (Table S4). For PER, activity peaked in June 2012, without showing any differences across sampling locations, or sampling day, after June 2012 (Table s4).

All six stepwise regression models yielded significant results, with the single strongest factor being the activity of another enzyme activity (Table 1). The most complex model was generated for NAG activity (3 factors); while the simplest model (1 factor) was generated for AP activity (Table 1). The most complex models was generated for PER (5 factors) and NAG (3 factors), while the single variable models were generated for AP and POX.

The PCA factor one explained 34.1% of the variation for the grass canopy and bare soil samples and factor two accounted for 27.1% (Fig 4). Factor one was positively loaded by by all six EEA rates and  $\text{NO}_3^-$  (Table 2). Factor two was positively loaded with by all soil variables, and PER rates, and negatively loaded with SOM, SWC, and POX activity (Table 2). From the beneath grass canopy and bare soils PCA, the correlation matrix shows an enzyme activity profile similar to the whole ecotone site profile, as the relationships among NAGase, BG, phosphatase and oxidase all ranged from  $63\% \leq r \leq 92.0\%$ , while for peptidase and POX,  $r = 79.0\%$  (Fig. 4, Table s11).

The PCA analysis of the shrub canopy and bare soil samples from the shrub-grass ecotone site accounted for 49.5% of the variability in this data sub-set, with factor one accounting for 27.7% of the variability, and factor two explaining 22.3% (Fig 5). Factor one was positively loaded by PER activity rates, and all soil variables except SOM, and  $\text{NO}_3^-$ , which both negatively loaded onto factor one, along with the activity of AAP, AP, BG, NAG and POX (Table 2). Finally, from the shrub and bare location PCA both the correlation matrix (Table s12), and the plot itself (Fig 5), show an EEA profile where NAGase, BG, AP, and peroxidase, all grouped together, and produced  $32.0\% \leq r \leq 54.0\%$ , while oxidase correlations with these three enzymes flipped signs and became weaker ( $-7.0\% \geq r \geq -32.0\%$ ) (Table s12).

### *3.4 Desert shrub*

The results from the desert shrub land show multiple significant interactions between the location, sampling date, for both EEA and physicochemical variables (Table S6). AAP was higher in bare soils than under shrub canopies, in June 2012, September 2012, and July 2013 (Table S6). In contrast BG, and NAG were higher in soils collected beneath shrub canopies than from bare soils (Table S6).

All six stepwise regression models yielded significant results, with the single strongest factor for five of the six enzyme activities being the activity rate of other enzymes (Table 1). The first factor in the AAP model was soil organic matter (SOM) content (Table 1). The most complex models were generated for AP and POX activity rates (4+ factors each) (Table 1).

The PCA factors one and two explained 45.6% of the total variation, with factor one explaining 29.4%, and factor two accounting for 16.2% (Fig 6). Factor one is positively loaded by all factors except SWC, SOM and canopy cover (Table 2). Factor two was positively loaded by all factors except pH,  $\text{PO}_4^{3-}$ , SOM, NAG and POX activity (Table 2). The PCA correlation matrix indicates that five of the six enzymes showed strong relationships that ranged from  $31\% \leq r \leq$  to  $91.0\%$  (Table s13, Fig 6). In contrast AAP showed negative associations with the activities of the other five enzymes that ranged from  $-21.0\% \leq r \leq -10.1\%$ , (Table s13, Fig 6). The potential EEA rates for AAP, AP, BG, NAG and POX all shared relationships that spanned a total range of,  $-53.0\% \leq r \leq 58.4\%$ , with soil  $\text{NH}_4^+$  availability, FBM, SOM, and sampling day (Table s13).

#### **4. Discussion:**

These results provide a general overview of the influence of seasonal rainfall patterns and disturbances on soil processes on the scale of individual plant rhizospheres, as well as across whole plots that feature a variety of plant functional types, and different kinds of ecosystem disturbances. Our results build upon others which showed that disturbance events could have significant and potentially adverse effects on both soil biogeochemical cycles, and soil SOM turnover rates (Acosta- Martinez et al., 2014a, b; Petrie et al., 2015) and possibly plant productivity (Acosta- Martinez et al., 2014a, b; Petrie et al., 2015; Thomey et al, 20115).

##### *4.1 Influences of different plant canopy cover types on individual EEA rates*

The EEA results largely supported our first hypothesis, with the exception of AAP from the burned grassland (Fig 3, and Table s3). First, in September 2012, AAP

was 168% higher under canopies than in bare soils (Table s3). Second, PCA analyses yielded correlations between AAP activity and canopy cover of (-) 48.6% at burned, and (+) 30.2% at unburned (Figs 2, 3, and Tables s8, s9). In contrast, the AAP results from the three unburned sites largely confirm our first hypothesis. At these sites, AAP rates were higher in bare soils relative to canopies, twice at ecotone, twice at unburned grassland, and three times at desert shrub (Tables s2, s4, s6). Further supporting this first hypothesis, were the results from our analyses of AP, BG, NAG, POX, and PER rates, which all showed multiple, positive PCA correlations between enzyme activity, and canopy cover (Figs 2-6).

Initially, we expected to see higher AAP rates in interspace soils, because of biocrusts (Collins et al., 2014; Green et al., 2008; Pointing and Belknap 2012; Sinsabaugh et al., 2015). A large fraction of the production from the cyanobacteria in these crusts is protein contributing to an SOM pool enriched in substrate compatible with AAP (Hofmockel et al., 2010; Pointing and Belknap 2012; Sinsabaugh et al., 2015). The 2009 fire at Sevilleta, likely caused significant damage to soil biocrusts (Miller et al., 2014; Vargas et al., 2012), potentially explaining the why AAP activity was greater beneath the plant canopy at these sites.

For AP, BG, NAG POX, and PER, we predicted that activity would generally be greater in soils collected beneath plant canopies relative to interspaces because results presented in Aguiar and Sala (1998); Kieft et al., (1998); Allington et al., (2014), and those discussed in Collins et al., (2014). Each fertility island supplies their root associated soil microbial community with organic matter rich in plant cell wall material (Aguiar and Sala 1999; Kieft et al., 1998; Allington et al., 2014; Green et al., 2008).



#### *4.2 Disturbance events disrupt overall EEA dynamics across individual field sites*

PCA analyses, and step wise regressions largely support our second hypothesis. First at the intact sites 17 of 24 possible PCA correlations between individual EEA rates, SWC, and FBM were positive ( $11.0\% > r > 45.0\%$ ), while at the disturbed sites, 23 of 24 analogous correlations were both negative, and more variable ( $-2.8\% < r < -71.8\%$ ) (Figs 2, 6, Tables s8, s10). With respect to soil nutrient concentrations and enzymes, only the relationships between EEA rates and  $\text{NO}_3^-$  concentrations at burned grassland refuted this hypothesis. At burned grassland, all six EEA and  $\text{NO}_3^-$  relationships were positive ( $54.0\% < r < 68.0\%$ ), while at the grassland all six were negative ( $-10.0\% < r < 46.0\%$ ) (Figs 2, 6, and Tables s8-s9), indicating a strengthening in relationships between  $\text{NO}_3^-$  and EEA after fire. In contrast the other mineral nutrient and EEA results largely supported this hypothesis (Figs 1-6, Tables s2-s13). Finally, our analyses illustrate the ability of both fires and shrub encroachment to each yield unique, biome-specific relationships between EEA expression profiles and various soil properties (as discussed below) (Figs 2-5, Tables 1, 2), without decreasing our over-all ability to explain the variability within these data (Tables 1, 2, and Figs 2-6).

The 2009 grassland fire tightened the correlation among hydrolytic activities while disrupting their associations with oxidative activities (Figs 2, 3 and Tables 2, s8 and s9). These findings suggest that the fire mineralized nutrients and reduced recalcitrant carbon. Soil pH also increased, which could also influence the absolute and relative activities, as well as microbial community composition (Baath et al., 1995; Hamman et al., 2007; Zhang et al. 2007), plant productivity (Vargas et al., 2012), and the chemical composition of the extant SOM pool (Vargas et al., 2012). These alterations in

soil ecosystem properties may have also disrupted the process of mining humic materials for P, via the reduction of both AP and POX rates at the burned grassland site (Tables s2, s3) possibly disrupting the combined mineralization activities of both enzymes (Figs 2, 3) (Sinsabaugh and Shah 2011; Sinsabaugh et al., 2012). Thus, as a result of this fire, the disturbed RAM communities obtain their C, N and P largely through the hydrolysis of the more labile components within the extant SOM pool (Fig 3).

The grass canopy associated samples collected from the shrub-grass ecotone site illustrate that one of consequences of the creosote invasion into arid grasslands might be a tightening of the couplings among the activity rates of the hydrolytic and oxidative enzymes involved in humus mining, through increases in PER (Grandy et al., 2008; Sinsabaugh and Shah 2011; Sinsabaugh et al., 2012) (Figs 2 – 5, Tables 1, s2-s5). This stronger integration among enzymes may be a side-effect of exposure to Creosote (*Larrea tridentata*). Creosote exposure is potentially subjecting native grass root systems, and their RAM associates, to both creosote root exudates (Elakovich and Stevens 1985; Mahall and Callaway 1991; 1992; Kieft et al., 1998; Hierro and Callaway, 2003, Schenk et al., 1999), and foliage litters (Aguilar and Sala 1999; Allington et al., 2014; Kieft et al., 1998), with differing chemical profiles than those originating from their native competitors. These potential alterations in SOM chemistry may be promote synergistic interaction stronger cooperation among enzymes (Figs 4-6), driven by increased PER rates (Table s4), which could potentially increase soil C losses at ecotone (Thomey et al., 2014).

Results from our analyses of the shrub canopy associated samples at the ecotone also showed evidence of a response to disturbance (Table 1, Figs. 5 and 6). First, EEA

vectors in the shrub associated PCA from the ecotone were more dispersed than the EEA vectors at desert shrub (Figs. 5 and 6). Additionally the step-wise regression results featured more models with an EEA as their first and most predictive component (Table 1), with three of the models, i.e., AP, BG, and NAG showing  $R^2$  values that are approximately 2x larger than those from the ecotone shrub and bare sub-set (Table 1). Thus, in contrast to the responses exhibited by the disturbed grass associated RAM communities at ecotone, the cohesiveness of the shrub associated EEA profiles, including the relationships indicative of humus mining, e.g., BG, AP NAG and Ox activity (Fig 5), appear to have been relaxed in response to the extended, 150 year expansion of creosote shrubs into terrain previously dominated by grasses (Archer et al, 1995; D'odorico et al., 2013; Van Auken 2000), perhaps indicating competitive release of creosote.

## **5. Conclusions:**

Collectively, our results show the unique nature of the responses exhibited by different microbial communities to various kinds of environmental perturbation. These differences may be associated with both the functional type/ species identity of their host plant (Hernandez and Hobbie 2010; Keift et al., 1998; Petrie et al., 2015; Thomey et al., 2011), as well as the particular variety of disturbance each individual plant community is facing (Ladwig et al., 2012; 2015; Hierro and Callaway, 2003; Mahall and Callaway 1991; 1992, Vargas et al., 2012). Further, our results make a meaningful contribution toward identifying the belowground mechanisms by which chronic droughts, fires, widespread plant mortality, and woody plant encroachment exert alter arid ecosystem processes, and potentially direct successional transitions in ecosystem state (Berryman et al., 2013; Krofcheck et al., 2014; Petrie et al., 2015; Anderson-Teixeira et al., 2011).

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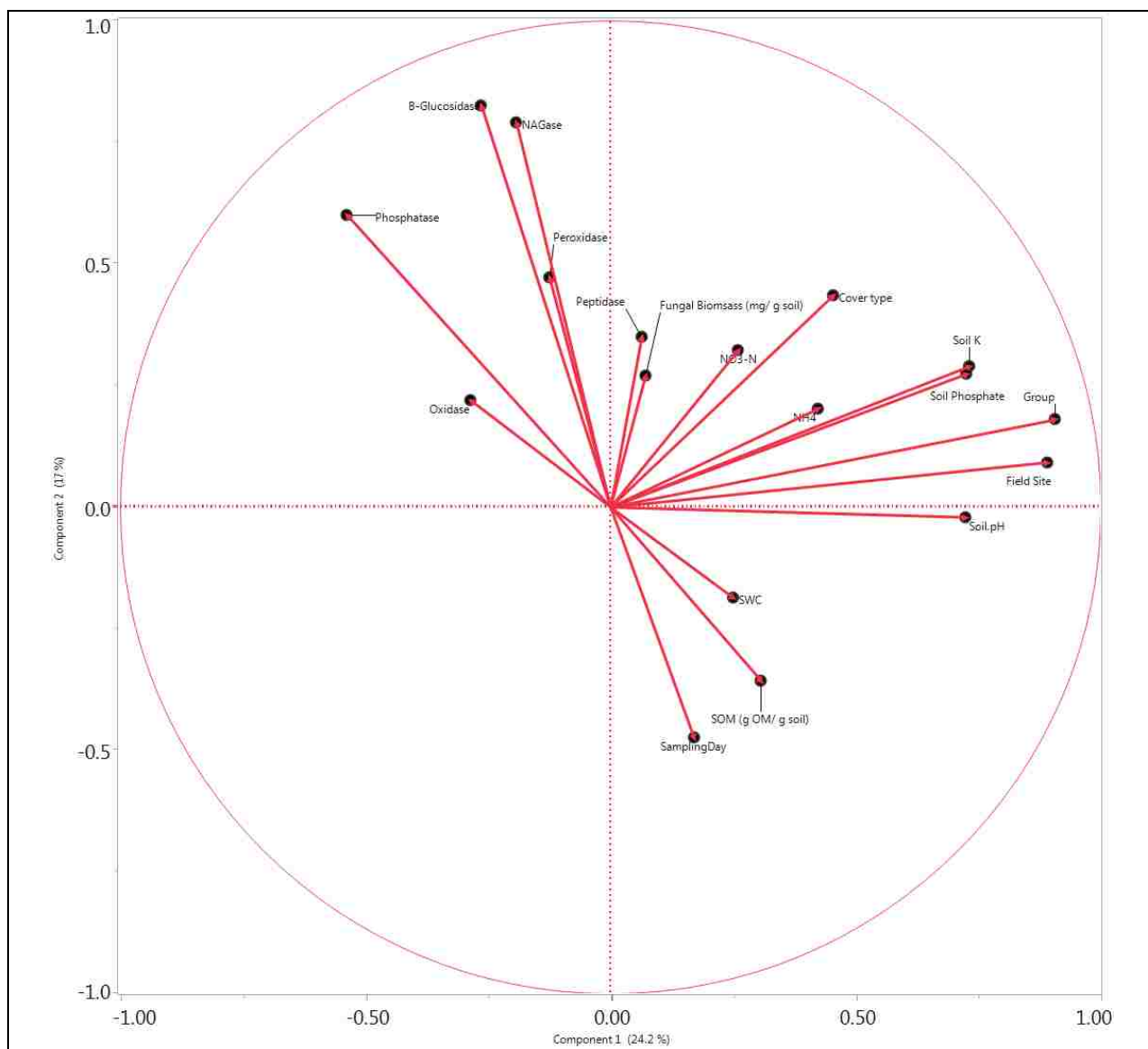
**Table 4.1.** Stepwise ANOVA results from soil property and EEA analyses of soil samples collected at the Sevilleta field sites in 2012 and 2013.

Full step-wise models						
Field Site Enzyme	All data, all sites	Grassland	Burned Grassland	Eco (Grass and Bare)	Desert Shrub	Eco (Shrub and Bare)
<b>AAP</b>	Perox, FBM, pH, Cover, Site #: R= 0.85, R <sup>2</sup> = 0.73	Location, AP: R= 0.85, R <sup>2</sup> = 0.73	AP, PO <sub>4</sub> <sup>3-</sup> , SOM: R= 0.70, R <sup>2</sup> = 0.49	Pox, Cover: R= 0.91, R <sup>2</sup> = 0.82	SOM, FBM: R= 0.72, R <sup>2</sup> = 0.52	SOM, AP: R= 0.84, R <sup>2</sup> = 0.71
<b>AP</b>	BG, Site #: R= 0.85, R <sup>2</sup> = 0.73	AAP, pH, SOM: R= 0.81, R <sup>2</sup> = 0.66	BG, Day, NH <sub>4</sub> <sup>+</sup> : R= 0.92, R <sup>2</sup> = 0.85	BG: R= 0.95, R <sup>2</sup> = 0.90	BG, NH <sub>4</sub> <sup>+</sup> , PO <sub>4</sub> <sup>3-</sup> , Day, NAG: R= 0.98, R <sup>2</sup> = 0.95	BG, Perox: R= 0.81, R <sup>2</sup> = 0.65
<b>BG</b>	NAG, AP, Site #, Day: R= 0.94, R <sup>2</sup> = 0.88	NAG, Day, Cover: R= 0.81, R <sup>2</sup> = 0.66	AP, Pox: R= 0.91, R <sup>2</sup> = 0.82	NAG, AP: R= 0.98, R <sup>2</sup> = 0.96	AP, soil K, NH <sub>4</sub> <sup>+</sup> : R= 0.95, R <sup>2</sup> = 0.90	AP, Pox, PO <sub>4</sub> <sup>3-</sup> , NH <sub>4</sub> <sup>+</sup> : R= 0.92, R <sup>2</sup> = 0.85
<b>NAG</b>	BG: R= 0.91, R <sup>2</sup> = 0.83	BG: R= 0.91, R <sup>2</sup> = 0.83	AP, Ox, soil K: R= 0.87, R <sup>2</sup> = 0.75	BG, NO <sub>3</sub> <sup>-</sup> , Cover: R= 0.97, R <sup>2</sup> = 0.95	AP, SWC, BG: R= 0.92, R <sup>2</sup> = 0.85	BG: R= 0.67, R <sup>2</sup> = 0.45
<b>Ox</b>	Day, AP, Perox, BG: R= 0.74, R <sup>2</sup> = 0.55	Day, Perox, NAG: R= 0.91, R <sup>2</sup> = 0.83	Day, Pox, NAG: R= 0.91, R <sup>2</sup> = 0.83	BG, soil pH: R= 0.90, R <sup>2</sup> = 0.81	BG, Ox, Day: R= 0.92, R <sup>2</sup> = 0.85	SWC: R= 0.65, R <sup>2</sup> = 0.42
<b>Pox</b>	SWC: R= 0.65, R <sup>2</sup> = 0.42	SWC: R= 0.65, R <sup>2</sup> = 0.42	SWC: R= 0.65, R <sup>2</sup> = 0.42	AAP, SWC: R= 0.94, R <sup>2</sup> = 0.86	BG, Ox, FBM, AAP: R= 0.47, R <sup>2</sup> = 0.21	Day, AAP, FBM, SOM, NAG: R= 0.97, R <sup>2</sup> = 0.95
Single best predictors						
Field Site Enzyme	All data, all sites	Grassland	Burned Grassland	Eco (Grass and Bare)	Desert Shrub	Eco (Shrub and Bare)
<b>AAP</b>	Perox: R= 0.74, R <sup>2</sup> = 0.55	Cover: R= 0.67, R <sup>2</sup> = 0.43	AP: R= 0.49, R <sup>2</sup> = 0.24	Perox: R= 0.87, R <sup>2</sup> = 0.75	SOM: R= 0.59, R <sup>2</sup> = 0.36	SOM: R= 0.76, R <sup>2</sup> = 0.56
<b>AP</b>	BG: R= 0.78, R <sup>2</sup> = 0.61	AAP: R= 0.67, R <sup>2</sup> = 0.45	BG: R= 0.87, R <sup>2</sup> = 0.76	AP: R= 0.95, R <sup>2</sup> = 0.90	BG: R= 0.94, R <sup>2</sup> = 0.88	BG: R= 0.67, R <sup>2</sup> = 0.45

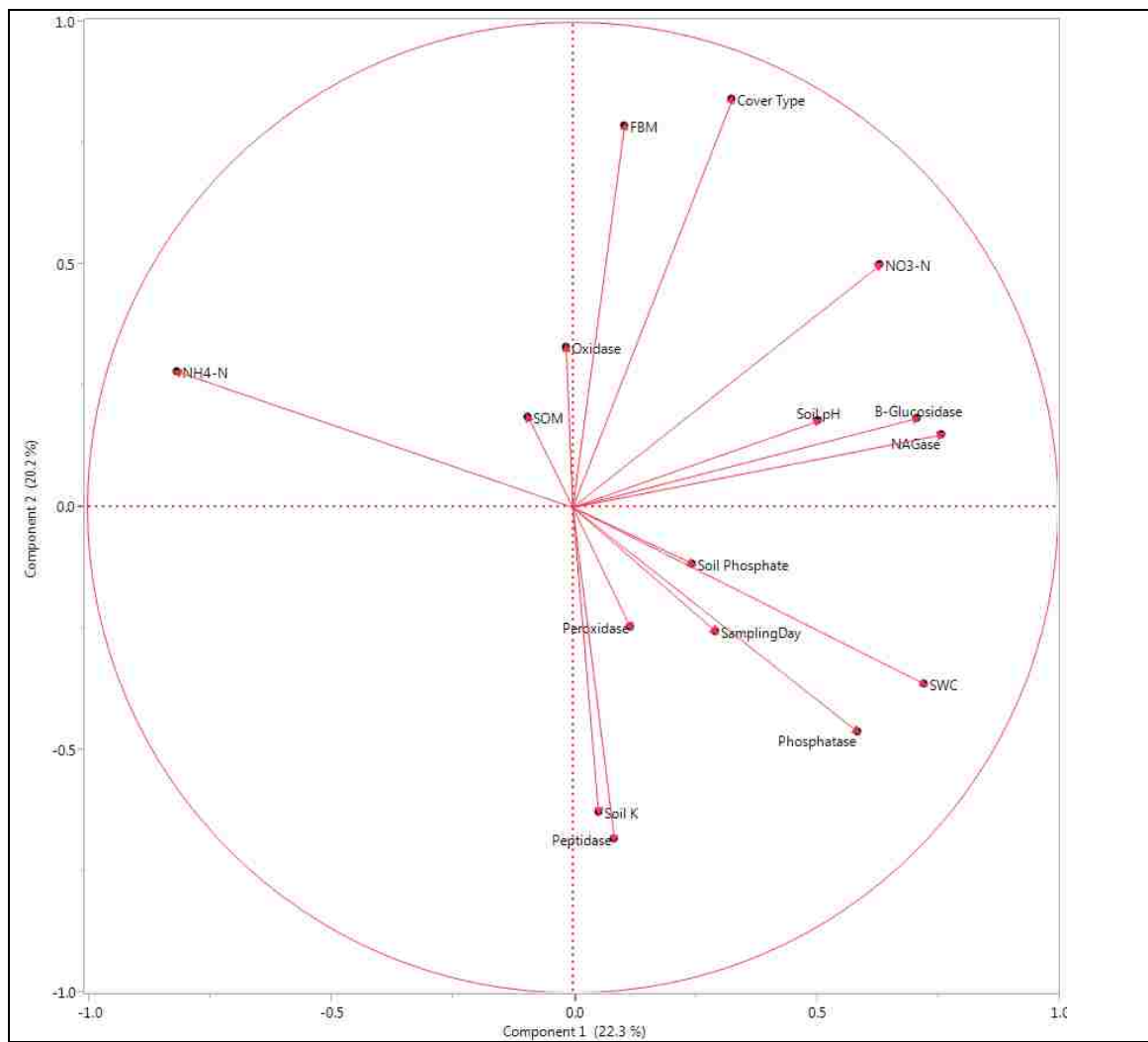
<b>BG</b>	NAG: R = 0.91, R <sup>2</sup> = 0.83	NAG R= 0.81 R <sup>2</sup> = 0.66	AP, Perox: R= 0.91, R <sup>2</sup> = 0.82	NAG: R= 0.95 R <sup>2</sup> = 0.90	AP R= 0.94, R <sup>2</sup> = 0.88	AP: R= 0.67, R <sup>2</sup> = 0.45
<b>NAG</b>	BG: R = 0.91, R <sup>2</sup> = 0.83	BG: R = 0.72 R <sup>2</sup> = 0.52	AP: R= 0.75 R <sup>2</sup> = 0.57	BG R= 0.95 R <sup>2</sup> = 0.90	AP R= 0.92 R <sup>2</sup> = 0.85	BG R= 0.67, R <sup>2</sup> = 0.45
<b>Ox</b>	Day: R = 0.53, R <sup>2</sup> = 0.29	Day: R= 0.62, R <sup>2</sup> = 0.39	Perox, R= 0.74, R <sup>2</sup> = 0.55	BG R= 0.86, R <sup>2</sup> = 0.73	SWC: R= 0.78 R <sup>2</sup> = 0.61	SWC R= 0.65, R <sup>2</sup> = 0.42
<b>Pox</b>	SWC R= 0.65, R <sup>2</sup> = 0.42	Day R= 0.62, R <sup>2</sup> = 0.39	Ox: R= 0.74 R <sup>2</sup> = 0.55	AAP R= 0.87, R <sup>2</sup> = 0.74	BG: R= 0.70, R <sup>2</sup> = 0.49	Day: R= 0.80, R <sup>2</sup> = 0.64

**Table 4.2.** Eigenvalues, and loading rates for PC factors one, and two, from all principle components anaylses performed on data generated from soil samples collected in 2012 and 2013.

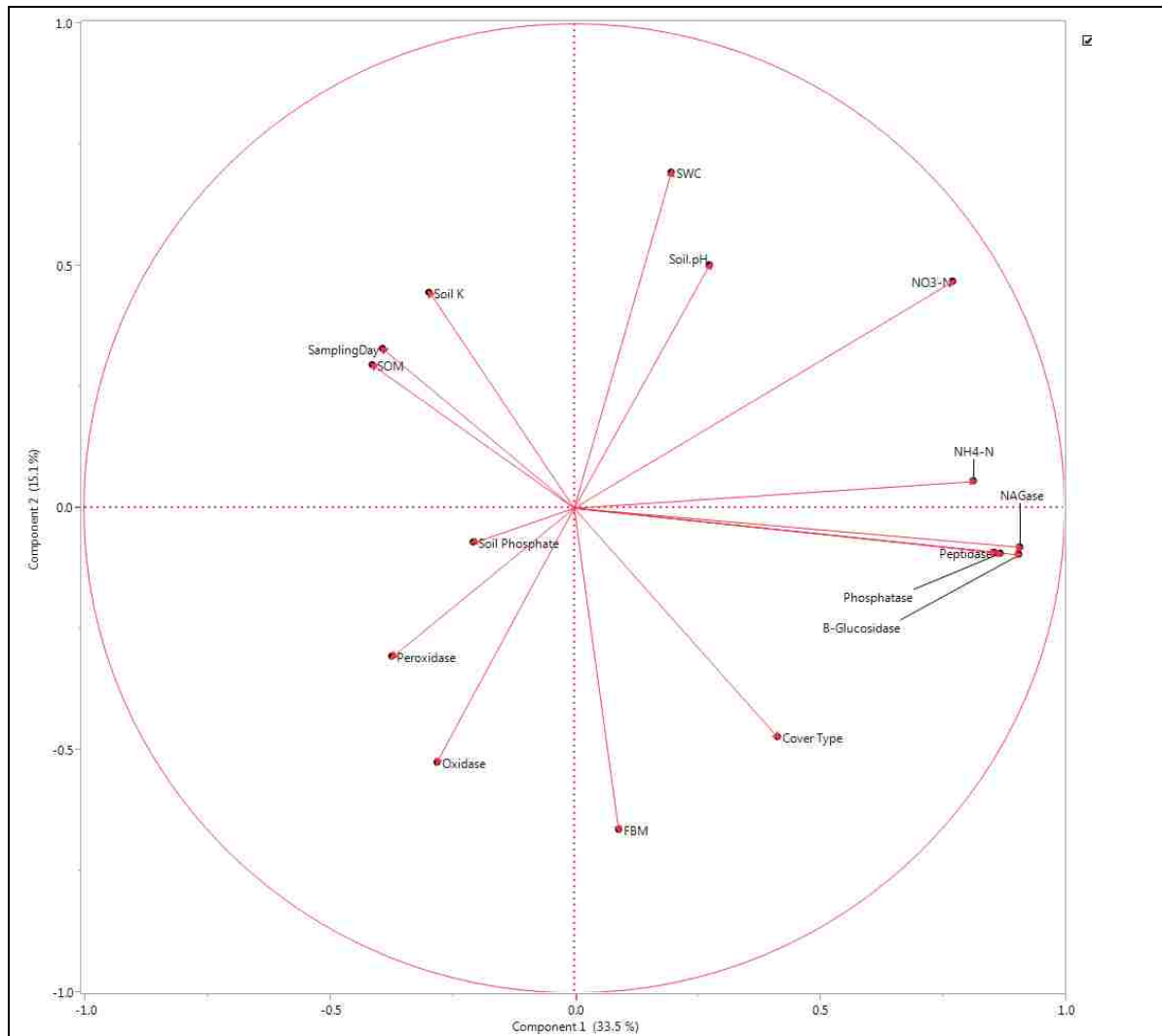
	Grassland		Burned Grassland		Ecotone (Grass and Bare)		Desert Shrub		Ecotone (Shrub and Bare)	
	Prin1	Prin2	Prin1	Prin2	Prin1	Prin2	Prin1	Prin2	Prin1	Prin2
<b>Eigenvalues</b>	3.86	2.95	5.36	2.42	5.46	4.34	4.71	2.59	4.43	3.56
<b>Field Site</b>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<b>Cover Type</b>	26.7%	-25.4%	-39.0%	32.9%	-57.5%	28.9%	-49.7%	51.5%	61.5%	-7.7%
<b>S. Day</b>	79.0%	-16.2%	19.8%	69.2%	-62.6%	62.5%	12.0%	72.7%	79.4%	-4.6%
<b>SWC</b>	40.2%	24.9%	27.6%	50.2%	-30.1%	-19.5%	-56.2%	2.1%	16.7%	-33.4%
<b>pH</b>	24.8%	-9.5%	-20.5%	-7.1%	14.5%	82.5%	57.0%	-41.7%	43.2%	69.5%
<b>PO<sub>4</sub><sup>3-</sup></b>	18.4%	-62.5%	-29.5%	44.5%	2.1%	72.7%	36.5%	-20.0%	45.4%	65.8%
<b>Soil K</b>	-80.9%	9.9%	81.5%	5.5%	-40.2%	48.3%	55.1%	35.4%	48.0%	51.7%
<b>NH<sub>4</sub>-N</b>	81.4%	25.2%	77.2%	46.7%	5.8%	64.0%	12.9%	27.8%	30.0%	26.2%
<b>NO<sub>3</sub>-N</b>	-8.5%	78.2%	9.0%	-66.3%	59.3%	54.2%	58.1%	5.6%	-21.6%	22.7%
<b>FBM</b>	-20.3%	15.2%	-41.1%	29.5%	-66.6%	56.8%	-8.6%	60.5%	79.3%	-13.6%
<b>SOM</b>	24.7%	-61.0%	85.6%	-9.1%	36.1%	-78.1%	-57.4%	-35.4%	-73.7%	-32.6%
<b>AAP</b>	72.1%	-26.0%	87.0%	-9.3%	83.7%	16.4%	80.9%	11.0%	-43.1%	68.5%
<b>AP</b>	62.5%	43.2%	90.7%	-9.6%	93.4%	5.9%	83.8%	2.8%	-64.6%	61.0%
<b>β-gluc</b>	66.0%	40.3%	91.0%	-8.1%	82.1%	18.8%	76.7%	22.2%	-45.9%	54.6%
<b>NAG</b>	1.4%	23.0%	-28.0%	-52.4%	64.2%	7.5%	36.8%	-72.3%	-16.0%	42.3%
<b>Ox</b>	36.1%	-18.7%	-37.1%	-30.7%	62.2%	-54.7%	43.6%	-40.2%	-83.9%	2.6%
<b>POx</b>	8.0%	90.3%	41.5%	-47.2%	56.5%	71.0%	63.9%	36.9%	20.3%	79.6%



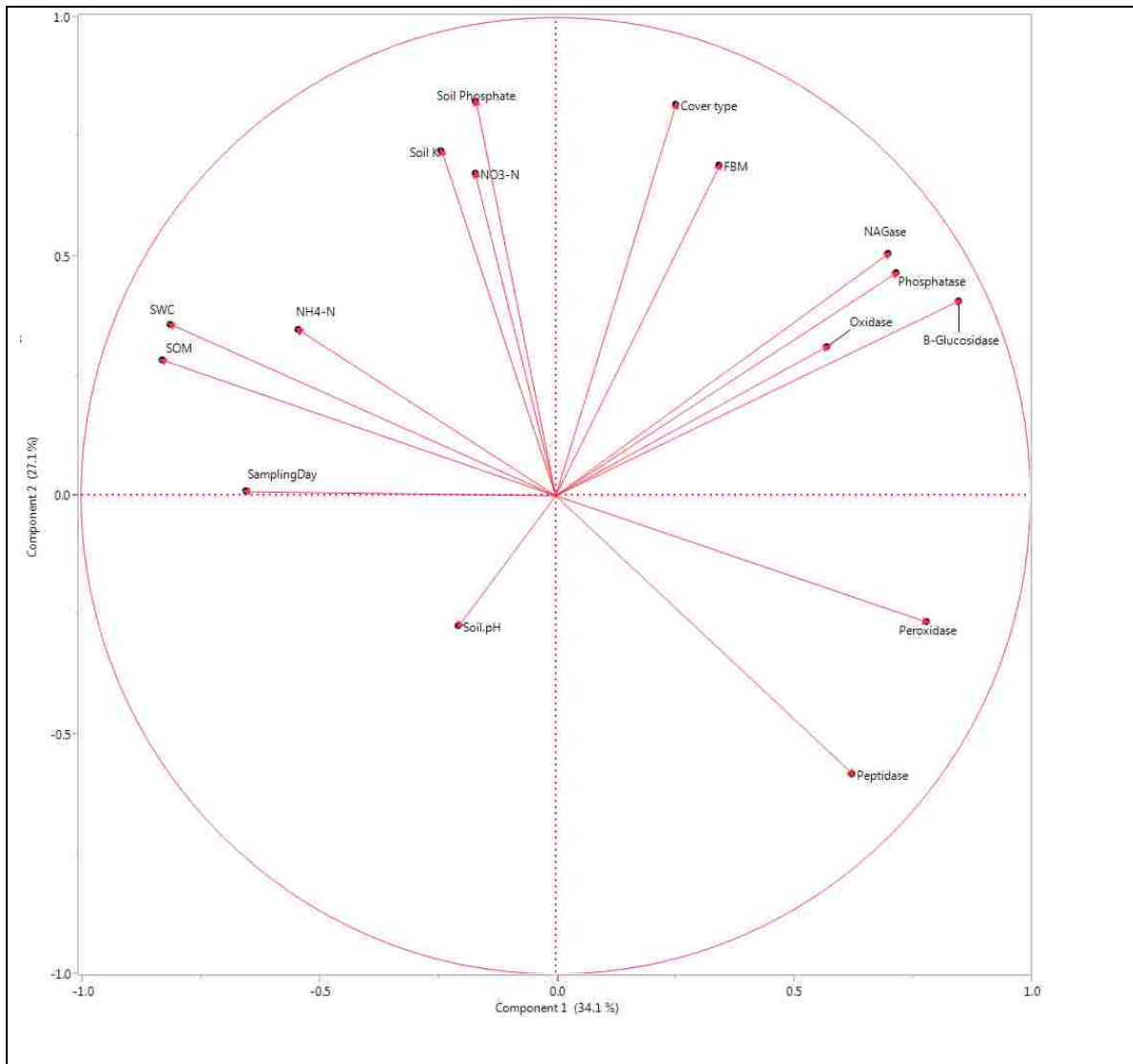
**Figure 4.1.** a) Soil extracellular enzyme activity rates from all four sites, for all four sampling dates



**Figure 4.2.** a) Soil extracellular enzyme activity rates from the grassland site for all four sampling dates.

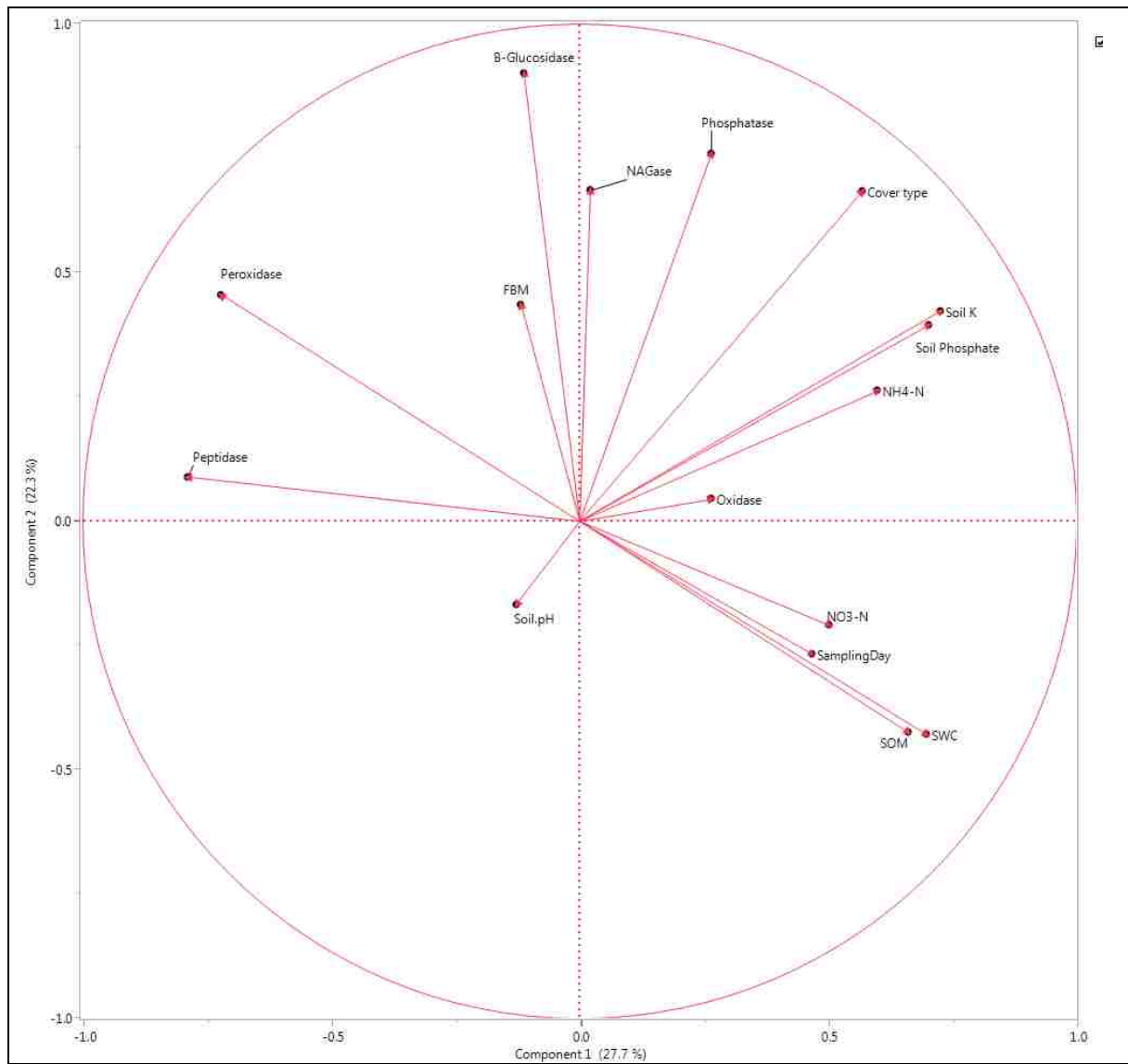


**Figure 4.3.** a) Soil extracellular enzyme activity rates from the burned grassland site for all four sampling dates and b) the PCA analysis of all grassland data considered together.

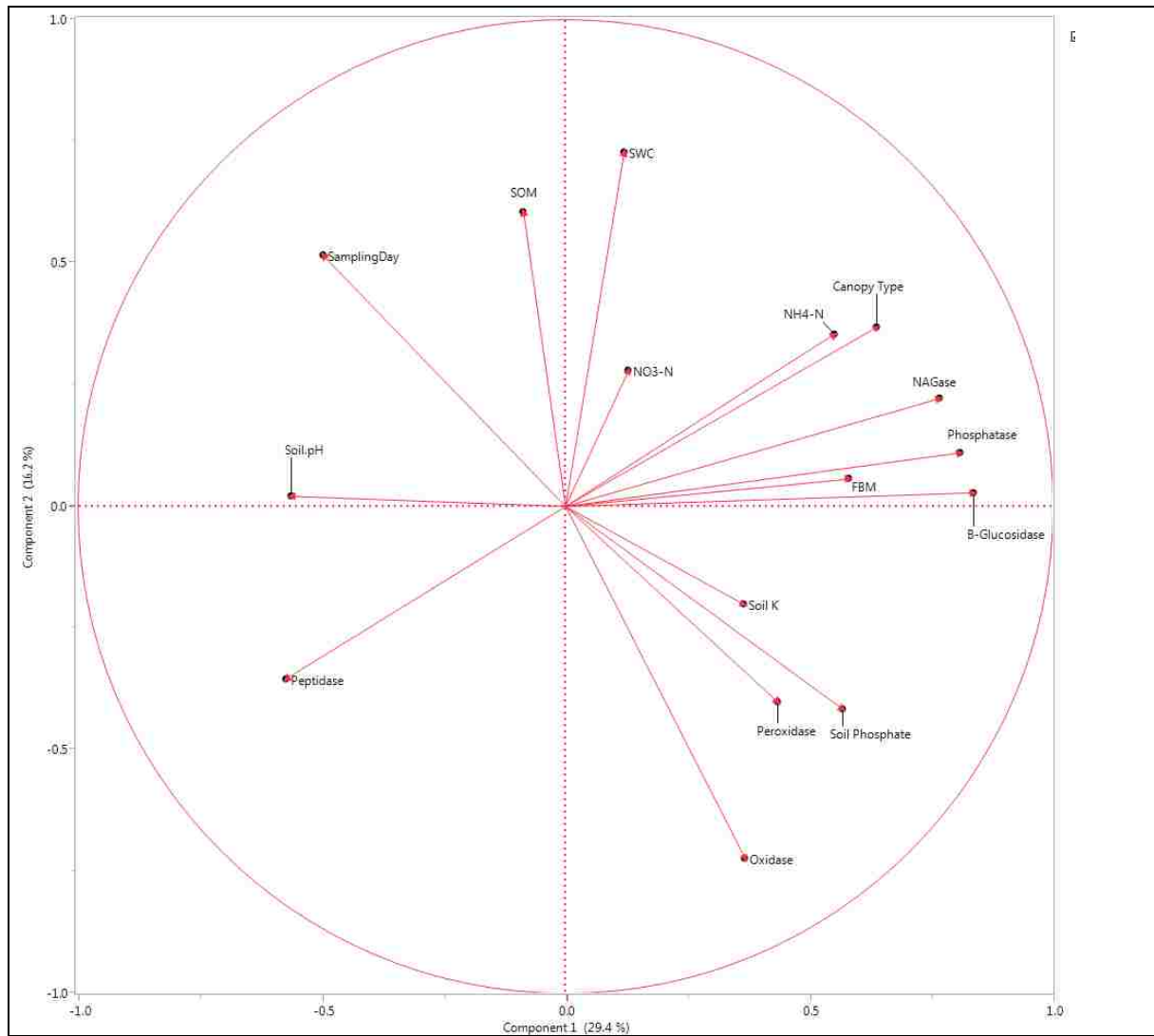


**Figure 4.4.** a) PCA results from all grass canopy and bare soil associated samples collected from the shrub/ grass ecotone site.





**Figure 4.5.** PCA results from all shrub canopy and bare soil associated samples collected from the shrub/ grass ecotone site.



**Figure 4.6.** Soil extracellular enzyme activity rates from the desert shrub site for all four sampling dates and b) the PCA analysis of all desert shrub land data considered together.

**Table 4.S1.:** Fungal biomasses from all 4 sites, for each of our 4 sampling dates. Bold text indicates a significant difference in parameter values across sampling locations, but from the same sampling period. Letters in superscript indicate significant differences in parameter values from samples collected at different times, but from the same location. Numbers in parentheses are equal to +/- 1 SD of the mean.

	Grassland		Burned Grassland		Ecotone			Shrub	
Sampling Date	Location		Location		Location			Location	
	Bare	Canopy	Bare	Canopy	Bare	Grass	Shrub	Bare	Canopy
<b>June 2012</b>	0.042 <sup>A</sup> (0.009)	<b>0.113<sup>A</sup></b> <b>(0.037)</b>	0.047 0.009	<b>0.130<sup>A</sup></b> <b>(0.053)</b>	0.049 (0.013)	0.283 (0.052)	<b>2.48</b> <b>(5.12)</b>	0.054 (0.031)	<b>0.204<sup>A</sup></b> <b>(0.037)</b>
<b>September 2012</b>	0.030 <sup>A</sup> (0.037)	<b>0.065<sup>B</sup></b> <b>(0.006)</b>	0.031 0.020	<b>0.067<sup>C</sup></b> <b>(0.019)</b>	0.068 (0.071)	0.173 (0.125)	0.203 (0.078)	0.037 (0.023)	<b>0.122<sup>B</sup></b> <b>(0.050)</b>
<b>July 2013</b>	0.029 <sup>A</sup> (0.007)	<b>0.111<sup>A</sup></b> <b>(0.016)</b>	0.052 0.018	<b>0.095<sup>B</sup></b> <b>(0.015)</b>	0.045 (0.039)	0.209 (0.042)	0.625 (0.984)	0.017 (0.005)	<b>0.208<sup>A</sup></b> <b>(0.154)</b>
<b>August 2013</b>	0.029 <sup>A</sup> (0.007)	<b>0.096<sup>A</sup></b> <b>(0.035)</b>	0.043 0.022	<b>0.075<sup>BC</sup></b> <b>(0.031)</b>	0.033 (0.010)	0.223 (0.073)	0.3310 (0.182)	0.025 (0.013)	0.075 <sup>B</sup> (0.036)
	$\chi=44.7, P<0.0001$		$\chi= 36.88, P<0.0001$		$\chi=54.7, P<0.0001$				
<b>Effect tests</b>								F	P
<b>Location</b>								40.3	<b>&lt;0.0001</b>
<b>Day</b>								3.86	0.014

**Table 4.S2.** Soil extracellular enzyme activity rates from the grassland site for all four sampling dates. Bold text indicates a significant difference in parameter values across sampling locations, but from the same sampling period. Letters in superscript indicate significant differences in parameter values from samples collected at different times, but from the same location. Numbers in parentheses are equal to +/- 1 SD of the mean.

Sample Date	Peptidase		Phosphatase		<sup>1</sup> BG		<sup>1</sup> NAase		<sup>1</sup> Oxidase		<sup>1</sup> Peroxidase	
	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy
<b>June 2012</b>	<b>7304.2<sup>A</sup></b> (1832.3)	3529.4 <sup>A</sup> (1748.9)	3169.5 <sup>B</sup> (1126.4)	3604.1 <sup>B</sup> (1179.4)	902.4 <sup>B</sup> (378.5)	<b>2540.9<sup>B</sup></b> (967.0)	135.5 <sup>B</sup> (50.3)	<b>331.4<sup>B</sup></b> (49.7)	410.8 <sup>B</sup> (317.5)	<b>1366.9<sup>A</sup></b> (330.2)	1059.7 <sup>BC</sup> (528.8)	485.7 <sup>B</sup> (382.0)
<b>Sept 2012</b>	7990.4 <sup>A</sup> (4124.2)	6107.2 <sup>A</sup> (1625.5)	7880.1 <sup>A</sup> (3296.3)	6162.5 <sup>A</sup> (2305.3)	4195.4 <sup>A</sup> (1568.5)	4801.2 <sup>A</sup> (2540.1)	591.2 <sup>A</sup> (217.6)	<b>865.3<sup>A</sup></b> (245.3)	210.6 <sup>B</sup> (150.3)	216.7 <sup>B</sup> (213.7)	<b>1852.5<sup>B</sup></b> (1228.5)	0 <sup>B</sup> 0
<b>July 2013</b>	6893.4 <sup>A</sup> (2662.7)	4924.4 <sup>A</sup> (4057.1)	6094.1 <sup>A</sup> (2727.3)	6079.4 <sup>A</sup> (1966.1)	716.9 <sup>B</sup> (171.2)	<b>2934.3<sup>AB</sup></b> (1099.3)	114.0 <sup>B</sup> (37.8)	<b>349.2<sup>B</sup></b> (184.5)	815.9 <sup>A</sup> (565.2)	1059.8 <sup>A</sup> (574.1)	3974.3 <sup>A</sup> (1165.2)	4171.5 <sup>A</sup> (2231.0)
<b>August 2013</b>	<b>8882.7<sup>A</sup></b> (4424.8)	3387.7 <sup>A</sup> (1924.1)	<b>5313.7<sup>B</sup></b> (1675.1)	3022.3 <sup>AB</sup> (2015.7)	933.7 <sup>B</sup> (538.7)	1328.7 <sup>B</sup> (634.6)	225.0 <sup>B</sup> (62.4)	216.4 <sup>B</sup> (121.7)	324.8 <sup>B</sup> (124.9)	273.0 <sup>B</sup> (111.4)	254.1 <sup>C</sup> (60.2)	182.9 <sup>B</sup> (68.6)
<b>Effect tests</b>	<b>F=</b>	<b>P=</b>	<b>F=</b>	<b>P=</b>								
Loc.	17.8	<.0001	2.58	0.11	$\chi=45.3, P<0.0001$		$\chi=44.4, P<0.0001$		$\chi=27.3, P=0.0003$		$\chi=39.6, P<0.0001$	
Day	0.83	0.4822	8.96	<.0001								
	<b>Soil moisture content</b>		<b>Soil organic matter content</b>		<b>Soil pH</b>		<b>Soil NO<sub>3</sub><sup>-</sup></b>		<b>Soil NH<sub>4</sub><sup>+</sup></b>		<b>Soil PO<sub>4</sub><sup>3-</sup></b>	
	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Bare	Bare	Canopy	Bare	Canopy	Bare	Canopy
<b>June 2012</b>	0.0091 <sup>B</sup> (0.003)	0.013 <sup>B</sup> (0.018)	0.013 (0.002)	0.013 <sup>AB</sup> (0.002)	7.54 (0.15)	7.74 (0.15)	3.00 <sup>B</sup> (0.00)	4.00 (0.76)	5.83 <sup>A</sup> (0.42)	4.68 (1.06)	39.57 (4.61)	41.75 (5.78)
<b>Sept 2012</b>	0.10 <sup>A</sup> (0.025)	0.10 <sup>A</sup> (0.020)	0.013 (0.003)	0.016 <sup>AB</sup> (0.004)	7.73 (0.25)	7.88 (0.17)	NO DATA	No Data	2.18 <sup>B</sup> (0.11)	2.42 (0.14)	43.06 (4.93)	46.10 (7.58)
<b>July 2013</b>	0.010 <sup>A</sup> (0.015)	0.084 <sup>A</sup> (0.018)	0.013 (0.003)	0.010 <sup>B</sup> (0.002)	No Data	No Data	NO DATA	No Data	NO DATA	No Data	No Data	No Data
<b>August 2013</b>	0.078 <sup>A</sup> (0.007)	0.085 <sup>A</sup> (0.007)	0.011 (0.002)	0.021 <sup>A</sup> (0.013)	7.73 (0.11)	7.84 (0.15)	3.59 <sup>C</sup> (0.55)	3.84 (0.20)	2.80 <sup>C</sup> (0.42)	3.34 (0.16)	47.07 (9.15)	44.35 (7.38)
<b>Effect tests</b>	<b>F=</b>	<b>P=</b>	<b>F=</b>	<b>P=</b>	<b>F=</b>	<b>P=</b>	<b>F=</b>	<b>P=</b>	<b>F=</b>	<b>P=</b>	<b>F=</b>	<b>P=</b>
Loc.	0.0188	0.8914	1.9659	0.1665	10.0	0.024	2.96	0.007	8.31	0.001	0.1649	0.6876
Day	97.9729	<.0001	1.9055	0.1394	4.26	0.021	2.96	0.007	14.6	<0.0001	1.2636	0.2977

**Table 4.S3:** Soil extracellular enzyme activity rates from the burned grassland site for all four sampling dates. Bold text indicates a significant difference in parameter values across sampling locations, but from the same sampling period. Letters in superscript indicate significant differences in parameter values from samples collected at different times, but from the same location. Numbers in parentheses are equal to +/- 1 SD of the mean.

Sample Date	Peptidase		Phosphatase		BG		NAGase		Oxidase		Peroxidase	
	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy
<b>June 2012</b>	11348.0 <sup>A</sup> (3588.9)	7296.4 <sup>B</sup> (5301.7)	2442.4 (1581.0)	2134.2 <sup>B</sup> (1610.4)	1761.1 (1464.9)	1389.1 <sup>B</sup> (1025.0)	218.1 (324.9)	200.7 <sup>B</sup> (200.3)	515.7 <sup>AB</sup> (473.8)	289.4 <sup>AB</sup> (353.0)	863.5 <sup>B</sup> (876.8)	48.2 <sup>B</sup> (113.2)
<b>Sept 2012</b>	10479.4 <sup>A</sup> (3760.9)	<b>17623.6<sup>A</sup></b> <b>(6257.3)</b>	2607.8 (1516.2)	<b>10283.9<sup>A</sup></b> <b>(2694.8)</b>	1782.2 (1380.3)	<b>6751.8<sup>A</sup></b> <b>(2987.6)</b>	270.4 (177.8)	<b>1095.3<sup>A</sup></b> <b>(435.8)</b>	68.7 <sup>B</sup> (73.7)	26.7 <sup>B</sup> (49.2)	0 <sup>B</sup> 0	0 <sup>B</sup> 0
<b>July 2013</b>	3622.1 <sup>B</sup> (2043.8)	7232.5 <sup>B</sup> (4269.0)	1236.4 (659.0)	<b>3105.4<sup>B</sup></b> <b>(1668.6)</b>	420.4 (305.3)	1930.7 <sup>B</sup> (1113.1)	32.6 (40.3)	234.9 <sup>B</sup> (177.9)	551.4 <sup>A</sup> (458.7)	357.0 <sup>A</sup> (294.5)	2383.6 <sup>A</sup> (1412.2)	2120.8 <sup>A</sup> (1227.3)
<b>August 2013</b>	4985.3 <sup>B</sup> (2101.3)	8953.8 <sup>B</sup> (2221.5)	2032.2 (826.3)	2469.5 <sup>B</sup> (1022.8)	1015.1 (676.2)	1174.5 <sup>B</sup> (888.0)	94.7 (33.3)	157.2 <sup>B</sup> (121.1)	78.0 <sup>AB</sup> (52.2)	87.6 <sup>AB</sup> (77.5)	69.3 <sup>B</sup> (39.4)	82.8 <sup>B</sup> (51.6)
<b>Effect tests</b>	<b>F=      P=</b>											
Loc. Day	10.8867 <.0001 5.316 0.0249		$\chi=26.8, P=0.0004$		$\chi=29.3, P<0.0001$		$\chi=29.7, P<0.0001$		$\chi=22.4, P=0.002$		$\chi=47.2, P<0.0001$	
	<b>Soil moisture content</b>		<b>Soil organic matter content</b>		<b>Soil pH</b>		<b>Soil NO<sub>3</sub><sup>-</sup></b>		<b>Soil NH<sub>4</sub><sup>+</sup></b>		<b>Soil PO<sub>4</sub><sup>3-</sup></b>	
	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Bare	Bare	Canopy	Bare	Canopy	Bare	Canopy
<b>June 2012</b>	0.0062 <sup>C</sup> (0.002)	0.0080 <sup>C</sup> (0.003)	0.013 (0.004)	0.060 (0.11)	8.24 <sup>B</sup> (0.079)	8.16 <sup>B</sup> (0.12)	5.17 (3.98)	4.14 (1.72)	5.37 <sup>A</sup> (1.50)	4.63 <sup>B</sup> (1.51)	45.5 (5.47)	48.71 (13.0)
<b>Sept 2012</b>	0.12 <sup>A</sup> (0.028)	0.11 <sup>A</sup> (0.016)	0.028 (0.012)	0.0010 (0.003)	8.38 <sup>A</sup> (0.036)	8.32 <sup>A</sup> (0.034)	No Data	No Data	4.20 <sup>A</sup> (1.02)	9.16 <sup>A</sup> (3.94)	48.85 (15.4)	51.37 (5.30)
<b>July 2013</b>	0.098 <sup>B</sup> (0.009)	0.088 <sup>B</sup> (0.013)	0.050 (0.050)	0.040 (0.061)	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
<b>August 2013</b>	0.076 <sup>B</sup> (0.008)	0.079 <sup>B</sup> (0.003)	0.019 (0.004)	0.020 (0.009)	8.25 <sup>B</sup> (0.091)	8.28 <sup>A</sup> (0.063)	3.51 (0.29)	3.46 (0.36)	2.98 <sup>B</sup> (0.42)	3.45 <sup>B</sup> (1.07)	62.71 (17.5)	54.22 (1.00)
<b>Effect tests</b>	<b>F=      P=</b>		<b>F=      P=</b>		<b>F=      P=</b>		<b>F=      P=</b>		<b>F=      P=</b>		<b>F=      P=</b>	
Loc. Day	0.0188 0.8914 97.9729 <.0001		1.9659 0.1665 1.9055 0.1394		5.6746 0.024 2.4462 0.1043		0.23 0.82 0.84 0.41		7.10 0.01 10.0 0.0003		0.1649 0.6876 1.2636 0.2977	

**Table 4.S4.** Soil extracellular enzyme activity rates from the shrub-grass ecotone site for all four sampling dates. Bold text indicates a significant difference in parameter values across sampling locations, but from the same sampling period. Letters in superscript indicate significant differences in parameter values from samples collected at different times, but from the same location. Numbers in parentheses are equal to +/- 1 SD of the mean.

Sampling Date	Peptidase			Phosphatase			BG		
	Location			Location			Location		
	Bare	Grass	Shrub	Bare	Grass	Shrub	Bare	Grass	Shrub
June 2012	<b>61309.7<sup>A</sup></b> (27913.4)	<b>36461.2<sup>A</sup></b> (10627.2)	9589 <sup>A</sup> (3435.9)	2701.3 <sup>A</sup> (1811.1)	<b>8173.1<sup>A</sup></b> (4304.7)	2411.1 <sup>B</sup> (628.4)	2459.6 <sup>A</sup> (957.6)	<b>8159.8<sup>A</sup></b> (5068.2)	2296.6 <sup>A</sup> (963.0)
September 2012	13681.1 <sup>B</sup> (5776.2)	9482.3 <sup>B</sup> (1839.4)	8946.4 <sup>AB</sup> (2765.4)	1964.5 <sup>A</sup> (316.3)	3344.7 <sup>B</sup> (903.4)	3009.3 <sup>AB</sup> (733.1)	1199.4 <sup>B</sup> (259.2)	2119.6 <sup>B</sup> (625.1)	1898.2 <sup>A</sup> (786.7)
July 2013	15750.3 <sup>B</sup> (4057.6)	7736.0 <sup>B</sup> (3587.8)	5018.6 <sup>B</sup> (1262.6)	1424.4 <sup>A</sup> (362.7)	<b>3947.1<sup>B</sup></b> (1286.0)	3122.8 <sup>AB</sup> (1027.6)	785.8 <sup>B</sup> (476.8)	2649.5 <sup>B</sup> (698.3)	1853.9 <sup>A</sup> (606.2)
August 2013	<b>21452.9<sup>B</sup></b> (15782.9)	9004.0 <sup>B</sup> (4449.0)	<b>11036.4<sup>A</sup></b> (2633.3)	1212.2 <sup>A</sup> (607.9)	2123.1 <sup>B</sup> (679.3)	<b>3929.9<sup>A</sup></b> (825.9)	535.1 <sup>B</sup> (468.2)	1988.8 <sup>B</sup> (471.5)	<b>2560.9<sup>A</sup></b> (1349.2)
Location	F= 17.4006 P <.0001			$\chi=48.1$ , P<0.0001			$\chi=45.8$ , P<0.0001		
Day #	F= 14.9469 P <.0001								
	NAGase			Oxidase			Peroxidase		
	Location			Location			Location		
	Bare	Grass	Shrub	Bare	Grass	Shrub	Bare	Grass	Shrub
June 2012	392.7 <sup>A</sup> (310.8)	<b>1257.2<sup>A</sup></b> (856.1)	293.75 <sup>B</sup> (122.8)	0 <sup>B</sup> (0)	<b>936.75<sup>A</sup></b> (466.8)	0 <sup>B</sup> (0)	12961.8 <sup>A</sup> (10504.2)	11315.9 <sup>A</sup> (3304.7)	10560.2 <sup>A</sup> (4926.0)
September 2012	282.6 <sup>AB</sup> (50.6)	450.7 <sup>B</sup> (92.3)	481.2 <sup>A</sup> (151.1)	0 <sup>B</sup> (0)	0 <sup>B</sup> (0)	0 <sup>B</sup> (0)	66.9 <sup>B</sup> (103.8)	495.5 <sup>B</sup> (265.6)	165.1 <sup>B</sup> (202.9)
July 2013	59.4 <sup>B</sup> (35.7)	473.2 <sup>B</sup> (298.7)	179.5 <sup>B</sup> (79.3)	223.1 <sup>A</sup> (142.2)	439.0 <sup>B</sup> (394.3)	<b>946.3<sup>A</sup></b> (705.5)	1745.5 <sup>B</sup> (888.7)	2884.0 <sup>BC</sup> (874.6)	683.7 <sup>B</sup> (484.4)
August 2013	147.7 <sup>A</sup> <sup>B</sup> (100.4)	285.6 <sup>B</sup> (107.8)	<b>511.0<sup>A</sup></b> (55.6)	51.6 <sup>B</sup> (66.3)	40.3 <sup>B</sup> (44.8)	102.3 <sup>B</sup> (63.5)	22.8 <sup>B</sup> (24.9)	63.6 <sup>C</sup> (39.5)	77.1 <sup>B</sup> (30.8)
	$\chi=44.5$ , P<0.0001			$\chi=52.9$ , P<0.0001			$\chi=54.6$ , P<0.0001		

**Table 4.S5.** Soil physicochemical properties from the shrub-grass ecotone site for all four sampling dates. Numbers in parentheses are equal to +/- 1 SD of the mean.

Sampling Date	Soil moisture content			Soil organic matter content			Soil pH		
	Location			Location			Location		
	Bare	Grass	Shrub	Bare	Grass	Shrub	Bare	Grass	Shrub
<b>June 2012</b>	0.0066 <sup>C</sup> (0.002)	0.0078 <sup>C</sup> (0.003)	0.0075 <sup>C</sup> (0.003)	0.0065 <sup>B</sup> (0.002)	0.0078 <sup>C</sup> (0.003)	0.022 <sup>B</sup> (0.007)	<b>8.34<sup>A</sup></b> <b>(0.049)</b>	8.13 <sup>A</sup> (0.052)	8.17 <sup>B</sup> (0.10)
<b>September 2012</b>	0.14 <sup>A</sup> (0.011)	0.13 <sup>A</sup> (0.018)	0.13 <sup>A</sup> (0.040)	0.038 <sup>A</sup> (0.008)	0.034 <sup>A</sup> (0.004)	0.044 <sup>A</sup> (0.011)	8.33 <sup>A</sup> (0.074)	8.27 <sup>A</sup> (0.039)	8.35 <sup>A</sup> (0.077)
<b>July 2013</b>	0.091 <sup>B</sup> (0.012)	0.095 <sup>B</sup> (0.015)	0.095 <sup>AB</sup> (0.023)	0.032 <sup>A</sup> (0.01Z)	0.023 <sup>B</sup> (0.003)	0.023 <sup>B</sup> (0.003)	No Data	No Data	No Data
<b>August 2013</b>	0.082 <sup>B</sup> (0.018)	0.090 <sup>B</sup> (0.009)	0.074 <sup>B</sup> (0.006)	0.025 <sup>A</sup> (0.009)	0.033 <sup>A</sup> (0.007)	0.024 <sup>B</sup> (0.004)	8.20 <sup>B</sup> (0.063)	8.23 <sup>B</sup> (0.039)	8.20 <sup>AB</sup> (0.063)
<b>Effect tests</b>	<b>F=</b> <b>P=</b>		<b>F=</b> <b>P=</b>			<b>F=</b> <b>P=</b>			
Location	0.5394 0.5857		1.3571 0.2645			4.4406 0.0187			
Day #	174.95 <.0001		33.2688 <.0001			6.1235 0.005			
	<b>Soil NO<sub>3</sub><sup>-</sup></b>			<b>Soil NH<sub>4</sub><sup>+</sup></b>			<b>Soil PO<sub>4</sub><sup>3-</sup></b>		
	Location			Location			Location		
	Bare	Grass	Shrub	Bare	Grass	Shrub	Bare	Grass	Shrub
<b>June 2012</b>	4.50 (3.40)	10.0 <sup>A</sup> (5.05)	12.5 <sup>A</sup> (5.10)	3.30 <sup>B</sup> (0.72)	3.70 (0.93)	3.77 <sup>B</sup> (1.67)	50.83 (5.00)	59.5 (10.9)	61.33 (9.11)
<b>September 2012</b>	No Data	No Data	No Data	11.0 <sup>A</sup> (7.20)	8.88 <sup>A</sup> (0.96)	16.82 <sup>A</sup> 7.86	61.0 (15.4)	69.53 (7.55)	90.25 (36.1)
<b>July 2013</b>	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
<b>August 2013</b>	3.50 (0.14)	5.06 <sup>B</sup> (0.71)	5.10 <sup>B</sup> (0.36)	5.54 <sup>B</sup> (1.67)	6.14 <sup>A</sup> (1.29)	16.4 <sup>A</sup> (8.20)	50.49 (2.24)	<b>66.74</b> <b>(5.31)</b>	<b>95.79</b> <b>(26.2)</b>
<b>Effect tests</b>	<b>F=</b> <b>P=</b>		<b>F=</b> <b>P=</b>			<b>F=</b> <b>P=</b>			
Location	6.27 0.005		8.67 0.001			5.6363 0.006			
Day #	14.8 0.0005		13.6 <0.0001			3.3243 0.0512			

**Table 4.S6.** Soil extracellular enzyme activity rates from the desert shrub site for all four sampling dates. Bold text indicates a significant difference in parameter values across sampling locations, but from the same sampling period. Letters in superscript indicate significant differences in parameter values from samples collected at different times, but from the same location. Numbers in parentheses are equal to +/- 1 SD of the mean.

Sample Date	Peptidase		Phosphatase		BG		NAGase		Oxidase		Peroxidase	
	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy	Bare	Canopy
June 2012	<b>17263.2<sup>A</sup></b> (5187.9)	9416.0 <sup>A</sup> (3845.2)	1580.6 <sup>A</sup> (177.1)	<b>3314.5<sup>AB</sup></b> (1621.3)	1168.3 <sup>AB</sup> (527.5)	<b>3143.0<sup>A</sup></b> (1943.3)	95.5 <sup>B</sup> (35.4)	<b>437.1<sup>AB</sup></b> (413.3)	792.3 <sup>A</sup> (247.2)	983.1 <sup>A</sup> (384.8)	1014.3 <sup>A</sup> (1344.2)	516.2 <sup>AB</sup> (797.2)
Sept 2012	<b>14401.3<sup>A</sup></b> (7112.2)	8327.1 <sup>A</sup> (3595.0)	2084.1 <sup>A</sup> (555.5)	<b>3504.1<sup>A</sup></b> (1470.7)	1385.0 <sup>A</sup> (682.5)	<b>2675.0<sup>A</sup></b> (1525.1)	334.0 <sup>A</sup> (160.5)	<b>570.8<sup>A</sup></b> (347.9)	39.1 <sup>B</sup> (86.8)	32.3 <sup>B</sup> (623.0)	290.1 <sup>AB</sup> (240.2)	408.4 <sup>B</sup> (370.2)
July 2013	<b>20165.0<sup>A</sup></b> (3459.6)	6131.4 <sup>A</sup> (1720.7)	890.2 <sup>B</sup> (301.1)	<b>1835.2<sup>BC</sup></b> (703.5)	602.0 <sup>BC</sup> (280.5)	<b>1946.0<sup>AB</sup></b> (1052.4)	24.1 <sup>B</sup> (41.4)	<b>258.6<sup>AB</sup></b> (199.2)	82.2 <sup>B</sup> (172.8)	<b>714.7<sup>A</sup></b> (389.8)	186.2 <sup>AB</sup> (229.8)	<b>1095.0<sup>A</sup></b> (446.3)
August 2013	13814.7 <sup>A</sup> (4908.0)	10026.8 <sup>A</sup> (5343.5)	811.1 <sup>B</sup> (290.2)	669.3 <sup>C</sup> (337.4)	376.3 <sup>C</sup> (177.3)	551.1 <sup>B</sup> (193.2)	29.9 <sup>B</sup> (28.4)	53.9 <sup>B</sup> (34.5)	14.5 <sup>B</sup> (25.8)	22.8 <sup>B</sup> (23.9)	8.8 <sup>B</sup> (10.2)	33.7 <sup>B</sup> (31.1)
Effect tests	F=	P=	F=	P=	F=	P=						
Loc.	37.0834 <.0001		15.6265 0.0002		18.3203 <.0001							
Day	0.428 0.7338		16.7232 <.0001		8.7247 <.0001		$\chi=40.7, P<0.0001$		$\chi=35.4, P<0.0001$		$\chi=19.1, P=0.001$	
	Soil moisture content		Soil organic matter content		Soil pH		Soil NO <sub>3</sub> <sup>-</sup>		Soil NH <sub>4</sub> <sup>+</sup>		Soil PO <sub>4</sub> <sup>3-</sup>	
	Location		Location		Location		Location		Location		Location	
	Bare	Canopy	Bare	Canopy	Bare	Bare	Bare	Canopy	Bare	Canopy	Bare	Canopy
June 2012	0.013 <sup>C</sup> (0.003)	0.0010 <sup>C</sup> (0.006)	0.021 (0.001)	0.022 <sup>B</sup> (0.006)	<b>8.34<sup>B</sup></b> (0.049)	8.22 <sup>B</sup> (0.041)	3.80 (1.33)	17.7 <sup>A</sup> (9.73)	3.98 (1.30)	4.15 <sup>B</sup> (0.88)	93.67 <sup>A</sup> (27.8)	106.33 <sup>A</sup> (27.4)
Sept 2012	0.13 <sup>A</sup> (0.021)	0.14 <sup>A</sup> (0.018)	0.026 (0.007)	0.033 <sup>AB</sup> (0.009)	No Data	No Data	No Data	No Data	5.56 (0.95)	13.45 <sup>A</sup> (5.97)	61.78 <sup>B</sup> (13.6)	<b>99.50<sup>AB</sup></b> (22.5)
July 2013	No Data	No Data	0.029 (0.006)	0.0278 <sup>AB</sup> (0.005)	<b>8.40<sup>A</sup></b> (0.00)	8.28 <sup>B</sup> (0.083)	No Data	No Data	No Data	No Data	No Data	No Data
August 2013	0.082 <sup>B</sup> (0.014)	0.091 <sup>B</sup> (0.019)	0.029 (0.006)	0.036 <sup>A</sup> (0.014)	8.35 <sup>B</sup> (0.041)	8.36 <sup>A</sup> (0.042)	4.073 (0.72)	4.67 (0.90)	3.42 (0.44)	4.5 <sup>B</sup> (1.59)	57.51 <sup>B</sup> (1.88)	77.8 <sup>B</sup> (9.18)
Effect tests	F=	P=	F=	P=	F=	P=					F=	P=
Loc.	0.9398 0.3385		1.572 0.2155		11.2244 0.0025							
Day	216.7 <.0001		4.8572 0.0047		4.2163 0.0259		$\chi=16.5, P=0.0009$		$\chi=21.4, P=0.0007$		6.8799 0.0144 5.3742 0.0111	



**Table 4.S8.** PCA correlations and loading values from the grassland site for all four sampling dates

	<b>Cover</b>	<b>S. Day</b>	<b>SWC</b>	<b>pH</b>	<b>PO<sub>4</sub><sup>3-</sup></b>	<b>Soil K</b>	<b>NH4-N</b>	<b>NO3-N</b>	<b>FBM</b>	<b>SOM</b>	<b>AAP</b>	<b>AP</b>	<b>β-gluc</b>	<b>NAG</b>	<b>Ox</b>	<b>POx</b>
<b>Cover</b>	100.0%	0.0%	-0.8%	41.5%	4.9%	-48.5%	1.2%	17.9%	76.8%	17.9%	-48.6%	-17.5%	32.3%	31.4%	20.9%	-15.1%
<b>S. Day</b>	0.0%	100.0%	58.4%	25.3%	26.3%	7.6%	-52.1%	-5.6%	-8.0%	14.6%	2.8%	5.2%	-26.9%	-22.5%	-11.0%	12.0%
<b>SWC</b>	-0.8%	58.4%	100.0%	28.7%	26.3%	19.4%	-73.5%	60.9%	-19.8%	11.1%	11.4%	43.7%	24.9%	34.1%	-21.3%	22.7%
<b>pH</b>	41.5%	25.3%	28.7%	100.0%	46.2%	18.7%	-34.6%	33.0%	12.3%	17.7%	-16.6%	2.3%	13.2%	15.5%	1.1%	3.4%
<b>PO<sub>4</sub><sup>3-</sup></b>	4.9%	26.3%	26.3%	46.2%	100.0%	32.7%	-15.9%	13.4%	-5.2%	-8.3%	-7.1%	-3.9%	-5.9%	-0.8%	-5.6%	3.6%
<b>Soil K</b>	-48.5%	7.6%	19.4%	18.7%	32.7%	100.0%	-7.6%	-2.9%	-39.7%	-17.4%	33.1%	21.1%	-12.3%	-8.9%	-14.1%	8.7%
<b>NH4-N</b>	1.2%	-52.1%	-73.5%	-34.6%	-15.9%	-7.6%	100.0%	-65.7%	10.6%	7.1%	-10.2%	-46.4%	-22.3%	-26.5%	-18.0%	-46.9%
<b>NO3-N</b>	17.9%	-5.6%	60.9%	33.0%	13.4%	-2.9%	-65.7%	100.0%	-4.5%	-5.2%	-7.0%	40.1%	58.7%	65.6%	6.4%	19.8%
<b>FBM</b>	76.8%	-8.0%	-19.8%	12.3%	-5.2%	-39.7%	10.6%	-4.5%	100.0%	-7.8%	-39.7%	-15.4%	19.6%	13.2%	40.4%	2.6%
<b>SOM</b>	17.9%	14.6%	11.1%	17.7%	-8.3%	-17.4%	7.1%	-5.2%	-7.8%	100.0%	-23.5%	-37.3%	-20.0%	-13.7%	-24.0%	-28.1%
<b>AAP</b>	-48.6%	2.8%	11.4%	-16.6%	-7.1%	33.1%	-10.2%	-7.0%	-39.7%	-23.5%	100.0%	61.8%	7.1%	8.3%	-1.3%	6.0%
<b>AP</b>	-17.5%	5.2%	43.7%	2.3%	-3.9%	21.1%	-46.4%	40.1%	-15.4%	-37.3%	61.8%	100.0%	49.3%	45.4%	10.6%	34.8%
<b>β-gluc</b>	32.3%	-26.9%	24.9%	13.2%	-5.9%	-12.3%	-22.3%	58.7%	19.6%	-20.0%	7.1%	49.3%	100.0%	86.1%	-6.4%	0.2%
<b>NAG</b>	31.4%	-22.5%	34.1%	15.5%	-0.8%	-8.9%	-26.5%	65.6%	13.2%	-13.7%	8.3%	45.4%	86.1%	100.0%	-16.5%	-10.8%
<b>Ox</b>	20.9%	-11.0%	-21.3%	1.1%	-5.6%	-14.1%	-18.0%	6.4%	40.4%	-24.0%	-1.3%	10.6%	-6.4%	-16.5%	100.0%	33.5%
<b>POx</b>	-15.1%	12.0%	22.7%	3.4%	3.6%	8.7%	-46.9%	19.8%	2.6%	-28.1%	6.0%	34.8%	0.2%	-10.8%	33.5%	100.0%

**Table 4.S9.** PCA correlations and loading values from the burned grassland site for all four sampling dates

	<b>Cover</b>	<b>S. Day</b>	<b>SWC</b>	<b>pH</b>	<b>PO<sub>4</sub><sup>3-</sup></b>	<b>Soil K</b>	<b>NH<sub>4</sub>-N</b>	<b>NO<sub>3</sub>-N</b>	<b>FBM</b>	<b>SOM</b>	<b>AAP</b>	<b>AP</b>	<b>β-gluc</b>	<b>NAG</b>	<b>Ox</b>	<b>POx</b>
<b>Cover</b>	100.0%	2.0%	-3.7%	-6.3%	-7.7%	-40.2%	23.1%	2.0%	63.3%	-10.4%	30.4%	43.1%	37.3%	38.3%	-17.4%	-8.8%
<b>S. Day</b>	2.0%	100.0%	44.0%	0.2%	38.2%	19.1%	-37.6%	-30.8%	-10.4%	15.1%	-40.9%	-22.6%	-27.9%	-28.1%	-17.0%	11.2%
<b>SWC</b>	-3.7%	44.0%	100.0%	51.4%	6.6%	14.9%	-5.7%	50.4%	-27.8%	15.2%	6.6%	25.2%	22.4%	21.8%	-26.2%	10.8%
<b>pH</b>	-6.3%	0.2%	51.4%	100.0%	-42.2%	6.6%	0.9%	38.4%	-11.8%	4.5%	27.5%	18.6%	18.4%	19.3%	-10.1%	-5.3%
<b>PO<sub>4</sub><sup>3-</sup></b>	-7.7%	38.2%	6.6%	-42.2%	100.0%	-10.5%	-8.9%	-15.8%	-13.3%	-2.1%	-25.1%	-3.0%	-9.2%	-4.9%	11.4%	10.4%
<b>Soil K</b>	-40.2%	19.1%	14.9%	6.6%	-10.5%	100.0%	-14.3%	-2.8%	-17.2%	9.3%	-21.3%	-26.6%	-27.1%	-28.9%	-6.3%	4.5%
<b>NH<sub>4</sub>-N</b>	23.1%	-37.6%	-5.7%	0.9%	-8.9%	-14.3%	100.0%	73.3%	-7.6%	-38.6%	62.1%	56.5%	65.1%	67.9%	-31.4%	-60.4%
<b>NO<sub>3</sub>-N</b>	2.0%	-30.8%	50.4%	38.4%	-15.8%	-2.8%	73.3%	100.0%	-22.4%	-21.6%	54.1%	54.1%	61.7%	64.3%	-38.1%	-38.3%
<b>FBM</b>	63.3%	-10.4%	-27.8%	-11.8%	-13.3%	-17.2%	-7.6%	-22.4%	100.0%	-9.3%	6.4%	11.7%	14.8%	9.5%	15.8%	9.3%
<b>SOM</b>	-10.4%	15.1%	15.2%	4.5%	-2.1%	9.3%	-38.6%	-21.6%	-9.3%	100.0%	-46.8%	-34.7%	-32.7%	-29.9%	-13.1%	-3.5%
<b>AAP</b>	30.4%	-40.9%	6.6%	27.5%	-25.1%	-21.3%	62.1%	54.1%	6.4%	-46.8%	100.0%	72.5%	76.9%	74.5%	-14.0%	-21.0%
<b>AP</b>	43.1%	-22.6%	25.2%	18.6%	-3.0%	-26.6%	56.5%	54.1%	11.7%	-34.7%	72.5%	100.0%	87.7%	88.2%	-12.8%	-14.5%
<b>β-gluc</b>	37.3%	-27.9%	22.4%	18.4%	-9.2%	-27.1%	65.1%	61.7%	14.8%	-32.7%	76.9%	87.7%	100.0%	93.3%	-10.7%	-12.6%
<b>NAG</b>	38.3%	-28.1%	21.8%	19.3%	-4.9%	-28.9%	67.9%	64.3%	9.5%	-29.9%	74.5%	88.2%	93.3%	100.0%	-8.1%	-15.2%
<b>Ox</b>	-17.4%	-17.0%	-26.2%	-10.1%	11.4%	-6.3%	-31.4%	-38.1%	15.8%	-13.1%	-14.0%	-12.8%	-10.7%	-8.1%	100.0%	66.0%
<b>POx</b>	-8.8%	11.2%	10.8%	-5.3%	10.4%	4.5%	-60.4%	-38.3%	9.3%	-3.5%	-21.0%	-14.5%	-12.6%	-15.2%	66.0%	100.0%

**Table 4.S10.** PCA correlations and loading values for all soil samples collected from the shrub/ grass ecotone site

	Cover	S. Day	SWC	pH	PO <sub>4</sub> <sup>3-</sup>	Soil K	NH <sub>4</sub> -N	NO <sub>3</sub> -N	FBM	SOM	AAP	AP	β-gluc	NAG	Ox	POx
Cover	100.0%	-0.0%	-2.7%	-18.0%	59.9%	67.9%	43.0%	19.3%	23.4%	6.6%	-45.5%	34.4%	25.4%	22.9%	20.6%	-6.4%
S. Day	-0.0%	100.0%	44.9%	-20.0%	27.4%	16.6%	27.6%	-39.3%	-15.8%	32.4%	-40.3%	-28.2%	-39.7%	-32.5%	-0.3%	-64.0%
SWC	-2.7%	44.9%	100.0%	35.2%	21.8%	26.1%	29.7%	45.9%	-20.2%	69.2%	-47.6%	-19.3%	-40.1%	-20.4%	-11.2%	-71.6%
pH	-18.0%	-20.0%	35.2%	100.0%	-14.4%	7.0%	-15.2%	22.0%	-35.0%	20.7%	22.8%	-19.8%	-26.2%	-5.2%	-25.7%	-14.2%
PO <sub>4</sub> <sup>3-</sup>	59.9%	27.4%	21.8%	-14.4%	100.0%	64.3%	76.1%	15.2%	-4.8%	11.4%	-37.4%	18.1%	11.8%	10.2%	11.7%	-28.5%
Soil K	67.9%	16.6%	26.1%	7.0%	64.3%	100.0%	45.2%	41.4%	-0.7%	19.5%	-43.6%	12.5%	5.9%	15.4%	12.6%	-28.6%
NH <sub>4</sub> -N	43.0%	27.6%	29.7%	-15.2%	76.1%	45.2%	100.0%	7.8%	-1.0%	17.2%	-33.8%	8.4%	-8.3%	-9.8%	14.0%	-30.0%
NO <sub>3</sub> -N	19.3%	-39.3%	45.9%	22.0%	15.2%	41.4%	7.8%	100.0%	8.7%	46.4%	-32.1%	1.8%	1.1%	16.1%	-4.3%	-8.6%
FBM	23.4%	-15.8%	-20.2%	-35.0%	-4.8%	-0.7%	-1.0%	8.7%	100.0%	-10.4%	-7.5%	10.0%	18.3%	3.6%	3.9%	33.7%
SOM	6.6%	32.4%	69.2%	20.7%	11.4%	19.5%	17.2%	46.4%	-10.4%	100.0%	-63.3%	-39.3%	-50.2%	-28.4%	-28.7%	-59.7%
AAP	-45.5%	-40.3%	-47.6%	22.8%	-37.4%	-43.6%	-33.8%	-32.1%	-7.5%	-63.3%	100.0%	5.0%	28.7%	19.7%	-6.2%	65.1%
AP	34.4%	-28.2%	-19.3%	-19.8%	18.1%	12.5%	8.4%	1.8%	10.0%	-39.3%	5.0%	100.0%	79.5%	68.3%	38.3%	19.8%
β-gluc	25.4%	-39.7%	-40.1%	-26.2%	11.8%	5.9%	-8.3%	1.1%	18.3%	-50.2%	28.7%	79.5%	100.0%	75.1%	29.4%	47.5%
NAG	22.9%	-32.5%	-20.4%	-5.2%	10.2%	15.4%	-9.8%	16.1%	3.6%	-28.4%	19.7%	68.3%	75.1%	100.0%	12.3%	29.3%
Ox	20.6%	-0.3%	-11.2%	-25.7%	11.7%	12.6%	14.0%	-4.3%	3.9%	-28.7%	-6.2%	38.3%	29.4%	12.3%	100.0%	4.6%
POx	-6.4%	-64.0%	-71.6%	-14.2%	-28.5%	-28.6%	-30.0%	-8.6%	33.7%	-59.7%	65.1%	19.8%	47.5%	29.3%	4.6%	100.0%

**Table 4.S11.** PCA correlations and loading values all grass canopy and bare soil associated samples collected from the shrub/grass ecotone site

	Cover	S. Day	SWC	pH	PO <sub>4</sub> <sup>3-</sup>	Soil K	NH <sub>4</sub> -N	NO <sub>3</sub> -N	FBM	SOM	AAP	AP	β-gluc	NAG	Ox	POx
Cover	100.0%	1.9%	7.0%	-31.2%	64.7%	39.8%	-3.5%	31.0%	79.9%	1.8%	-35.6%	55.3%	52.4%	47.9%	35.9%	-6.3%
S. Day	1.9%	100.0%	46.8%	-29.4%	10.5%	5.8%	15.7%	-31.9%	-6.4%	48.7%	-53.4%	-50.7%	-53.7%	-48.7%	-22.3%	-61.5%
SWC	7.0%	46.8%	100.0%	19.7%	34.3%	37.6%	59.0%	49.7%	-8.7%	77.4%	-65.5%	-32.6%	-50.5%	-33.3%	-34.2%	-71.8%
pH	-31.2%	-29.4%	19.7%	100.0%	-5.7%	2.6%	16.8%	11.9%	-28.4%	5.3%	27.1%	-26.7%	-28.4%	-20.4%	-44.7%	5.6%
PO <sub>4</sub> <sup>3-</sup>	64.7%	10.5%	34.3%	-5.7%	100.0%	78.0%	37.1%	56.7%	49.3%	27.7%	-46.4%	12.6%	15.2%	27.0%	3.9%	-22.3%
Soil K	39.8%	5.8%	37.6%	2.6%	78.0%	100.0%	45.8%	65.9%	25.0%	30.7%	-38.6%	5.0%	9.9%	23.6%	-5.5%	-22.5%
NH <sub>4</sub> -N	-3.5%	15.7%	59.0%	16.8%	37.1%	45.8%	100.0%	46.4%	-10.4%	45.4%	-40.6%	-14.4%	-27.2%	-21.6%	-12.7%	-40.3%
NO <sub>3</sub> -N	31.0%	-31.9%	49.7%	11.9%	56.7%	65.9%	46.4%	100.0%	13.7%	39.0%	-33.2%	17.4%	10.2%	37.1%	1.9%	-13.9%
FBM	79.9%	-6.4%	-8.7%	-28.4%	49.3%	25.0%	-10.4%	13.7%	100.0%	-4.0%	-25.5%	51.2%	53.5%	50.4%	38.2%	0.9%
SOM	1.8%	48.7%	77.4%	5.3%	27.7%	30.7%	45.4%	39.0%	-4.0%	100.0%	-72.1%	-47.9%	-54.5%	-41.8%	-37.6%	-68.0%
AAP	-35.6%	-53.4%	-65.5%	27.1%	-46.4%	-38.6%	-40.6%	-33.2%	-25.5%	-72.1%	100.0%	8.5%	31.1%	20.1%	2.3%	78.8%
AP	55.3%	-50.7%	-32.6%	-26.7%	12.6%	5.0%	-14.4%	17.4%	51.2%	-47.9%	8.5%	100.0%	82.5%	71.4%	54.9%	33.0%
β-gluc	52.4%	-53.7%	-50.5%	-28.4%	15.2%	9.9%	-27.2%	10.2%	53.5%	-54.5%	31.1%	82.5%	100.0%	81.0%	59.2%	54.1%
NAG	47.9%	-48.7%	-33.3%	-20.4%	27.0%	23.6%	-21.6%	37.1%	50.4%	-41.8%	20.1%	71.4%	81.0%	100.0%	43.6%	41.8%
Ox	35.9%	-22.3%	-34.2%	-44.7%	3.9%	-5.5%	-12.7%	1.9%	38.2%	-37.6%	2.3%	54.9%	59.2%	43.6%	100.0%	26.7%
POx	-6.3%	-61.5%	-71.8%	5.6%	-22.3%	-22.5%	-40.3%	-13.9%	0.9%	-68.0%	78.8%	33.0%	54.1%	41.8%	26.7%	100.0%

**Table 4.S12.** PCA correlations and loading values all shrub canopy and bare soil associated samples collected from the shrub/grass ecotone site

	<b>Cover</b>	<b>S. Day</b>	<b>SWC</b>	<b>pH</b>	<b>PO<sub>4</sub><sup>3-</sup></b>	<b>Soil K</b>	<b>NH4-N</b>	<b>NO3-N</b>	<b>FBM</b>	<b>SOM</b>	<b>AAP</b>	<b>AP</b>	<b>β-gluc</b>	<b>NAG</b>	<b>Ox</b>	<b>POx</b>
<b>Cover</b>	100.0%	7.3%	1.3%	-17.7%	60.0%	76.5%	44.4%	17.2%	15.2%	2.0%	-39.7%	50.1%	42.5%	36.6%	26.4%	-12.1%
<b>S. Day</b>	7.3%	100.0%	41.9%	-41.2%	22.5%	29.9%	28.1%	-29.1%	-9.9%	18.5%	-43.5%	-1.8%	-23.5%	-12.5%	23.3%	-59.9%
<b>SWC</b>	1.3%	41.9%	100.0%	24.3%	23.5%	29.6%	31.9%	-31.5%	-17.5%	66.2%	-49.1%	-4.1%	-30.7%	-8.8%	10.7%	-67.9%
<b>pH</b>	-17.7%	-41.2%	24.3%	100.0%	-17.2%	-1.6%	-24.2%	-28.9%	-36.8%	8.5%	28.1%	0.3%	-3.6%	18.1%	-15.7%	-2.7%
<b>PO<sub>4</sub><sup>3-</sup></b>	60.0%	22.5%	23.5%	-17.2%	100.0%	63.5%	77.2%	-3.8%	-12.5%	19.4%	-31.7%	41.8%	24.2%	17.7%	15.5%	-27.9%
<b>Soil K</b>	76.5%	29.9%	29.6%	-1.6%	63.5%	100.0%	45.2%	26.3%	-6.3%	13.1%	-45.7%	39.0%	24.4%	29.0%	34.2%	-33.2%
<b>NH4-N</b>	44.4%	28.1%	31.9%	-24.2%	77.2%	45.2%	100.0%	-8.2%	-10.6%	21.6%	-32.5%	32.7%	11.0%	13.7%	9.6%	-31.1%
<b>NO3-N</b>	17.2%	-29.1%	-31.5%	-28.9%	-3.8%	26.3%	-8.2%	100.0%	26.3%	-16.0%	0.2%	-4.0%	29.5%	19.4%	-17.2%	39.7%
<b>FBM</b>	15.2%	-9.9%	-17.5%	-36.8%	-12.5%	-6.3%	-10.6%	26.3%	100.0%	-16.6%	-0.7%	9.4%	38.3%	11.0%	6.5%	34.9%
<b>SOM</b>	2.0%	18.5%	66.2%	8.5%	19.4%	13.1%	21.6%	-16.0%	-16.6%	100.0%	-58.0%	-29.0%	-49.0%	-19.8%	-14.0%	-52.6%
<b>AAP</b>	-39.7%	-43.5%	-49.1%	28.1%	-31.7%	-45.7%	-32.5%	0.2%	-0.7%	-58.0%	100.0%	-2.1%	31.1%	25.0%	-22.4%	72.1%
<b>AP</b>	50.1%	-1.8%	-4.1%	0.3%	41.8%	39.0%	32.7%	-4.0%	9.4%	-29.0%	-2.1%	100.0%	66.2%	52.6%	19.8%	-4.0%
<b>β-gluc</b>	42.5%	-23.5%	-30.7%	-3.6%	24.2%	24.4%	11.0%	29.5%	38.3%	-49.0%	31.1%	66.2%	100.0%	64.7%	-6.4%	48.1%
<b>NAG</b>	36.6%	-12.5%	-8.8%	18.1%	17.7%	29.0%	13.7%	19.4%	11.0%	-19.8%	25.0%	52.6%	64.7%	100.0%	-24.1%	24.3%
<b>Ox</b>	26.4%	23.3%	10.7%	-15.7%	15.5%	34.2%	9.6%	-17.2%	6.5%	-14.0%	-22.4%	19.8%	-6.4%	-24.1%	100.0%	-17.8%
<b>POx</b>	-12.1%	-59.9%	-67.9%	-2.7%	-27.9%	-33.2%	-31.1%	39.7%	34.9%	-52.6%	72.1%	-4.0%	48.1%	24.3%	-17.8%	100.0%

**Table 4.S13.** PCA correlations and loading values from the desert shrub site for all four sampling dates

	<b>Cover</b>	<b>S. Day</b>	<b>SWC</b>	<b>pH</b>	<b>PO<sub>4</sub><sup>3-</sup></b>	<b>Soil K</b>	<b>NH<sub>4</sub>-N</b>	<b>NO<sub>3</sub>-N</b>	<b>FBM</b>	<b>SOM</b>	<b>AAP</b>	<b>AP</b>	<b>β-gluc</b>	<b>NAG</b>	<b>Ox</b>	<b>POx</b>
<b>Cover</b>	100.0%	7.4%	14.5%	-46.7%	27.8%	20.8%	33.9%	37.5%	60.9%	22.6%	-66.4%	31.6%	40.6%	33.3%	15.3%	7.7%
<b>S. Day</b>	7.4%	100.0%	34.3%	29.5%	-32.8%	4.2%	-18.3%	-15.2%	-19.0%	36.5%	-3.4%	-53.0%	-42.6%	-36.4%	-46.6%	-26.2%
<b>SWC</b>	14.5%	34.3%	100.0%	21.4%	-28.5%	4.4%	41.8%	-12.7%	-2.2%	24.7%	-11.5%	27.2%	23.6%	42.4%	-50.6%	-2.8%
<b>pH</b>	-46.7%	29.5%	21.4%	100.0%	-53.4%	-11.3%	-41.6%	-37.6%	-27.4%	-11.5%	43.4%	-26.5%	-24.1%	-11.8%	-21.3%	-12.7%
<b>PO<sub>4</sub><sup>3-</sup></b>	27.8%	-32.8%	-28.5%	-53.4%	100.0%	73.3%	25.6%	-21.9%	20.2%	-3.6%	-26.9%	22.1%	30.2%	17.0%	38.9%	33.3%
<b>Soil K</b>	20.8%	4.2%	4.4%	-11.3%	73.3%	100.0%	14.9%	-59.0%	8.9%	2.7%	-20.4%	13.2%	22.5%	20.2%	17.4%	15.5%
<b>NH<sub>4</sub>-N</b>	33.9%	-18.3%	41.8%	-41.6%	25.6%	14.9%	100.0%	13.4%	-3.6%	3.4%	-21.8%	58.4%	36.6%	46.9%	-14.4%	10.8%
<b>NO<sub>3</sub>-N</b>	37.5%	-15.2%	-12.7%	-37.6%	-21.9%	-59.0%	13.4%	100.0%	51.4%	14.6%	-29.5%	4.0%	-12.9%	-12.8%	3.5%	-14.1%
<b>FBM</b>	60.9%	-19.0%	-2.2%	-27.4%	20.2%	8.9%	-3.6%	51.4%	100.0%	7.6%	-55.4%	29.1%	37.6%	29.2%	45.2%	16.7%
<b>SOM</b>	22.6%	36.5%	24.7%	-11.5%	-3.6%	2.7%	3.4%	14.6%	7.6%	100.0%	-47.5%	-23.8%	-23.6%	-14.2%	-35.7%	-20.7%
<b>AAP</b>	-66.4%	-3.4%	-11.5%	43.4%	-26.9%	-20.4%	-21.8%	-29.5%	-55.4%	-47.5%	100.0%	-21.5%	-30.7%	-27.6%	-13.7%	-23.3%
<b>AP</b>	31.6%	-53.0%	27.2%	-26.5%	22.1%	13.2%	58.4%	4.0%	29.1%	-23.8%	-21.5%	100.0%	87.0%	90.3%	6.8%	20.9%
<b>β-gluc</b>	40.6%	-42.6%	23.6%	-24.1%	30.2%	22.5%	36.6%	-12.9%	37.6%	-23.6%	-30.7%	87.0%	100.0%	91.4%	16.6%	34.9%
<b>NAG</b>	33.3%	-36.4%	42.4%	-11.8%	17.0%	20.2%	46.9%	-12.8%	29.2%	-14.2%	-27.6%	90.3%	91.4%	100.0%	-4.1%	22.7%
<b>Ox</b>	15.3%	-46.6%	-50.6%	-21.3%	38.9%	17.4%	-14.4%	3.5%	45.2%	-35.7%	-13.7%	6.8%	16.6%	-4.1%	100.0%	53.6%
<b>POx</b>	7.7%	-26.2%	-2.8%	-12.7%	33.3%	15.5%	10.8%	-14.1%	16.7%	-20.7%	-23.3%	20.9%	34.9%	22.7%	53.6%	100.0%

## Chapter 5

### Conclusions

The studies described in this document make a significant contribution to our understanding of how multiple, different types of disturbance events can affect various kinds of RAM communities, and ultimately the soil processes that contribute to carbon storage within arid ecosystems.

In chapter two, I demonstrated how proximity to nearest neighbors that are members of the same species, the co-dominant species, or are dead, can significantly affect soil microbial community activity profiles within individually lining piñon, dead piñon, and juniper supported RAM communities. Additionally, in this chapter, I showed the ability of disturbance events, such as wide spread mortality among mature piñons, can influence soil microbial community activity patterns, at multiple scales, which span from individual rhizosphere associated microbial (RAM) communities, to whole field sites. Lastly, with the combination of the piñon and juniper sap-flow data, I was able to demonstrate the ability of specific RAM communities to respond more rapidly than others, to increases in host physiological activity via large increases in EE production. In one specific case the increased production of AAP from beneath the trees in LP/LP at the gridled site may be tied to an increased demand for N from the increasingly active piñons, as they respond to seasonal moisture pulses by performing more photosynthesis, which requires the use of the N rich protein, RuBisCO.

In chapter three, I revisited the PJ woodland data from the previous chapter, and expanded on those statistical analyses to include all assessed soil physico-chemical

variables, as well as all soil biotic variables I had data for. Data from a nearby juniper savannah site were incorporated in this study to serve as a reference regarding the steady state from what below ground RAQM community activity profiles may begin to look like, as piñon mortality affected PJ woodlands transition from an ecosystem featuring two co-dominant tree species, to a system primarily dominated by junipers. The results generated from these analyses, which include SEM, PCA, and step wise ANOVA all showed that the below ground function behaviors at the girdled PJ woodland site are still more similar to those at the control PJ woodland site, than those that are ongoing at the juniper savannah site. Further, a side-by-side comparison of the results from two different model types, e.g., SEM and step-wise ANOVA, shows the capacity of widespread piñon mortality to disrupt multiple, direct relationships between soil parameters, and EEA rates, while causing multiple, indirect pathways to gain in importance. Thus, the increased model complexity from the girdled PJ woodland comes at the expense of the overall ability of many of the statistical models to account for the variance within each individual soil EEA rate.

For chapter four of this dissertation, I expanded my research focus to include three additional lower elevation arid biomes, which are all represented by four different field sites that were located within the boundaries of the Sevilleta long-term ecological research site. This study focused on four different eddy-flux covariance tower sites, these sites included unburned grassland, burned grassland, a desert shrub land, and a shrub-grass ecotone site. Previous research conducted at these sites has shown evidence for some alterations within below ground ecosystem process rates, e.g., soil respiration,



which are likely related to GCC related disturbances such as drought, fire, and shrub encroachment. My work from these four sites expands on these previous studies. First, I present results from two different multivariate data analysis methods that show how the relationships between individual EEA rates, various soil parameters, and the rates of other individual EEs are all affected by disturbance events. Second, we show that grass supported, and shrub supported RAM communities, each demonstrate unique response patterns to different kinds of disturbance, e.g., fire, vs. shrub encroachment. Thus, our analyses of the alterations in expressed EEA profiles in various disturbed arid field sites may help explain the patterns of shifting ecosystem process rates, e.g., soil respiration, which have been revealed in previous studies.