EXPERIMENTAL WARMING CHANGES MICROBIAL ENZYME ACTIVITY IN SEMIARID SOILS

By

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Abstract:

Climate change is altering the function of ecosystems across the planet, including the semi-arid soil ecosystems like those found in the Arizonan desert. Soil enzymes produced by microbes are essential to these ecosystems, and their productivity is threatened by the predicted rise in temperature. In this study, seven different soil enzymes were studied to observe how seasonality, soil water content, and plant cover affected the activity of these enzymes through experimental warming. It was concluded that seasonality and plant cover have direct effects on enzymatic activity, while the soil water content cannot be correlated with the activity of all soil enzymes. The results of these different variables when being measured with one anther and the soil enzyme activity did not provide any conclusive results, further asserting the intricacy of these soil enzyme interactions and how future studies need to be conducted in order to better understand the complexities of these soil variable interactions.

Introduction:

The Southwest is both the warmest and driest region within the United States with current projections of warmer and drier conditions (Theobald et al. 2013) and more variable precipitation (Hoerling et al. 2013,). The years between 2001-2010 were measured to be the hottest years on record, with an increase in the annual average temperature by 0.9^{C} in the Southwest from 1901-2010 (Hoerling et al. 2013). Drylands (defined as arid and semiarid ecosystems) like the American Southwest account for about 40% of the world's terrestrial land surface; these drylands are also responsible for 40% of the world's net primary productivity (Wang et al. 2012). Even if global emissions were to decrease to a low-emission scenario, the projected temperature increase in the southwest ranges between $1.4\text{-}1.9^{C}$ by 2021-2050 (Cayan et al. 2013). This substantial change in ecosystem conditions brings uncertainty regarding how these ecosystems will respond. A significant amount of research has focused on how ecosystems will adapt to the progressive increase in temperature, highlighting the critical importance of understanding how soils and soil microbes influence their response.

Soil contains both microbes and organic material that microbes consume for energy and building biomass. Soil microbes include a wide variety of organisms, such as bacteria, fungi, archaea, and rhizobia (Van Der Heijden et al. 2008). These microbes use extracellular enzymes to metabolize larger molecules in order to obtain necessary nutrients that can be assimilated into the cell. The activities of these microbes and their extracellular enzymes are crucial for soil function and for maintaining biodiversity and food web structure. The presence and diversity of microbes within the soil influences soil ecosystem stability, as well as microbial productivity and resilience when exposed to ecosystem stress and disturbance (Torsvik ,Øvreås 2002). Soil microbes have also been found to be a main regulator of ecosystem functions such as plant productivity. This is especially the case in nutrient-poor environments, where plant symbionts are responsible for plants' ability to acquire certain nutrients (Bell et al. 2013). Soil microbes are recognized as drivers in multiple ecosystem processes, including decomposition, stages of the Nitrogen cycle and stages of the Phosphorous cycle (Olander et al. 2000). Furthermore, soil microbes such as microbial pathogens largely influence plant community dynamics and plant diversity, like determining plant species abundance and possibly assisting in the invasion of foreign plant species (Van Der Heijden et al. 2008). A vast majority of the complex interactions between the soil, microbes and other organisms are still unknown, and less than 1% of microorganisms that are observed within soil samples are identified (Torsvik ,Øvreås 2002). Even so, we can still observe the different extracellular enzymes that these microbes are

producing and under what circumstances these enzymes are being produced in order to understand the timing and rates of biogeochemical processes in soils.

To a certain threshold, the reaction rate of enzymes increases with temperature, which breaks down larger molecules into smaller molecules at a faster rate. However, enzymes also have an optimal temperature range at which they function and will begin to denature or become less efficient above that optimal temperature (Copeland 2000). Q_{10} temperature coefficient values have been used in previous studies in order to measure the rate of change in soil enzymatic activity to establish when soil enzyme are most sensitive to temperature changes. Seasonal patterns and activities of these enzymes varied between sampling dates (Wallenstein et al. 2009), so variability among soil enzyme activity is not unusual. Within the complex matrix of soil there are many factors that influence enzyme efficiency, including soil moisture, clay content, pH, and other chemical and physical characteristics (Sinsabaugh and Shah 2012). If temperature increases change the rate and functioning of soil enzymes, this will have significant implications for rates of Nitrogen, Phosphorous, and other nutrient cycling in soils and will influence Carbon storage capacity.

Factors like water availability can have a large impact on the activity of soil enzymes. It has been found that enzymatic activity in warming conditions generally plateau or decrease in dry soil conditions with lower values of activity for some soil enzymes in comparison to soils with higher soil water content (Suseela et al. 2014). Even though temperature plays such a key role in the rate of enzymatic reactions, many factors contribute to how a change in temperature affects enzyme activities under field conditions (Wallenstein et al. 2010). This is especially true in arid ecosystems, where low soil moisture has strong controls over activity. For some soil enzymes in dry soils, enzymatic activity in warming conditions have patterns of plateau or decrease, but when the system has a high water content the activities of these enzymes have been found to increase up to about 3300 nmol $h^{-1}g^{-1}$ between the driest and wettest conditions tested (Suseela et al. 2014). The soil water content can help buffer effects that increasing temperature might have on the soils enzymes through evaporative cooling. Water also serves to make necessary compounds more accessible for enzyme activity, thus making the soil more inhabitable for soil biota.

Areas in the Southwestern United States have special rain seasons, noted as monsoon season, in which heavy rainfall occurs mainly between the months of July to early September (Maddox et al. 1995). During these monsoon seasons, the soil moisture reaches its peak as a result of the high average rainfall during this time of the year, as Arizona receives over half of its annual rainfall during its monsoon season (McHugh et al. 2014). As a result, during this wet season of the year is when water is most available for soil enzyme activity. Soil moisture is pivotal to the functioning of enzymes, as it allows the enzymes to maintain natural structure conformations, interaction with enzyme reactants and products, and act as a reactant or product for certain reactions. In order for enzymes to function optimally, the ideal amount of water needs to be available to those enzymes (Rezaei et al. 2007). Studies have shown that a 21% decrease in soil moisture can result in a decrease in soil enzymatic activity in up to 80% for certain enzymes (Sardans and Penuelas 2005). With too little or too much water availability, the enzyme will not function properly or will function at a slower rate than it is capable of.

The type and amount of plant cover over the soil have also both been found to positively influence the microbial community in the plant rhizosphere and bulk soil. Higher plant diversity has been found to increase microbial growth and microbial biomass, both of which are linked to how the plant cover significantly affects soil enzyme activity. It has been found that high plant

diversity can buffer some effects of temperature increase (like decreasing the water lost in a soil system), but can also increase other effects by intensifying the effects on certain soil enzymes (Steinauer et al. 2015). It has also been found that as plant cover has declined, soil enzymatic activity has also decreased (Garcia et al. 2002). These feedbacks are critically important to Carbon, Nitrogen, and Phosphorous cycling.

In this study, two soils common to Arizona known as the Chiricahua and Hathaway soils (as described in Rasmussen et al. (2015)), were studied with warming and non-warming treatments then evaluated using Extracellular Enzyme Assay (EEA) to assess the activity of seven different soil enzymes involved in Carbon, Nitrogen, and Phosphorous breakdown. The results from this study will be adding to our growing understanding of how soil microbial activity responds to warming in ecosystems with low and fluctuating available soil moisture.

Hypotheses:

We hypothesize that season, soil moisture, and plant cover will significantly influence the response of extracellular enzymes to warming by mitigating the increasing temperature affects and allowing enzymes to operate within optimal temperature conditions longer. It has been found that higher plant diversity may be a leading control of soil processes in the face of climate change. Some enzyme activity has been found to be dependent on plant diversity and higher soil enzyme activities have been observed when these enzymes were in plots with high plant diversities and higher temperatures (Steinauer et al. 2015). It has also been observed that certain soil enzymes have increased activity in moist soil conditions and warm temperatures (Suseela et al. 2014). Based on these findings, it is believed that soils with a higher percentage of plant cover, higher moisture, and higher temperatures will result in higher enzymatic activity while soil plots will lower plant cover, lower moisture, and higher temperatures will result in the lowest soil enzyme activity.

Methodology:

General Setup

This project is part of a larger warming experiment that measures the effects of soil amendments on carbon storage and nutrient cycling in semiarid grasslands, but this project specifically focuses on non-amended soils. This experiment was conduced outdoors, located in a field site at the Campus Agricultural Center at the University of Arizona, and has been ongoing since October 2013. Infrared reflecting mirrors passively warm 1x1 m soil plots that are 0.5 m deep, which increase the temperature of the soil by about 1.5°C to a depth of 5 cm (Rasmussen et al. 2015). The remaining soil plots are controls that are not receiving the additional reflected sunlight. Each plot is lined with porous geotextile fabric, which enabled water to drain but inhibited the mixing of the experimental soil with the existing soil.

Image 1: Experimental field site located at the Campus Agricultural Center in Tucson, Arizona. Both control and mirrored plots are shown. Photo taken in October 2015.

Of the 4 different mulch types that are available on the experimental site, only the control (non-mulch added) for the Hathaway and Chiricahua soils was utilized to limit the variables in this experiment. The Chiricahua soil was created using of a mixture of metamorphic rocks, while the Hathaway soil consists of a combination of sandstone and other sedimentary rock (Rasmussen et al. 2015). For the plots, there were 8 samples for each treatment (either the mirror or no mirror), for a total of 16 plots observed (n = 16).

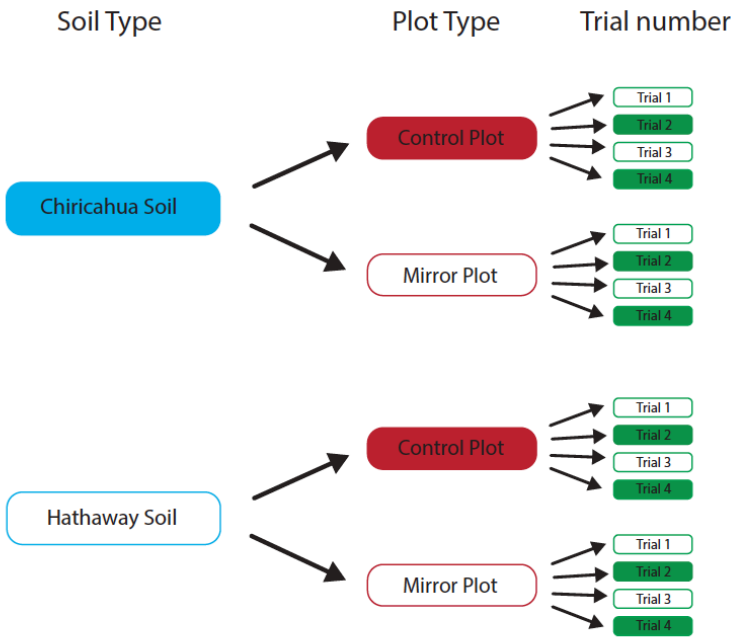


Image 2: A visual representation of the soil types, their mirror and control plots, and the number of mirror and control plots for each soil type.

Soils were sampled bi-annually in April and November to assess long-term temporal responses to the experimental warming. Since the monsoon season generally takes place between July and early August, November is considered the wet sampling season while April is the dry sampling season. As a result of this, it is expected that the soil moisture will be higher in November sampling events than April sampling events. Soil respiration was measured *in-situ* prior to soil collection. Samples were then collected from each plot by using a hand spade to remove the top 5 cm of soil and place it into a 1-quart Ziploc bag until full. These samples were placed into an insolated cooler and then transported back into the lab. After each plot is sampled, the soil is then sieved with a 2 mm sieve to remove any large rocks and organic matter. The sieved plot samples are stored at 4^{C} to preserve the soil properties and the microbial community until processing.

Fluorescence Enzyme Assay

The activities of seven different soil enzymes were measured in order to understand the effects of the temperature increase and how the interactions with the plant cover, soil moisture, and seasonality affected the enzymatic activities. The enzymes studied included 4-MUB-β-D-cellobioside (CB), 4-MUB-a-D-glucopyranoside (AG), 4-MUB-β-D-glucoside (BG), L-leucine-7-amido-4-methylcoumarin hydrochloride (LAP), 4-MUB-N-acetyl-β-D-glucosaminide (NAG), 4-MUB phosphate (PHOS) and 4-MUB-β-D-xyloside (XYL). Four of these enzymes are involved in carbon metabolism (CB, BG, XYL, and AG), two in nitrogen metabolism (LAP, NAG), and one in phosphorous metabolism (PHOS). Each of these enzymes has important roles in soil microbial metabolism. The function of CB is cellulose degradation, AG and BG specialize in sugar degradation, LAP functions in protein degradation, NAG specializes in chitin degradation, PHOS specialized in phosphorus mineralization, and XYL functions in hemicellulose degradation (Zhang et al. 2015).

In the Fluorescence Enzyme Assay (adapted from Saiya-Cork et al. 2002), a soil slurry was created by blending the 2.75 g of soil with 91 mL of 50 mM Sodium Acetate Buffer (pH equivalent to the average of the pH of the soil group assayed). Substrates were added to the slurries, being a standard curve of the used fluorescent indicators 7-amino-4-methylcoumarin (MUC) or 4-methylumbelliferone (MUB) or one of the seven soil enzymes. The MUC and MUB plates incubated at 25^{C} for 40 minutes. The enzyme plates incubated at 25^{C} and 35^{C} for 40 minutes and 15^{C} for 4 hours. The enzymes were measured using a Biotek plate reader (with an excitation wavelength of 365 nm and an emission wavelength of 450 nm) to assess the fluorescence of each soil-substrate slurry, indicating the activity level of the enyme.

Gravimetric Water Content (GWC)

About 5 grams of soil from each plot was weighed into a pre-weighed crucible and the exact weight of the soil was recorded. These samples were then placed in an over at 75-80^{C} over duration of 48 hours. The crucibles with the soil were immediately reweighed to find the exact weight of water evaporated from the sample.

Total Organic Carbon (TOC)

After the completion of GWC (or after the samples had the water weight evaporated out of the sample), each plot sample was placed into a muffle furnace where it was heated to 500^{C} for duration of 5 hours. The samples were then immediately weighted to find the lost weight of the carbon that was vaporized from the soil sample.

Soil pH

Five grams of each plot sample was weighted out to 0.1 accuracy and transferred to a 50 mL tube. Each sample had distilled water added to them and place in a shaker for 15 minutes and 160 RPM then let stand for 30 minutes. After this, a calibrated pH meter was used to determine the pH of each soil sample.

Analysis

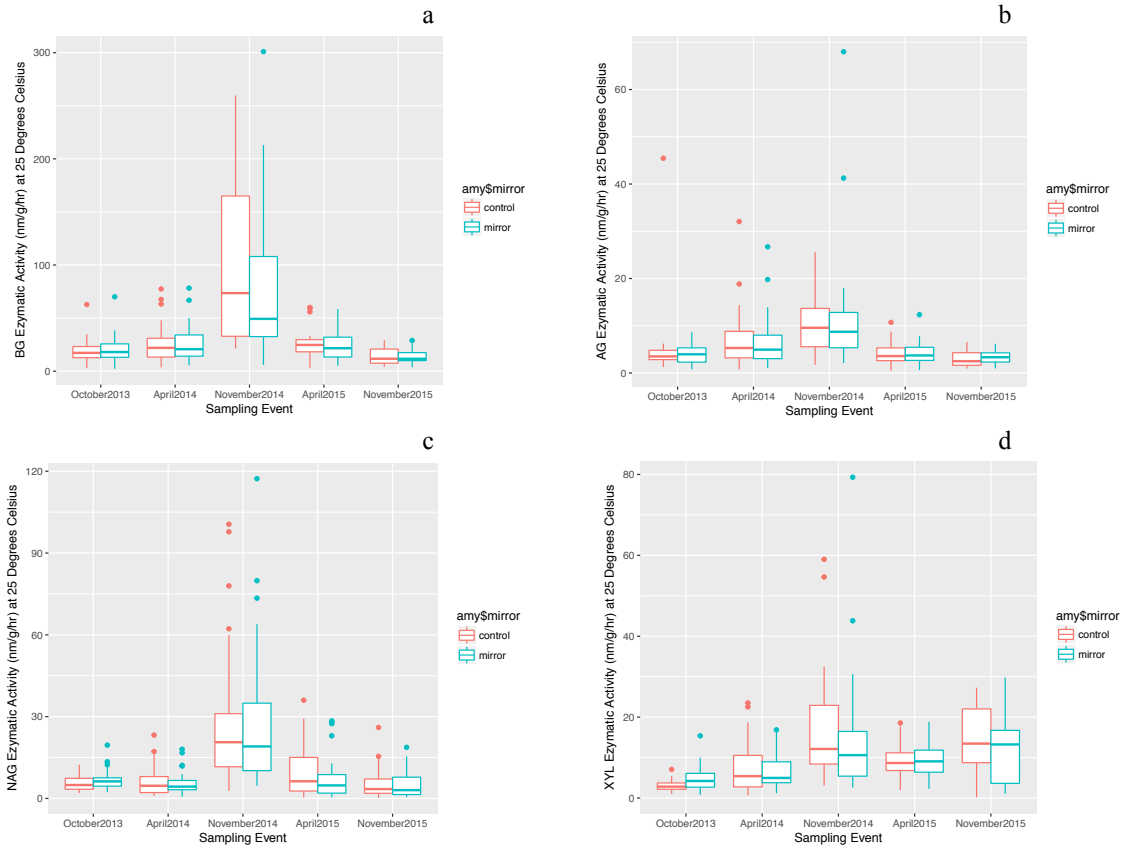
Q_{10} temperature coefficient values were utilized in order to measure the change in the enzymatic activity rate as a result of temperature increase. The data from the Fluorescent Enzyme Assays were analyzed using the Q_{10} temperature coefficient in order to measure the rate of change in the enzymatic activity as a result in the temperature increase between the 15^C and 35^C assay plates for each of the seven soil enzymes focused on. Q_{10} was calculated as:

$$Q_{10} = (Rate_{35}/Rate_{15})^{(1/2)}$$

where $Rate_{35}$ and $Rate_{15}$ were the activity rates of the enzyme at 35^C and 15^C. All data from the experiments were analyzed and graphed using R.

Results:

Figure 1 a-g shows enzyme activity from October 2013 - November 2015. Enzymatic activity varies among enzymes and years, which was expected as a result of each enzyme having different functions and properties. For many of the enzymes sampled, there is greater variability in activity from the November 2014 sampling date.



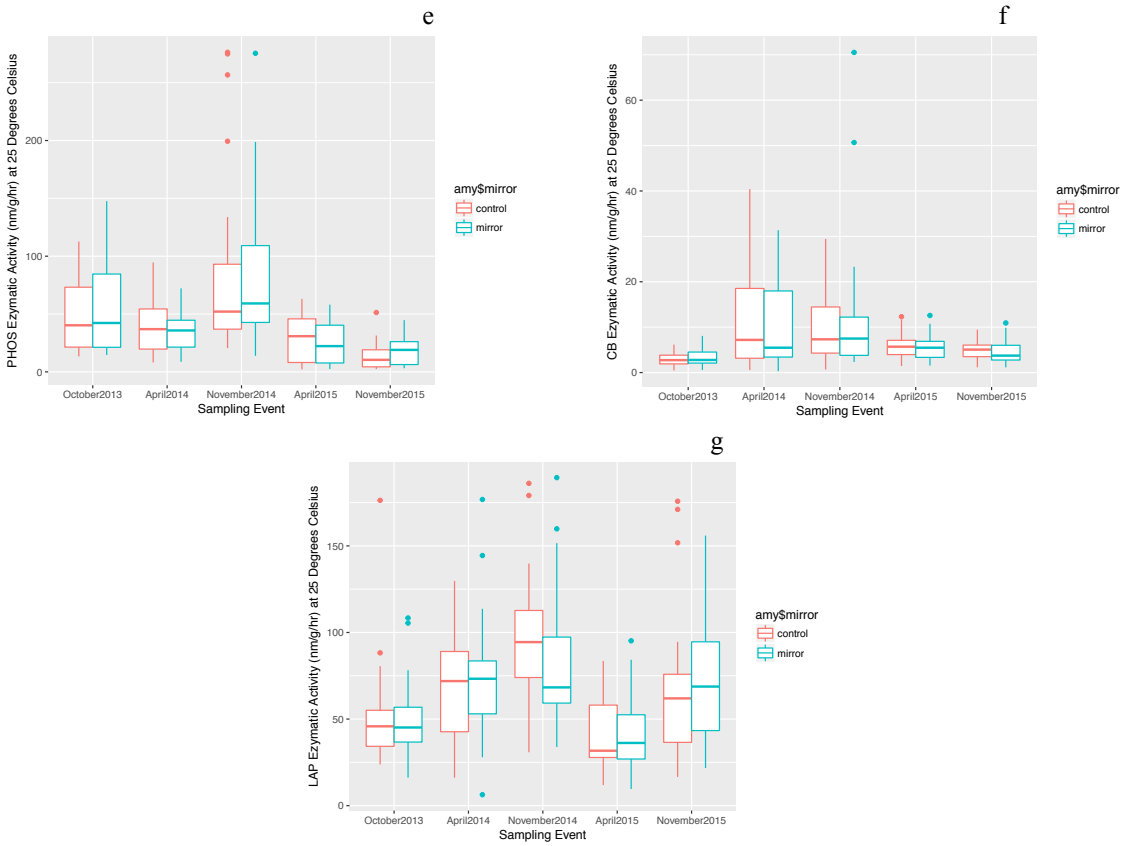
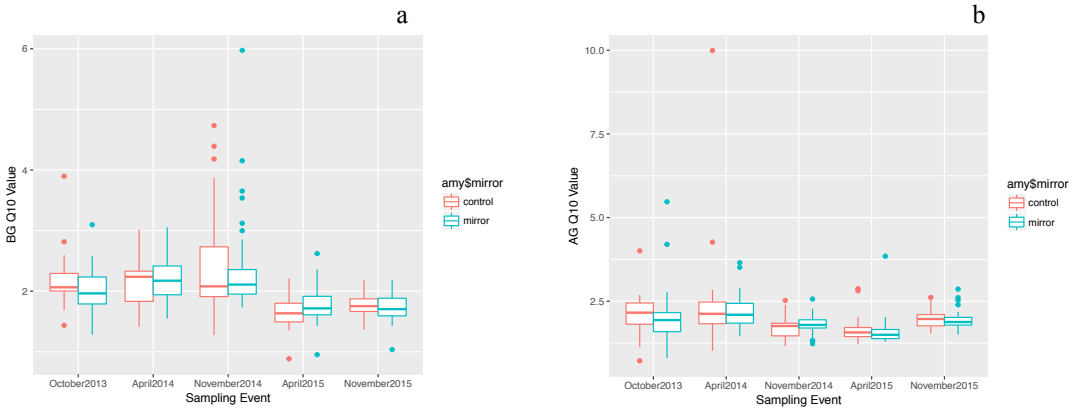


Figure 1 a-g displays the average enzymatic activity of all seven of the experimental soil enzymes in both the mirror and control plots throughout the sampling events, allowing the direct comparison of the enzyme activities between the two treatments. Box plots represent mean values with ± SE.



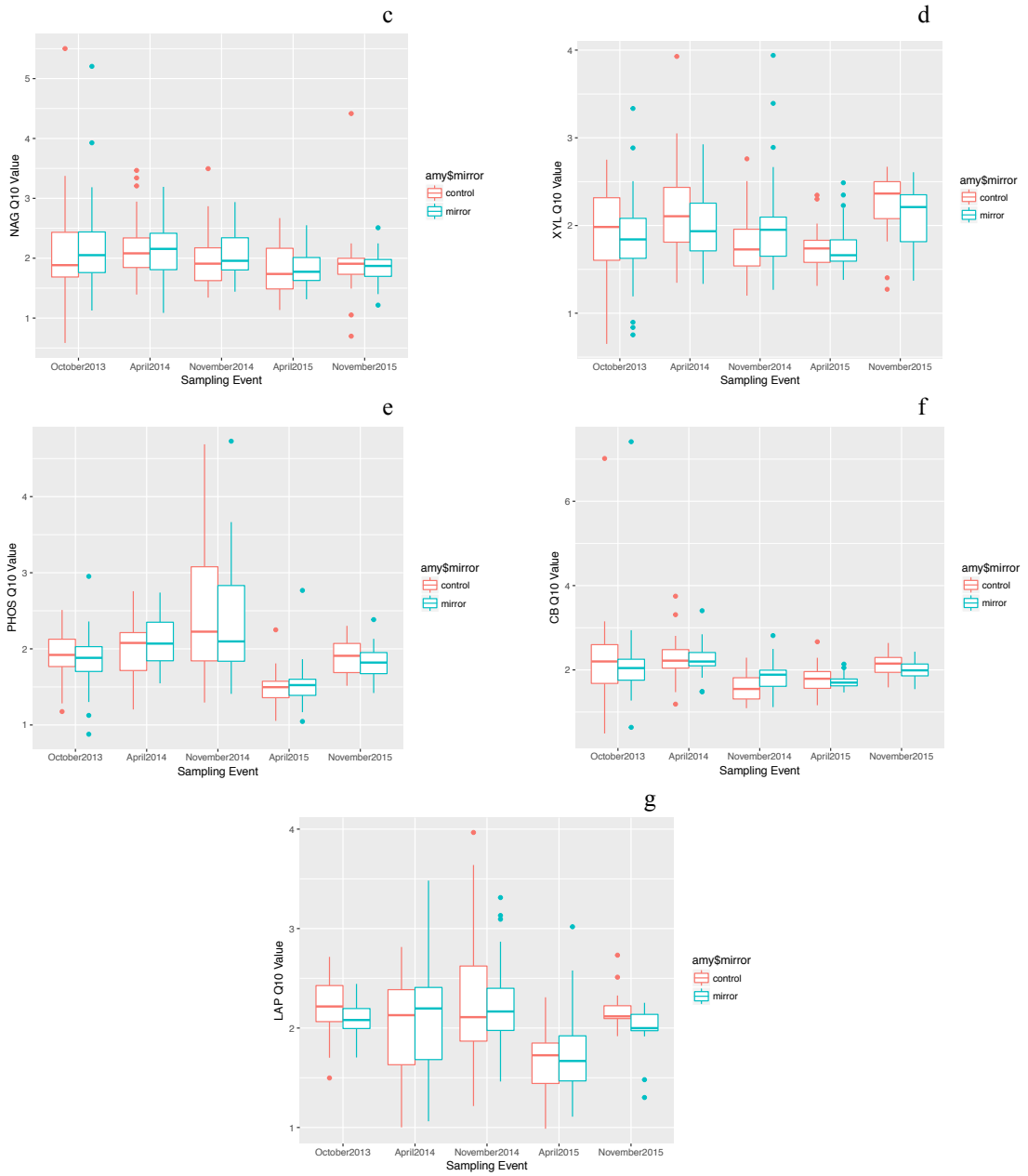


Figure 2 a-g illustrates the Q_{10} values of all seven enzymes at 25^{C} for both mirrored and non-mirrored plots throughout the sampling events, allowing for the comparison of Q_{10} values between mirror and control plots, enzymes and sampling events. Box plots represent mean values with ± SE.

Figure 2 a-g illustrates the variation in the Q_{10} values across the different enzymes and their sampling events. A larger Q_{10} value indicates a greater change in activity in a given set of circumstances. The BG, PHOS, and LAP (Figure 2 a, e, and g) appear to have had significant Q_{10} values for the November 2014 sampling event while the other enzymes all had similar Q_{10} patterns of the October 2013, April 2014, and November 2015 being the more significant sampling events. Table 1 below illustrates the numberic properties of the Q_{10} values calculated for each enzyme. Most of the enzymes, especially AG and CB, are shown to have a large range of values.

Enzyme	Min.	1st Quar.	Mean	3rd Quar.	Max.
BG	0.88	1.70	2.05	2.24	5.97
AG	**0.72**	1.57	1.95	2.13	**9.99**
CB	**0.49**	1.65	1.99	2.21	**7.41**
XYL	0.65	1.64	1.94	2.22	3.94
NAG	0.58	1.70	2.02	2.27	5.50
LAP	0.99	1.78	2.05	2.30	3.97
PHOS	0.88	1.58	1.96	2.15	4.73

Table 1 shows the numeric properties (minimum, 1st quartile, mean, 3rd quartile, and maximum) of the Q_{10} values for each of the enzymes studied, allowing for the comparison of values between the enzymes.

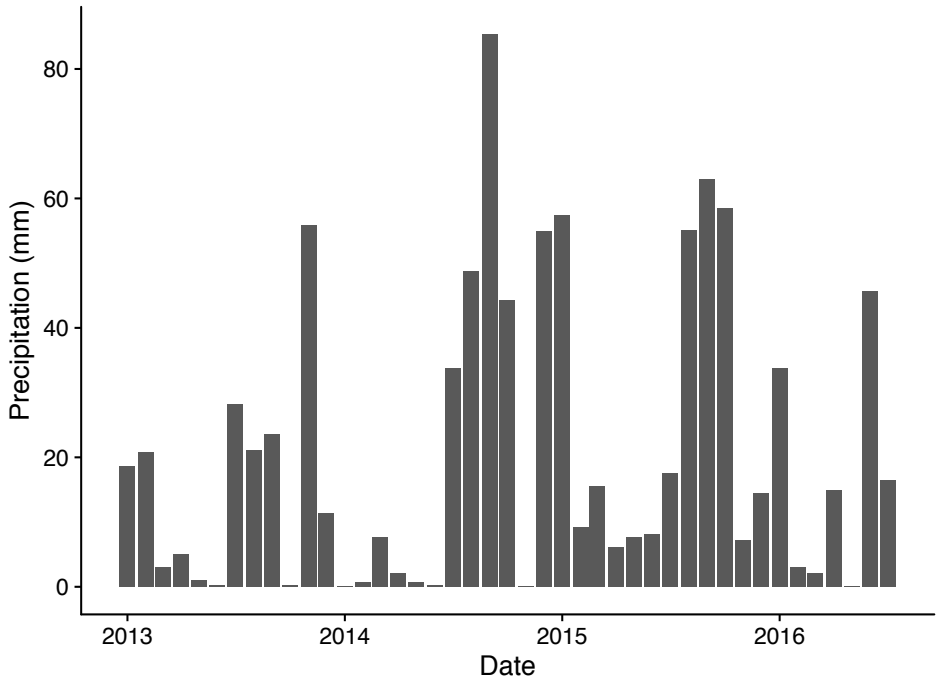
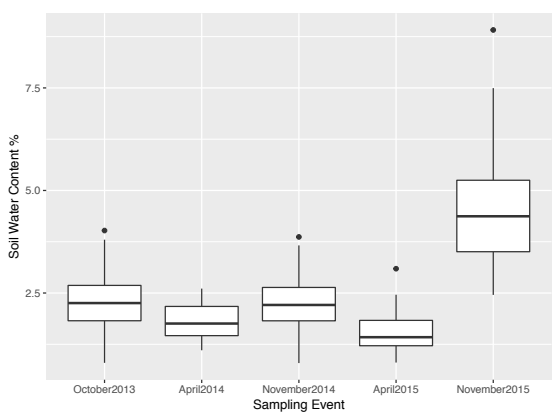


Figure 3 shows precipitation over the course of the experiment to August 2016. This provides information as to which seasons (and collection events) had higher precipitation levels.

Figure 3 displays the millimeters of rainfall that occurred over the years that this ongoing experiment has been preformed. This information is pivotal to understanding the moisture of the plots not only when the sampling events took place but also weeks before it too incase the precipitation events might have had any effect on the soil moisture in the following month. Due to the monsoon seasons lasting from July-September, it was assumed that the wet season sampling event would correspond with the November samplings. When observing the precipitation between the November and April sampling events of each year since 2013, it can be seen that this assumption is not necessarily accurate, so the moist season sampling events cannot be assumed to be November in each year and should be determined based on the rainfall charts above. However, the peak in rainfall in November 2014 does correspond to higher and more variable enzyme activity in November 2014.



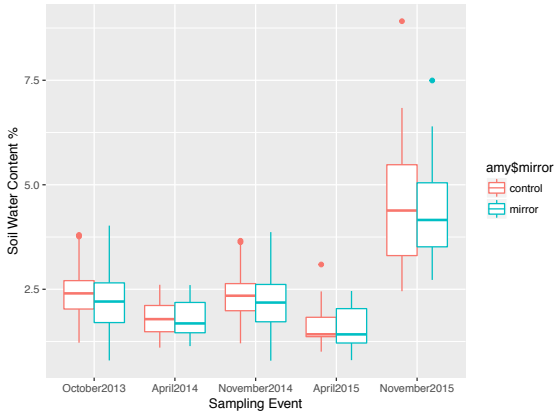
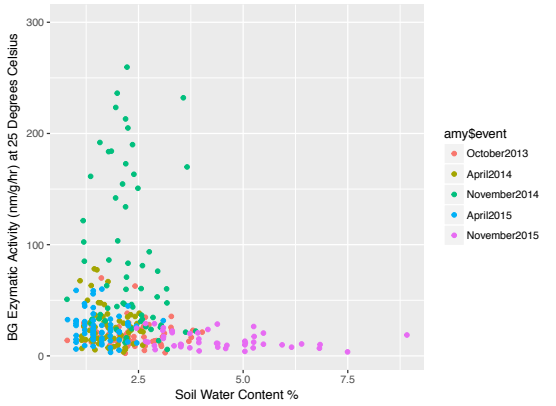


Figure 4 (top left) shows the average soil water content of all plots from the different sampling events as time progresses. This allows the comparison of general precipitation throughout the time on the experiment. Box plots represent mean values with ± SE.
Figure 5 (top right) illustrates the soil water content of mirrored and control plots at different seasons over time. This allows the comparison of values between the control and variable conditions. Box plots represent mean values with ± SE.

In correspondence with the precipitation values displayed in Figure 3, Figure 4 displays the averaged GWC of the sampled plots from each sampling event. It can be observed that even though Figure 3 displayed information that lead to the conclusion that November was not always the wet sampling event of the year, both Figures 4 and 5 above illustrate the post-monsoon sampling events to have higher soil water content than the pre-monsoon collection events. Figure 5 allows for the comparison of the GWC between the control and the mirror plots, revealing the average GWC of the mirror plots to be lower than the average of the control plots. The exception to this is the April 2015 sampling event, in which the two averages were extremely close to one another.

It is seen in Figure 1 a-g above that the enzymatic activity of each of the seven enzymes focused on in the study varies significantly even though each of the plots were exposed to the same amounts of precipitation. It was expected that there would be a correlation between the amounts of precipitation that a plot received with the amount of enzymatic activity that was measured. Referring back to Figure 4 and comparing the higher GWC sampling events with the enzymatic activities of the sampling events in Figure 1, no clear overall correlation between the soil water content and the enzyme activity of the soil enzymes can be determined.



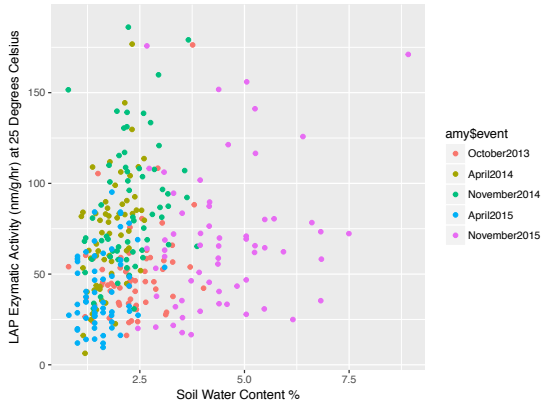


Figure 6 (left) shows the activity of the BG enzyme at the 25^{C} treatment as a function of the soil GWC for all of the plots sampled during each sampling event.
Figure 7 (right) shows the activity of the LAP enzyme at the 25^{C} treatment as a function of the soil GWC for all of the plots sampled during each sampling event.

As seen in Figure 1 a-g, the timing of soil sampling, which correlates with GWC seems to have differing effects on the different soil enzymes. Figures 6 and 7 further reinforce this observation by showing a more direct relationship between the GWC and enzymatic activity of specific soil enzymes. Figure 6 reveals that for the BG enzyme, which is involved with the breakdown of cellulose, GWC appears to have no clear correlation with this enzymes activity, showing it to be an indeterminate measure of this enzymes activity in the soil. There is higher variability in BG activity with lower GWC. In contrast, Figure 7 reveals that the activity of the LAP enzyme, which is important for protein acquisition, has a weak positive correlation with the soil's GWC. With the difference between these two figures, it can be stated that soil moisture has a differential effects on the activity of certain enzymes.

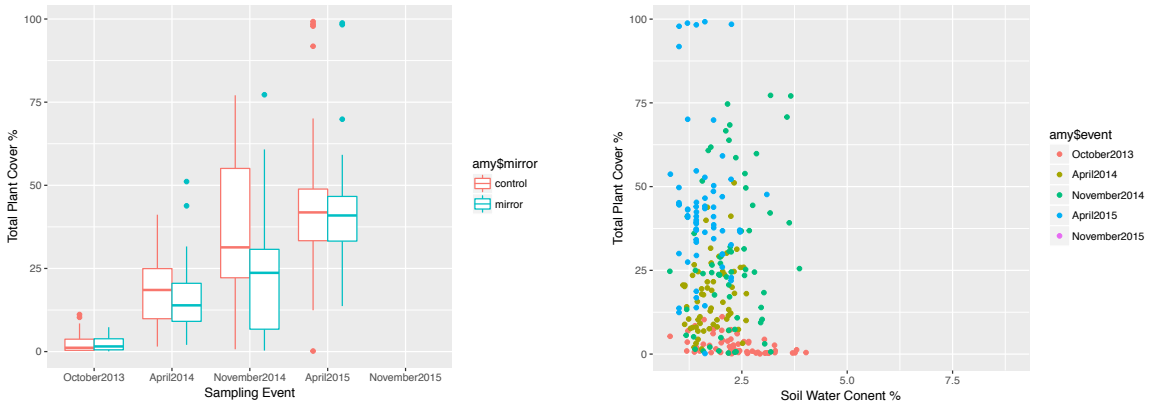


Figure 8 (left) shows the average plant cover of the mirror and control plots in each sampling event. Box plots represent mean values with ± SE.
Figure 9 (right) illustrates the amount of plant cover in each plot as a function of the plot's GWC during each sampling event.

Results from Figure 8 show that plant cover increases over time in both the mirror and control plots from October 2013 to April 2015. As a result of the information gathered in Figure 8, it can be observed that the average total plant cover of the plots decreased in the mirrored plots in comparison to the control plots. This shows that the heat treatment does have a negative effect on plant growth.
There is no plant cover data from November 2015, which had the highest GWC (Figures 4-7). Figure 9 shows that within the narrow range of GWC variation for the years sampled, there is no clear correlation between the plot's GWC and the amount of total plant cover that it has. Plant cover varies from 0-100% among the plots.

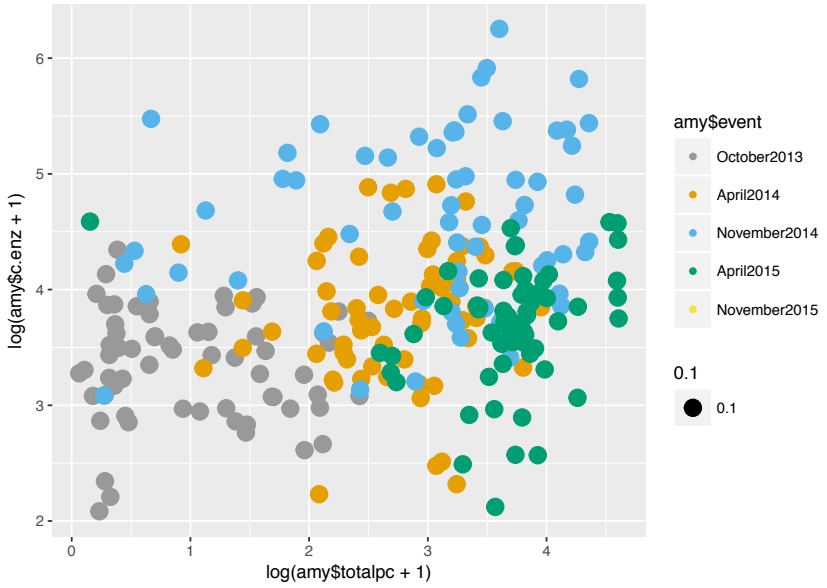


Figure 10 illustrates the logarithmic relationship carbon metabolizing enzymes (BG, AG, XYL, and CB) activity of different sampling events as a function of the total plant cover during those sampling events. A weak positive trend can be observed in the scatter plot, suggesting that the enzymatic activity in the soil has a positive association with the soil's total plant cover.

The enzyme activity in in Figure 10 above exhibits a positive trend between the total plant cover and the enzymatic activity of carbon-metabolizing enzymes.

Discussion

We hypothesized that season, soil moisture, and plant cover would significantly influence the response of extracellular enzymes to warming by mitigating the increasing temperature affects and allowing enzymes to function under normal temperature conditions longer.

Effects of Season

The amount of precipitation that the overall season has produced appears to have a significant effect on the enzymatic activity. As exemplified by Figure 1 a-g, all enzymes with the exception of CB (Figure 1 f) experienced a large spike in activity in the November 2014 sampling event. It was hypothesized that increased soil moisture would have a positive effect on enzymatic activity, and this was supported in November 2014. However, precipitation in November 2015 was higher than in 2014 (Figures 4 and 5), but overall enzyme activities were not. This could be a result of the sampling event occurring at variable times of precipitation during the sampling month. A large precipitation event occurring over a week before the sampling event may not be reflected in the soil water content. The higher activity in November 2014 may not be a result of the direct soil moisture measured, but from prolonged, high precipitation that the plots experienced over the entire wet season of November 2014 in comparison to the other sampling events.

As previously discussed, the November sampling events are generally considered to be the wet sampling event since it occurs just after Arizona's monsoon season in which a majority of the annual rainfall occurs. When referring to Figure 3, it can be observed that all November sampling events occurred after a large amount of precipitation in at least the 3 prior months.

Even though the November 2014 sampling event saw nearly no rainfall that month, it experienced a significant amount of a total of ~215 mm of rainfall over 4 months prior. In comparison, November 2015 did have ~10 mm of rainfall that month but only a significant precipitation for the prior 3 months with a total of ~178 mm before the months of sampling. As a result, this data supports the hypothesis that seasonality does have a significant effect on the activity of soil enzymes by providing more moisture over a longer period of time, but does not necessarily support the hypothesis that soil moisture can have a direct effect on enzymatic activity. This could also account for the large Q_{10} value increase in the BG, PHOS, and LAP enzymes (Figures 2 a, e, and g) during the November 2014, signifying a significant increase in the enzymatic activity in those soil enzymes during that sampling event.

Effects of Soil Moisture and Temperature

The effects of soil moisture on prolonging the productivity of the studied soil enzymes appears to vary depending on the enzyme. Our hypothesis that soil moisture would significantly influence the activity of these enzymes was only true for specific enzymes within the experiment. When observing Figure 5, we see a general decrease in soil moisture in the mirrored plots as opposed to the control plots, which was expected as a result of greater evaporation in warmer conditions. Also referring to Figure 5, it can be observed that the largest moisture event by far was the November 2015 event, therefore it was expected based on our hypothesis that there should be an increase in enzymatic activity in the soil enzymes observed specifically for that sampling event in comparison to the others. This hypothesis was not be supported by the results shown in Figure 1 a-g, as only the XYL enzyme (Figure 1d) experienced a significant increase in its activity for the November 2015 sampling event. All other enzymes exhibited either no significant increase in activity (Figure 1 g) or a decrease in activity (Figure 1 a-c, e-f). This data is inconsistent with other studies, which were able to show a direct relationship between soil moisture and enzymatic activity. The study by Suseela et al., (2014) shows that BG had the highest activity at all temperatures when the most water was available to it while NAG experienced higher activity in wetter soils at all temperatures in comparison to the driest soil tested. The third enzyme tested in that study (Phenol oxidase, which was not focused on in this study) showed varying results of activity in different soil moistures at different temperatures. This illustrated how diverse enzymes will respond differently to their environment. The results from Suseela et al. (2014), which focus on may of the same enzymes, contradict our results. While both of these studies were conducted in grasslands, the differences might be driven by the different types of microbial communities in these different ecosystems.

The Q_{10} values displayed in Figure 2 appear to reflect more consistently the increase in enzymatic activity in response to moisture, as all of the enzymes with the exception of BG (Figure 2 a) and PHOS (Figure 2 e) illustrate a more consistent pattern of the November 2015 sampling event to have a higher Q_{10} value that is similar to the October 2013 and April 2014 Q_{10} values. This corresponds better with the Suseela et al. (2014) study's results of higher moisture generally correlating with higher enzymatic activity. Even so, only the XYL enzyme (Figure 2 d) had the highest Q_{10} value during the highest soil-sampling event of November 2015. These Q_{10} values had a further point of interest, being the wide range of minimum and maximum values for the enzymes found, shown in Table 1. Results show that enzymes like AG and CB had very wide ranges of Q_{10} values (being 0.72-9.99 and 0.49-7.14 respectively). This was very different from the widest range reported by Stone et al. (2011), with their widest Q_{10} range in their soil enzymes studied being 1.64-2.27 across all of their enzymes. This study was preformed in the Artic tundra,

so it is possible that the change in temperature between the two different sampling sites can account for the change in activity, as the average temperature during the samplings that occurred in the Stone et al. (2011) study were between 3-12^{C} while the range of temperatures our study's Q_{10} values were derived from were between 15-35^{C}. Our temperature range was much larger than theirs (9^{C} versus 20^{C}), which may account for such a wider Q_{10} range in our study than in the Stone et al. (2011) study.

To further illustrate the effects of the soil moisture on the differing enzymes, scatter plots were created to see the correlations between the soil moisture and the enzymatic activity. The LAP enzyme (Figure 8) was found to have a positive correlation between the GWC and its activity, while the BG enzyme (Figure 9) was found to have no real correlation, though there was greater variability in activity with lower GWC. As a result, it can be stated that soil moisture (GWC) cannot be a consistent measure for soil enzyme activity since it affects enzymes differently. These results coincide with those published in the Steinauer et al. (2014) study, which show a variety of enzymatic activity among soil enzymes in correlation with the soil water content. The soil water content only appeared to have correlations with some of the enzymes in that study (such as PHOS), while others did not appear to have any correlation between the enzyme activity and soil water content (such a BG), which supports the data that is presented in our study.

Effects of Plant Cover and Temperature

In both the control and mirror plots, plant cover increased over the course of the experiment (Figure 8). With the decreased amount of plant cover in the mirrored plots as displayed by Figure 8, it is expected that this will have a negative effect on the enzymatic activities of these plots. With the information given in Figures 1 a-g, there seems to be no clear conclusion as to whether plant cover negatively or positively affects the activity of the enzymes tested. The mirrored plots generally have less plant cover (Figure 8) though do not necessarily have lower enzyme activities for the enzymes tested. Each enzyme graphed in Figure 1 a-g has mirrored plots with both more and less measured enzymatic activity than the its control counterpart. However, as exemplified in Figure 10, there is a positive association between carbon-metabolizing enzymes and the amount of plant cover, showing plots that had more plant cover to have higher carbon enzyme activity. Taking the logarithm of the enzymatic activity values and the plant cover values (Figure 10) allows for a more consistent statistical analysis of to relationship between these two variables by lessening the impact that the outliers have on the numeric results. The outcome of this is a weak positive correlation between the total plant cover and the activity of the carbon-interacting enzymes. This analyzed correlation is consistent with the results of Garcia et al. (2002), which found enzymatic activity to decline when less plant cover is present.

We predicted that soils with a higher percentage of plant cover, higher moisture, and higher temperatures should support higher enzymatic activity while soil plots will lower plant cover, lower moisture, and higher temperatures should support the lowest soil enzyme activity. In response to this prediction, we expected data from the mirrored plots that had higher plant cover and higher soil moisture content to have higher enzymatic activities and Q_{10} values. When referring back to Figure 8, the mirrored plots have less plant cover in general. Even so, the plant cover continually increased over sampling events (Figure 8), so we would expect to see mirrored plots in later November sampling events, which is generally the wetter season, to produce more

enzymatic activity than earlier sampling events. When referring to Figure 4 and 5, we see that the highest soil moisture sampling event is November 2015, which allows for easy comparison. This is a later sampling event, so it is expected that the mirror plots from this sampling event will have an obvious increase in enzymatic activity and Q_{10} values. When observing Figures 1 a-g and 2 a-g, we see that this is not necessarily the case. As previously discussed, the most impactful sampling event across the board for enzymatic activity was November 2014, and multiple Q_{10} value peaks are seen in the in the same sampling event. The only enzyme that appears to support this hypothesis is XYL (Figure 1 c and 2 c), which peaks in November 2015 for both its enzymatic activity and Q_{10} value. This prediction is therefore not supported by the results from this experiment, as both GWC and plant cover is highest in November 2015 but enzyme activity is not. This is in contradiction to previously published information, which provided that the highest enzyme activities are seen with warmer temperatures paired with wetter conditions (Suseela et al. 2014) and with higher plant cover (Garcia et al. 2002).

Alternatively, we expected the opposite pattern of enzymatic activity for plots with the lowest soil moisture and plant cover, which were found in April. Although April 2014 has one of the lowest soil water content values of the sampling events (seen in Figures 4 and 5) and a lower plant cover amount compared to the future three sampling events (as seen in Figure 8), there is no consistency with the values of the enzymatic activity across the seven enzymes during this sampling event. When observing the Q_{10} values for the enzymes during this sampling event, they appear to stay consistently near the values of the October 2013 event even though there was less water in this sampling event. Like our hypothesis concerning conditions predicting the highest enzymatic activates, these results do not support the prediction that lowest enzyme activities are correlated with lowest plant cover and soil water content. Once again, the results observed in this study are contradictory to those previously published. Low enzymatic activity values were correlated with low soil moisture and warmer temperatures (Suseela et al. 2014) and lower plant cover (Garcia et al. 2002). The results found in this study contradicts these previously published enzymatic activities when all of these variables being measured in the same experimental plots. This difference in the expected results from those that were observed might be tied to the complex interactions that this study sought out to better understand. With multiple variables occurring in a single experimental set up, it can be difficult to tie direct relationships between the individual variables. This study and its results are inefficient in drawing direct conclusions between some interactions that are present with the microbial enzymes in the soil plots observed.

Conclusion and Further Work

The results of this experiment provide further evidence that the enzymatic activities and relationships of soil microbes are extremely complex and have many factors that affect their overall productivity. This study was able to provide evidence in support of the hypothesis that in semiarid soils, seasonality and plant cover have direct effects on the enzymatic activities of soil microbes. This suggests that these factors will directly influence how well these soil enzymes continue to function with the rising temperatures resulting from climate change. The soil moisture content was found to have a much less direct effect on these enzyme activities, as it correlated with these activities for only certain enzymes. Even though soil water content will continue to be an important factor in activity, it cannot be a direct indicator of enzyme activity. Even though the results in this study provided evidence for these conclusions, it was not able to provide enough evidence to make any conclusions about the complex interactions that these variable have when interacting with one another in the soil enzyme activity. This study was

insufficient in pinpointing the indirect causes of certain outcomes and the important interactions among the numerous variables such as soil type, season, plant cover, and soil water content. Future studies should further explore the complex interactions of the variables explored in this study and attempt to better understand the outcomes that the presence or absences of each variable can have on others.

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