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**Introduction**

Climate change can be viewed as humanity’s defining problem of the 21st century (citation needed). Due to the multi-faceted nature of the problem, tackling this issue involves multiple interdisciplinary approaches in terms of both solutions and effects of climate change. For one, the different feedbacks between climate and the rest of the Earth system needs to be considered in order to understand and project the pace at which the climate is changing (citation needed). Multiple feedbacks are at play with some being positive feedbacks while others are negative feedbacks (citation needed). It is also worthwhile to compare the sizes of different reservoirs of carbon in the Earth system. As soil carbon is larger than either land plants or the atmosphere, a small change in this reservoir, depending on the direction of the change, can either greatly exacerbate or curb climate change (citation needed). Therefore, the stability of soil as a reservoir of carbon needs to be studied.

The flux of carbon from soils to the atmosphere is mediated primarily by microbes via the sum of processes known as “decomposition” (citation needed). Historically, studies of decomposition primarily considered climatic abiotic factors – such as precipitation, temperature, evapotranspiration – in studying decomposition while neglecting the role of microbial community composition and function (citation needed). Only more recently, towards the beginning of the 21st century, did biogeochemists consider the role of soil microbes in carbon cycling in terrestrial ecosystems. This bias was seen not just in empirical studies (citation needed) but also models that range from the ecosystem scale to the global scale (citation needed). Even to this day, amongst the Earth system models from CMIP6, only one explicitly considers soil microbes in decomposition (citation needed).

This study aims to fill some of the knowledge gaps regarding the role(s) of microbes in biogeochemistry and climate change. Responses of microbes based on their physiology and ecology will influence the nature of feedbacks between soils and climate (citation needed). As a result, studying the responses of microbes to the varying effects of climate change (e.g. from drought, rising temperatures, increasingly intense precipitation) is crucial in projecting future climate change. Microbial-explicit ecosystem models predict fairly different results from microbial-implicit ecosystem models (citation needed). On the one hand, the response of microbes depend on their temperature sensitivity, with soils predicted to sequester more carbon as temperatures increase if microbes have high temperature sensitivity and so microbial mortality increases with temperature; however, if microbes have low temperature sensitivity, then as temperature increases, soils will store less carbon due to lower microbial mortality and, hence, increased decomposition (citation needed).

However, what is still lacking from these models is the influence of drought on soil microbial communities, hindering projections of precipitation changes can affect decomposition (citation needed?). The symptoms of climate change are varied and includes increasing drought (citation needed), extreme precipitation (citation needed), rising temperatures (citation needed). There have been empirical studies on the influence of the aforementioned changes on climate on either microbial community composition or function. For example, studies along a Mediterranean climate gradient in California indicated that microbial enzyme activity in cold alpine ecosystems will increase faster with temperature than warm semi-arid/arid ecosystems (Baker and Allison, Nisson and Allison). Specifically, Baker and Allison found that the amount of enzymes – measured by an enzyme’s Vmax – in wet seasons shows this temperature sensitivity, while only one enzyme – leucine aminopeptidase (LAP) – shows this temperature sensitivity during the dry season.

There have been a variety of empirical studies on the effects of changes in precipitation – and the interactions between precipitation and other factors such as vegetation type and nitrogen input – on soil microbes. Some results suggests that the responses of litter microbes to drought differs across vegetation type, with microbes in grasslands showing a decrease in expression of resource acquisition traits and an increase in the expression of stress tolerance traits while microbes from a chaparral ecosystem showing no significant changes in either classes of traits (Malik et al. 2019? Or 2020?). Other results involving transplants of litter and microbes found that leaf litter inoculated with microbes from drought environments show significantly less mass loss during the dry season of a Mediterranean climate, and that this can be attributed to the smaller microbial biomass of drought-derived microbes (Allison et al. 2013). These serve as powerful pieces of evidence of the role of soil microbes in regulating soil carbon while also showing that microbial responses to changes in precipitation – which is already being witnessed across the globe and is projected to continue with climate change – influences decomposition. However, a study in the same system as Allison et al found that while there is no significant change in litter mass loss between two time points of a dry season, there is a significant increase in the amount of enzymes in the latter time point, indicating a significant decoupling between amount of enzymes and mass loss (Alster et al. 2013). In contrast, studies in temperate woodlands and forests show that enzyme activity and mass loss are positively correlated (Sinsabaugh et al. 1993; Sinsabaugh et al. 2002). These varied characteristics of extracellular enzyme activity across ecosystems warrant further investigation.

This experiment studies how extracellular enzyme activity in leaf litter varies across ecosystems and by precipitation. Microbes decompose organic matter via the secretion of extracellular enzymes (citation needed), which have been modeled by microbial ecologists using Michaelis-Menten kinetics (citation needed, although see Tang and Riley 2013 or so for a different formulation of microbial enzyme kinetics). The Michaelis-Menten enzyme parameters are Vmax – defined as the maximum reaction velocity when the amount of substrates are abundant – and Km – Michaelis-Menten constant, defined as the ratio between the rate constants of the breakdown of the enzyme-substrate complex and the formation of the enzyme-substrate complex. Vmax, in the context of biogeochemistry and microbial ecology, is a proxy measurement of the amount of a particular enzyme where higher values indicate higher enzyme amounts (citation needed). This practical definition stems from the derivation for the Michaelis-Menten equation, where as a substrate becomes abundant and enzymes become fully saturated, reaction velocity is limited by the amount of enzymes rather than the amount of substrates (citation needed). Km, on the other hand, is used as a measure of the amount of enzymatic products (citation needed). This practical definition of Km stems from products having been shown to be competitive inhibitors of substrates for the same enzyme such that high product concentrations correlate with high Km, and that high Km is correlated with low amounts of substrates (citation needed). Thus, Km is a function of two processes: (1) the production of products and (2) microbial uptake of enzymatic products.

This experiment investigates how microbial enzyme activity varies by ecosystem and precipitation. Specifically, it seeks to answer the following questions

1. How will microbial investment of resources in enzyme production change under drought with climate change?
2. How will microbial responses to drought differ by ecosystems?
3. How will changes in amount of enzymes due to drought responses affect amounts of products?

**Methods**

*Study site and field experiment*

The study was conducted at the Loma Ridge Global Change Experiment near Irvine, California (33°44’N, 117°42’W, 365 m elevation). The 2 ecosystem types at this site are (1) a variety of chaparral endemic to southern California and Baja California known as coastal sage scrub (hereafter, CSS) and (2) a grassland filled with exotic plants. The climate is Mediterranean, with a rainy season from November to March (which is also the growing season for plants) and a dry season for the rest of the year. The soil is remarkably coarse, with the fine earth fraction consisting of at least 80% sand across both vegetation types (Scot Parker dissertation). The site delineates plots of coastal sage scrub and grassland and manipulates precipitation to both vegetation types, with treatments for grassland plots having been in place since 2006 and treatments for CSS plots in place since 2008. The precipitation treatments are (1) plots where precipitation is reduced by being covered with clear polyethylene tarps during rainstorms, reducing mean annual precipitation by approximately 40% and simulating drought, and (2) plots where precipitation is not manipulated and are not covered by polyethylene during rainstorms. Each treatment combination (vegetation x precipitation) has 4 replicate plots. Thus, there are a total of 16 plots involved in this experiment (2 vegetation types x 2 precipitation treatments x 4 replicate plots/treatment combination). In addition, the site was burned once over 2007. Therefore, the relevant treatments start in 2008.

The experiment took place from 2017 to 2019. Litter was sampled from each treatment combination on August 30, 2017 and hand mixed, with litter from each treatment combination being kept separate from other combinations. After hand mixing, 15 cm x 15 cm bags with 1 mm mesh were filled with 6 g of litter from a specific treatment combination. Bags were then deployed on September 12, 2017 in plots with the same treatment combination as litter inside the bags. Litter bags were then sampled in 4 time points: time point 1 litter bags were collected towards the end of November of 2017; time point 2 litter bags were collected on April 11, 2018; time point 3 bags were collected during November of 2018; and time point 4 bags were collected during February of 2019. Note the seasonality of the time points: time points 1 and 3 were at the beginning of their respective wet seasons; time point 2 was at the beginning of the dry season; and time point 4 was in the latter half of the wet season. In total, 64 bags were collected, with litter bags from the 16 plots being collected 4 times (16 plots per time point x 4 time points = 64 bags). Once collected, litter from each litter bag was grinded down in coffee grinders and then stored in freezers at -80°C for later laboratory analysis.

*Extracellular enzyme (EE) assays and description of enzymes*

Extracellular enzyme assays were conducted as a way to measure the microbial resource acquisition trait of enzyme production (Malik et al. 2019). The hydrolytic enzymes that were assayed are α-glucosidase (AG), (acid) phosphatase (AP), β-glucosidase (BG), β-xylosidase (BX), cellobiohydrolase (CBH), leucine aminopeptidase (LAP), and N-acetyl-β-D-glucosaminidase (NAG). The oxidative enzymes that were assayed are polyphenol oxidase (PPO) and peroxidase.

Hydrolytic enzymes were assayed with fluorimetric methods described in Baker and Allison (2017) and German et al (2011). A homogenate suspension was made for each litter bag with ratios of 0.4 g litter per 150 mL of buffer, with the buffer solution being 25 mM maleate with pH 6. Homogenates were homogenized in 50 mL test tubes using a tissue tearor for four 30-second intervals, with at least 30 seconds of rest in between each interval. After homogenizing, a test tube and the tissue tearor is cleaned with more buffer until the homogenate is 150 mL in volume. In addition, serial dilutions were made of fluorogenic substrates that are either bonded with 4-methylumbilleffirone (MUB) – which assays for AG, AP, BG, BX, CBH, and NAG – or 7-amino-4-methylcoumarin (AMC) – which assays for LAP. Solutions of 62.5 µM AMC or 25 µM MUB were used as standards and were plated with homogenates and substrates in 96 microplate wells and left to incubate for 4 hours at room temperature in the dark before having their fluorescence read in a microplate reader. Each homogenate has three sets of controls: (1) a homogenate control in which only the inherent fluorescence of the homogenate is recorded; (2) a quench control in which the degree to which the homogenates decreases the fluorescence of either AMC or MUB is read; (3) and a substrate control in which the inherent fluorescence of a substrate is recorded (German et al. 2011). Each well has a volume of 250 µL, with 125 µL comprising of either the homogenate or plain buffer and the remaining 125 µL comprising of either the substrate, a standard solution, or water. Raw fluorescence values were then converted into reaction velocity using formulas described in German et al (2011).

Oxidative enzymes were assayed with colorimetric methods as described in German et al (2011) and sharing many of the same steps as fluorimetric assays of hydrolytic enzymes described above. In this case, both PPO and peroxidase used pyrogallol as the substrate, while peroxidase also uses H2O2 as a second substrate (Bach et al). Pyrogallol serial dilutions were made from a stock solution of 1 mg pyrogallol per 7.9 mL of water. Homogenates were vacuum filtered, and the subsequent filtrates were plated with pyrogallol to assay for PPO or both pyrogallol and H2O2 to assay for total oxidase activity. Two sets of controls were used: (1) a homogenate control in which only the inherent absorbance of the filtrate is read and (2) a substrate control in which the inherent absorbance of pyrogallol or pyrogallol and H2O2 were read. Calculations of PPO and total oxidase activity were calculated using equations from German et al (2011), and peroxidase activity was calculated by subtracting PPO activity from total oxidase activity. However, because peroxidase activity is consistently negative, it is assumed that peroxidase activity is negligible in these two ecosystems and so peroxidase is not analyzed.

*Description of enzymes*

Oxidative EEs (in this case, the only relevant enzyme is PPO) uses polyphenols – e.g. lignin and tannins – as their substrate. In this case, PPO activity is used to primarily gauge the degradation of lignin rather than other polyphenols. Long-term decomposition studies have found that lignin as a proportion of the remaining mass in litter increases with time (Schlesinger 1985; Schlesinger and Hasey 1981), and lignin content has been found to be negatively correlated with decomposition rates in many field studies (Adair et al. 2008; Bontti et al. 2009; Cornwell et al. 2008). Thus, it is expected that PPO will play an increasingly large role in decomposition with time.

AG primarily catalyzes the hydrolysis of starch (German et al. 2011). The hydrolysis of cellulose – a key structural component in plant cells – is conducted by BG and CBH, with CBH hydrolyzing 1, 4-β-D glucosidic linkages in cellulose while BG hydrolyzing terminal glycosidic linkages in the latter steps of cellulose degradation (German et al. 2011; Romero-Olivares et al. 2017). BX catalyzes the breakdown of hemicellulose (German et al. 2011; Romero-Olivares et al. 2017), which is not a major structural component of plant cells but is still a major polymer in the chemical composition of litter. Altogether, AG, BG, CBH, and BX breaks down carbon containing compounds and, therefore, serve as carbon sources for microbes, and BG activity had been used as a measure of the rate of carbon acquisition by soil microbes (Allison and Vitousek 2005; Sinsabaugh et al. 2008).

AP mineralizes organic phosphorus, and its activity had been used to measure the rate of phosphorus immobilization by soil microbes (Allison and Vitousek 2005; Sinsabaugh et al. 2008). In addition, its activity has been linked to the level of phosphorus in soils, with activity increasing in soils with low levels of phosphorus and when exposed to high concentrations of organic carbon and organic phosphorus (Allison and Vitousek 2005; Ali et al. 2019; Sinsabaugh et al. 2008). LAP catalyzes the hydrolysis of amino acids from the N-terminus of polypeptides (German et al. 2011). Its activity has been shown to be negatively correlated with the amount of other hydrolytic enzymes in the field (Baker and Allison 2017), suggesting that LAP also catalyzes the breakdown of other enzymes. NAG mineralizes chitin, a structural component of fungi cell walls. Taken together, AP, LAP, and NAG mineralizes organic nutrient sources, with AP mineralizing organic sources of phosphorus and LAP and NAG mineralizing nitrogen. NAG mineralizes fungal sources of nitrogen while LAP mineralizes proteins, which can be of plant origin (if proteins originated from the litter itself) or of microbial origin (if proteins are extracellular enzymes secreted by microbes). Activities of LAP and NAG had been used as a measure of the rate of nitrogen immobilization by soil microbes (Sinsabaugh et al. 2008).

*Hypotheses*

In trying to answer the three questions that motivate this experiment, the following hypotheses were formulated and will be tested.

H1: Enzyme amount controls product concentrations in such a way that they are positively correlated with each other.

* If this hypothesis is true, then Vmax and Km of an enzyme should be positively correlated in a linear regression. In addition, the treatments should have similar effects for both Vmax and Km.

H2: Microbes will increase investment of a particular enzyme when exposed to higher concentrations of substrates for that enzyme (Allison et al. 2005?).

* Predictions for this hypothesis are based on initial litter chemistry from Esch et al (2019). Litter from the coastal sage scrub ecosystem is of “lower quality” (citation needed) due to lower nitrogen content, higher lignin content, and lower cellulose and hemicellulose content (Esch et al. 2019) and so should see lower Vmax of enzymes that degrade cellulose (BG and CBH), hemicellulose (BX), and organic nitrogen (LAP, NAG) and higher PPO Vmax.

H3: Lignin acts as a competitive inhibitor of hydrolytic enzymes (Sewalt et al. 1997; Senior et al. 1991).

* Coastal sage scrub leaf litter, which has higher lignin, should see lower Vmax of hydrolytic enzymes and higher Vmax of polyphenol oxidase.

H4: In a high resource environment, there is a tradeoff between resources and energy between resource acquisition traits and stress tolerance traits where increased investment of resources in one class (e.g. stress tolerance) will lead to decreased investment of resources in another (e.g. resource acquisition) (Malik et al. 2019).

* This hypothesis is based on the YAS framework (Malik et al. 2019) that frames microbial life history in terms of 3 strategies (the relevant ones in this hypothesis are stress tolerance and resource acquisition). The stress in this experiment is the reduced precipitation treatment. Grassland litter is viewed as of “higher quality”. In this litter type, compared to ambient precipitation, microbes will decrease investment in enzymes (as indicated by lower Vmax) when under drought.

H5: In a low-resource environment, enzyme production is primarily constitutive (citation needed).

* Vmax does not significantly vary across the two precipitation treatments in coastal sage scrub.

*Data analysis*

All analysis and calculations were conducted on Python. After being converted from raw fluorescence or absorbance readings into enzyme activity, the activity of each enzyme from each litter sample is plotted against their respective substrate concentrations and judged visually for substrate inhibition or negative activity. While Michaelis-Menten kinetics predict that reaction velocity reaches a maximum value at infinite substrate concentrations, the phenomenon of substrate inhibition is common in many enzymes (Reed et al. 2010; response to German et al. 2011). As substrate concentrations increase, reaction velocity reaches a maximum level before decreasing again, contrary to Michaelis-Menten kinetics. This can lead to underestimates of Vmax, leading to lower enzyme amounts than there might actually be (response to German et al. 2011). As a result, data points in which substrate inhibition is observed are removed. The final enzyme activity, after having negative activity set to 0 and substrate inhibition data points are removed, are then fitted against the Michaelis-Menten equation using the *curve\_fit()* function from the *optimization* module of the *scipy* package to obtain Michaelis-Menten parameters.

Normality of these parameters were checked using the Shapiro-Wilk test, which was conducted using the *stats* module from *scipy*. Michaelis-Menten parameters were then log10 transformed to improve normality. While some of the data still shows a lack of normality under the Shapiro-Wilk test, the transformation overall greatly improved normality. Further data analysis was then conducted with the transformed data.

In order to test hypothesis 1, linear regression was conducted between transformed Vmax and Km of all enzymes using *scipy*’s *stats* module, and the significance of each regression was analyzed using Wald’s test. Further testing of hypothesis 1 and all other hypotheses were conducted using a factorial multivariate analysis of variance (MANOVA), factorial analysis of variance (ANOVA)s, and followed by Tukey post-hoc tests of significant interactions from ANOVAs or significant main effects that are not part of significant interactions from ANOVAs. These subsequent forms of statistical analyses were conducted using the *statsmodels* package. Given that there were 8 enzymes under analysis (peroxidase was not analyzed due to consistently negative activity) and the Michaelis-Menten equation has 2 parameters, there were a total of 16 dependent variables under analysis.

A factorial MANOVA was ran as a form of exploratory data analysis with time points, vegetation, and precipitation treatments as “between-subjects” factors (i.e. independent variables) and with the dependent variables being the Vmax and Km of all enzymes. Afterwards, factorial ANOVAs were ran on each of these dependent variables using the same factors as the MANOVA. Type III factorial ANOVAs were ran repeatedly, with nonsignificant interactions removed each time. When there are no significant interactions associated with a dependent variable, type II factorial ANOVAs were ran repeatedly on main effects with nonsignificant main effects removed after each iteration.

Tukey’s Honest Significant Difference tests were then conducted as post-hoc tests on dependent variables with significant interactions and significant main effects that were not part of significant interactions from ANOVAs. Interestingly, some significant interactions/main effects predicted by ANOVAs were then showed to be non-significant under pairwise comparisons of the same interactions/main effects by Tukey post-hoc testing.

**Results**

Of particular note is the small influence that precipitation has on either Vmax or Km. MANOVA results, on the surface, indicate that precipitation is significant at α = 0.05 in three-way interactions and also as part of an interaction with vegetation. Diving deeper into ANOVAs and subsequent Tukey post-hoc comparisons reveal that the effect of precipitation on either Vmax or Km are all relatively minor, either as a main effect alone or as part of an interaction with either time, vegetation, or both. For example, precipitation only influenced Vmax as part of a three-way interaction for PPO and does not have an effect on any other enzyme’s Vmax either as part of an interaction with vegetation or time or either as a main effect (Table 1). In contrast, the effect of precipitation on Km is somewhat larger; its interaction with vegetation is significant for AP, and its interaction with time is significant for CBH. As a main effect, precipitation influences the Km of only AP.

In contrast, vegetation has more influence on V­max and Km than precipitation. Its influence on Vmax is significant as an interaction with time on CBH and NAG and as a three-way interaction on PPO, while its influence as a main effect is significant for BG (Table 2). On Km, vegetation is part of a significant interaction with precipitation for AP, and its interaction with time is significant for AP, CBH, NAG, and PPO (Table 2).

In testing hypothesis 1, it appears that the linear regressions between Vmax and Km are significant for all 8 enzymes under analysis (Figure 1), which seems to support H1. However, the spread of the data points is especially wide for some enzymes, such as BX, CBH, and LAP, indicating that this relationship is not extremely strong. Indeed, H1 predicts that the treatments should exert similar changes on both of an enzyme’s Vmax and Km. However, this is not necessarily true. For example, in considering the enzyme AP, its Km has a significant interaction between vegetation and precipitation; however, vegetation and precipitation does not exhibit similar effects on its Vmax (AP Km and Vmax vegetation x precipitation boxplots).

**Discussion**

Some of the predictions from H2 seems to be validated. Because coastal sage scrub litter has lower cellulose content than grassland litter, H2 predicts that microbes that originate from coastal sage scrub should invest less resources into production of enzymes that degrade cellulose (i.e. BG and CBH). Indeed, BG Vmax is higher in grasslands (BG Vmax figure), and CBH Vmax is higher in grasslands across all time points (CBH Vmax figure). However, results from PPO and BX do not support the same predictions for lignin and hemicellulose, respectively. While hemicellulose is at a higher content in grassland than coastal sage scrub (Esch et al. 2019), there is no significant difference in BX production (Table 1). In addition, while coastal sage scrub litter has higher lignin content than grassland litter (Esch et al. 2019), production of PPO tends to be higher in grassland plots than coastal sage scrub plots, although these differences tend to be non-significant with exception for the drought treatment of CSS and grassland during the initial time point.

These results seem to indicate that microbes primarily use cellulose as a carbon and energy source rather than hemicellulose or lignin, and that changes in hemicellulose or lignin might not microbial community composition or functioning. The increase in hemicellulose in grassland litter does not cause a significant change in production of BX, indicating that this increase in hemicellulose does not incentivize microbes in acquiring more carbon or energy from hemicellulose.

In addition, H2 also predicted that production of enzymes that degrade organic nitrogen (NAG and LAP) should be higher in grassland litter (due to its higher level of nitrogen) than CSS litter. These results are partially validated, as production of LAP does not significantly differ by vegetation, either as a main effect or as an interaction with time or precipitation (Table 1). However, production of NAG is notably higher in grassland plots across all time points than CSS plots (NAG Vmax time x vegetation). This higher production of NAG is also accompanied by higher NAG products – although in a non-significant manner – in grassland plots across all time points than CSS plots. This correlation between NAG Vmax and Km is especially strong (Figure 1), indicating that the production of NAG reaction products is the main factor that controls concentrations of NAG reaction products rather than the microbial uptake of NAG reaction products. This implies that microbial uptake of NAG reaction products is high, indicating that chitin is a key source – if not the primary source – of nitrogen for soil and litter microbes rather than proteins. The slightly higher level of nitrogen in grassland litter, therefore, seems to lead to higher nitrogen immobilization by microbes.

Noteworthy is the lack of an effect of precipitation on enzyme production of all enzymes across all ecosystems except for a three-way interaction with PPO. Precipitation still has some significance on product concentrations of some enzymes, such as an interaction with vegetation in AP and an interaction with time for CBH (Table 2). However, when CBH Km is visualized as a function of time and precipitation, the effect of precipitation becomes even more unclear and seems to diminish even more (CBH Km time x precipitation box plot). On the other hand, when AP Km is visualized as a function of vegetation and precipitation, ambient CSS plots have higher Km than the other 3 combinations (drought CSS and both grassland precipitation combinations) (AP Km vegetation x precipitation box plot). Given the lack of an effect precipitation has on production of almost all enzymes, including AP, this indicates that microbes in drought CSS plots decrease uptake of AP reaction products compared to all other vegetation x precipitation combinations. Therefore, it is possible that the rate of phosphorus immobilization decreases in CSS plots under drought.

The higher rate of nitrogen immobilization by grassland litter microbes, higher rate of carbon acquisition from cellulose in grassland litter microbes, along with the lower rate of phosphorus uptake by CSS drought microbes, might have led to slightly higher production of oxidative enzymes that degrade lignin in grassland liter microbes (PPO Vmax box plot). Thus, with increasing drought due to climate change (citation needed), the “recalcitrance” of lignin in Mediterranean grasslands might decrease, potentially leading to faster decomposition and higher heterotrophic respiration in Mediterranean grasslands as lignin content decreases (Cornwell et al). However, note that the difference in production of PPO between CSS and grassland is, for the most part, insignificant by Tukey comparisons, and so this conclusion should be tempered (PPO Vmax box plot).

In addition, the lack of an effect of precipitation on enzyme production (Table 1) and reaction product concentrations (Table 2) is present across both ecosystem types and seems to contradict H5. H5 predicts that enzyme production is primarily constitutive only in low-resources environment (in this case, coastal sage scrub). These results indicate that enzyme production in both coastal sage scrub and grasslands might be constitutive and relatively robust to environmental change in the form of precipitation. These results reflect some of the findings from recent studies conducted across a California climate gradient (Baker and Allison, 2017; Nisson and Allison, 2020) that found that enzymes from colder-and-wetter ecosystems tend to be more responsive to environmental change via temperature increases than warm, semiarid ecosystems. Thus, while these results indicate that lignin in Mediterranean grasslands might become less recalcitrant with increasing drought, increases in decomposition will be negligible, and soils and litter carbon pools in Mediterranean ecosystems will remain relatively stable even with increased drought.

In addition, as documented so far, vegetation influences enzyme production and product concentrations more than precipitation.

**Figures and tables**

Chart, scatter chart

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***Figure 1.*** Linear regressions of log10-transformed Vmax and Km of all enzymes.

Chart, box and whisker chart

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***Figure 2.*** AP Vmax as a function of time.

Chart, box and whisker chart

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***Figure 3.*** AP Km as a function of time and vegetation.

Chart, box and whisker chart

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***Figure 4.*** AP Km as a function of vegetation and precipitation.

Chart, box and whisker chart

Description automatically generated

***Figure 5.*** BG Vmax as a function of vegetation.

Chart, box and whisker chart

Description automatically generated

***Figure 6.*** BX Km as a function of vegetation

***Table 1***. MANOVA and ANOVA results of Vmax, updated with non-significant interactions and main effects from Tukey post-hoc comparisons. (blank cells indicate lack of significance; \* 0.01 ≤ p < 0.05; \*\* 0.001 ≤ p < 0.01; \*\*\* p < 0.001).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Enzyme** | Time | Vegetation | Precipitation | Time x Precipitation | Time x Vegetation | Vegetation x Precipitation | Three-way |
| MANOVA | \*\*\* | \*\*\* | \* |  | \*\*\* | \*\* | \* |
| AG |  |  |  |  |  |  |  |
| AP | \*\* |  |  |  |  |  |  |
| BG |  | \*\*\* |  |  |  |  |  |
| BX |  |  |  |  |  |  |  |
| CBH | \*\*\* | \*\*\* |  |  | \*\*\* |  |  |
| LAP |  |  |  |  |  |  |  |
| NAG | \*\*\* | \*\*\* |  |  | \*\* |  |  |
| PPO |  | \*\* |  |  |  |  | \* |

***Table 2.*** MANOVA and ANOVA results of Km, updated with non-significant interactions and main effects from Tukey post-hoc comparisons. (blank cells indicate lack of significance; \* 0.01 ≤ p < 0.05; \*\* 0.001 ≤ p < 0.01; \*\*\* p < 0.001).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Enzyme** | Time | Vegetation | Precipitation | Time x Precipitation | Time x Vegetation | Vegetation x Precipitation | Three-way |
| MANOVA | \*\*\* | \*\*\* | \* |  | \*\*\* | \*\* | \* |
| AG |  |  |  |  |  |  |  |
| AP | \* | \*\*\* | \* |  | \* | \* |  |
| BG |  |  |  |  |  |  |  |
| BX |  | \* |  |  |  |  |  |
| CBH | \*\*\* | \*\*\* |  | \* | \*\*\* |  |  |
| LAP |  | \*\*\* |  |  |  |  |  |
| NAG | \* | \*\*\* |  |  | \*\*\* |  |  |
| PPO | \*\* | \*\* |  |  | \* |  |  |