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Drought effects on soil enzyme activity

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DROUGHT EFFECTS ON SOIL ENZYME ACTIVITY

By

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ABSTRACT

Soil extracellular enzyme activity (EEA) is a strong predictor for soil health. EEA cycle nutrients within terrestrial systems, processing carbon, nitrogen, and phosphorous, while also mineralizing and stabilizing gas. These processes are susceptible to disruption from global change drivers. How EEA responds to global change drivers remains poorly understood, however. My objectives were to examine how EEA is affected by drought treatment.

Here I conduct a global meta-analysis to observe the EEA of 7 enzymes in response to drought using 384 paired observations from 37 studies. These studies are globally distributed and encompass multiple ecosystems. I then calculated natural log response ratios of EEA values under drought treatment to the control. I tested whether the natural log response ratios differed from zero, and whether they were influenced drought intensity, drought duration, soil depth and aridity. Within this analysis, I evaluated the response of enzymes by distinguishing class, nutrient cycle, and individual identity. This allowed for the comparison between hydrolytic and oxidative functioning while also examining how specific nutrient cycles were impacted.

On average across all studies, EEA did not show a significant response to drought treatments. When analyzed by individual groups, the responses of neither hydrolytic nor oxidative enzymes to drought were statistically significant on average. Similarly, there was no significant responses when EEA were classified by element cycles, i.e., carbon, nitrogen, and phosphorous. Among all individual enzymes studied, only alkaline phosphomonoesterase displayed the significant response to drought treatment, showing reduced average alkaline phosphomonoesterase activity under drought than in the control. Further, contrary to our hypothesis, drought intensity and drought duration on average did not significantly influence EEA response to drought. However, the responses of EEA were dependent on soil depth and

aridity EEA in the topsoil's (<10 cm) experienced decreases in activity, whereas those in subsoil (>10 cm in depth) experienced significant increases. Across a global gradient of aridity index (0.092 to 2.28), the responses of EEAs to drought treatments decreased as climatic humidity increased, showing null or even positive responses in arid climates but negative responses in humid climates.

My finding showed the evidence that responses of EEA to drought are EEA type-, soil depth- and aridity-dependent responses. This study indicates a stimulation of enzyme activity in deeper soil layers under drought conditions. Furthermore, this increase in EEA response to drought is exacerbated by aridity, wherein more arid regions showed higher susceptibility to increases in EEA under drought. Therefore, arid regions can be expected to be most adversely affected by drought, through the potential vulnerability of soil organic matter loss due to an increase in EEA.

Key words: soil enzyme, enzyme, drought, terrestrial nutrient cycling

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CHAPTER ONE: GENERAL INTRODUCTION

Anthropogenic activity has contributed to increases in land use intensity (Herold et al. 2014), nitrogen deposition (Fenn et al. 2003), biodiversity loss (Duran et al. 2020), and has impacted global climate systems leading to altered precipitation patterns . In response to these increased pressures to natural systems, the Intergovernmental Panel on Climate Change created the IPCC 2015 report, which generated multiple predicted climate scenarios in response to anthropogenic activities (Pachauri et al. 2014). These climatic predictions anticipate changes in regional precipitation patterns and temperatures that may adversely impact terrestrial systems (Pachauri et al. 2014). Recent research has focused on the impact these projected precipitation changes will have on terrestrial ecosystem functioning (Alster et al. 2013, Knapp et al. 2015, Hedo de Santiago et al. 2016, Li 2018, Hinojosa et al. 2019).

Following the release of the IPCC 2015 report, projected climate scenarios, including increases in atmospheric CO_2, and elevated mean temperatures (Pachauri et al. 2014), were examined across multiple sites globally. Additionally, nitrogen deposition rates are expected to increase as a result of anthropogenic activities (Pachauri et al. 2014). In response, new experimental designs such as FACE (Free-air CO_2 enrichment) have been developed to examine ecosystem processes under projected climatic conditions (Souza et al. 2017). Collectively, this work has contributed to a better understanding of global change drivers on net primary production, plant biomass, and respiration rates (Sardans et al. 2017). Studies investigating global change drivers on soil microbes have focused on traits such as microbial biomass carbon, fungi to bacteria ratio, and relative microbial abundance (Zhang et al. 2018). However, an oversight exists between studies examining global change drivers and the effects that they have on soil extracellular enzyme activity (EEA).

Global change drivers threaten natural systems by disrupting historical climate patterns and biological diversity, leading to altered biological functions responsible for nutrient cycling (Pachauri et al. 2014, Bouskill et al. 2016). Alterations to natural systems disrupt established biological processes and result in decreases in process activity (Li et al. 2018b). Degradation of organic matter and nutrient mobilization by extracellular enzymes are one such biological process that play a pivotal role in terrestrial nutrient cycling. Soil microbial communities use extracellular enzymes to metabolize complex molecules, releasing nutrients into the soil where they can be accessed by plants and other terrestrial lifeforms such as microbes (Kennedy and Smith 1995, Balser and Firestone 2005). Microbial communities further contribute to mineralization and stabilization of CO_2 (Allison 2005, Ficken and Warren 2019), as well as decomposition of plant litter (Nguyen et al. 2018). Nutrient cycling functions performed by the soil microbial community are achieved through extracellular enzymes (Bouskill et al. 2016). Enzyme function, however, is dependent on the microbial community along with soil properties (outlined below) (Balser and Firestone 2005, Chaer et al. 2009).

Extensive research has focused on characterizing soil microbial communities. This work has largely explored the influence of abiotic and biotic factors on the composition and function of soil microbes. Abiotic factors which have been previously examined include soil properties such as nitrogen and carbon pools, dissolved organic carbon, soil pH, soil moisture, and soil temperature (Banerjee et al. 2018). Previously explored biotic factors include above-ground plant community dynamics, including species richness and evenness, presence of plant functional groups, and plant litter inputs (Leloup et al. 2018, Porazinska et al. 2018, Boeddinghaus et al. 2019). Focused on the soil environment, this research provides a foundational understanding of the surrounding soil environment's influence in determining the composition and function of the

soil microbial community, though how increasing CO_2 levels, reduced precipitation, and nitrogen deposition alter the composition and function of microbial communities remains unclear.

Disturbances to the soil environment disrupt soil microbial community composition and impact soil processes (Bastida et al. 2017a). Soil microbial communities are susceptible to disturbances such as land-use intensity (Allan et al. 2014) and increases in N-deposition (Ramirez et al. 2010). Additionally, rising atmospheric CO_2 concentrations (Edwards and Zak 2011) and naturally occurring extreme weather events, such as drought (Bouskill et al. 2016), have been shown to impact soil communities. How the disrupted microbial community functions as a result of these factors, however, remains unclear. With increasing frequency of drought events and continued pressure on natural systems from anthropogenic activities, the implications of global change factors on soil processes need to be understood.

Previous meta-analyses have amassed recent data on experiments examining global change drivers on soil process (Xiao et al. 2018, Abbasi et al. 2020). These analyses demonstrate a contemporary focus on literature about responses of increased temperature, increased CO_2, and nitrogen deposition on microbial determinants and soil chemical properties. Despite their limited inclusion in previous meta-analyses, soil microbial processes during drought conditions have received more recent research attention. New experimental designs such as the DRI-Grass experiment in Power et al. (2016) have been designed to approach the question of ecosystem function under projected climatic conditions. Although the repurposing of older designs, such as BACE (Boston-Area Climate Experiment) in Steinweg et al. (2013), and LTER (Long-term Ecological Research) in Knapp et al. (2015), have allowed for the examination of soil processes under predicted climatic conditions, this work has presented no consistent response of soil processes to predicted climate conditions such as increased droughts.

Drought, which represents a significant disturbance to soil microbial communities, is projected to increase in intensity and duration (Pachauri et al. 2014, de Vries et al. 2018). Soil microbial communities, even those in regions with regular drought patterns (Bouskill et al. 2016, Ochoa-Hueso et al. 2018), have been shown to be susceptible to drought. Drought not only directly impacts soil microbes, but further alters and affects soil physiochemical properties (Hartmann et al. 2013, Juckers and Watmough 2014). Altered soil physiochemical properties can be long lasting and impact EEA further (Sofi et al. 2016). Thus, increased drought intensity and duration represents firstly an immediate disturbance to the microbial community, and secondly a prolonged hinderance to EEA. Expanding our understanding of how drought will influence soils in the proposed climate conditions is then paramount to accurately assessing soil functions.

CHAPTER TWO: GLOBAL ACTIVITY RESPONSE OF SOIL MICROBES IN RESPONSE TO DROUGHT

Introduction

Microbial enzymes are crucial to nutrient cycling in terrestrial ecosystems (Bouskill et al. 2016). Nutrient specific extracellular enzymes within soil facilitate nutrient cycles involving carbon, nitrogen, and phosphorus (Sinsabaugh et al. 2009). Therefore, extracellular enzyme activities (EEA) are frequently studied as predictors of ecosystem health (German et al. 2011). EEA are sensitive to change and have been demonstrated to be impacted by global change drivers such as nitrogen deposition and precipitation alterations **(**Saiya-Cork et al. 2002**,** Chaer et al. 2009**,** Alster et al. 2013**).** Studying the response of EEA to global change drivers allows insights into how soil functions will be impacted by future global climatic conditions. Recent studies, however, have focused on the impact global change drivers have had on these soil activities, with conflicting results.

Identified by the IPCC (Intergovernmental Panel on Climate Change), severe weather events such as drought, are projected to occur more frequently and with greater intensity (Pachauri et al. 2014).. How EEA will respond to drought remains unclear. For example, Moreno et al. (2019) found that β-1,4,-glucosidase (BG) activity increased under drought conditions, while Bastida et al. (2017b) found decreasing BG activities in response to drought. Understanding why EEA do not have a consistent response to drought is important for creating more accurate models of ecosystems, thus facilitating better carbon budgeting. Furthermore, as extracellular enzymes are used as indicators of soil health, an understanding of how EEA responds to drought would allow for accurate predictions of soil vitality.

Several reasons can help explain these divergent responses. First, individual enzymes can respond differently to droughts. For instance, polyphenol oxidase (PPO) has been shown to respond positively under drought conditions, but urease (URE) decreased in activity (Alster et al. 2013, Moreno et al. 2019). Second, for each given enzyme, its responses to drought can differ with drought intensity and drought duration. For instance, Li (2018) reports increased activities for BG under elevated drought conditions relative to lower drought conditions. Further, Ochoa-Hueso et al. (2018) reports divergent EEA at two different lengths of time, indicating that drought duration impacts enzyme response. Third, the responses of EEA can differ with background conditions such as soil depth and aridity **(**Webster et al. 2014**,** Ochoa-Hueso et al. 2018**,** Moreno et al. 2019**).** Regions which are already dry may be more sensitive to drought, as there are established moisture constraints on the system. Decreasing moisture availability to a system with established moisture limitations may result in too much strain being exerted on the system to maintain normal function. Finally, enzyme class determines the required conditions to function, leading to possible divergent EEA between hydrolytic and oxidative enzymes **(**Alster et al. 2013**)**. Examining these factors may provide invaluable insight into why soil EEA is not uniformly impacted by drought.

Divergent responses to drought conditions have been observed amongst soil enzymes. Activities of PPO increased under an imposed precipitation reduction as shown in Alster et al. (2013). Similarly, peroxidase (PER) activities showed an increase in activity when soils were subjected to a precipitation reduction in Su et al. (2020). PPO and PER both facilitate the oxidation of their respective substrates and so are classified as oxidative enzymes (German et al. 2011, Matulich et al. 2015). While the activities of acid phosphatase (AP) and BG were demonstrated to decrease when subjected to drought conditions; both enzymes are hydrolytic and utilize water to lyse their respective substrates(Jeoh et al. 2005, Manrubia et al. 2019).

Furthermore, a decrease in activity of URE, N-acetyl-glucosaminnidase (NAG), and leucine-amino peptidase (LAP) was observed when exposed to drought conditions, aligning with the decreased activities of AP and BG (Li et al. 2018a, Nickel et al. 2018, Monokrousos et al. 2020). AP, BG, URE, NAG, and LAP comprise a representative sample of hydrolyzing enzymes, which accordingly are classified as hydrolytic enzymes (German et al. 2011). Possible reasoning for the divergent response of EEA to drought conditions includes enzyme class. Enzyme class is determined by the mechanism through which an enzyme functions; either hydrolytic or oxidative.

Examining EEA by enzyme class offers further explanation for the plausible mechanisms driving the response activity. Drought reduces soil moisture and changes water potential (Ψ) (Bouskill et al. 2013, Bouskill et al. 2016), thus impacting the mechanisms for which both oxidative and hydrolytic enzymes function. The mechanism by which hydrolytic enzymes function requires the presence of water in order to hydrolyze their targeted substrate (Jeoh et al. 2005). Similarly, oxidative enzymes require oxygen to oxidize their respective substrates (Koval et al. 2006). The efficiency of hydrolytic enzymes under drought conditions decreases, which could be attributed to mechanism limitations for hydrolytic enzymes under reduced water availability (Alster et al. 2013).

The goal of this research is to examine and synthesize current research on altered global precipitation patterns, here droughts, and their impacts on EEA. Drought is determined regionally by a reduction in seasonal or annual mean precipitation totals more than two standard deviations below the long-term average (Hogg et al. 2008, Dai 2011, Sheffield et al. 2012). Here, we collected 386 experimental observations from 37 studies encompassing most terrestrial ecosystems and examined the effects of regional drought on the activities of β-1,4- glucosidase (BG), N-acetyl-glucosaminidase (NAG), L-leucine aminopeptidase (LAP), acid phosphatase (AP), alkaline phosphomonoesterase (APA), p-phenol oxidase (PPO), urease (URE), and

peroxidase (PER). These enzymes were examined as they are commonly used as indicators of soil health **(**Weedon et al. 2011**)**. Furthermore, we examined the EEA by nutrient cycle association to further isolate drought effects. Our working hypotheses are as follows: 1) Drought negatively affects soil extracellular enzyme activity, and is more pronounced on hydrolytic class enzymes compared to oxidative class enzymes. 2) The negative effects of droughts on extracellular enzyme activity are amplified by the intensity of the drought and the duration of the drought. 3) Droughts effects on both hydrolytic and oxidative enzymes will be exacerbated by regional aridity. Regions which are more arid will show a larger decrease in EEA compared to more humid regions, as determined by aridity index values.

Methods

Data collection

A systematic search of peer-reviewed journal articles that examined the impacts of drought on soil enzyme activity was conducted using *Web of Science* and *Google Scholar*. Various keyword combinations were used such as (enzyme OR soil enzyme) AND (plant diversity OR monoculture OR mixed OR plant biodiversity OR water reduction). Subject field used was grassland OR drought. Inclusion criteria for selecting studies is as follows: (a) accessible peer-reviewed articles published in scientific journals, (b) studies were designed to test the effects of drought on EEA, (c) they had at least one drought treatment and a corresponding control, and (d) they had the same initial climatic and soil properties in the drought and control treatment plots. A total of 37 publications meeting exclusion criteria were retrieved and retained (Figure 1). The last search was conducted on October 20, 2020. The literature search was conducted following the

guidelines of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Moher et al. 2009); Figure 1.

Data extracted from these 37 studies was quantified in the following way: 'Enzyme activity' for BG, NAG, LAP, AP, URE, APA, PPO, and PER were recorded as reported in their source material. Activities reported as 0, were included as 0.001 to accommodate detection limitations of equipment; 'geographical location' with longitudinal and latitudinal coordinates; and 'climatic factors' including mean annual precipitation (MAP) in millimeters and mean annual temperature in degrees Celsius. Aridity for each experimental site was calculated using open source data and sample site coordinates **(**Trabucco and Zomer 2009**)**. 'Soil physio-chemical properties' are in their respective units (grams carbon per cubic kilogram soil).

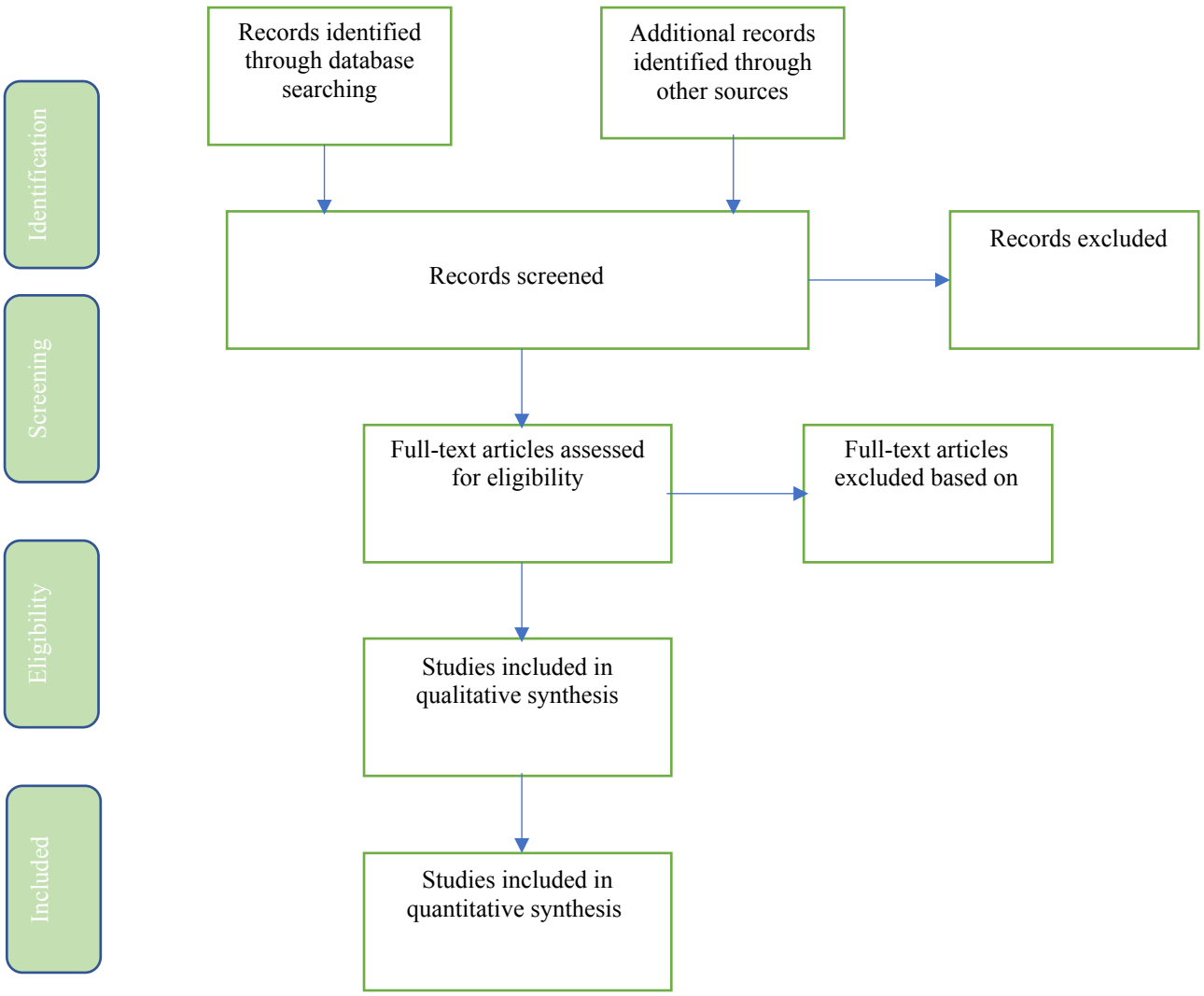

Figure 1. PRSIMA selection criteria for meta-analysis data.

Study Sites

To encompass a global scale, data was collected from 37 different studies representative of North America, Europe, Asia, and Australia. The studies selected within this research comprise a wide range of ecosystem types, mean annual temperature and annual aridity indices. European and Asian regions included in this study offer a range of aridity. This allows for a comprehensive

examination of not only region-specific response trends but allows for an analysis of global trends, despite geographic location.

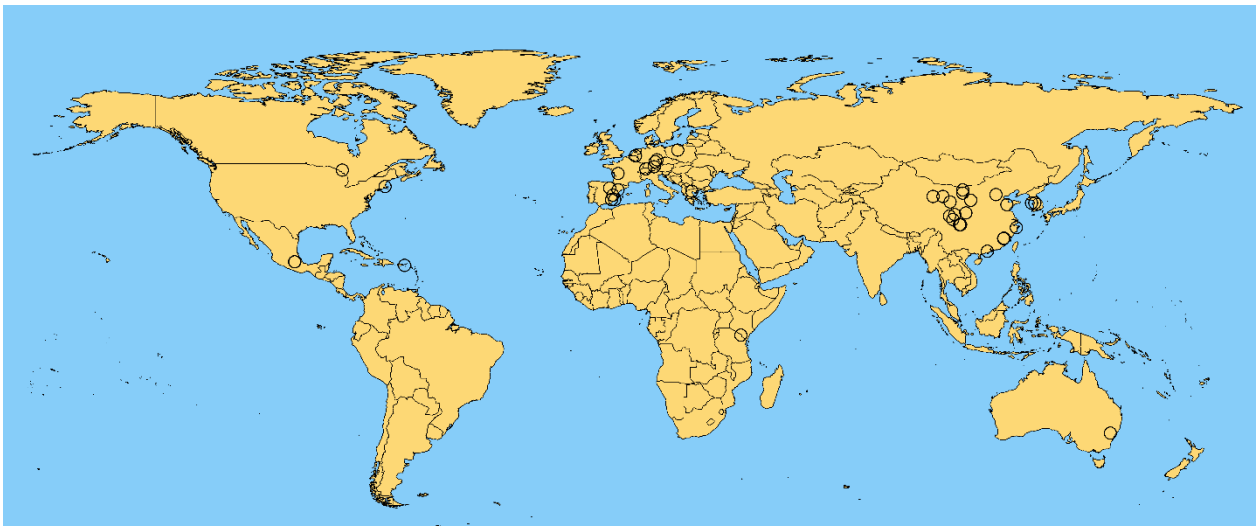

Figure 2. Global distribution of sampling sites.

Data Analysis

Calculations

The response of soil microbial EEA to drought was examined, with drought intensity, drought duration, aridity, and soil depth as explanatory factors. A natural log-transformed response ratio (ln*RR*) was used as the effect size to examine the impact of drought on soil EEA. The response ratio was calculated as follows:

(1)

where X_t and X_c are the observed and expected values, respectively, in a study (Chen et al. 2019). Variance within meta-analyses can consist of between study variance and within study variance (Hedges et al. 1999). These variations can be accounted for by including an error value for studies (equation 3) and the weighting of individual study estimates. The methodology in this

study adopted those of Chen et al. (2019). To avoid few observations being assigned substantial importance, weighting was determined by the number of observations within each study:

(2)

where W_r is the weight for each observation and N_t and N_c are the numbers of replicates in treatment and controlled conditions, respectively (Chen et al. 2019).

Statistical Analyses

The EEA of each enzyme, class, and nutrient affiliation in response to drought was examined to determine if it differs significantly from zero. The effects of drought intensity (*Dt*), duration (*t*) in days (to accommodate for studies less than one year in length), soil depth (*SD*), and aridity index (*AI*) were examined in combination through a linear mixed effects model to examine their influence in determining the natural log response ratio (Equation 3):

(3)

where β is the coefficient being determined; is the random effect of study that accounts for autocorrelation among observations within each study; ε is total sampling error. Analysis was conducted using R 3.5.2 with the lme4 package. W_r was used for the weight of each corresponding observation. Predictor values were scaled (minus mean and divided by one standard deviation) as done in (Chen et al. 2019). Aridity index values were generated using the method described in Trabucco and Zomer (2009),.. The model (equation 3) was retained as it met the criteria of the core hypothesis to keep *Dt, t, SD,* and *AI*. Correlation of predictor values was assessed to ensure independence. To isolate individual predictor effects on enzyme activity, the natural log response ratio of enzyme activity was plotted using a linear model with one fixed effect. ln*RR* and its confidence intervals (CI) were transformed back to percentage change as:

(4)

for ease of interpretation graphically. Where CIs do not encompass zero, the predictor effect is significant at $\alpha=0.05$ between controls and treatment.

Results

The Average Effect of Drought on Enzyme Activity by Class

When evaluated by class, EEA did not show significant reductions in activity. Activities of hydrolytic enzymes did not change with drought ($P = 0.864$) while oxidative enzyme activity nonsignificantly decreased by a mean of 13% (95% confidence interval, -57.5 to 30%; $P = 0.146$) (Figure. 3).

Effect of Drought on Nutrient Specific Extracellular Enzyme Activity

When assessed by nutrient affiliation, EEA remained stable for carbon and nitrogen, with nonsignificantly decreased activity for phosphorous ($P = 0.708$, 0.497, 0.206, respectively for carbon, nitrogen, and phosphorous; Figure 3). Phosphorous related enzymes decreased in activity

by a mean of 11.7% (-48.3 to 24.9%) (Figure 3).

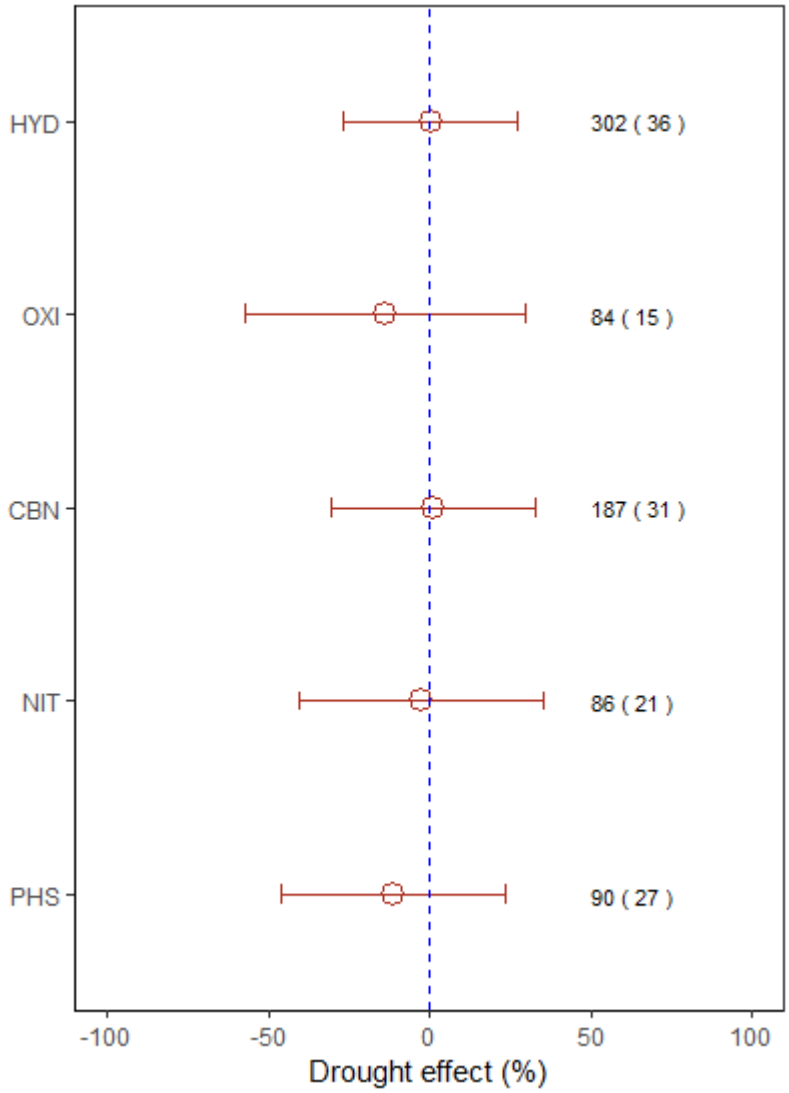

Figure 3. Enzyme activity percentage change by class and nutrient cycle. Values are mean ± 95% confidence intervals of the percentage effects between the drought and control treatments. The number of observations is outside parentheses, the number of studies within parentheses. HYD, OXI, CBN, NIT, and PHS represent hydrolytic, oxidative, carbon, nitrogen, and phosphorous enzymes, respectively.

Table 1. The mean effect of each predictor variable on extracellular enzyme activity by class and nutrient affiliation. Where Lower CI is "lower confidence interval" and Upper CI is "upper confidence interval. Dt, t, SD, and AI represent "Drought intensity", "Duration", "Soil depth", and "Aridity index" respectively. Bold mean effect values were significant at P=0.05.

Predictor	Trait	Observations	Journal articles	Lower CI	Upper CI	Mean effect (%)
Dt						
	Hydrolytic	302	36	-27.92	16.84	-5.54
	Oxidative	84	15	-49.19	66.23	8.52
	Carbon	187	31	-31.99	16.52	-7.73
	Nitrogen	86	21	-46.22	9.33	-18.44
	Phosphorous	90	27	-49.97	16.02	-16.98
t						
	Hydrolytic	302	36	-33.66	6.30	-13.68
	Oxidative	84	15	-20.20	188.09	83.95
	Carbon	187	31	-38.75	8.21	-15.27
	Nitrogen	86	21	-36.09	21.46	-7.23
	Phosphorous	90	27	-44.58	22.68	-10.95
SD						
	Hydrolytic	302	36	16.21	57.46	36.84
	Oxidative	84	15	-45.87	22.51	-11.68
	Carbon	187	31	-26.90	11.99	-7.45

	Nitrogen	86	21	13.71	78.24	45.97
	Phosphorous	90	27	1.34	82.49	41.91
AI						
	Hydrolytic	302	36	-6.59	40.02	16.71
	Oxidative	84	15	-69.71	-21.27	-45.49
	Carbon	187	31	-20.32	24.90	2.29
	Nitrogen	86	21	-24.81	34.79	4.99
	Phosphorous	90	27	-24.44	49.99	12.77

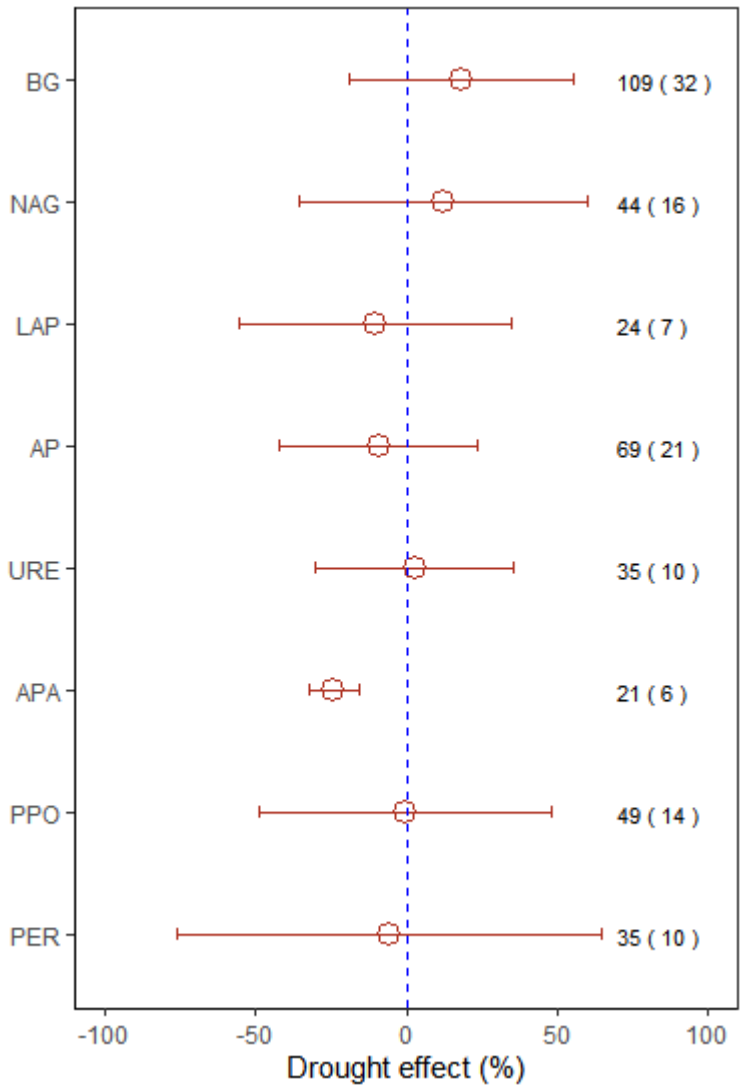

Figure 4. Enzyme activity percentage change by enzyme. Values are mean ± 95% confidence intervals of the percentage effects between the drought and control treatments. The number of observations is outside parentheses, the number of studies within parentheses. BG, NAG, LAP, AP, URE, APA, PPO, and PER represent β-1-4 glucosidase, N-acetyl-glucosaminidase, L-leucine aminopeptidase, acid phosphatase, urease, alkaline phosphomonoesterase, P-phenol oxidase, and peroxidase, respectively.

Individual Extracellular Enzyme Activity

No significant effect of drought on the activities of BG and NAG was observed. URE and PPO remained relatively unchanged with 2.77% (-30.0 to 35.6%; P= 0.883) and -0.55% (-49.1 to 48%; P=0.606) respectively (Figure 4). While LAP, AP, and APA all experienced decreases in activity, only APA was found to be significant (-55.4 to 35.1%; P= 0.382, -42.1 to 23.4%; P= 0.410 and -35.6 to -15.8%; P<0.005, respectively)(Figure 4).

Effect of drought intensity and duration on enzyme activity

Contrary to our second hypothesis wherein drought intensity and duration would amplify negative affects of drought on EEA drought intensity did not significantly impact enzyme activity regardless of class, nutrient affiliation, or enzyme (P>0.05; Table. 1). Similar to drought intensity, duration of drought, which ranged from 4 to 2190 days, did not result in significant changes in enzyme activity (P>0.05; Table. 1).

Effect of soil depth on enzyme activity

Soil depth was a significant predictor for enzyme activity. The activity of hydrolytic enzymes experienced an increase by an average of 36% (16.8-57.5%; P<0.005; Table 1, Figure 6a) as soil depth increased, whereas those enzymes involved in phosphorous cycling experienced an average increase of 41.9% (1.34-82.5%; P<0.05; Table 1) (Figure 6b). Nitrogen cycling enzymes significantly increased (P=0.03; Table 1) (Figure 6c), with NAG having increased by an average of 63.6% (18-109.2%; P<0.05) (Figure 6d) and LAP increased on average by 115.4% (8.81-222.1%; P=0.03) (Figure 6e).

Aridity index effect on the responses of enzyme activity to drought

Aridity had a significant effect on oxidative enzyme response activities to drought, averaging a negative impact of 45% (-69.7 to -21.3%; $P = 0.03$) (Table 1, Figure 7a). The activities of APA were decreased by 11.7% on average (-21.6 to -1.82%; P=0.04; Figure. 7b).

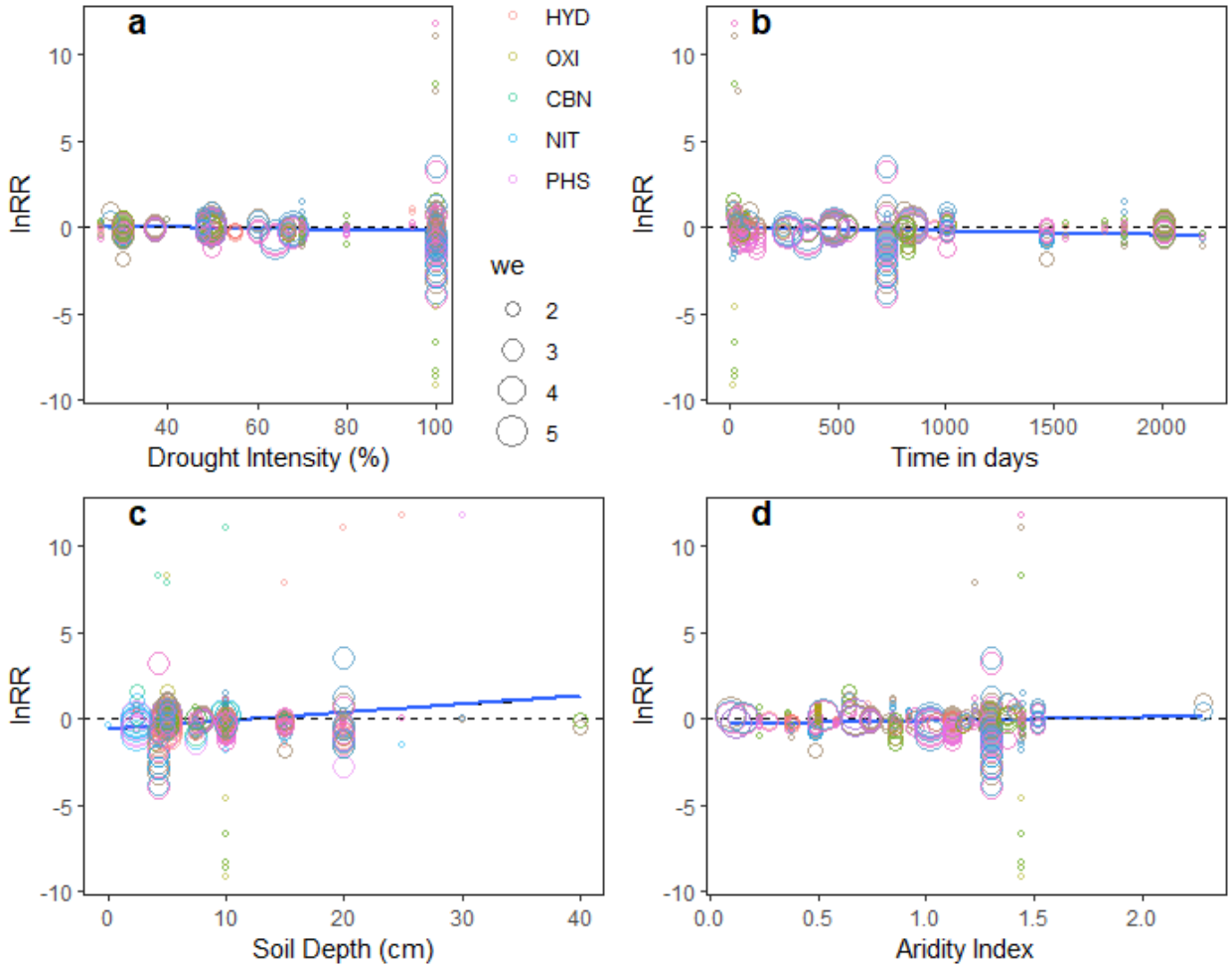

Figure 5. Natural log response ratio (lnRR) of extracellular enzyme activity in relation to (a) lnRR of EEA to drought intensity; (b) lnRR of EEA to drought duration; (c) lnRR of EEA to soil depth; (d) lnRR of EEA to aridity index. Enzymes are identified by class and nutrient cycle affiliation through colour. The weights of each observation on the linear regression are indicated by circle size. See Figure 3. for abbreviations.

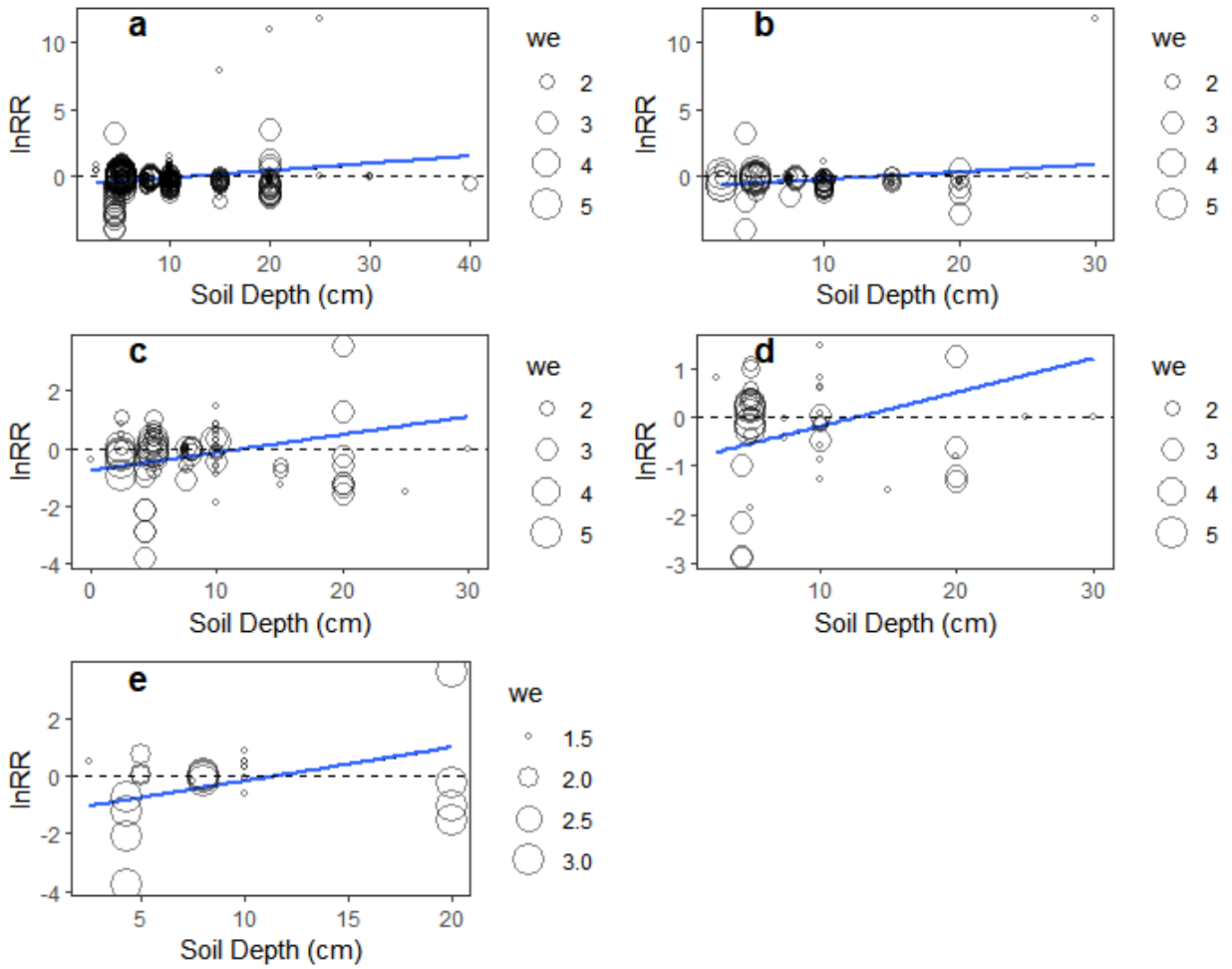

Figure 6. Natural log response ratio (lnRR) of extracellular enzyme activity in relation to soil depth: (a) lnRR of HYD EEA to soil depth; (b) lnRR of PHS EEA to soil depth; (c) lnRR of NIT EEA to soil depth; (d) lnRR of NAG EEA to soil depth; (e) lnRR of LAP EEA to soil depth. The weights of each observation on the linear regression are indicated by circle size. See Figure 3. for abbreviations.

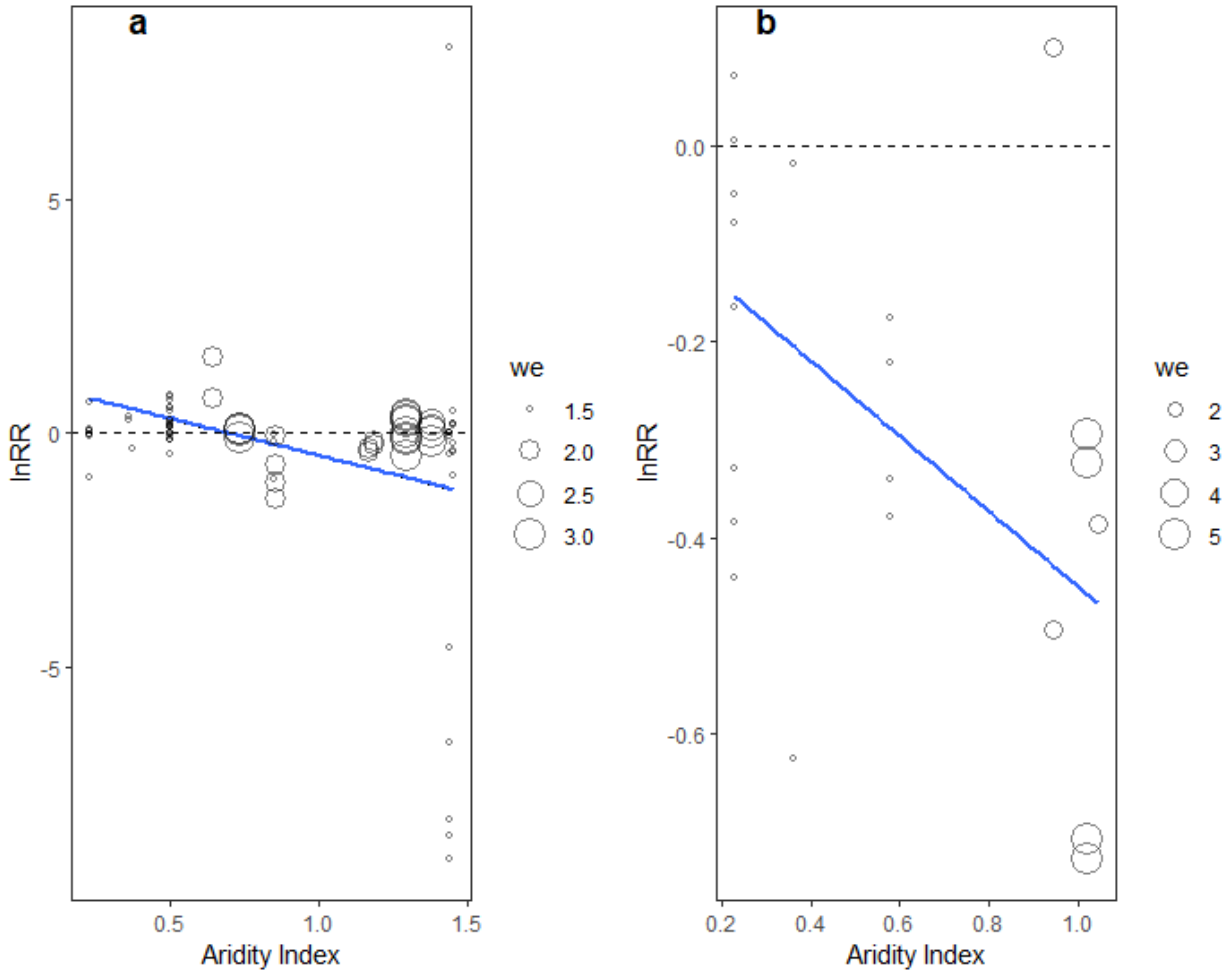

Figure 7. Natural log response ratio (lnRR) of extracellular enzyme activity to aridity index. (a) lnRR of OXI EEA to aridity index; (b) lnRR of APA EEA to aridity index. The weights of each observation on the linear regression are indicated by circle size. See Figure 3. for abbreviations**.**

Discussion

Global trends in response to drought

By encompassing a global distribution and a range of drought intensities, soil depths, regional aridity and duration of drought, our data represents a robust sample of available EEA responses. With few exceptions, and across all matrices examined within our work, soil enzyme

activity did not significantly respond to increased drought. EEA in response to increased drought remained statistically nonsignificant for all but APA. This contradicts our initial hypotheses in which we projected an decrease in oxidative enzyme activities along with more pronounced decreases in hydrolytic activity. These findings differ from those reported by Xiao et al. (2018), which is further discussed below. Our analysis showed that background condition significantly influenced the response of EEA to drought. Soil depth and aridity were stronger determinants of EEA response than average drought effect, drought intensity or drought duration.

Average response to drought by EEA remained stable (Figure 3). Enzyme responses to drought reported within individual studies include increases in activity surpassing 100%, along with decreases in activity exceeding 70% (Steinweg et al. 2013, Nickel et al. 2018). When assessed collectively, globally distributed enzyme responses maintained a net neutral activity. Observed increases in activity within one study were balanced by an observed decrease in a separate study. Our findings indicate that arid regions were predominantly more positively impacted by drought conditions compared to their humid region counterparts. This could be attributed to arid regions frequent experience of moisture limitation and stress. As a result soil, microbial communities developed over evolutionary time scales, selecting for life history traits to compensate for moisture stress, resulting in stability under drought conditions (Ochoa-Hueso et al. 2018). Humid regions, however, do not posses the same stability as their arid counterparts to drought (Bouskill et al. 2016). While regional variation in extracellular enzyme activity response to drought exists, our findings suggest enzyme activity remained constant at a global scale.

Background conditions remain strongest determinants of EEA response

A departure in our findings from previous work can be attributed to research focus. Xiao et al. (2018) examined multiple global change factors, including drought effect on the activities of soil microbes. They found significant deceases in activities of URE, PPO and oxidative class

enzymes in response to drought. The analysis featured a random effects model to determine whether the effects of different global change factors were significant or not (Xiao et al. 2018). However, the model did not include expressions for background conditions such as soil depth or aridity. The exclusion of variables reflective of background conditions, removes the importance these conditions have in determining EEA. Further, differences in our results can be attributed to limitations on site dispersion. Xiao et al. (2018) featured ten sites examining drought effect on EEA, with forty observations primarily within North America, Europe, and East Asia.

Unsurprisingly, aridity index was a significant predictor of EEA response. Soil depth, while not part of our initial hypothesis, further proved to be a strong predictor. It is generally accepted that enzyme activity varies within soil layers, with upper layers containing higher biological activity due to high substrate quality (Webster et al. 2014). Upper layers of soil are subject to wider fluctuations in conditions such as drying and oxygenation, allowing for an adjustment of EEA to such conditions (Balser and Firestone 2005, Reiche et al. 2009). Deeper layers, previously anaerobic in condition, exhibit increases in activity when desiccated (Reiche et al. 2009, Bonnett et al. 2017). These responses were observed within our study, supporting previous work (Webster et al. 2014).

Our ability to asses a divergent enzyme activity response based on soil depth is due to incorporating deep soil studies (Webster et al. 2014, Wang et al. 2017, Nickel et al. 2018) despite the general methodology of limited coring to a depth of 10 cm. While 10 cm is representative of the active soil layer, it omits enzyme activity which occurs in deeper soils. As demonstrated by Webster et al. (2014), soils in excess of 10 cm at depth, possess EEA which is susceptible to change under drought conditions. These findings are further supported by Nickel et al. (2018), who identified enzyme activity extending to depths of 30 cm which were altered under

drought conditions. The limited literature evaluating altered EEA within deep soil, however, creates a barrier to fully understanding drought response by EEA.

Explanations for the observed increase in EEA within deeper soil levels of peatlands include the transition from anerobic to aerobic conditions (Webster et al. 2014). Under new aerobic conditions, enzyme activity increases (Freeman et al. 2001). Freeman et al. (2001) suggests releasing oxygen limitation allows for the catabolism of enzyme inhibiting compounds and the metabolism of new enzymes. Moreover, oxidative stress caused by oxygen and nutrient availability, can induce the production of peroxidases (Rabinovich et al. 2004, Sinsabaugh 2010). Thus, deep soils transitioning from anerobic conditions to aerobic conditions resulting from drying allow for increased EEA.

In our research, arid conditions significantly influenced EEA response to drought. EEA follows a trend of decreasing activity along the aridity gradient. Arid regions under drought conditions experience an increase in enzyme activity (Ochoa-Hueso et al. 2018, Bastida et al. 2019), while semi-arid regions remain insignificantly impacted (Moreno et al. 2019). Humid regions remain largely contradictory as EEA can both increase in activity (Sanaullah et al. 2011, Bouskill et al. 2013, Bouskill et al. 2016) or decrease in activity (Steinweg et al. 2012, Nickel et al. 2018, Su et al. 2020). Explanations for this phenomenon include the largely accepted theory of the "iron gate". This theory posits that iron in the form of Fe(II) limits the activity of phenol oxidase in organic, humid soils (Freeman et al. 2001, Wang et al. 2017).Our findings align with these emerging trends. While data limitations exist for arid regions, those included within the analysis featured an increased enzyme activity.

This increased enzyme activity contradicts our hypothesis that arid regions would show a larger decrease in soil enzyme activity. Potential explanations for this contradiction include adaptations of the soil microbial community to changes in osmotic pressures. Detailing these

adaptations Schimel et al. (2007) provides a summary of microbial adaptations to osmotic stress. In brief, four general "functional" groups of microbes exist classified by their ability to produce osmolytes; solutes used to alter their internal water potential to avoid desiccation (Harris 1981). Arid soils experience regular osmotic pressures, and thus selective pressures on the microbial community would favour life history strategies best suited for moisture limitation and osmotic stress (Allison and Martiny 2008).

Enzyme class and nutrient cycle

Class determines soil enzyme function along with the optimal conditions under which enzymes normally operate (German et al. 2011). Interestingly, when divided by class, no significant difference in soil enzyme response to drought was detected. These findings contradict Xiao et al. (2018) who showed class along with nutrient cycle affiliation influences enzyme response to drought. Furthermore, our findings found no such association between nutrient affiliation to be significant. Specialized enzyme function, such as those involved in nitrification and denitrification are of particular interest in this case as they are synthesized by "specialists" within the microbial community (Chaer et al. 2009, Herzog et al. 2013). While the average activities of general enzymes (those widely produced by multiple microbial species)remains unchanged, the potential for specialised enzyme activities to be masked by general enzyme activity, remained plausible. The enzyme PPO is considered to be a specialist enzyme involved in the oxidation of phenolic compounds to quinones (Bukh et al. 2006). When examined however, PPO did not show a significant response to drought. Thus, our work suggests that total enzyme function, regardless of class, nutrient affiliation, or specialized function, remains unaffected globally on average.

Implications of drought on EEA

The response of EEA to drought is type-, soil depth-, and aridity-dependent. Individual EEA response to drought is independent of nutrient cycling. Decreases in an individual EEA does not adversely impact the affiliated nutrient cycle, as demonstrated with APA and phosphorous. Our research further demonstrates that EEA response to drought is soil depth dependent, with subsoils (>10 cm) exhibiting increased EEA. This increased activity is further exacerbated by aridity, wherein arid regions displayed an elevated EEA response to drought compared to humid regions. Collectively, these results demonstrate that the effects of drought on EEA are not uniform. Thus, EEA in response to drought conditions predicted by the IPCC 2015 report will differ globally, with arid regions experiencing the largest increase in EEA in subsoils.

Future considerations and general conclusions

Through our research, we have identified the need for further investigation into the effects of drought on soil extracellular enzyme activities. Current available research within the literature is limited and thus provides equally limiting insight. Within our study, we identified and assessed 37 applicable published articles examining the effects of drought on EEA. Comparatively, meta-analyses examining other global change drivers (nitrogen deposition), were successful in identifying 151 studies (Zhang et al. 2018). Furthermore, meta-analyses explicitly examining global change drivers on enzyme activity were able to identify 132 studies, with only six examining moisture limitation (Xiao et al. 2018). Limited available peer-reviewed articles within the literature remains a barrier to clear understanding. Increased available peer reviewed articles would improve the statistical strength of analyses, providing clearer mean trends.

Resulting from our analyses, we identified the potential vulnerabilities of soil systems to drought. Increases in EEA were observed following a trend of decreasing aridity index values. Regions which have lower aridity index values, and therefore are arid, are susceptible to increases in EEA. Discussed above are possible explanations for this observation, however, these

increased activities can result in a loss of stored soil organic matter (Acosta-Martinez et al. 2014). Soil organic matter is strongly correlated to soil fertility and health, and therefore, the loss of stored organic matter may be detrimental. The potential loss of stored soil organic matter in arid regions as a result of increased EEA due to drought, makes arid regions vulnerable to drought.

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SUPPLEMENTARY INFORMATION:

Table S1. References used in the meta-analysis

First Author	Title	Year	Publication
Acosta-Martinez, V.	Soil enzyme activities during the 2011 Texas record drought/heat wave and implications to biogeochemical cycling and organic matter dynamics	2014	Applied Soil Ecology 75, 43-51
Banerjee, S.	Linking microbial co-occurrences to soil ecological processes across a woodland-grassland ecotone	2018	Ecology and Evolution 8, 8217-8230
Bastida, F.	Differential sensitivity of total and active soil microbial communities to drought and forest management	2017	Global Change Biology 23, 4185-4203
Bastida, F.	When drought meets forest management: Effects on the soil microbial community of a Holm oak forest ecosystem	2019	Science of The Total Environment 662, 276-286
Bouskill, N. J.	Belowground Response to Drought in a Tropical Forest Soil. I. Changes in Microbial Functional Potential and Metabolism	2016	Front Microbiol 7, 525
Diaz-Guerra, L.	Effects of UV radiation and rainfall reduction on leaf and soil parameters related to C and N cycles of a Mediterranean shrubland before and after a controlled fire	2018	Plant and Soil 424, 503+
Hammerl, V.B.	Seasonal Effects of Extreme Weather Events on Potential Extracellular Enzyme Activities in a Temperate Grassland Soil	2019	Frontiers in Environmental Science 6, 10
He, D.	Diversity and co-occurrence network of soil fungi are more responsive than those of bacteria to shifts in precipitation seasonality in a subtropical forest	2017	Soil Biology and Biochemistry 115, 499-510
Herzog, C.	Drought and air warming affects abundance and exoenzyme profiles of *Cenococcum geophilum* associated with *Quercus robur, Q. petraea and Q. pubescens*	2013	Plant Biology 15, 230-237

Jassey, V.E.J.	Tipping point in plant–fungal interactions under severe drought causes abrupt rise in peatland ecosystem respiration	2018	Global Change Biology 24, 972-986
Jiang, M.	Effects of warming and precipitation reduction on soil enzyme activity in a young *Cunninghamia lanceolata* plantation	2018	Chinese Journal of Ecology, 37, 3210-3219
Li, S.J.	Effects of Throughfall Exclusion on Soil Microbial Biomass and Enzyme Activities in a Natural *Castanopsis carelesii* Forest in Subtropical China	2018	Journal of Subtropical Resources and Environment 13, 17-25
Li, G.	Precipitation affects soil microbial and extracellular enzymatic responses to warming	2018	Soil Biology and Biochemistry 120, 212-221
Manrubia, M.	Soil functional responses to drought under range-expanding and native plant communities	2019	Functional Ecology 33, 2402-2416
Mganga, K.Z.	Phenological Stage, Plant Biomass, and Drought Stress Affect Microbial Biomass and Enzyme Activities in the Rhizosphere of *Enteropogon macrostachyus*	2019	Pedosphere 29, 259-265
Monokrousos, N.	The effects of plant type, AMF inoculation and water regime on rhizosphere microbial communities	2020	European Journal of Soil Science 71, 265-278
Moreno, J.L.	Land use shapes the resistance of the soil microbial community and the C cycling response to drought in a semi-arid area	2019	Science of The Total Environment 648, 1018-1030
Moreno-Espíndola, I.P.	The Bacterial Community Structure and Microbial Activity in a Traditional Organic Milpa Farming System Under Different Soil Moisture Conditions	2018	Frontiers in Microbiology 9
Mengyin, N.	Seasonal response of extracellular enzyme activity to precipitation exclusion in a subtropical *Cunninghamia lanceolata* plantation	2018	ACTA ECOLOGICA SINICA, 38, 2129-2127
Na, X.	Vegetation biomass and soil moisture coregulate bacterial community succession under altered precipitation regimes in a desert steppe in northwestern China	2019	Soil Biology and Biochemistry 136, 107520
Nickel, U.T.	Quantitative losses vs. qualitative stability of	2018	Global Change Biology 24,

	ectomycorrhizal community responses to 3 years of experimental summer drought in a beech–spruce forest		e560-e576
Ochoa-Hueso, R.	Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents	2018	Global Change Biology 24, 2818-2827
Olatunji, O.A.	The responses of soil microbial community and enzyme activities of Phoebe zhennan cultivated under different soil moisture conditions to phosphorus addition	2018	iForest - Biogeosciences and Forestry 11, 751-756
Olatunji, O.A.	Influence of phosphorus application and water deficit on the soil microbiota of N2-fixing and non-N-fixing tree	2018	Ecosphere 9, e02276.
Ouyang, Y.	Effect of repeated drying-rewetting cycles on soil extracellular enzyme activities and microbial community composition in arid and semi-arid ecosystems	2020	European Journal of Soil Biology 98, 103187
Reiche, M.	Impact of manipulated drought and heavy rainfall events on peat mineralization processes and source-sink functions of an acidic fen	2009	Journal of Geophysical Research: Biogeosciences 114
Sanaullah, M.	Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition	2011	Applied Soil Ecology 48, 38-44
Sardans, J.	Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex L.* forest	2005	Soil Biology & Biochemistry 37, 455-461.
Steinweg, J.	Microbial responses to multi-factor climate change: effects on soil enzymes	2013	Frontiers in Microbiology 4
Su, X.	Drought accelerated recalcitrant carbon loss by changing soil aggregation and microbial communities in a subtropical forest	2020	Soil Biology and Biochemistry 148, 107898
Sun, D.	Effects of organic amendment on soil aggregation and microbial community composition during drying-rewetting alternation	2017	Science of The Total Environment 574, 735-743
Vo, N.X.Q.	Regulation of soil enzyme activities in constructed wetlands under a short-term drying period	2013	Chemistry and Ecology 29, 146-165

Wang, Y.	Iron-mediated soil carbon response to water-table decline in an alpine wetland	2017	Nature Communications 8, 15972
Wang, Z.-y.	A study of soil-dynamics based on a simulated drought in an alpine meadow on the Tibetan Plateau	2013	Journal of Mountain Science 10, 833-844
Webster, Kara L.	Potential Vulnerability of Deep Carbon Deposits of Forested Swamps to Drought	2014	Soil Science Society of America Journal 78, 1097-1107
Yan, Z.	Different responses of soil hydrolases and oxidases to extreme drought in an alpine peatland on the Qinghai-Tibet Plateau, China	2020	European Journal of Soil Biology 99, 103195
Zhao, Q.	Spring drying and intensified summer rainfall affected soil microbial community composition but not enzyme activity in a subtropical forest	2018	Applied Soil Ecology 130, 219-225

Table S2. The effect (*P* values) of drought (intercept) and predictors (where Dt is drought intensity, t is time in days, SD is soil depth, and AI is aridity index) on Natural log response ratios (ln*RR*) of soil enzymes. Where hydrolytic (HYD), oxidative (OXI), carbon (CBN), nitrogen (NIT), and phosphorous (PHS) enzymes. Individual enzymes include beta -1,4-glucosidase (BG), N-acetyl-glucosaminnidase (NAG), L-leucine aminopeptidase (LAP), acid phosphatase (AP), alkaline phosphomonoesterase (APA), p-phenol oxidase (PPO), urease (URE), and peroxidase (PER). Bold values indicate P≤0.05. Wald test was used for degrees of freedom (df).

Fixed effects	**Estimate**	**Standard error**	***df***	***t***	***P***
HYD					
(Intercept)	-0.02	0.11	29	-0.17	0.864
Dt	-0.09	0.12	47	-0.70	0.489
t	-0.17	0.12	43	-1.45	0.154
SD	0.30	0.08	237	3.90	**0.000**
AI	0.13	0.10	51	1.30	0.200
BG (nmol h^{-1} g^{-1} soil)					
(Intercept)	0.12	0.17	21	0.69	0.495
Dt	-0.07	0.19	35	-0.40	0.692
t	-0.33	0.18	37	-1.81	0.079
SD	0.20	0.13	103	1.55	0.124
AI	0.20	0.15	35	1.29	0.206
NAG (nmol h^{-1} g^{-1} soil)					
(Intercept)	0.01	0.23	9	0.06	0.956
Dt	-0.34	0.24	17	-1.45	0.165

t	-0.07	0.23	14	-0.30	0.768
SD	0.45	0.15	32	3.10	**0.004**
AI	-0.07	0.19	21	-0.37	0.718
LAP (nmol h^{-1} g^{-1} soil)					
(Intercept)	-0.25	0.28	19	-0.90	0.328
Dt	-0.77	0.37	19	-2.09	0.050
t	-0.48	0.49	19	-0.99	0.337
SD	0.63	0.28	19	2.27	**0.035**
AI	0.35	0.48	19	0.72	0.479
AP (nmol h^{-1} g^{-1} soil)					
(Intercept)	-0.17	0.19	8	-0.87	0.410
Dt	-0.41	0.30	24	-1.36	0.188
t	-0.29	0.27	25	-1.06	0.300
SD	0.36	0.20	59	1.84	0.071
AI	0.10	0.22	24	0.46	0.653
URE (nmol h^{-1} g^{-1} soil)					
(Intercept)	-0.03	0.17	4	-0.16	0.883
Dt	0.20	0.20	5	1.02	0.358
t	-0.19	0.19	5	-1.01	0.357
SD	0.03	0.11	16	0.30	0.772
AI	-0.10	0.17	4	-0.57	0.598
APA (umol h^{-1} g^{-1} soil)					
(Intercept)	-0.28	0.06	16	-4.99	**0.000**
Dt	-0.09	0.10	16	-0.97	0.346
t	-0.13	0.10	16	-1.37	0.189
SD	0.06	0.05	16	1.02	0.323
AI	-0.13	0.06	16	-2.28	**0.037**

OXI					
(Intercept)	-0.30	0.21	79	-1.47	0.146
Dt	-0.08	0.30	79	-0.28	0.781
t	0.42	0.33	79	1.27	0.207
SD	-0.21	0.21	79	-0.99	0.327
AI	-0.72	0.24	79	-2.94	**0.004**
PPO (umol h^{-1} g^{-1} soil)					
(Intercept)	-0.14	0.27	44	-0.52	0.606
Dt	-0.12	0.46	44	-0.27	0.785
t	0.02	0.49	44	0.05	0.961
SD	-0.17	0.28	44	-0.60	0.549
AI	-0.30	0.31	44	-0.97	0.340
PER (umol h^{-1} g^{-1} soil)					
(Intercept)	-0.47	0.49	3	-0.95	0.419
Dt	0.03	0.67	4	0.05	0.961
t	0.76	0.67	4	1.15	0.314
SD	-0.63	0.44	12	-1.42	0.182
AI	-1.24	0.60	3	-2.09	0.130
CBN					
(Intercept)	-0.04	0.11	182	-0.37	0.708
Dt	-0.12	0.14	182	-0.85	0.398
t	-0.21	0.15	182	-1.42	0.158
SD	-0.10	0.11	182	-0.92	0.359
AI	0.00	0.12	182	-0.02	0.983
NIT					
(Intercept)	-0.11	0.16	11	-0.70	0.497
Dt	-0.27	0.18	18	-1.47	0.160
t	-0.13	0.16	21	-0.77	0.448
SD	0.35	0.12	81	3.08	**0.003**

AI	0.01	0.15	21	0.05	0.964
PHS					
(Intercept)	-0.20	0.15	11	-1.34	0.206
Dt	-0.27	0.22	29	-1.27	0.215
t	-0.19	0.20	32	-0.95	0.349
SD	0.31	0.15	57	2.05	0.045
AI	0.06	0.18	27	0.36	0.723

R Codes

Codes were written for use with RStudio 1.1.463 with R 3.5.2

```
library(broom)
library(car)
library(cowplot)
library(data.table)
library(FD)
library(emmeans)
library(gginnards)
library(ggpmisc)
library(ggplot2)
library(ggpubr)
library(ggthemes)
library(gridExtra)
library(grid)
library(gtable)
library(Hmisc)
library(jtools)
library(labeling)
library(lme4)
library(lmerTest)
library(lsmeans)
library(MASS)
library(multcomp)
library(MuMIn)
library(pastecs)
library(pdp)
library(plyr)
library(raster)
library(rgdal)
```

```
#####Read in data####
SEA<-fread('DEA 2.csv',select=c('t', 'Xc','CSE','Xt','TSE','SD','Dt','nc','Study','Line','AI', "Trait", "Enzyme"))
SEA[,rr:=log(Xt/Xc)]
SEA[,we:=nc*nc/(nc+nc)]
SEA$ID<-as.factor(SEA$Line)
setnames(SEA, c ('t', 'Xc','CSE','Xt','TSE','SD','Dt','nc','Study','Line','AI','Trait','Enzyme','rr','we','ID'))
SEA$Trait<-factor(SEA$Trait, levels = c("HYD", "OXI", "CBN", "NIT", "PHS"))
SEA$Enzyme<-factor(SEA$Enzyme, levels = c( 'BG','NAG','LAP','AP','URE','APA','PPO','PER'))



###Figure 2###
##########WORLD MAP##############


nrow(unique(Arid[,c("Lat", "Lon")]))


world_map <- map_data("world")
p <- ggplot()+
  coord_fixed() +
  xlab("") + ylab("")+
  geom_map(dat=world_map,map=world_map,
           aes(map_id=region),
           colour="black", fill="#fed976",size=0.2)+
  expand_limits(x = c(-170,177), y = c(-54,90))+
  theme_map()+
  theme(
    panel.background = element_rect(fill = 'lightskyblue', colour = 'lightskyblue'),
    axis.line = element_blank(),
    legend.position  = c(0.01,0.03),
    legend.text = element_text(size = 9),
```

```
    legend.key.height=unit(0.4,"line"),
    legend.key.width =unit(0.5,"line"),
    legend.background = element_blank(),
    legend.key = element_blank(),
    axis.ticks=element_blank(),
    axis.text.x=element_blank(),
    axis.text.y=element_blank())+
  geom_point(data = Arid,aes(x=Lon,y=Lat),size=3,shape=21,col="black")+
  scale_fill_manual(name  ="",values=c("magenta","forestgreen","purple","red","yellow","pink"))+
  scale_color_manual(name  ="",values=c("magenta","forestgreen","purple","red","yellow","pink"))+
  guides(fill=guide_legend(nrow=3,byrow=TRUE))

ggsave(filename="Plot_Map20200605.tiff",dpi =600, plot=p,width = 9.93, height =4.12, units = "in")

########################ARIDITY Index####################
Arid<-fread('DEA.csv',select=c('Lat','Lon'))

Location<-data.table(read.csv("DEA.csv"))

ai<-readGDAL("ai_yr/hdr.adf")
ai<-raster("ai_yr/hdr.adf")

data.table(Location)
Location$Lat<-c(7.7)
Location$Lon<-(13.883)
### Turned all lat, lon into 7.7 and 13.883

data.table(Location)
Location$Lat
Location$Lon
#### Kept original Lon Lat data
```

```
lats<-Location$Lat
lons<-Location$Lon

coords <- data.frame(x=lons,y=lats)
points <- SpatialPoints(coords, proj4string = ai@crs)
values <- extract(ai,points)
df <- cbind.data.frame(coordinates(points),values)
write.csv(df,"Weather-Aridity index3.csv")
### Produced AI for each sample location

###HYD Simp####
HYD<-subset(SEA, Trait=="HYD")
FULL_HYD<-lmer(rr~scale(Dt) + scale(t) + scale(SD) + scale(AI) + (1|Study),
        weights=we,
        na.action = na.fail,
        data=HYD)
summary(FULL_HYD,ddf="Kenward-Roger")
HYD_KEN<-data.frame(summary(FULL_HYD,ddf='Kenward-Roger')$coefficients)
write.csv(HYD_KEN,'HYD_KEN.csv')

####OXI Simp####
OXI<-subset(SEA, Trait=="OXI")
FULL_OXI<-lmer(rr~scale(Dt) + scale(t) + scale(SD) + scale(AI) + (1|Study),
        weights=we,
        na.action = na.fail,
        data=OXI)
summary(FULL_OXI,ddf="Kenward-Roger")
OXI_KEN<-data.frame(summary(FULL_OXI,ddf='Kenward-Roger')$coefficients)
write.csv(OXI_KEN,'OXI_KEN.csv')
```

```
####CBN Simp####
CBN<-subset(SEA, Trait=="CBN")
FULL_CBN<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                 (1|Study),
               data=CBN,
               weights=we,
               na.action = na.fail)
summary(FULL_CBN,ddf="Kenward-Roger")
CBN_KEN<-data.frame(summary(FULL_CBN,ddf='Kenward-Roger')$coefficients)
write.csv(CBN_KEN,'CBN_KEN.csv')

###NIT Simp####
NIT<-subset(SEA, Trait=="NIT")
FULL_NIT<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                 (1|Study),
               data=NIT,
               weights=we,
               na.action = na.fail)
summary(FULL_NIT,ddf="Kenward-Roger")
NIT_KEN<-data.frame(summary(FULL_NIT,ddf='Kenward-Roger')$coefficients)
write.csv(NIT_KEN,'NIT_KEN.csv')

####PHS Simp####
PHS<-subset(SEA, Trait=="PHS")
FULL_PHS<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                 (1|Study),
               data=PHS,
               weights=we,
               na.action = na.fail)
summary(FULL_PHS,ddf="Kenward-Roger")
PHS_KEN<-data.frame(summary(FULL_PHS,ddf='Kenward-Roger')$coefficients)
```

```
write.csv(PHS_KEN,'PHS_KEN.csv')


####BG Simp####
BG<-subset(SEA, Enzyme=="BG")
FULL_BG<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
               (1|Study),
             data=BG,
             weights=we,
             na.action = na.fail)
summary(FULL_BG,ddf="Kenward-Roger")
BG_KEN<-data.frame(summary(FULL_BG,ddf='Kenward-Roger')$coefficients)
write.csv(BG_KEN,'BG_KEN.csv')


####NAG Simp####
NAG<-subset(SEA, Enzyme=="NAG")
FULL_NAG<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                (1|Study),
              data=NAG,
              weights=we,
              na.action = na.fail)
summary(FULL_NAG,ddf="Kenward-Roger")
NAG_KEN<-data.frame(summary(FULL_NAG,ddf='Kenward-Roger')$coefficients)
write.csv(NAG_KEN,'NAG_KEN.csv')


####LAP SImp####
LAP<-subset(SEA, Enzyme=="LAP")
FULL_LAP<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                (1|Study),
              data=LAP,
              weights=we,
              na.action = na.fail)
```

```
summary(FULL_LAP,ddf="Kenward-Roger")
LAP_KEN<-data.frame(summary(FULL_LAP,ddf='Kenward-Roger')$coefficients)
write.csv(LAP_KEN,'LAP_KEN.csv')

####AP Simp####
AP<-subset(SEA, Enzyme=="AP")
FULL_AP<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                (1|Study),
              data=AP,
              weights=we,
              na.action = na.fail)
summary(FULL_AP,ddf="Kenward-Roger")
AP_KEN<-data.frame(summary(FULL_AP,ddf='Kenward-Roger')$coefficients)
write.csv(AP_KEN,'AP_KEN.csv')

####APA Simp####
APA<-subset(SEA, Enzyme=="APA")
FULL_APA<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                 (1|Study),
               data=APA,
               weights=we,
               na.action = na.fail)
summary(FULL_APA,ddf="Kenward-Roger")
APA_KEN<-data.frame(summary(FULL_APA,ddf='Kenward-Roger')$coefficients)
write.csv(APA_KEN,'APA_KEN.csv')

####URE Simp####
URE<-subset(SEA, Enzyme=="URE")
FULL_URE<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
                 (1|Study),
               data=URE,
```

```
            weights=we,
            na.action = na.fail)
summary(FULL_URE,ddf="Kenward-Roger")
URE_KEN<-data.frame(summary(FULL_URE,ddf='Kenward-Roger')$coefficients)
write.csv(URE_KEN,'URE_KEN.csv')

####PPO####
PPO<-subset(SEA, Enzyme=="PPO")
FULL_PPO<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
             (1|Study),
            data=PPO,
            weights=we,
            na.action = na.fail)
summary(FULL_PPO,ddf="Kenward-Roger")
PPO_KEN<-data.frame(summary(FULL_PPO,ddf='Kenward-Roger')$coefficients)
write.csv(PPO_KEN,'PPO_KEN.csv')

####PER####
PER<-subset(SEA, Enzyme=="PER")
FULL_PER<-lmer(rr~scale(Dt)+scale(t)+scale(SD)+scale(AI)+
             (1|Study),
            data=PER,
            weights=we,
            na.action = na.fail)
summary(FULL_PER,ddf="Kenward-Roger")
PER_KEN<-data.frame(summary(FULL_PER,ddf='Kenward-Roger')$coefficients)
write.csv(PER_KEN,'PER_KEN.csv')



##AIC of models###
dEpI<-cbind(rbind(AIC(FULL_HYD),AIC(FULL_OXI),AIC(FULL_CBN),AIC(FULL_NIT),AIC(FULL_PHS)))
```

```
data.frame(dEpI)
dEpI<-format(dEpI, digits=1)

write.csv(dEpI,"DEPI6.csv",row.names=c("Hydrolytic","Oxidative","Carbon","Nitrogen","Phosphorus"))

DEPI<-read.csv("DEPI6.csv")
DEPI



###Figure 3###
###Enzyme class & Nutrient Cycle###
eric_boot<-function(model,nsim=1000){
  storefix<-bootMer(model,fixef,nsim)##bootstrap the fixed effects - more stable
  return(t(apply(storefix$t,2,function(x)quantile(x,c(0.025,0.5,0.975)))))##take the lower %2.5, %50 (here, the mean), and 97.5%
}
Df_F<-rbind(eric_boot(FULL_HYD,nsim=1000)[1,],##NOTE - if running too slow, try lowering nsim. default is 1000;
HOWEVER be careful - need enough simulations to get representative sample
          eric_boot(FULL_OXI)[1,],
          eric_boot(FULL_CBN)[1,],
          eric_boot(FULL_NIT)[1,],
          eric_boot(FULL_PHS)[1,])

Df_F<-data.table(Df_F)
Df_F<-setNames(Df_F,c("cl","mean","cu"))



DT<-data.table(SEA)
N_C<-DT[,.N,by=Trait]
N_S<-DT[,.(NS=length(unique(Study))),by=Trait]
NCS<-merge(N_C,N_S)
Df_F<-cbind(Df_F,NCS)
```

```
Df_F$st<-paste(Df_F$N,"(",Df_F$NS,")")


Df_F$cl_p<-(exp(Df_F$cl)-1)*100
Df_F$cu_p<-(exp(Df_F$cu)-1)*100
Df_F$mean_p<-(Df_F$cl_p+Df_F$cu_p)/2
Df_F
str(Df_F)


Df_F$Att<-c("B","B","B","B","B")


SF1<-ggplot(Df_F)+geom_point(aes(x=Trait, y=mean_p, col=Att),stat="identity",shape=21,size=4)+
  geom_errorbar(aes(x=Trait,ymin=cl_p,ymax=cu_p,col=Att),width=.1,size=0.5)+
  geom_text(aes(label=st,x=Trait,y=50,hjust=0),size=3)+
  coord_flip(ylim=c(-100,100))+
  scale_x_discrete(limits=rev(levels(Df_F$Trait)))+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="blue")+
  xlab("")+ylab("Drought effect (%)")+
  theme_bw()+theme(axis.line=element_line(colour="black"), panel.grid.major=element_blank(),
                   panel.grid.minor = element_blank(),
                   panel.background=element_blank())+
  theme(strip.background=element_blank(),strip.placement = "outside")+
  scale_colour_hue(l=40)+theme(legend.position="none")


SF1


pdf("Simple Fig 1.pdf",width=4,height=5,paper='special')
SF1
dev.off()


###Figure 4#####
#####Individual Enzyme Response#####
```

```
Df_F2<-rbind(confint(FULL_BG, method="Wald")[3,],confint(FULL_NAG, method="Wald")[3,],confint(FULL_LAP,
method="Wald")[3,],
        confint(FULL_AP, method="Wald")[3,],confint(FULL_URE, method="Wald")[3,],confint(FULL_APA,
method="Wald")[3,],
        confint(FULL_PPO, method="Wald")[3,],confint(FULL_PER, method="Wald")[3,])

Df_F2<-data.frame(Df_F2)

Df_F2$mean<-(Df_F2$X2.5..+Df_F2$X97.5..)/2
Df_F2<-setNames(Df_F2,c("cl","cu","mean"))



DT2<-data.table(SEA)
N_C2<-DT2[,.N,by=Enzyme]
N_S2<-DT2[,.(NS=length(unique(Study))),by=Enzyme]
NCS2<-merge(N_C2,N_S2)
NCS2<-NCS2[-c(1),] ###Removed NA (Non labled enzyme)
Df_F2<-cbind(Df_F2,NCS2)
Df_F2$st<-paste(Df_F2$N,"(",Df_F2$NS,")")

Df_F2$cl_p<-(exp(Df_F2$cl)-1)*100
Df_F2$cu_p<-(exp(Df_F2$cu)-1)*100
Df_F2$mean_p<-(Df_F2$cl_p+Df_F2$cu_p)/2
Df_F2
str(Df_F2)

Df_F2$Att<-c("B","B","B","B","B","B","B","B")



F2<-ggplot(Df_F2)+geom_point(aes(x=Enzyme, y=mean_p, col=Att),
```

```
                stat="identity",shape=21,size=4)+geom_errorbar(aes(x=Enzyme,ymin=cl_p,ymax=cu_p,col=Att),
                                                width=.1,size=0.5)+geom_text(aes(label=st,x=Enzyme,y=70,hjust=0),
                                                                             size=3)+coord_flip(ylim=c(-
100,100))+scale_x_discrete(limits=rev(levels(Df_F2$Enzyme)))+geom_hline(aes(yintercept=0),linetype="dashed",colour="blue"
)+xlab("")+ylab("Drought effect (%)")+
  theme_bw()+theme(axis.line=element_line(colour="black"), panel.grid.major=element_blank(),
                   panel.grid.minor = element_blank(),
                   panel.background=element_blank())+theme(strip.background=element_blank(),strip.placement =
"outside")+scale_colour_hue(l=40)+theme(legend.position="none")
F2

######ANOVA#####

anova(FULL_CBN)

anova_Full_Dt<-rbind(anova(FULL_HYD)[1,],anova(FULL_OXI)[1,],
                     anova(FULL_CBN)[1,],anova(FULL_NIT)[1,],
                     anova(FULL_PHS)[1,])
anova_Full_Dt
write.csv(anova_Full_Dt, 'anova_FUll_Dt.csv')



anova_Full_t<-rbind(anova(FULL_HYD)[2,],anova(FULL_OXI)[2,],
                    anova(FULL_CBN)[2,],anova(FULL_NIT)[2,],
                    anova(FULL_PHS)[2,])
anova_Full_t
write.csv(anova_Full_t, 'anova_FUll_t.csv')



anova_Full_SD<-rbind(anova(FULL_HYD)[3,],anova(FULL_OXI)[3,],
                     anova(FULL_CBN)[3,],anova(FULL_NIT)[3,],
```

```
                anova(FULL_PHS)[3,])
anova_Full_SD
write.csv(anova_Full_SD, 'anova_FUll_SD.csv')



anova_Full_AI<-rbind(anova(FULL_HYD)[4,],anova(FULL_OXI)[4,],
                anova(FULL_CBN)[4,],anova(FULL_NIT)[4,],
                anova(FULL_PHS)[4,])
anova_Full_AI
write.csv(anova_Full_AI, 'anova_Full_AI.csv')



####WALD SUMM#####
summary(FULL_BG, method='Wald')
BG_WAL<-data.frame(summary(FULL_BG)$coefficients)

summary(FULL_NAG, method='Wald')
NAG_WAL<-data.frame(summary(FULL_NAG)$coefficients)

summary(FULL_LAP, method='Wald')
LAP_WAL<-data.frame(summary(FULL_LAP)$coefficients)

summary(FULL_AP, method='Wald')
AP_WAL<-data.frame(summary(FULL_AP)$coefficients)

summary(FULL_URE, method='Wald')
URE_WAL<-data.frame(summary(FULL_URE)$coefficients)

summary(FULL_APA, method='Wald')
APA_WAL<-data.frame(summary(FULL_APA)$coefficients)
```

```
summary(FULL_PPO, method='Wald')
PPO_WAL<-data.frame(summary(FULL_PPO)$coefficients)

summary(FULL_PER, method='Wald')
PER_WAL<-data.frame(summary(FULL_PER)$coefficients)

summary(FULL_HYD, method='Wald')
HYD_WAL<-data.frame(summary(FULL_HYD)$coefficients)

summary(FULL_OXI, method='Wald')
OXI_WAL<-data.frame(summary(FULL_OXI)$coefficients)

summary(FULL_CBN, method='Wald')
CBN_WAL<-data.frame(summary(FULL_CBN)$coefficients)

summary(FULL_NIT, method='Wald')
NIT_WAL<-data.frame(summary(FULL_NIT)$coefficients)

summary(FULL_PHS, method='Wald')
PHS_WAL<-data.frame(summary(FULL_PHS)$coefficients)

###Partial Dependence plots###
FULL<-lmer(rr~scale(Dt) + scale(t) + scale(SD) + scale(AI) + (1|Study),
        weights=we,
        na.action = na.fail,
        data=SEA)

Trait<-subset(SEA, select=Trait)

Dt_PDP<-partial(FULL, pred.var = "Dt",
            train = SEA, plot = TRUE,
```

```
            smooth = TRUE,
            plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Drought Intensity (%)'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=SEA, shape=21, alpha=0.5, aes(x=Dt, y=rr, size = we, weight=we, color=Trait))

Dt_PDP

t_PDP<-partial(FULL, pred.var = "t",
            train = SEA, plot = TRUE,
            smooth = TRUE,
            plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Time in days'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=SEA, shape=21, alpha=0.5, aes(x=t, y=rr, size = we, weight=we, color=Trait), show.legend = FALSE)

t_PDP

SD_PDP<-partial(FULL, pred.var = "SD",
            train = SEA, plot = TRUE,
            smooth = TRUE,
            plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Soil Depth (cm)'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=SEA, shape=21, alpha=0.5, aes(x=SD, y=rr, size = we, weight=we, color=Trait), show.legend = FALSE)
```

```
SD_PDP


AI_PDP<-partial(FULL, pred.var = "AI",
                train = SEA, plot = TRUE,
                smooth = TRUE,
                plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Aridity Index'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=SEA, shape=21, alpha=0.5, aes(x=AI, y=rr, size = we, weight=we, color=Trait), show.legend = FALSE)


AI_PDP


###Figure 5####
plot_grid(Dt_PDP,
          t_PDP,
          SD_PDP,
          AI_PDP,
          labels=c('a','b','c','d'),
          label_x=0.2,
          ncol=2)


###Highlight PDP###
HYD_PDP<-partial(FULL_HYD, pred.var = "SD",
                 train = HYD, plot = TRUE,
                 smooth = TRUE,
                 plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Soil Depth (cm)'))+
```

```
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=HYD, shape=21, alpha=0.5, aes(x=SD, y=rr, size = we, weight=we))

HYD_PDP

PHS_PDP<-partial(FULL_PHS, pred.var = "SD",
                 train = PHS, plot = TRUE,
                 smooth = TRUE,
                 plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Soil Depth (cm)'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=PHS, shape=21, alpha=0.5, aes(x=SD, y=rr, size = we, weight=we))

PHS_PDP

NIT_PDP<-partial(FULL_NIT, pred.var = "SD",
                 train = NIT, plot = TRUE,
                 smooth = TRUE,
                 plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Soil Depth (cm)'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=NIT, shape=21, alpha=0.5, aes(x=SD, y=rr, size = we, weight=we))

NIT_PDP
```

```
NAG_PDP<-partial(FULL_NAG, pred.var = "SD",
                 train = NAG, plot = TRUE,
                 smooth = TRUE,
                 plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Soil Depth (cm)'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=NAG, shape=21, alpha=0.5, aes(x=SD, y=rr, size = we, weight=we))

NAG_PDP

LAP_PDP<-partial(FULL_LAP, pred.var = "SD",
                 train = LAP, plot = TRUE,
                 smooth = TRUE,
                 plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Soil Depth (cm)'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=LAP, shape=21, alpha=0.5, aes(x=SD, y=rr, size = we, weight=we))

LAP_PDP

APA_PDP<-partial(FULL_APA, pred.var = "AI",
                 train = APA, plot = TRUE,
                 smooth = TRUE,
                 plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Aridity Index'))+
```

```
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=APA, shape=21, alpha=0.5, aes(x=AI, y=rr, size = we, weight=we))

APA_PDP

OXI_PDP<-partial(FULL_OXI, pred.var = "AI",
                 train = OXI, plot = TRUE,
                 smooth = TRUE,
                 plot.engine = "ggplot2")+
  geom_hline(aes(yintercept=0),linetype="dashed",colour="black")+
  ylab("lnRR") + xlab(bquote('Aridity Index'))+
  theme_bw()+theme(panel.background = element_blank(),panel.grid.major = element_blank(),panel.grid.minor =
element_blank())+
  geom_point(data=OXI, shape=21, alpha=0.5, aes(x=AI, y=rr, size = we, weight=we))

OXI_PDP

####Figure 6#####
plot_grid(HYD_PDP,
          PHS_PDP,
          NIT_PDP,
          NAG_PDP,
          LAP_PDP,
          labels=c('a','b','c','d','e'),
          label_x=0.2,
          ncol=2)

###Figure 7#####
plot_grid(OXI_PDP,
APA_PDP,labels=c('a','b'),
```

```
label_x=0.2,
ncol=2)
```