DISSERTATION

SENSITIVITY OF MICROBIAL COMMUNITY PHYSIOLOGY TO SOIL MOISTURE AND TEMPERATURE IN AN OLD FIELD ECOSYSTEM

Submitted by

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ABSTRACT

SENSITIVITY OF MICROBIAL COMMUNITY PHYSIOLOGY TO SOIL MOISTURE AND TEMPERATURE IN AN OLD FIELD ECOSYSTEM

There is little consensus on how soil systems will respond to climate change over the long-term. The relationship between temperature, moisture and decomposition rates is driven by underlying microbial processes. Aggregate measurements of microbial function, such as carbon dioxide and methane production, have been used to demonstrate the critical role of temperature and moisture on soil organic matter decomposition rates. However, a more mechanistic understanding of decomposition has been elusive due to the small spatial and temporal scales at which decomposition occurs.

Enzymes catalyze the extracellular breakdown of organic material into smaller subunits that can be assimilated by organisms. The majority of organic inputs to soils are complex, high molecular weight compounds that must be broken down by enzymes in order for them to be assimilated by the microbial community. Enzymatic depolymerization may be the rate limiting step in decomposition, thus the rate of enzymatic reactions affected by soil temperature and moisture can greatly influence decomposition rates. In addition, shifts in substrate utilization and allocation by microorganisms due to climate change will affect the type and rate of substrates

decomposed, as well as the amount of respiration produced. Microbial functional responses to climate change through altered enzyme production and carbon utilization profiles can lead to large changes in decomposition and nutrient cycling.

The goal of this research was to provide a more mechanistic understanding of how microbial community function may change under different climate regimes by using enzyme activity and substrate utilization profiles as indicators of microbial physiology. I collected soils from the Boston Area Climate Experiment, a multi-factor climate manipulation using three levels of precipitation and four levels of warming. Enzyme activity was assayed at several temperatures and soil moistures to measure the sensitivity of activity to these two variables. In addition, carbon utilization profiles and temperature sensitivity of respiration were determined by incubating soils with several different substrates at multiple temperatures. I hypothesized that both enzyme activity and substrate induced respiration would be lower under drought because the stress of drought conditions would reduce microbial biomass. Following warming treatments I hypothesized an increased use of complex substrates because the depolymerization rate of complex compounds is more sensitive to warming than that of simple compounds. In addition potential enzyme activity would be lower under the warmed treatment because of increased enzymatic efficiency, reaction rates, under warmer temperatures resulting in fewer enzymes needed to meet microbial nutrient demands.

My results from analyzing hydrolytic enzyme activity indicated a stable enzyme pool under drought conditions despite reduced microbial biomass and field respiration. This result was counterintuitive because nutrient limitations would likely be greater

under drought, due to diffusion constraints, resulting in fewer nutrients available for enzyme production. Based on my results, I hypothesized that drought resulted in decreased enzyme turnover because of increased clay adsorption and tannin complexation of enzymes resulting in increased protection from proteolysis. Clay adsorption may have increased under drought because water films become thinner increasing the frequency of enzyme-clay interactions. Tannin complexation may also have increased due to increased tannin concentrations measured at the BACE (Tharayil et al. 2011).

In my second study, using microbial community level physiology profiles, there was a shift in community substrate utilization towards an increased use of tannins and vanillin under dry, hot conditions. This shift may have been due to increased production of plant tannins, and thus availability of these substrates in the field leading the community to shift to organisms better adapted to use these substrates. In addition, when more complex substrates were used the amount of respiration produced per unit biomass was lower indicating an increased carbon utilization efficiency of the microbial community and perhaps a shift towards slower growing, more efficient organisms.

Last, I used enzyme temperature and moisture sensitivity measurements in conjunction with field temperature and soil moisture data to estimate *in situ* activity. The moisture model predicted high activity in the drought plots due to large increases in activity as diffusion limitations were relieved when soil moisture increased. Temperature was the main driver when estimating *in situ* activity with both temperature and moisture sensitivity equations combined into a single model, except

when soil moisture went below a certain threshold, about 7% soil moisture. The results of this study suggest that, under intensified precipitation regime, this old-field ecosystem may experience pulses of rapid decomposition of simple and complex carbon compounds following precipitation events. Further study is needed to elucidate whether the patterns observed here are generalizable to other ecosystems that differ in climate, soils and vegetation.

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1 INTRODUCTION

Belowground responses to climate change are uncertain over the long-term (Baveye, 2007). Many studies investigate the individual effect of warming or precipitation change on decomposition, but the effect of the two variables in combination has been understudied (Garten et al. 2009). The relationship between temperature, moisture and decomposition rates is driven by underlying microbial processes (Ekschmitt et al. 2005). Respiration measurements have been used as a proxy for microbial activity and growth. However, rates of microbial growth and respiration are differentially affected by temperature, nutrient availability, and microbial community structure seasonally and over long time periods (Bradford et al. 2008; Steinweg et al. 2008). Often microbial processes are not explicitly defined when assessing soil carbon loss (Figure 1.1). For example, carbon utilization efficiency (CUE) is a measure of how efficiently microorganisms immobilize versus mineralize carbon. CUE is a fixed parameter in ecosystem models such as CENTURY (Parton et al. 1987), however Steinweg et al. (2008) demonstrated that carbon utilization efficiency is temperature dependent, with a lower CUE at higher temperatures. A more mechanistic understanding of microbial influences on decomposition is important in dynamic systems but is difficult to ascertain due to small spatial and temporal scales at which microorganisms function.

Microorganisms are phylogenetically diverse and inhabit every system currently explored on earth (Torsvik et al. 2002). Their ability to adapt to changing environments is rapid, through changes in physiology and/or community composition. Changes in microbial activity due to environmental disturbance can be directed by two mechanisms, (1) the active community composition shifts because some organisms are not well adapted to the new conditions (Zogg et al. 1997; Zhang et al. 2005) or (2) the active community remains the same but alters their physiology to better adapt (Cooper et al. 2001; Bennett & Linkski, 2007). Microbial functional responses to climate change through altered enzyme production and carbon utilization profiles can lead to large changes in decomposition and nutrient cycling (Fierer et al. 2003; Collins et al. 2008). Looking at how both microbial enzyme activity and carbon utilization profiles are influenced by climate provides a higher resolution understanding of decomposition processes than measuring CO_2 alone (Figure 1.2).

Microorganisms produce extracellular enzymes to breakdown organic matter polymers into smaller subunits that can be transported into their cells (Burns, 1982). Enzymatic depolymerization has been hypothesized as a rate limiting step in decomposition, thus the temperature and moisture sensitivity of enzymatic reactions can greatly influence decomposition rates (Schimel & Bennet, 2004; Allison et al. 2010). Enzyme activity has been assayed in soils for over sixty years (Skujins, 1976) and used as an indicator of substrate use and nutrient cycling, and to provide a mechanistic understanding of decomposition in natural and disturbed systems (Nannipieri ,1994; Dilly & Nannipieri, 1998). Enzymatic reactions, like all biogeochemical reactions, are

affected by temperature (Trasar-Cepeda et al. 2007). The Arrhenius equation, $k= A*e^{(-Ea/RT)}$ where k is the reaction rate, A is the frequency factor, E_a is the activation energy, R is the universal gas constant and T is the temperature in kelvin, provides a mathematical understanding of how decomposition and enzyme activity in particular are affected by temperature and moisture. The temperature response of activity is calculated by looking at how changes in T affect the Arrhenius equation. As temperature rises, the T in the denominator increases causing the exponent value to become less negative resulting in increased activity. In addition to temperature effects, soil moisture affects the frequency factor, A, in the Arrhenius equation by controlling the number of substrate-enzyme interactions. As soil moisture varies, the size and connections between water films changes leading to different contact frequencies between substrates and enzymes.

The Arrhenius equation also takes into account the quality, or complexity, of the substrate involved in the reaction. The E_a, activation energy, increases with substrate complexity resulting in slower reaction rates. With climate change, plant community composition is predicted to change along with litter chemistry (Aerts, 1997; Woodward et al. 2004). Microbial communities are well adapted to breakdown plant products, however different enzymes are required for different compounds and not all microbes produce these (Marsden & Gray 1986; Kirk & Farrell 1987). As litter quality and quantity changes the substrate utilization profiles of microbial communities will also shift because of changes in substrate availability. Microorganisms allocate carbon to growth, maintenance, enzyme production and respiration, with the relative amount of nutrient allocation changing between these four processes because of altered environmental

conditions. Understanding what types of compounds microorganisms preferentially use and how efficiently they use them provides a picture of what types of substrates are used to provide carbon for microbial processes.

The Boston Area Climate Experiment, established in 2007 in an old field ecosystem, is a multi-factor climate change experiment that has provided an opportunity to study microbial functional responses under multiple temperature and precipitation manipulation levels both individually and in combination. In my first chapter I assessed the sensitivity of hydrolytic enzyme activity to climate variables, drought in particular. In addition, I used calculations of enzyme stoichiometry as another metric to assess shifts in enzyme production based on nutrient needs over the course of a year (Sinsabaugh et al. 2009). My second chapter is dedicated to better understanding microbial community level physiology. Substrates of varying quality were used to determine how microorganisms may have shifted substrate utilization over the course of the year and with climate variables in the field. Last, I attempted to more closely approximate *in situ* enzyme activity. Our ability to estimate *in situ* activity is hampered by the current methodology, so here I modified the assay and used field soil moisture and temperature data to better approximate how these two variables influence activity in the field. These three studies aim to provide a more mechanistic understanding of how decomposition will be affected by climate change.

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1.2 FIGURES

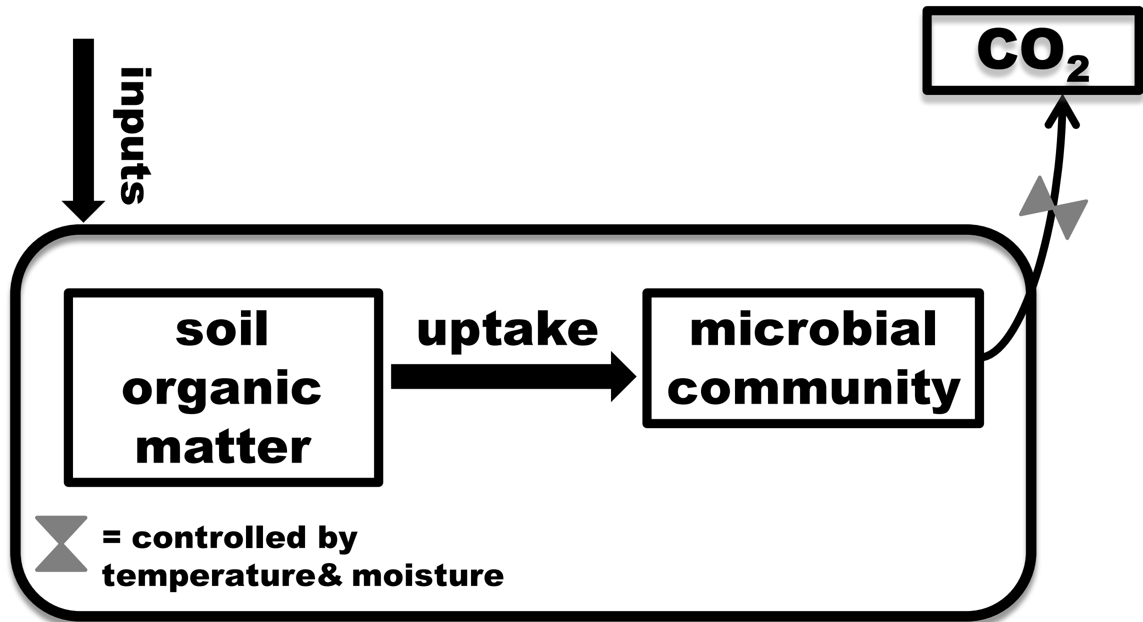

Figure 1.1 Soil organic matter decomposition including the influence of temperature moisture on respiration.

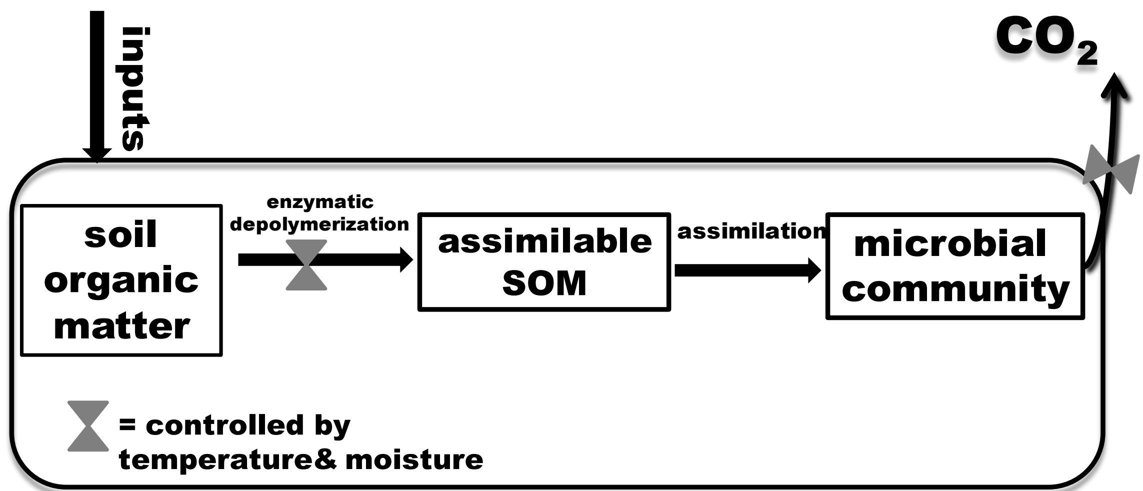

Figure 1.2 Microbial decomposition pathway including two processes influenced by changes in soil temperature and moisture (modified from Conant et al. in review).

2 DROUGHT STABILIZES ENZYME ACTIVITY IN AN OLD FIELD ECOSYSTEM: INSIGHTS INTO CONTROLS ON ENZYME TURNOVER

2.1 ABSTRACT

Much of our current understanding of belowground responses to climate change comes from the measurement of respiration, an aggregate metric which is the net result of many microscale processes performed by microorganisms. Enzymes are produced by microorganisms in response to nutrient requirements and availability. Changes in enzyme activity are indicative of microbial substrate use and are important for understanding nutrient cycling. Enzyme activity is sensitive to temperature like all chemical reactions, however there is limited understanding of how concurrent changes in soil temperature and moisture will affect enzyme activity. I used the Boston Area Climate Experiment, a multi-factor climate change experiment in an old field ecosystem, to address how soil enzyme activity is affected by climate variables.

Enzyme activity was measured in soils from plots exposed to a combination of three levels of precipitation (ambient, +50% ambient (wet), -50% ambient (drought)) and four levels of warming (no change, +1, +2 and +3˚C above ambient) over the course of a year. I assayed six enzymes involved in carbon, nitrogen and phosphorus cycling to determine if they were affected in a similar fashion by moisture and temperature manipulations.

Warming and season had very little effect on potential enzyme activity. The lack of temperature effect may have been due to the low level of warming exposed to the plots. In addition, enzyme activity was not significantly affected by moisture when calculated on a per g soil basis, indicating no change in the enzyme pool size, which was surprising because of a reduced microbial biomass under drought in June 2009. Enzyme activity is governed by pool size which is a function of enzyme production and turnover. Nutrient limitations were likely greater under drought because of diffusion constraints. The production of enzymes is energetically costly to microorganism, which could be detrimental if nutrient returns are less than enzyme production costs, likely resulting in decreased enzyme production. I hypothesized that the lack of change in enzyme pool size despite a decline in microbial biomass under drought was due to a decrease in turnover rate instead of increased production.

Even though enzyme pools remained the same under drought, the *in situ* activity would still be quite low. Diffusion constraints on enzymes, substrates and products are likely quite strong leading to lower activity as soil moisture declines. However, precipitation events are predicted to decrease in frequency but increase in intensity, leading to longer periods where soils are dry and enzymes may stabilize. On release from diffusion limitations these soils will be primed for rapid enzymatic depolymerization, resulting in rapid changes of nutrient pools and loss of carbon.

2.2 INTRODUCTION

Aggregate measurements of microbial function, such as CO_2 and CH_4 production, have been used to demonstrate the critical role of temperature and moisture on soil

organic matter (SOM) decomposition rates (Davidson et al. 1998; Schimel et al. 1998). However, the relationship between these abiotic drivers and decomposition rates are driven by underlying microbial processes (Ekschmitt et al. 2005). For example, enzymatic depolymerization of organic matter has been hypothesized as the rate-limiting step in SOM decomposition (Schimel and Bennet, 2004; Allison et al. 2010). The majority of organic matter entering the soil system is not readily accessible for microorganisms, requiring fragmentation and enzymatic breakdown (Dighton & Boddy 1989). Enzymes are involved in the extracellular break down of organic material into smaller subunits that can be assimilated by organisms (Burns, 1982; Nannipieri et al. 2002). The rate of *in situ* enzyme activity is sensitive to several biotic and abiotic factors, such as temperature, moisture and soil texture (Allison, 2006; Trasar-Cepeda et al 2007; Wallenstein & Weintraub, 2008).

Production and turnover of enzymes control the size of the enzyme pool and thus the rate of activity (Figure 2.1). The rate of enzyme production may be affected by microbial demand for nutrients (Sinsabaugh & Moorhead, 1994; Allison & Vitousek, 2005), substrate concentration (German et al. 2011; Allison et al. 2011) and potentially by the composition and aggregate traits of the active microbial community. To maintain the stoichiometry of their biomass (driven by the fixed stoichiometry of cellular components), microbes produce enzymes targeting specific compounds that are rich in either C, N or P (Sinsabaugh et al. 2008; Sinsabaugh et al. 2009). The requirements of nutrients can vary between fungi and bacteria (Strickland & Rousk, 2010) or even between different microbial taxa, resulting in fluctuating enzyme pool sizes as the

community changes. Enzyme stoichiometric ratios provide a metric to assess shifts in enzyme production based on nutrient needs (Sinsabaugh et al. 2009). However, enzyme production declines for many substrates when their concentration is low (German et al. 2011). Wallenstein et al (2009) hypothesized that observed declines in enzyme activity may be related to reduced nitrogen (N) availability in the Arctic, because the N requirement for enzyme production is high. Turnover of enzymes occurs because of structural breakdown of the enzyme over time, but also due to protease activity. Proteases release organic N from proteinaceous compounds, and extracellular enzymes can be broken down in times of N limitations (Weintraub & Schimel, 2005a).

Seasonality can also influence *in situ* enzyme activity through changes in substrate inputs, temperature and soil moisture (Kshattriya et al. 1992). When plants are actively growing, root exudation can lead to rapid acceleration of nutrient cycling, indicated by respiration and enzyme activity measurements (Weintraub et al. 2007; Hernandez & Hobbie, 2010; Zhu & Cheng, 2010). Plant allocation of nutrients above- and belowground alters the quality and accessibility of nutrients for enzymatic breakdown. Since C and N mineralization are closely coupled, it is not surprising that enzyme activity changes with inputs over the course of the year (McGill & Cole, 1981; Weintraub & Schimel, 2005a,b).

Seasonal changes in temperature can directly affect *in situ* enzyme activity due to the thermal sensitivity of enzymatic reactions (Trasar-Cepeda et al., 2007; Koch et al. 2007). Enzymatic reactions proceed faster as temperatures increase (up to an optimal temperature that is usually above maximum soil temperatures in any particular

environment), increasing the depolymerization efficiency per enzyme (Koch et al. 2007; Wallenstein et al. 2010). As a result of increased enzyme activity of the extant enzyme pool, microorganisms may allocate fewer resources to enzyme production with increasing temperature (Allison & Vitousek, 2005). Consistent with this feedback, Bell et al. (2010) measured increases in potential enzyme activity in the winter, which they attributed to enhanced enzyme production in response to decreased reaction efficiency under cold temperatures. Varying enzyme concentrations may not be the only response to increasing temperatures. Shifts in the thermal optima of enzyme activity have been measured, with the optima shifting up or down based on the seasonal ambient temperatures (Fenner et al. 2005). Changing enzyme temperature sensitivity could be explained by the production of isoenzymes, enzymes with the same substrate specificity, but different temperature optima (Wallenstein & Weintraub, 2008; Loveland et al. 1994).

Increasing temperatures and altered precipitation patterns due to climate change could have a large impact on soil moisture. Diffusion of substrates, enzymes and products are necessary in order for organisms to recoup nutrient losses from maintenance, growth and enzyme production. Drought conditions could impose diffusion limitations on enzymes and substrates (Allison, 2005; Stark & Firestone, 1995). The result of drought in already oxic soils could be a decrease in enzyme production as biomass declines or an increase in production to satisfy nutrient requirements of the biomass (Sardans & Penuelas, 2005; Sowerby et al. 2005; Allison, 2005). Additional precipitation would release diffusion limitation, but in some areas would increase

anaerobic conditions, altering the efficiency of oxidative enzymes, resulting in an increase in phenolic compounds and carbon storage (Freeman et al. 1997; Freeman et al. 2001).

The purpose of this experiment was to assess the sensitivity of hydrolytic enzyme activity and enzymatic resource allocation to different climate variables. To separate the influence of soil temperature and moisture on enzyme activity from seasonal effects, I measured the activity and stoichiometry of six enzymes involved in carbon, nitrogen and phosphorus cycling, from the Boston Area Climate Experiment (BACE) four times over the course of a year. BACE is a multifactorial climate change manipulation on an old field ecosystem, providing three levels of precipitation and four levels of warming. Potential enzyme activity is a metric for soil microbial functional response to disturbance (Henry et al 2005) and indicates shifts in metabolic requirements (Caldwell 2005). I hypothesized 1) a reduction in microbial biomass under drought conditions would results in reduced potential enzyme activity, 2) reduced potential activity under warming because of increased enzyme reaction efficiency resulting in fewer enzymes required for the same number of reactions to be performed and 3) greater enzyme activity in the growing season compared to the winter because of increased nutrient availability stimulating enzyme production.

2.3 METHODS

2.3.1 Study Site

The soils were obtained from the Boston-Area Climate Experiment (BACE), an old field ecosystem, located in Waltham, Massachusetts at the University of Massachusetts

Agricultural Experiment Stations. Mean annual precipitation and temperature in nearby Boston, MA is 1054 mm yr^{-1} and 10.3˚C. Soils are classified as mesic Typic Dystrudepts and the upper 30 cm consists of loam soils (45% sand, 46% silt, and 9% clay), with a pH 5.5. The site was previously an apple orchard, but was abandoned over 40 years ago. Current vegetation includes about 38 non- native grasses and forbs.

2.3.2 Field Experimental Design

The BACE exposes thirty-six plots to one of three precipitation treatments and four warming levels. The three precipitation treatments, no change, plus 50% precipitation (wet), and minus 50% precipitation (drought) and four temperature treatments, no change, +200 W m^{-2} (warm), +600 W m^{-2}(medium), and +1000 W m^{-2} (hot) are arranged in a full-factorial design with three replicates for each treatment. Precipitation is controlled by clear partial roofs in the drought plots and additional precipitation is added after natural precipitation events in the wet plots. During the winter, drought plots are maintained, but additional water is not added to the wet plots. Warming is achieved by ceramic infrared heaters, mounted 1m above the ground at each corner of a plot. Air temperature is monitored in each plot to maintain target temperatures. In the warmest plots, air temperatures are limited to no more than four degrees above ambient air temperature. Soil moisture is measured weekly during the non-freezing months, usually beginning in April and ending in December, whereas soil temperature is monitored daily throughout the year. Precipitation treatments began in July 2007 and warming treatments began June 2008. Field respiration measurements were taken using a LI-COR 6400-09 soil CO_2 flux chamber attached to a 6400 portable

photosynthetic system once a month from within a 25cm PVC collar installed in each plot.

2.3.3 Soil Sampling and Pre-processing

Soils were first collected from all plots in June 2008, one year after precipitation manipulations began and prior to warming treatments initiated at BACE. Additionally, soil samples were taken three times (August 2008, January 2009, and June 2009) following the initiation of the warming treatment in June 2008. Two cores (5 cm diameter) were collected from each plot at 0-5 and 5-15cm depths. Soils were packaged on ice and shipped to the laboratory overnight, where the cores from each plot were 2mm sieved, rocks and roots removed, homogenized and frozen at -10˚C until analysis.

2.3.4 Soil Characterization

Subsamples from each plot were taken for determination of percent soil moisture, pH, total C and N concentrations. Soil moisture was determined after field moist soils were weighed and dried for 48 hours at 60˚C and then reweighed. Soil pH was determined using the supernatant of soil mixed with water (1:5 by volume). Soil subsamples were dried at 60˚C and ground to measure total C and N concentrations on the LECO CHN-1000 autoanalyzer (LECO Corporation, St. Joseph, MI, USA).

2.3.5 Microbial Biomass Measurement

Substrate induced respiration (SIR) was used to estimate microbial biomass (Anderson & Domsch 1978), using a deep-well microplate setup called, MicroResp™(Aberdeen, UK; Campbell et al. 2003). Soils were removed from the freezer and a 20g subsample was warmed to about 20˚C. All samples from June and August

samplings had water added to bring soil moisture up to 55% water holding capacity. After water addition, samples were covered for one hour, homogenized and measured into 96 –well deep-well plates. For January 2009, 20g subsamples were dried to 55% water holding capacity at room temperature, about 20°C, for 6-36 hours. Following drying, samples were homogenized and measured into 96-well deep-well plates.

Three wells on a plate were used per sample, with about 0.2-0.3g of moist soil added to each well, using the MicroResp manufacturer's protocol. After samples were added to the deep-well plate, they were covered with sealing film and placed at 4˚C for about 18h prior to addition of glucose. Samples were then incubated at 25˚C for 6 hours following addition of 25µl 1M glucose solution (determined to saturate demand in preliminary assays). The CO_2 indicator plates were read on a Tecan Infinite M500 microplate reader at 625nm prior to being placed on deep-well plates. The indicator plate and deep-well plate were attached to one another using the MicroResp apparatus and allowed to incubate. Following the six hour incubation the indicator plates were removed from the deep-well plates and read again on the Tecan microplate reader at 625nm.

Indicator plates were made one week in advance of the assay according to the manufacturer's guidelines. Standard curves were generated by incubating indicator plates in jars filled with known concentrations of CO_2. The amount of CO_2 produced from the water addition wells was subtracted from the respiration in the glucose addition wells. Microbial biomass was calculated from respiration produced from the

glucose amended wells at 25˚C and using the following equation from Anderson & Domsch (1978):

$$\text{mg MBC 100 g soil}^{-1} = 40.04y + 0.37$$

where y is the amount of CO_2 produced under glucose amendment.

2.3.6 Enzyme Assays

Enzyme assays were performed on samples from all plots at each collection date. Each sample was assayed for the activity of six different hydrolytic enzymes involved in C, N and P acquisition (Table 2.1). The assay protocol was modified from Sinsabaugh et al. (1992). The assays were run at 25˚C for 3 hours using one deep-well 96-well plate. Two additional plates were used to create standard curves for each sample at 25˚C. The reference standard for the leucine amino peptidase assay was 7-amino-4-methylcoumarin (MUC) and for the remaining substrates it was 4-methylumbelliferone (MUB). The standard curve plates had a column for each sample and different concentrations in each of the wells of the MUB or MUC standards, 0, 2.5, 5, 10, 25, 50 and 100µM.

Soils were removed from the freezer and a 2.75g subsample was taken and warmed to about 20˚C. The subsample was homogenized with 50mM sodium acetate (pH 5.5) for one minute on high in a Waring blender. Each column on the deep-well 96-well plates corresponded to one sample. After homogenization, 800µl of suspension was aliquoted into each of the eight wells of a column on all five plates. Following addition of twelve samples into their respective columns the MUB substrates were

added. Each substrate was added to one well in each column, so that all twelve samples received each of the six substrates once.

The plates were incubated for three hours at 25˚C and then centrifuged for three minutes at 350g. Afterwards, 250µl of supernatant from each well was placed into the corresponding well on a 96-well black plate. Fluorescence was measured immediately following 5µl addition of NaOH to each well to terminate the reaction. A Tecan Infinite M500 spectrofluorometer was used to measure fluorescence with wavelengths set at 365nm and 450nm for excitation and emission, respectively. The plates with the standards were used to calculate a linear standard curve and determine enzyme activity for each sample as µmol h^{-1} g dry $soil^{-1}$and µmol h^{-1} g C^{-1}.

2.3.7 Calculations & Statistical Analysis

Ratios for C and N cycling were calculated as BG: (NAG+LAP) and C:P ratios as BG:PHOS using activity for each sample as a µmol h^{-1} g C $soil^{-1}$ (Sinsabaugh et al. 2009). The ratio of activity for different enzymes is a metric for understanding microbial nutrient demand. Mass specific enzyme activity, was calculated by dividing the enzyme activity by the microbial biomass estimated from substrate induced respiration (Hassett & Zak, 2005). There was no calculation of mass specific enzyme activity for June 2008 samples because microbial biomass could not be estimated due to lack of soil. The field respiration rate was divided by enzyme activity to estimate the amount of respiration produced per unit enzyme activity for carbon cycling enzymes.

Potential enzyme activities were log transformed in order to normalize the variance prior to analysis using SAS PROC GLIMMIX with Tukey's adjustment, $\alpha = 0.05$

(SAS Institute, Cary, NC). Block, precipitation treatment and season were selected as random effects, temperature as a fixed effect and enzyme activities were designated as dependent variables. Next, PROC GLIMMIX was used to determine significant field treatment effects within each season and contrasts between treatments or seasons that were deemed significant in GLIMMIX. For mass specific enzyme activity all plots under each precipitation treatment were used unless GLIMMIX indicated a significant effect of temperature or temperature x precipitation interaction.

2.4 RESULTS

2.4.1 Experimental Climate Effects

Warming treatments raised soil temperatures on average by 0.70, 2.05 and 2.70˚C above ambient at 2cm in both years for the warm, medium and hot treatments respectively (Figure 2.2a). Soil temperatures at 10cm were also affected by warming, increasing the temperature 0.12, 1.64 and 2.84˚C above ambient in both years for plots with warm, medium and hot treatments. Precipitation treatments altered soil moisture substantially, with soil moisture in drought-only treatments being on average 75% of ambient at 0-30cm in 2008 and 50% of ambient moisture at 0-10cm in 2009 (Figure 2.2b). There was no effect of additional water on the soil moisture of wet plots.

In the interaction plots, the warming treatment increased the soil temperature in drought and ambient plots, with the largest soil warming occurring in the drought x hot plot, by 4.0 and 3.5˚C compared to the ambient plots in 2008 and 2009, respectively. Soil moisture in wet plots was not affected by the addition of heating in 2008 or 2009. There was no measurable change in total soil carbon, nitrogen or C:N

ratio due to treatment or seasonal effects. The average total soil carbon and nitrogen values were 57 and 4.7 for 0-5cm, and 42 and 3.6 for 5-15cm.

2.4.2 Warming and Precipitation Field Treatments

Average potential enzyme activities and standard errors are presented by season, precipitation and warming treatments in Appendix 1 for main treatment and interaction plots (Table A.1-A.4). In all plots, PHOS and BG potential activities were the highest and the remaining enzymes exhibited similar activities, usually under 200 nmol activity g dry soil^{-1} h^{-1} (Figure 2.3). Precipitation manipulations had no significant effect on enzyme activity when calculated per g dry soil, however there was a trend towards increased activity in drought plots in June 2009, which was significant for PHOS at 0-5cm ($P<0.05$).There was no significant effect of temperature alone on potential enzyme activity at 0-5cm, but NAG in June 2008, LAP in January 2009 and CB in June 2009 were affected by precipitation x temperature treatments ($P<0.05$). The interaction effect at 0-5cm always resulted in decreased activity in drought plots with warming. At the 5-15cm depth warming tended to decrease activity in the medium-warmed plots compared to the ambient plots, and this effect was statistically significant for XYL and LAP ($P<0.01$).

SIR biomass estimates were negatively affected by drought in June 2009 ($P=0.1$), but the highest warming treatment significantly increased biomass in ambient and drought plots (Figure 2.4, $P<0.05$). In August 2008 there was a precipitation effect, with microbial biomass significantly lower under the wet and drought treatments compared to ambient ($P<0.05$), however this effect was not measured in the 2009 samples.

Mass specific enzyme activity (nmol activity h^{-1} μg microbial biomass $carbon^{-1}$) indicated significant changes in the amount of enzyme per unit biomass over the three seasons and by precipitation treatment (Figure 2.5, $P<0.05$). The mass specific enzyme activity in January 2009 was significantly higher than that of August 2008 and June 2009 ($P<0.05$). In August there was a trend towards increased mass specific enzyme activity under drought compared to ambient and wet treatments, and became significant in June 2009 for all enzymes ($P<0.05$).

Field respiration per unit enzyme activity for carbon cycling enzymes was significantly affected by precipitation treatments in January 2009 and June 2009 (Figure 2.6, $P<0.05$). In January 2009 the drought and ambient plots had higher respiration per unit enzyme activity than wet plots. However, in June 2009 respiration per unit BG, CB and XYL enzymes was significantly lower in the drought plots compared to the ambient and wet plots.

2.4.3 Seasonal Patterns

At both soil depths, NAG, XYL and LAP were significantly affected by season. Activities were significantly lower in January 2009 at both depths for NAG, XYL and LAP compared to August 2008 (Figure 2.3, $P<0.05$). Microbial biomass was significantly lower in January 2009 than August 2008 and June 2009 ($P<0.05$). Season affected the C:N acquiring enzyme stoichiometry at both depths, with a significant increase in enzyme C:N in winter 2009 compared to the two June samples ($P<0.01$, Figure 2.7). The C:N enzyme ratio increased from June 2008 to January 2009 and then declined in June 2009, whereas C:P enzyme ratios show no seasonality. There is also a significant depth effect

in the C:N enzyme ratio for June 2008 and January 2009, with 5-15cm depth have a higher ratio than 0-5cm ($P<0.05$).

2.5 DISCUSSION

2.5.1 Precipitation and Warming Effects

Temperature and moisture are widely observed to be the primary drivers of SOM decomposition rates across space and time (Schimel et al. 1994; Davidson & Janssens, 2006; Gabriel & Kellman, 2011), but the underlying microbial mechanisms that drive these patterns are complex and not fully understood. In particular, the effects of climate on soil enzyme activities involve not only short-term changes in activity driven by thermodynamics (Trasar-Cepeda et al. 2007), but also long-term changes in enzyme pools due to direct and indirect effects on microbial activity (Sowerby et al. 2005; Schimel et al. 2007). The BACE allowed me to assess the interactive effects of temperature and precipitation on soil enzymes. I predicted that enzyme activity would decrease in response to drought due to decreased microbial activity. On the contrary, there was no change in the potential activity for any of the enzymes involved in C, N and P cycling in any precipitation treatment. The lack of change in enzyme pool size was surprising, since both microbial biomass and field respiration declined under drought (Suessela et al. in review). It is widely assumed that enzyme pools are strongly correlated to microbial biomass, and thus most soil ecosystem models do not explicitly incorporate enzymes.

The maintenance of the enzyme pool size despite a smaller microbial biomass under drought could be a result of increased resource allocation towards enzyme

production. Nutrient limitation may have been amplified as soils dried thus more enzymes were produced to compensate for resource demand (Harder & Dijhuizen, 1983), although this response would likely be short-lived as these resources are required for enzyme production (Allison & Vitousek, 2005). However, the most parsimonious explanation for a stable enzyme pool size with reduced microbial activity under drought is decreased enzyme turnover rate (Figure 2.1). As soils dry, enzymes may become stabilized on clay and organic residues. Water films are maintained for longer periods of time in the micropores of clay soils. Enzymes remain in water films and have a greater chance of becoming adsorbed to clays (Nannipieri et al. 2002). Proteases, which degrade other enzymes, are also subject to diffusion limitations and would have reduced activity if their target enzymes were not accessible. Adsorption to clay minerals may also lower turnover rates by protecting enzymes from proteolytic enzymes (Ensminger & Gieseking, 1942; Skujins, 1976; Allison, 2006). Another form of protection is enzyme complexation with tannins (Joanisse et al. 2007). Complexed enzymes can continue with reactions as long as the enzyme is not bound to the clay/tannin at the active site (Pflug, 1981; Kandeler, 1991). Tharayil et al. (2011) measured increased plant tannins under the most water stressed conditions at BACE, which correlated with a significant increase in litter β-glucosidase complexation capacity. These three mechanisms, reduced proteolysis, clay adsorption and tannin complexation, could reduce enzyme turnover rates and allow for a more stable enzyme pool despite declining microbial biomass and possibly enzyme production.

Suessela et al. (in review) measured a 21% reduction in heterotrophic respiration under drought at BACE, suggesting that decomposition rates are lower in drought plots despite potential enzyme activity similar to ambient conditions. Potential enzyme activity as measured in laboratory assays does not necessarily directly correlate with *in situ* activity under field conditions (Wallenstein and Weintraub, 2008). For example, the potential enzyme activities that I measured in drought treatments may not translate to the same rates of enzymatic degradation in ambient field plots. When field respiration was considered on a per unit enzyme activity basis, there was a decline in the ratio under drought and wet treatments. In the wet plots the response was driven by both high respiration and enzyme activity, but in the drought plots the response was driven by low respiration and high enzyme activity. Under low soil moisture conditions, the diffusion of enzymes and substrates will be limited to thin water films and pockets of moisture with low connectivity (Stark and Firestone, 1995). The lack of diffusion of substrates to enzymes and products to microbes could be the rate limiting step in decomposition under drought instead of enzymatic depolymerization as evidenced by high mass specific enzyme activity but low field respiration. In addition, root biomass did not increase under drought at the BACE as has been seen in other grass dominated systems (Williams & Black, 1994; Kalapos et al. 1996; Burke et al. 1998). Without an increase in root biomass it appears that rhizodeposition, which supplies some labile substrates likely decreased or remained the same under drought. Reduced rhizodeposition might also decrease the microbial production of enzymes associated

with priming (Fontaine et al. 2003); however, I did not see any evidence for this mechanism.

In a peatland, drought conditions that reduced the water table resulted in increased enzyme activity (Freeman et al. 1998). Even though that system was very different from the old field studied here, Freeman et al. (1998) hypothesized that increased activity was due to stimulation of existing enzymes and not *de novo* production because the microbial respiratory activity did not increase. However, most other studies have detected declines in hydrolytic and oxidative enzyme activity under drought conditions in already oxic soils (Sardens & Penuelas, 2005; Sardens et al. 2008; Sardens & Penuelas, 2010; Toberman, 2008). Most of these studies have been in Mediterranean systems which are drought-prone and may contain drought adapted microorganisms, whereas drought is a rare feature at the BACE location and in peatlands.

In contrast to several other studies, there was no detectable effect of warming alone on potential enzyme activity (Sowerby et al. 2005; Sardens et al. 2008). The lack of temperature response may have been due in part to the relatively small temperature change induced by warming. The highest degree of soil warming was around 4°C above ambient, which may not have been great enough to induce a change in enzyme activity because soil temperatures vary intra-annually by over 25°C at the BACE site. Even without a change in enzyme pools, warming should result in increased enzyme activity *in situ* because of the inherent temperature sensitivity of enzyme activity (Trasar-Cepeda et al. 2007).

Increased enzyme activity due to warming should increase the supply of assimilable substrates to microbes, which should increase their growth rate and thus microbial biomass (Allison et al. 2010). Consistent with this mechanism, microbial biomass increased in the warmest plots under ambient and drought treatments. If enzymatic efficiency increases as temperature rises, fewer enzymes are required for the reactions to proceed and nutrient requirements to be satisfied (Koch et al. 2007; Allison et al. 2010). Thus, microbes may allocate fewer resources to enzyme production as temperature increases, resulting in lower mass specific enzyme activity.

2.5.2 Seasonal Trends

The most striking response of enzymes to season was a change in enzymatic stoichiometry. Sinsabaugh et al. (2009) reported an average enzyme C:N ratio (BG activity:NAG+LAP activities) close to 1.41 for soils from 40 ecosystems. When ratios were averaged across seasons and depths, the C:N enzyme ratio in BACE soils was about 1.74 which is driven primarily by the high ratios at 5-15cm depth. A change in BG:NAG+LAP is indicative of a change in the ratio of C:N acquiring enzymes. During winter there was more BG, CB and XYL activities compared to the two mid-summer samples at 0-5cm, indicating increased C acquisition in the winter. Biomass did not increase during the winter, however maintenance costs continue and may increase with freezing events (Methe et al. 2005), resulting in a continual need for C substrates, without a corresponding increase in N demand. Also, enzyme turnover could be slower in colder temperatures, allowing the enzymes that are produced to remain in the soil longer even though production has slowed or halted. However, the increase in enzyme

C:N from June 2008 to January 2009 was driven by both the rise in C acquiring enzymes and a drop in the potential activity of both N acquiring enzymes in the winter, indicating a reduction in organic N acquisition in the winter compared to the growing season. The reduction in organic N acquiring enzymes could possibly be due to increased dissolved nitrogen (Chrost, 1991), which was measured in the winter at the BACE (data not shown). The average BG:PHOS ratios at BACE, 0.73, were similar to the reported average of 0.62 for soils (Sinsabaugh et al. 2009).The regularity of enzymatic C:P ratios demonstrate a consistent phosphorus requirement over the year. Even though there may be the consistent potential enzyme activity in the winter and summer for some enzymes, it is unlikely that *in situ* activity is the same (Bell et al. 2010). Low soil temperatures would result in slower reaction rates and frozen soils would limit diffusion of substrates resulting in reduced *in situ* activity.

2.6 CONCLUSION

Precipitation regimes are likely changing in most parts of the world, but the direction and magnitude of the change is uncertain (Planton et al. 2005). In areas where drought is uncommon, such as the northeast US, the decreased turnover and resultant increased stability of enzyme pools could have large implications for nutrient cycling rates, especially if rates for enzymes involved with different nutrients become uncoupled. It is important to note that potential enzyme activity is not representative of the *in situ* activity but provides an index of enzyme pools. Under drought conditions, it is likely that *in situ* activity is much lower than laboratory estimates due to reduced diffusion of enzymes and substrates, thus decomposition rates may be governed

primarily by substrate and product diffusion instead of enzymatic depolymerization rates. However, soils that contain a stable pool of enzymes due to reduced turnover under drought will be primed for rapid cycling of nutrients following a rewetting period when diffusion and substrate availability are no longer limiting.

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2.8 TABLES

Table 2.1. Enzymes assayed, abbreviations, nutrient cycle and substrates

Enzyme Name	Abbreviation	Nutrient Cycle	Substrate
β-glucosidase	BG	carbon	cellobiose
Cellobiohydrolase	CB	carbon	cellulose
Xylosidase	XYL	carbon	xylan
N-acetyl glucosaminidase	NAG	nitrogen	cell wall hydrolysis
Leucine-amino peptidase	LAP	nitrogen	protein
Phosphatase	PHOS	phosphorus	organic phosphorus

2.9 FIGURES

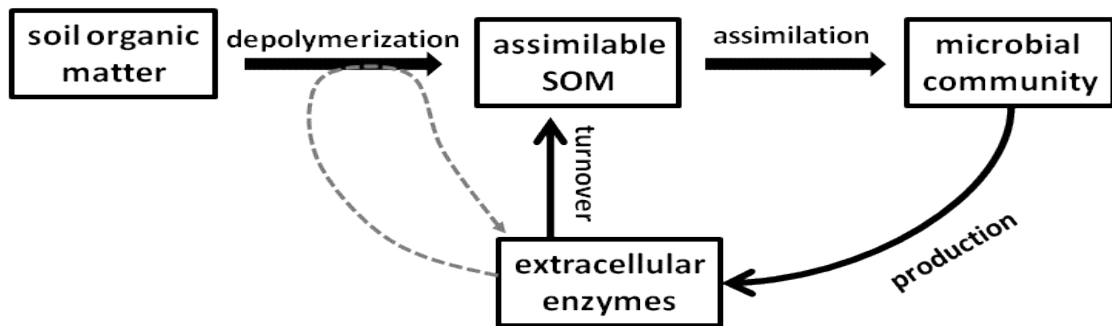

Figure 2.1 Microbial decomposition pathway (modified from Conant et al. in review). The black arrows indicate the flow of resources. The gray dotted line is the involvement of enzymes in the depolymerization step when complexed to a substrate and then movement back into the enzyme pool when not involved in depolymerization.

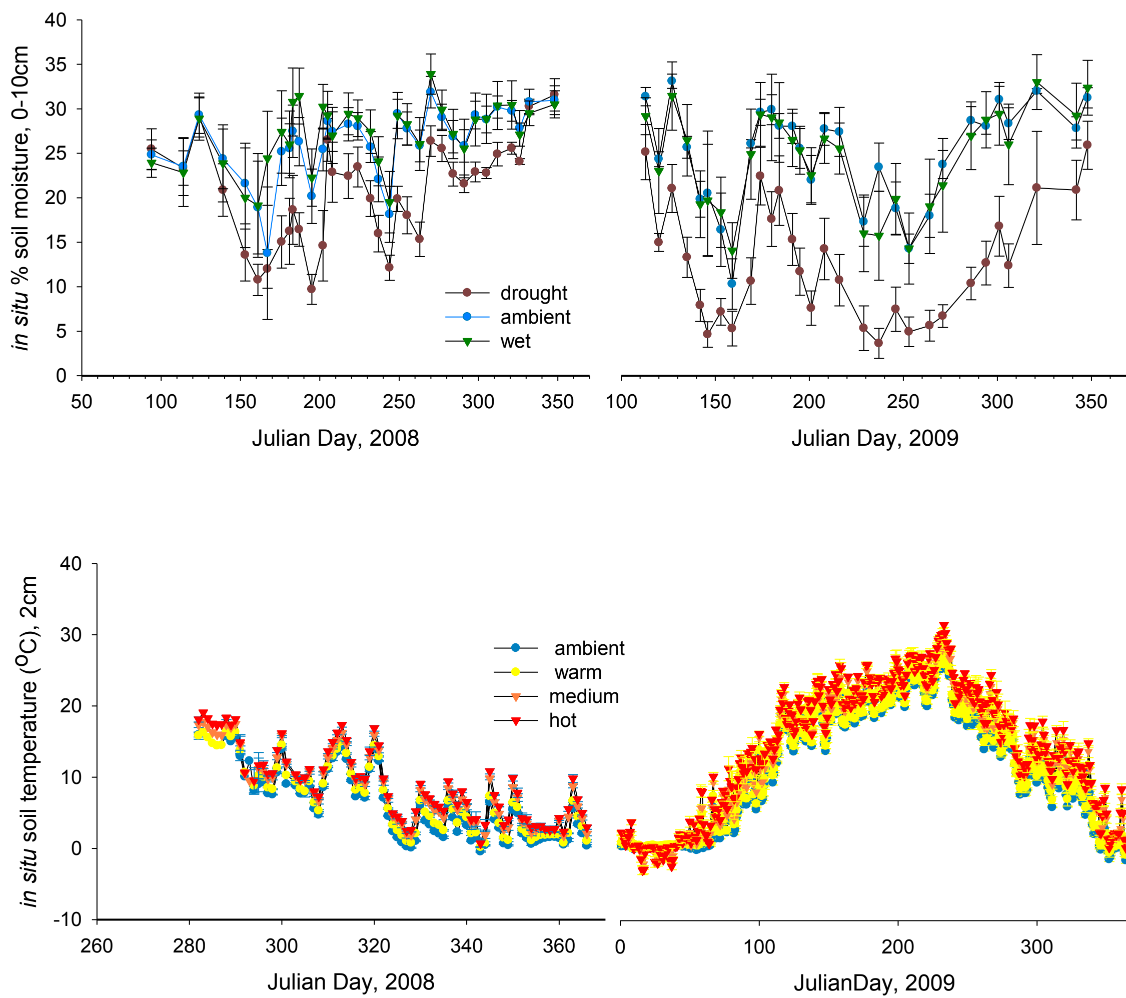

Figure 2.2 Measurements of (a) soil moisture in precipitation manipulation only plots and (b) soil temperature in temperature manipulation only plots. Soil moisture averages in 2008 are from 0-30cm and in 2009 from 0-10cm. The bars represent standard error for a sample size of three at each treatment.

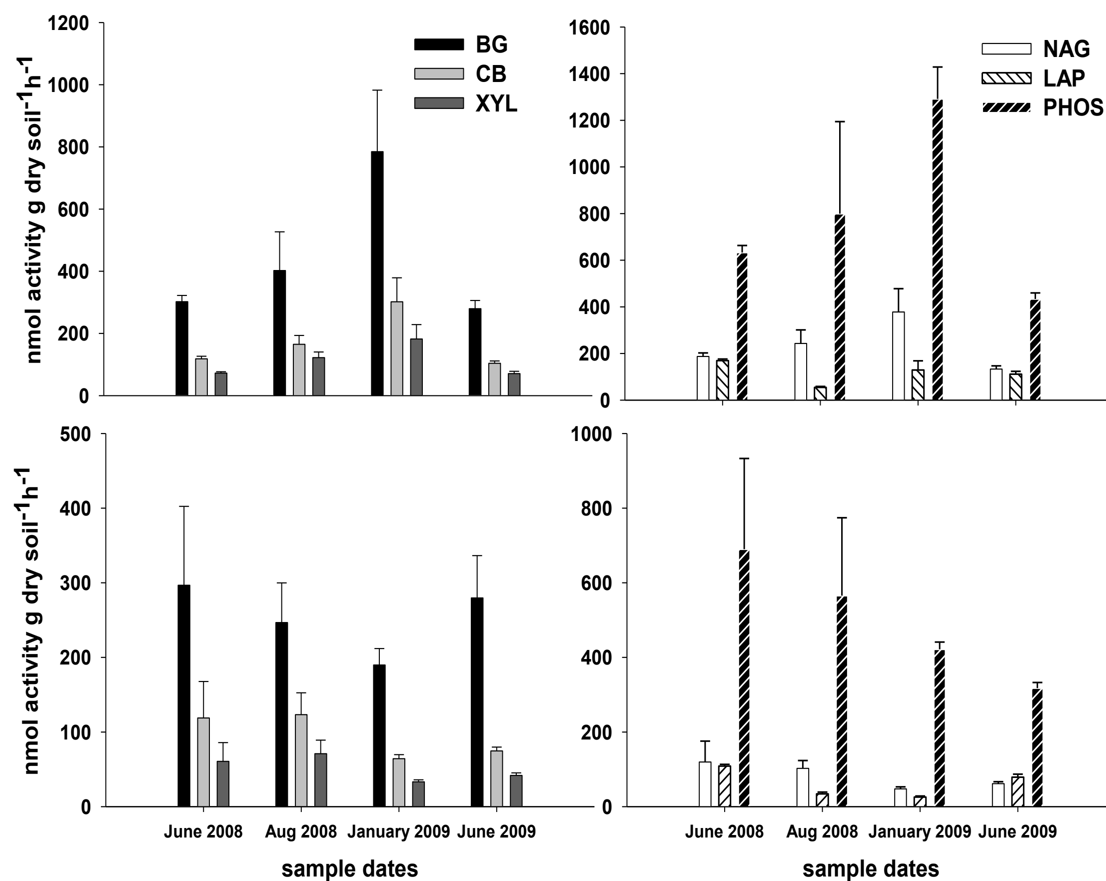

Figure 2.3 Potential enzyme activity, expressed as μmol activity g dry soil^{-1} h^{1} at each date for (a) 0-5cm C enzymes, (b) 0-5cm N and P enzymes, (c) 5-15cm C enzymes and (d) 5-15cm N and P enzymes. The averages and standard errors were estimated using all 36 plots due to the lack of treatment effects.

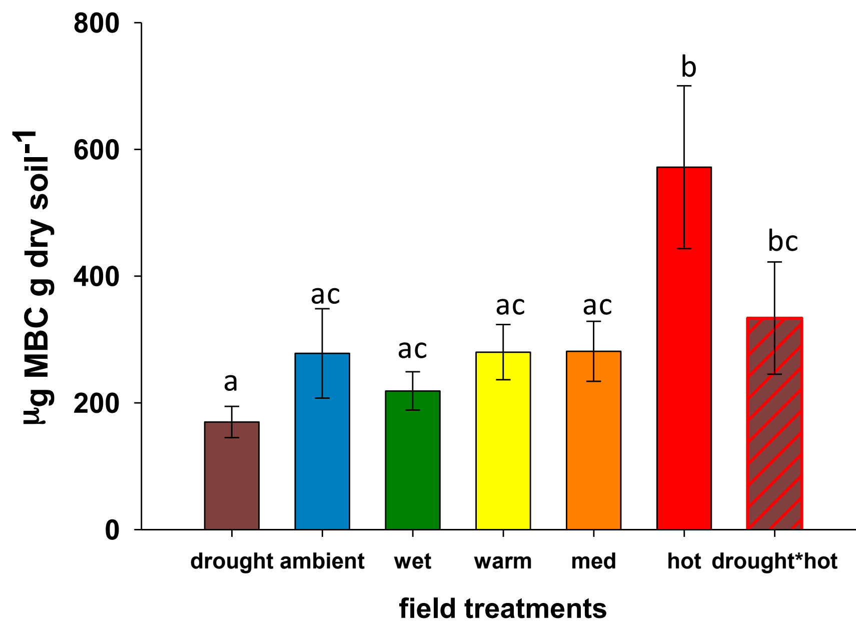

Figure 2.4 Microbial biomass in June 2009 determined by substrate induced respiration using glucose additions. Lower case letters indicate significant differences between field treatments (P<0.05).

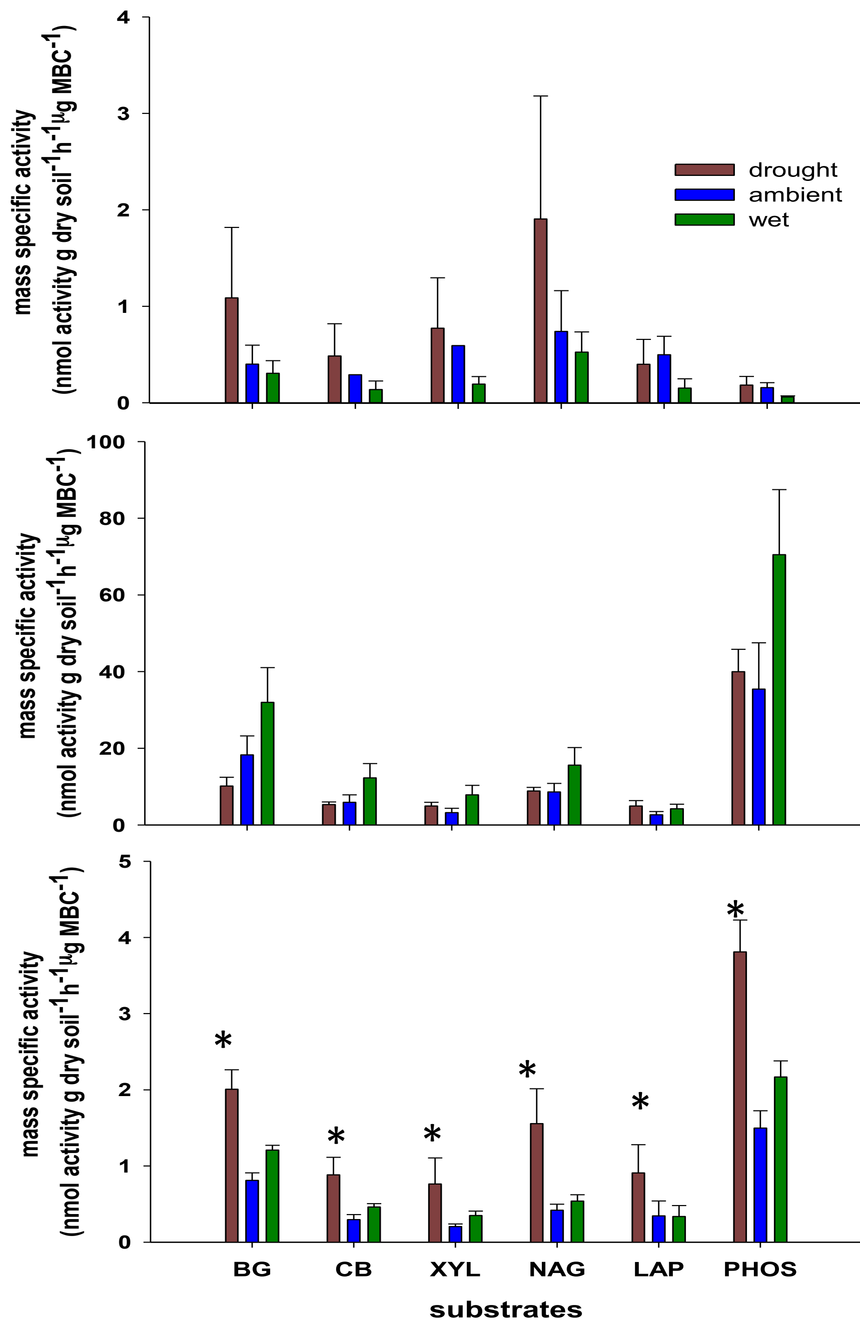

Figure 2.5 Mass specific enzyme activity in precipitation manipulation plots only for (a) August 2008, (b) January 2009 and (c) June 2009. Significant difference in mass specific activity ($P<0.05$) between drought plots and ambient and wet plots indicated by an asterisk for each substrate. Averages and standard errors were calculated using all the

plots under a precipitation treatment, regardless of temperature treatment, n=12. There is no June 2008 mass specific enzyme activity calculation because microbial biomass was not measured on those samples due to lack of soil.

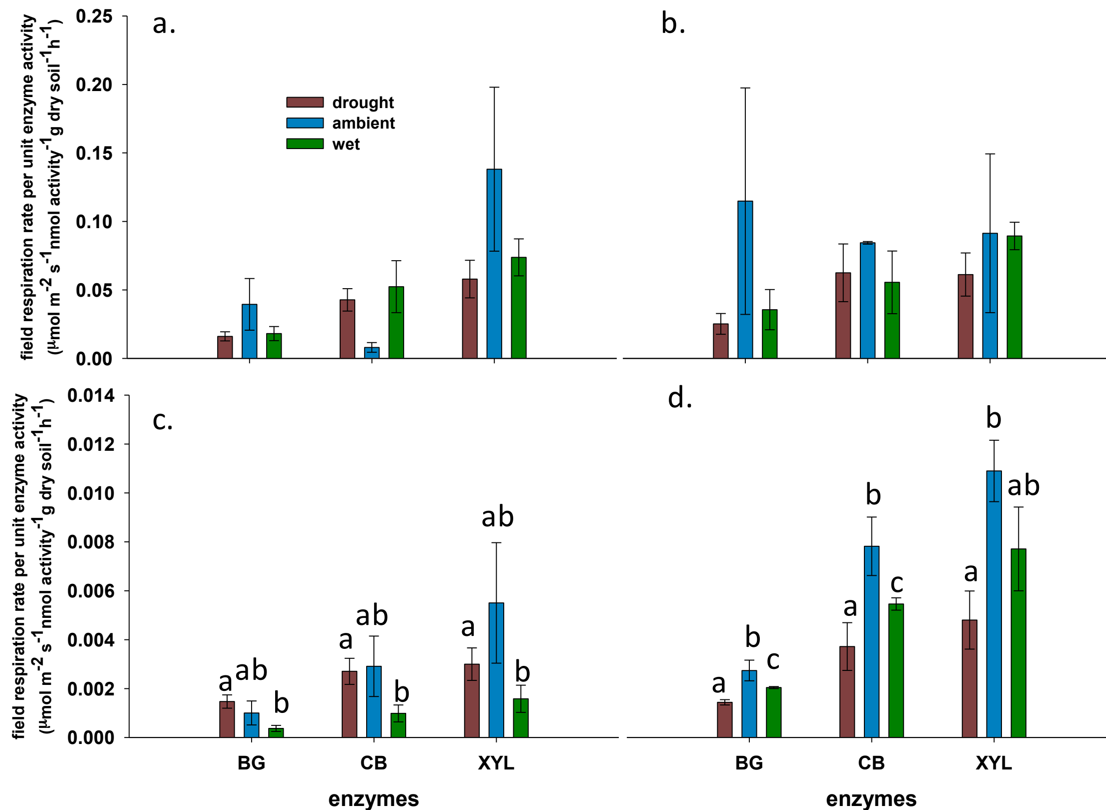

Figure 2.6 Respiration per unit enzyme activity for (a) June 2008, (b) August 2008, (c) January 2009 and (d) June 2009. Significant difference in rates (*P*<0.05) for each date between precipitation treatments are indicated by a lower case letter. Averages and standard errors were calculated using the plots in precipitation only manipulations (n=3).

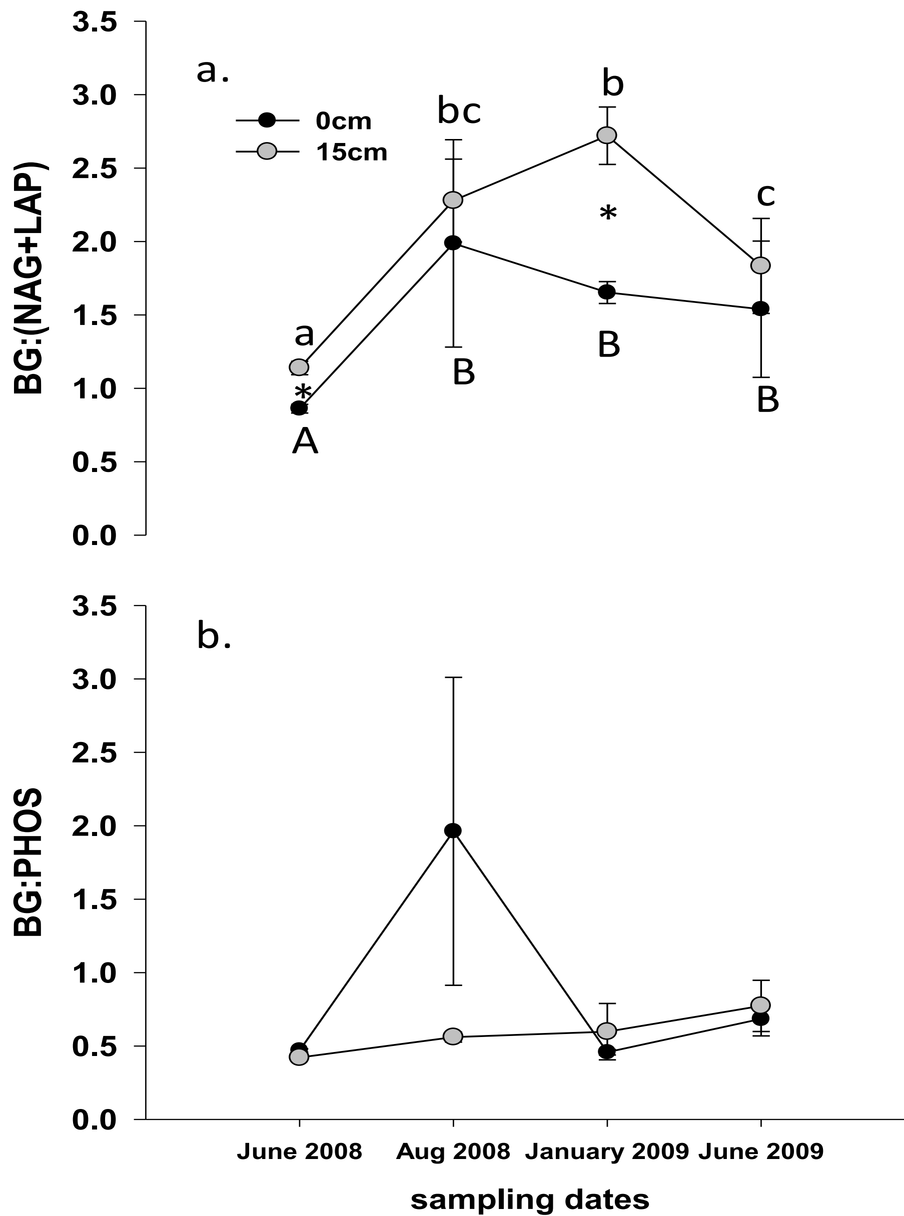

Figure 2.7 Stoichiometric enzyme ratios for both depths, (a) C:N and (b) C:P. Capital letters indicate significant differences ($p<0.05$) between sampling dates at 0-5cm depth, and an asterisks indicates significant difference by depth. Averages and standard errors were calculated using all the plots for each date due to the lack of treatment effect, n=36.

3 ALTERED MICROBIAL SUBSTRATE UTILIZATION PROFILES UNDER A MULTI-FACTOR CLIMATE MANIPULATION

3.1 ABSTRACT

Soil organic matter is a heterogeneous mix of compounds ranging from simple to complex in structure. Microbial communities are able to utilize these various compounds and allocate the nutrients to respiration, growth, maintenance and enzyme production. Climate change has the potential to alter microbial carbon allocation and utilization through a variety of mechanisms. For example, climate change may alter substrate availability to microbial communities by plant litter chemistry and plant community structure. Altered enzyme activity, which is sensitive to temperature, may also result in changes in reaction rates and accessibility of substrates to microbes. The objective of my study was to quantify the effects of temperature, moisture and season on the physiology of the microbial community.

Soils were collected from all temperature (ambient, +1,2 and 3˚C) and moisture (ambient, drought and wet) manipulation plots at the Boston Area Climate Experiment in August 2008, January 2009 and June 2009, and subjected to additions of multiple substrates of varying quality. Microbial biomass carbon measurements were made using both the substrate induced respiration (SIR) and chloroform-fumigation extraction methods. The SIR measurements using multiple substrates were made at three

temperatures (15, 25 and 35˚C) to determine whether the temperature sensitivity of respiration had been altered by field warming treatments or season. Substrate utilization profiles began to shift by field treatments in June 2009 and by season. Under warming x drought there was increased utilization of tannin and vanillin substrates, which is likely due to the concurrent increase in plant tannin concentration shifting the community towards using complex substrate. Expectedly, there was greater respiration in the summer months for all substrates compared to January, possibly due to greater microbial biomass in the summer. When respiration was normalized for biomass, mass-specific respiration (R_{mass}), there was greater R_{mass} in summer for the labile substrate additions but little change for the more complex substrates. Organisms that utilize the complex substrates are characterized by having slow growth rate, so that changes in moisture and temperature with season may have little impact on their metabolism. Despite R_{mass} being similar across the seasons for complex substrates I could not determine with this method if the mechanisms behind respiration and nutrient allocation were similar. It is likely that maintenance respiration would be higher in winter compared to summer due to the physiological changes needed to survive at near freezing conditions. Community level physiological profiles provide an assessment of how substrate preference and respiration change with different field variables; however I was unable to ascertain whether these changes are due to altered physiology of the same active community or a different active community using this method.

3.2 INTRODUCTION

A fundamental uncertainty in predictive carbon cycle models is the long-term effect of climate change on soil respiration (Baveye, 2007). At the ecosystem scale, soil respiration aggregates CO_2 efflux derived from catabolism of a highly diverse suite of substrates that compose detritus and soil organic matter (SOM). Although temperature and moisture are the primary rate determinants of soil respiration, predictions of soil respiration under climate change are complicated by the potential for changes in quality and quantity of SOM and the potential for changes in microbial community function driven by adaptation to temperature, moisture or substrate availability (Potts, 1994; Aerts, 1997; Koch et al. 2007). Until recently there has been a dearth of research on the interactive effects of warming and soil moisture changes on soil carbon cycling (Bardgett et al. 2008). Assessment of these two factors individually using aggregating measurements such as respiration have shown opposite trends, with respiration rates increasing under warming but decreasing under drought in the field (Suessela et al. *in review*). With these opposing responses it is difficult to estimate how respiration will change under different climate regimes (Bardgett et al. 2008). In order to predict how respiration will change with climate it is necessary to better understand the underlying processes involved in respiration production.

Heterotrophic respiration is driven by a consortium of microorganisms utilizing different substrates at various rates (Marsden & Gray, 1986; Kirk & Farrell, 1987). Soil microbial communities consist of bacteria, fungi, and archaea with different life strategies, substrate utilization, and growth rates. These microorganisms have been

characterized into r- vs K-strategists at the phyla level, though this is a simplification of a continuous range of metabolisms (Gerson & Chet, 1981; Fierer et al. 2007). r-strategists are adapted for maximal potential growth rates, have quick turnover times and utilize easily degradable compounds, whereas K-strategists tend to grow slowly, longer life spans and degrade more complex materials (Gerson & Chet, 1981). In soils, microbial communities likely contain taxa that encompass the range of r and K strategies, but the relative abundance of taxa with these contrasting strategies may determine the catabolic potential for specific components of SOM that differ in complexity (Fierer & Schimel, 2002; Liu et al. 2009), which can be assessed using community level physiological profiles (CLPP).

Microbial community physiology is affected by temperature and moisture through changes in substrate availability and substrate accessibility. Substrate availability is defined here as the presence of a substrate regardless of quality, while accessibility refers to the likelihood for microbes and their extracellular enzymes to encounter a substrate. Inputs of substrates are governed by plant production and microbial biomass turnover. Substrate availability, provided by plant inputs, has been shown to be sensitive to temperature and precipitation manipulations through alterations in species composition and litter quality (Aerts, 1997; Woodward et al. 2004). Even if substrates are available, moisture and temperature can further affect CLPP through accessibility of the substrate.

Under some conditions, substrate availability and accessibility are equivalent, but most soil conditions reduce accessibility through diffusional constraints. Soil

moisture is a strong control on diffusion (Koch, 1990), such that if diffusion is limited then available substrates are not made accessible for enzymatic depolymerization and microbial assimilation (Stark & Firestone, 1995). Drought can lead to periods of low substrate accessibility, shifting the community to slower growing microbes that may be more efficient with substrates because of substrate limitation. Also, shifts in substrate utilization patterns without changes in community structure have been measured under drought conditions (Griffiths et al. 2003). Garten et al. (2009) found that soil moisture was the main determinant of soil carbon dynamics, with reduced CO_2 efflux under drought. The reduction in carbon cycling rates could be attributed to lower microbial biomass and/or shifts in carbon allocation. Microbial communities may change in community structure or shift allocation of resources from enzyme production and growth to drought stress responses, such as osmolyte production (Schimel et al. 2007).

Temperature also influences substrate accessibility through enzymatic depolymerization rates. The majority of compounds in soil require enzymatic breakdown because they are not in a readily accessible form (Nannipieri et al. 2002). Enzymatic reactions are temperature sensitive such that substrates become more accessible as enzyme activity increases with temperature (Koch et al. 2007; Wallenstein et al. 2008; Steinweg Ch. 1 & 4). In addition, the depolymerization of complex, high molecular weight substrates is theoretically more sensitive to temperature than that of low molecular weight substrates leading to increased breakdown of complex compounds (Bosatta & Agren, 1999). As climate warms, this mechanism could shift the

community towards dominance by K-strategist microbes that utilize complex substrates, thus reducing the respiratory response to temperature.

In addition to substrate effects on microbial physiology there can also be a direct effect of climate change on microbial community composition and thus physiology. Moisture stress requires specific physiological adaptations for survival such as osmolyte production to maintain osmotic balance with the soil solution (Harris, 1981; Csonka, 1989; Witteveen & Visser, 1995), which are energetically expensive and reduce growth (Killham & Firestone, 1984). Not all microbes have adaptations for drought stress, especially in systems where drought is uncommon, leading to changes in dominant taxa as a direct result of drought conditions (Potts, 1994; Nazih et al. 2001). Temperature has also been shown to alter microbial community composition (Zogg et al. 1997; Zhang et al. 2005), however it is not well understood if the shifts were due directly to warming or indirectly through changes in substrate availability and accessibility with warming. Cooper et al. (2001) demonstrated the ability of a single bacterial population to acclimate to a higher temperature regime. Currently most microbes are living under sub-optimal temperatures, so that a rise in temperature by a few degrees may not directly shift community composition.

The Boston Area Climate Experiment provides a multi-factor climate experiment in which to assess the effects of different warming and precipitation manipulations individually and in combination on microbial substrate utilization. I used CLPP to determine how microbial carbon utilization would be affected by temperature and precipitation manipulations in the field over the course of a year. I hypothesized that

carbon utilization profiles would shift in the following five ways with temperature, moisture and season: (1) a larger proportion of respiration in warmed plots will be derived from utilization of complex substrates, (2) drought will result in reduced respiration for all substrates because of a reduced microbial biomass, (3) mass-specific respiration will be lower under drought because of a shift towards a community dominated by K-strategists due to diffusion constraints on substrate accessibility, (4) the combination of warming and drought will result in a reduction in respiration because diffusion constraints on substrate accessibility imposed by low soil moisture will nullify any increase in enzymatic efficiency with temperature and (5) unfavorable environmental conditions in winter will select for a smaller microbial biomass dominated by K-strategists leading to a reduction in respiration compared to summer. There are several methods of CLPP available to determine microbial substrate utilization. I chose to use the MicroResp™ technique for my analysis because it allows measurement of the total active microbial community respiration, both bacteria and fungi, and an estimation of the biomass.

3.3 METHODS

3.3.1 Study Site

Soils (mesic Typic Dystrudepts, 45% sand, 46% silt, 9% clays, pH 5.5) were collected from the Boston-Area Climate Experiment (BACE), an old field ecosystem, located in Waltham, Massachusetts at the University of Massachusetts Agricultural Experiment Stations. Mean annual temperature and precipitation in nearby Boston, MA is 10.3˚C and 1054 mm yr^{-1}. The site was previously an apple orchard, but was

abandoned over 40 years ago. Current vegetation includes 38 species of primarily non-native grasses and forbs.

3.3.2 Field Experimental Design

The BACE exposes thirty-six plots to one of three precipitation treatments and four warming levels in a full-factorial design with three replicates per treatment. The three precipitation treatments are, no change, plus 50% precipitation (wet), and minus 50% precipitation (drought) and four temperature treatments, no change, +200 W m^{-2} (warm), +600 W m^{-2}(medium), and +1000 W m^{-2} (hot). Precipitation is controlled by clear partial roofs in the drought plots and additional precipitation is added after natural precipitation events in the wet plots. During the winter, drought and warming treatments are maintained, but additional water is not added to the wet plots. Warming is achieved by ceramic infrared heaters, mounted 1m above the ground at each corner of a plot. Air temperature is monitored in each plot to maintain target temperatures. In the warmest plots, air temperatures are limited to no more than four degrees above ambient air temperature. Precipitation treatments began in July 2007 and warming treatments began June 2008.

3.3.3 Soil Sampling and Pre-processing

Soils were first collected from all 36 plots in August 2008, one year after precipitation manipulations began and six weeks after warming treatments were initiated at BACE. Additionally, soil samples were taken in January 2009 and June 2009. Two cores (5 cm diameter) were collected from each plot at two depths, 0-5 and 5-15cm. Soils were packaged on ice and shipped to the laboratory overnight, where the

cores from each plot were 2mm sieved, rocks and roots removed, and soil cores were homogenized and frozen at -10˚C until analysis.

3.3.4 Soil Characterization

Subsamples from each plot were used to determine water holding capacity, pH, total C and N concentrations. Water holding capacity was assessed after field moist soils were weighed and saturated with water for four hours. The water was drained and samples remained covered for an additional twelve hours. Subsequently the soils at field capacity were weighed and then placed in a 60˚C drying oven for 48 hours and weighed again. Soil pH was determined using the supernatant of soil mixed with water (1:5 by volume). Soil subsamples were dried at 60˚C and ground to measure total C and N concentrations on the LECO CHN-1000 autoanalyzer (LECO Corporation, St. Joseph, MI, USA).

3.3.5 Substrate Induced Respiration (SIR)

Substrate induced respiration was measured using the MicroResp™ (Aberdeen, UK) apparatus and technique with a few modifications (Campbell et al. 2003). Soils were removed from the freezer and a 20g subsample was warmed to about 20˚C. All samples had water added to bring soil moisture up to 55% water holding capacity in August 2008 and June 2009. After water addition, samples were covered for one hour, homogenized and measured into 96 –well deep-well plates. For January 2009, the soils were completely saturated, so 20g subsamples were air-dried to 55% water holding capacity at 4°C, for 6-36 hours. Following drying, samples were homogenized and measured into 96-well deep-well plates.

Three microplate columns were filled per sample, with a known mass (about 0.2-0.3g) of moist soil added to each well, using the MicroResp™ manufacturer's protocol (Aberdeen, UK). Incubation of soils with substrates occurred at three temperatures, 15, 25 and 35˚C, so three plates had the same sample layout. After addition of samples to the three plates, they were covered with sealing film and placed at 4˚C for about 18h.

After 18 hours, samples were warmed to about 15˚C and 25µl of substrates were added to each well. Substrates added were in order from the top to bottom row of the plate: bovine serum albumin+tannic acid, glucose, sucrose, tannic acid, vanillin, yeast extract, water, and no addition, labeled as BSAT, GLUC, SUCR, TA, VAN, YST, WATER and SOIL respectively. These substrates were chosen because of their range of molecular weight and similarity to compounds used in soil nitrogen and carbon cycling. Each soil sample had three analytical replicates in each row for each substrate. The indicator plates, described below, were read on a Tecan Infinite M500 microplate reader at 625nm prior to being placed on deep-well plates. The plates were attached to one another using the MicroResp™ apparatus and allowed to incubate at one of three temperatures, 15, 25, or 35˚C, for 6 hours. Following the six hour incubation the indicator plates were removed from the deep-well plates and read again on the microplate reader at 625nm.

Substrate concentrations were determined during preliminary trials. Various concentrations of substrates were used starting with the highest concentration attainable by solubility, or 2M, and then diluted. Subsamples were taken from BACE soils, two from 0-5cm and 5-15cm depths in ambient and drought plots. Each substrate

concentration was added to all soils and respiration measured after six hours of incubation at 35˚C. Actual substrate concentrations used for the assays were decided when cumulative respiration plateaued, indicating no substrate limitation.

Indicator plates were made one week in advance of the assay according to the manufacturer's guidelines (MicroResp™, Aberdeen UK). To make one liter of indicator solution, 18.75mg cresol red, 16.77g KCl, and 0.315g $NaHCO_3$ were added to 900ml deionized water over heat (~45˚C) and then diluted to 1000ml. A 3% agar solution was made and autoclaved at 121˚C to ensure complete melting of the agar and then cooled to 65˚C. The indicator solution was added to the agar at a 2:1 concentration, stirred and kept at 65˚C. 150µl of indicator solution was pipetted into clear 96-well plates. Once the agar in the plates cooled they were left on the bench top overnight and then placed in desiccators with soda lime and a beaker of water to maintain humidity. Plates were then stored in the dark in the desiccators six days before use. Indicator plates remained in desiccators throughout the experiment except for use during the substrate induced respiration assay.

Standard curves were created using known CO_2 concentrations in jars with indicator plates. The absorbance values from the indicator plates were plotted against the known percent of CO_2 in the jars, to calculate an equation for sample plates. The equation was used to calculate the amount of CO_2 released following substrate additions g dry soil^{-1} hr^{-1}. The amount of CO_2 produced from the water addition wells was subtracted from the respiration in the substrate wells to accurately calculate the

substrate induced respiration response and not the additional moisture respiration response.

3.3.6 Microbial Biomass, Dissolved Organic Carbon and Nitrogen Measurements

Microbial biomass was measured using two difference techniques, SIR and soil microbial chloroform-fumigation and extraction (CFE). SIR and CFE have been shown to estimate similar microbial biomass (Wardle & Parkinson 1991), however SIR estimates the active microbial biomass while CFE estimates the total biomass. SIR microbial biomass was calculated from respiration produced from the glucose amended wells at 25˚C and using the following equation from Anderson & Domsch (1978):

$$\text{mg MBC 100 g soil}^{-1} = 40.04y + 0.37$$

where y is the amount of CO_2 produced under glucose amendment.

The chloroform fumigation and extraction method followed the protocol from Vance et al (1987). In brief, one-6g subsample from each plot, labeled the fumigated sample, was exposed to chloroform for five days in a fumigation chamber and then shaken with 42mL of 0.5M K_2SO_4 for four hours. Following shaking, the sample was gravity filtered and the extract kept for analysis on the Shimadzu TOC-V Total Organic Carbon analyzer for total extractable organic carbon. A second 6g subsample from all plots, the unfumigated sample, was shaken for four hours with 42mL of 0.5M K_2SO_4 and gravity filtered. The extract from the unfumigated sample was also analyzed on the Shimadzu for extractable organic carbon and dissolved nitrogen. The difference

between the organic carbon extracted from the fumigated sample and the unfumigated sample for each plot is indicative of microbial biomass carbon.

3.3.7 Calculations and Statistics

The mass-specific respiration, R_{mass}, was calculated using the respiration produced from all substrates divided by the SIR biomass estimate from glucose addition (Bradford et al 2008). SIR biomass estimates were used because they give a better approximation of the active community, the biomass which is producing the CO_2, whereas CFE estimates include active and dormant microbes (Wardle & Parkinson, 1991; Lipson et al. 1999). Temperature sensitivity was assessed using the Q_{10} function:

$$Q_{10} = \frac{\text{respiration at } T_2}{\text{respiration at } T_1}^{\left(\frac{10}{T_2 - T_1}\right)}$$

where T is the temperature of incubation. Determination of field treatment and seasonal effects on respiration, temperature sensitivity, biomass and R_{mass} was made using PROC GLIMMIX (SAS Institute, Cary, NC).

3.4 RESULTS

Soil temperature and moisture were altered by field manipulations (Chapter 1). Warming resulted in increased soil temperatures from 0.70˚C above ambient in the warmed only plots up to 4˚C above ambient in the drought x hot plots. Soil moisture on average was about 38% lower in the drought plots compared to ambient and there was little effect of additional water on soil moisture in wet plots.

Extractable organic carbon (EOC) and dissolved nitrogen (DN) were significantly different by season and depth (Figure 3.1, $P<0.05$), with the greatest EOC at both depths

and DN at 0-5cm in January 2009. There were no treatment effects on EOC except in January 2009 where warming resulted in decreased EOC.

The microbial biomass estimations using SIR at 25˚C and total extractable microbial carbon using CFE were similar in August 2008 and June 2009, about 300-400 µg microbial biomass carbon g dry soil^{-1}, with no effect of warming or precipitation treatments (Figure 3.2). However, the two methods yielded significantly different estimates January 2009, with the CFE method estimated significantly higher extractable microbial biomass carbon than the SIR estimate in the winter (P<0.0001).

Addition of substrates resulted in positive respiratory responses for the majority of time points and treatments at 25˚C (Figure 3.3). August 2008 and June 2009 respiration in the 25˚C incubation was stimulated the most by glucose, sucrose and yeast, followed by BSA+tannic acid, tannic acid and vanillin, respectively. However, in January 2009 there was very little substrate induced respiration and for two substrates, sucrose and yeast, there was a negative respiratory response.

In June 2009 the drought versus wet plots in combination with warming exhibited opposite trends in SIR with TA and VAN. Drought x heated plots consistently had a strong trend towards more respiration for TA and VAN substrates than the wet x heated plots for all warming treatments (Figure 3.4, P =0.20). The temperature sensitivity of SIR was not affected by field treatments or season for both the 25˚C vs. 15˚C and 35˚C vs. 25˚C comparisons (Figure 3.5).

Respiration per unit microbial biomass, R_{mass}, using sucrose, yeast, BSAT , TA and VAN did not differ between season and was not related to substrate quality(Figure 3.6).

R_{mass} was greatest in August 2008 under sucrose addition, but under yeast addition in June 2009. In August 2008 and June 2009 R_{mass} declined as the quality of added substrates declined. In January 2009, R_{mass} was represented by BSAT, TA and VAN additions only because respiration was overall slightly negative for sucrose and yeast additions after subtracting respiration from water additions. There was no difference in R_{mass} between seasons for BSAT, TA and VAN.

3.5 DISCUSSION

Community-level physiological profiles were relatively unaffected by temperature manipulations, but did demonstrate some responses to changes in moisture and season. The lack of temperature sensitivity response was likely due to the low level of warming to which the soils were exposed. Also, it may take longer under a low level of warming to measure a change in respiration temperature sensitivity. Suseela et al. (in review) measured a decline in the apparent Q_{10} of field respiration in the hottest plots at the BACE site in the fall of 2009, over a year after warming began, whereas all of my measurements occurred within a year of initiating warming.

Experimental drought did not induce a shift in substrate utilization, but the combination of drought with warming resulted in an increased respiration response to tannic acid and vanillin additions. This response was not seen in the warmed only or wet x warmed plots, which had no change in soil moisture with warming. In the drought plots warming exacerbated already dry conditions, likely resulting in more extreme conditions where substrate accessibility may have been diffusion-limited. In addition, Tharayil et al. (2011) measured increased plant tannin concentrations under drought x

hot conditions at the BACE. The increased availability of tannins under the driest conditions could have shifted the community towards K-strategists able to utilize this complex substrate resulting in increased losses of soil carbon from phenolic compounds. Field respiration declined with drought, so if decomposition of phenolic compounds increased with drought then a larger portion of the CO_2 efflux would be from phenols.

Seasonal substrate utilization profiles were consistent with my *a priori* hypothesis of higher respiration in the summer compared to the winter. The high rate of SIR in summer samples was likely due to the large estimated active microbial biomass compared to the reduced active biomass in winter. Despite summer soils having higher respiration, the relative amount of respiration from complex substrates compared to labile was lower than it was in winter. There was a distinct shift in substrate utilization profiles between seasons, which could be a result of field substrate availability, accessibility, and/or a community composition shift. Substrate availability in terms of DOC was quite high during the winter. However, freezing conditions would result in decreased accessibility through low diffusion and enzyme reaction rates. Lipson and Schmidt (2004) measured a difference in bacterial community structure under snowpack and following snowmelt in alpine tundra. In the same alpine system, fungi dominated the winter biomass (Schadt et al. 2003), which could alter substrate utilization profiles (Rinnan & Baath, 2009). Fungi are often characterized as K-strategist organisms, indicative of slower growth and utilization of substrates with a lower energy yield (Gerson & Chet, 1981; Fierer et al. 2007). A shift to a K-strategist dominated system in

the winter could account for the greater respiration from the additions of complex substrates.

Assessing microbial physiology based on SIR responses can be misleading. Respiration responses need to be considered relative to active microbial biomass, which is called mass-specific respiration (R_{mass}). R_{mass} was similar between the two summer months with the greatest amount of respiration per unit biomass coming from the labile compounds. The R_{mass} values for sucrose and yeast were similar to those reported by Bradford et al. (2008). R_{mass} could not be compared between all three sample dates for labile compounds because of the lack of respiration induced in winter. The microbial communities at all three sample dates had similar abilities to use complex substrates. These results contrast with Bradford et al. (2008) who measured a decline in R_{mass} with warming and Steinweg et al. (2008) who measured an increase in substrate respiration with warming. The main difference between this study and the other two is the type of substrate used. Here I assessed R_{mas} based on respiration from labile and complex substrates, whereas Steinweg et al. (2008) and Bradford et al. (2008) used labile compounds. The types of organisms, K-strategists, involved in the decomposition of complex substrates tend to have a lower R_{mass} than organisms using predominantly labile compounds and may be less affected by seasonal temperature shifts. However, this is not to say that the physiology of microbes in these different seasons is exactly the same despite having similar respiration responses. Winter in particular can impose a large stress on microorganisms and their ability to maintain function would require additional physiological changes. Near-freezing conditions can mimic drought which

would require increased solute concentrations inside the cell (Mindock et al. 2001; Schimel & Mikan, 2005; Schimel et al. 1989) and membrane fluidity decreases with temperature (Methe et al. 2005), possibly altering allocation of nutrients to different metabolic pathways.

A surprising result from the SIR assays was the overall lack of a respiratory response to any of the substrates in January 2009, which could have been due to a small microbial biomass pool. Measurements of microbial biomass tend to correlate well with respiration when optimal conditions are present (Wardle & Parkinson, 1991). Total extractable microbial carbon from CFE was similar to SIR microbial biomass estimates in August 2008 and June 2009 but there was a disparity in biomass estimates for the winter sample which may be attributed to the way the two methods assess community size. CFE estimates the entire community, dormant and active, while SIR is based on the respiration of the active community (Wardle & Parkinson, 1991). In the summer months MBC turns over rapidly due to favorable soil temperature and moisture, so that the active biomass is similar to the total biomass in size. In the winter, microbial turnover slows down because of unfavorable temperatures limiting reaction rates, so that dormant biomass may be quite large, although the number of organisms that remain active is small. Contrary to my results, Lipson et al. (1999) measured a significantly larger microbial biomass in the winter compared to the spring using both CFE and SIR. Both my study and Lipson et al. (2000) measured a large EOC pool in the winter that declined into the summer, however the winter EOC was significantly greater. The concentrations of my added substrates may not have been large enough to increase the

EOC pool and stimulate respiration in the winter resulting in underestimated microbial biomass

3.6 CONCLUSION

Changes in microbial function due to drought will alter substrate availability which in turn affects the rate of carbon cycling. The interaction of temperature and substrate quality has been examined in numerous studies (Fierer et al. 2005; Wetterstedt et al. 2010); however there is a lack of understanding for moisture and substrate quality interactions. This experiment demonstrates a shift towards increased microbial utilization of complex compounds with drought, highlighting the need to focus on how substrate quality changes with moisture. Future work on soil respiration responses to temperature and moisture need to focus not only on the total respiratory response but the functional capacity of the microbial community and how substrate utilization is shifting. A shift in substrate utilization from labile to more resistant compounds could alter the rate of carbon cycling occurring and the stability of soil carbon storage.

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3.8 FIGURES

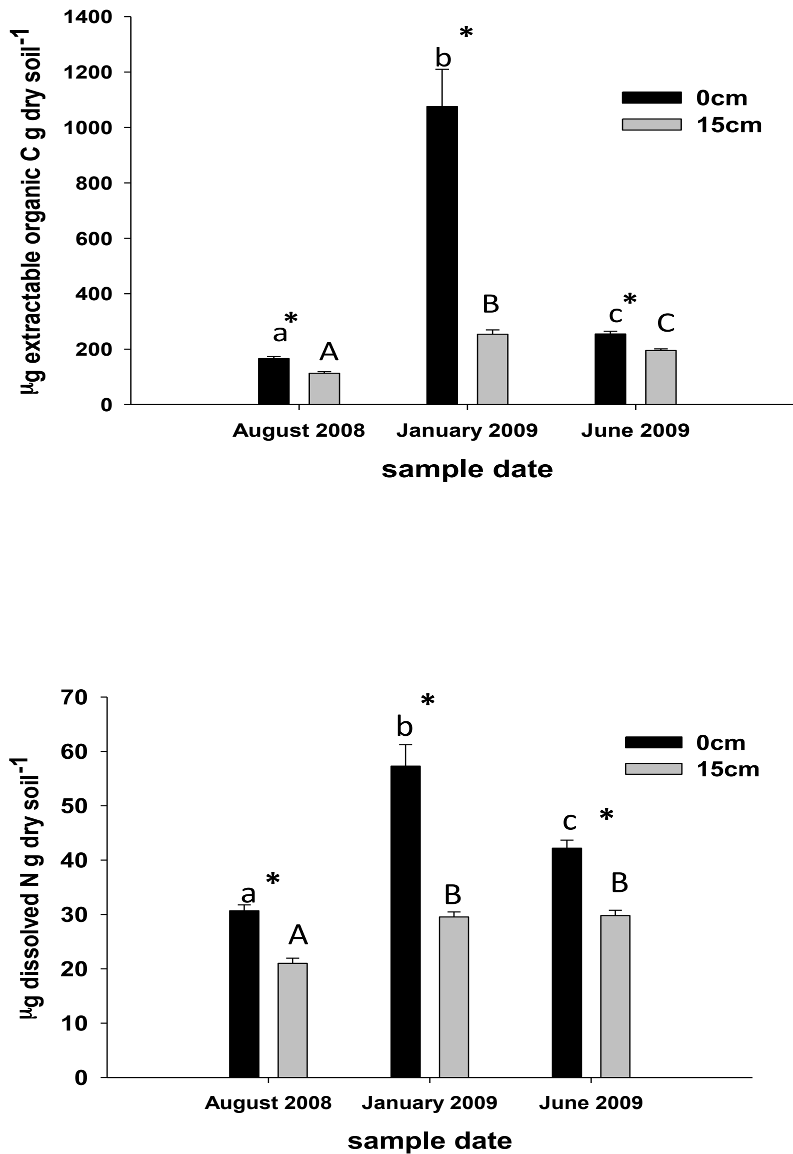

Figure 3.1. The amount of (a) K_2SO_4 extractable organic carbon and (b) dissolved nitrogen for the three sampling dates at both depths. Significant differences (p<0.05) by depth are indicated '*' and differenced by depth are indicated by lower case letters for 0-5cm and upper case letters for 5-15cm depth.

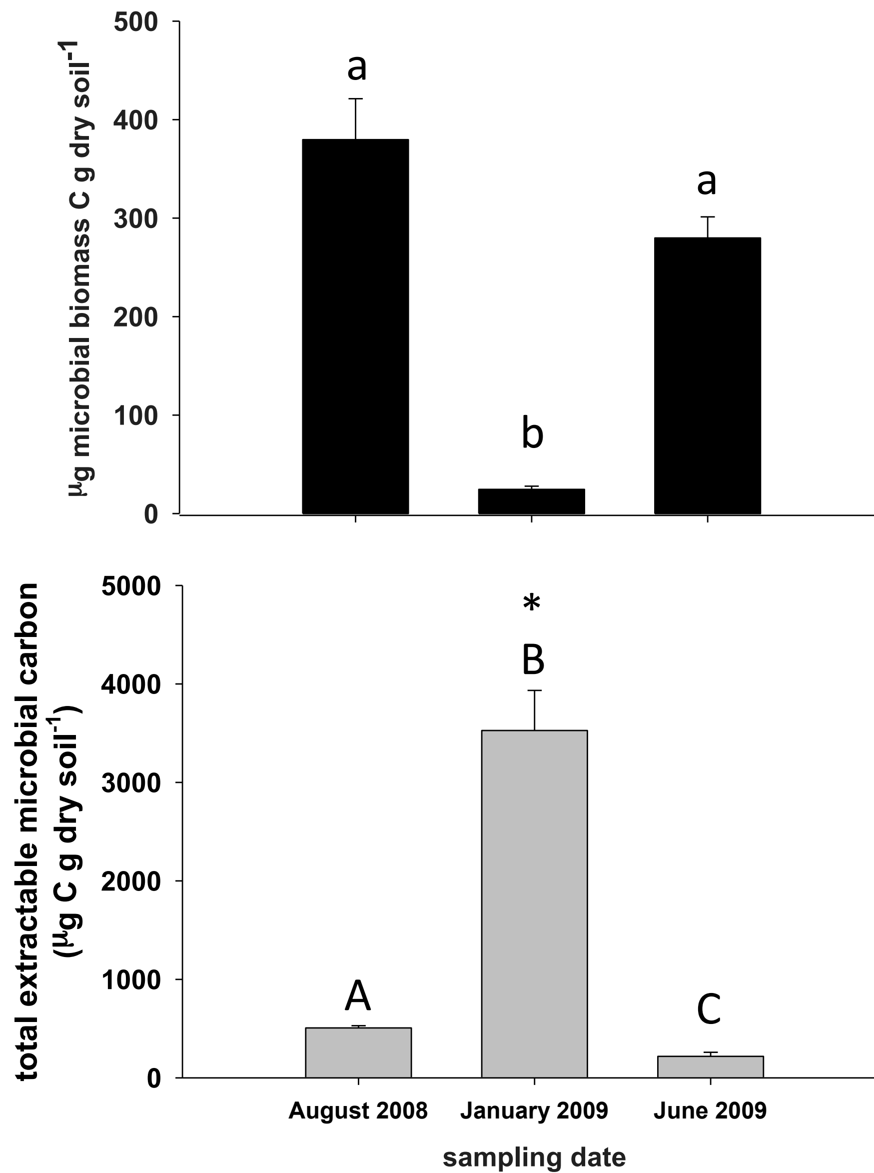

Figure 3.2 Microbial biomass estimates from (a) substrate induced respiration and (b) total extractable microbial carbon from fumigation. Significant differences(P<0.05) between methods are indicated by '*' , differences between dates for SIR biomass estimates are indicated by small letters and differences between dates total extractable microbial carbon estimates are indicated by capital letters.

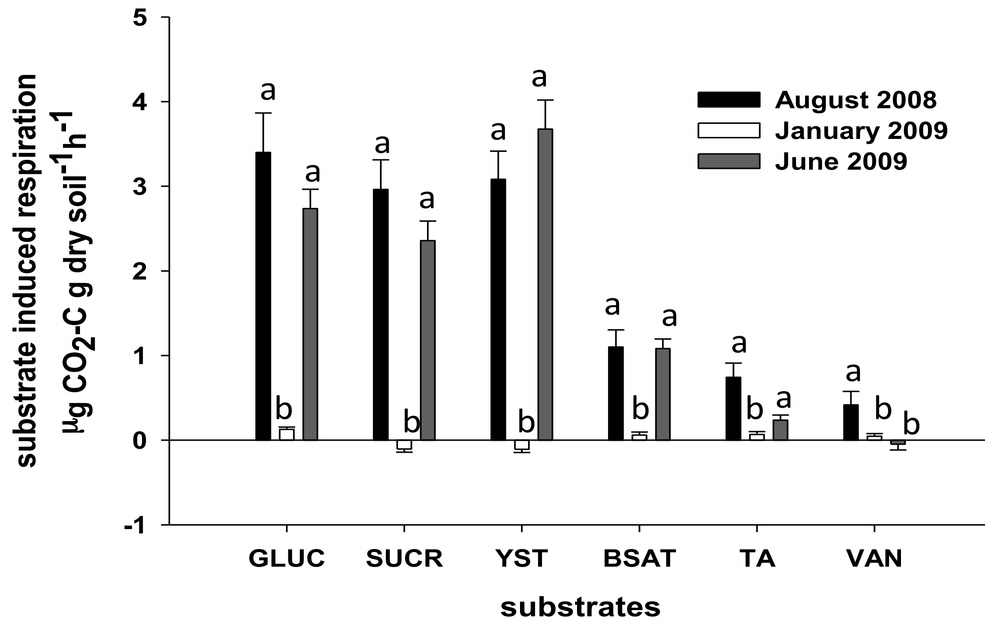

Figure3.3. Substrate induced respiration (SIR) six hours after the addition of six different substrates, glucose=GLUC, sucrose = SUCR, yeast=YST, BSA+tannic acid =BSAT, tannic acid = TA, vanillin =VAN. Significant differences in SIR by season are indicated by lower case letters (*P*<0.05).

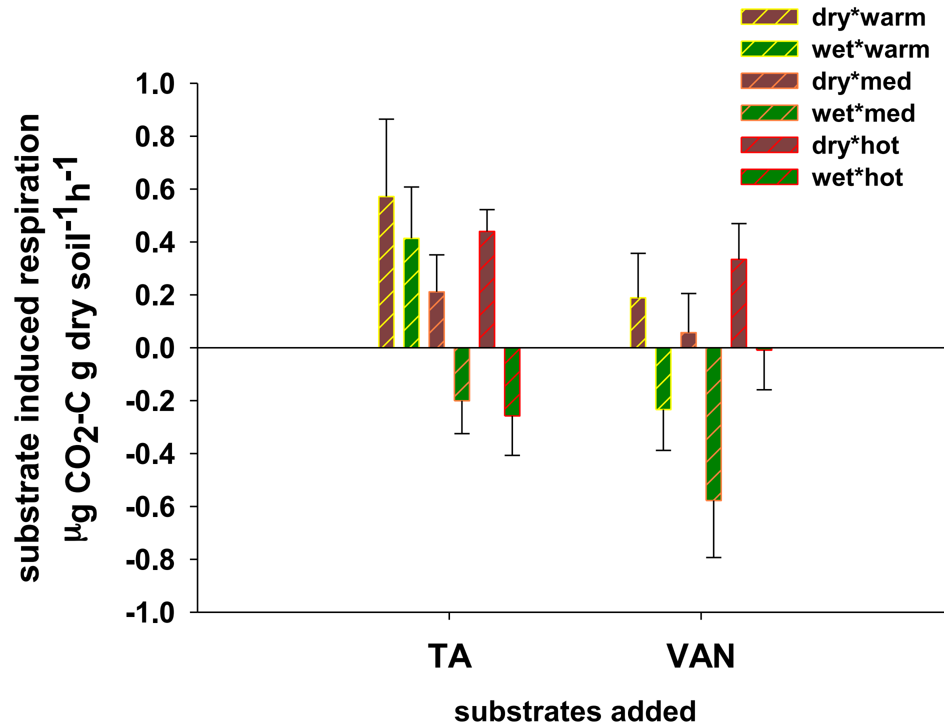

Figure 3.4. SIR from tannic acid and vanillin in June 2009 from soils under drought and additional water treatments in combination with warming. Brown bars are drought manipulation and green bars are additional water manipulations. The hatch mark color is indicative of the level of warming treatment imposed on the precipitation manipulations.

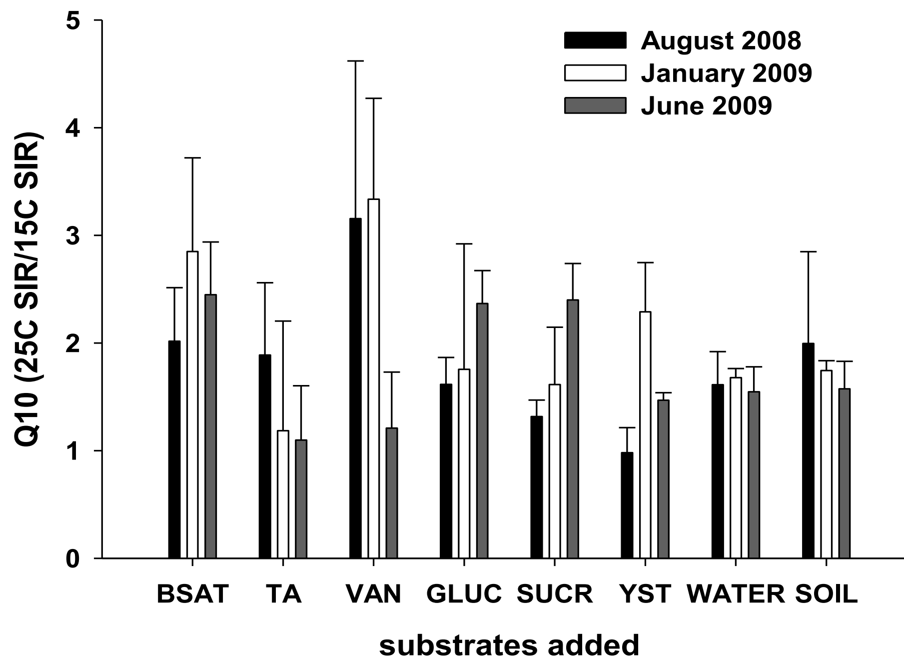

Figure 3.5. Temperature sensitivity (Q_{10}) of substrate induced respiration for each season by substrates. Respiration from the 25 and 15˚C incubations were used to calculate Q_{10}.

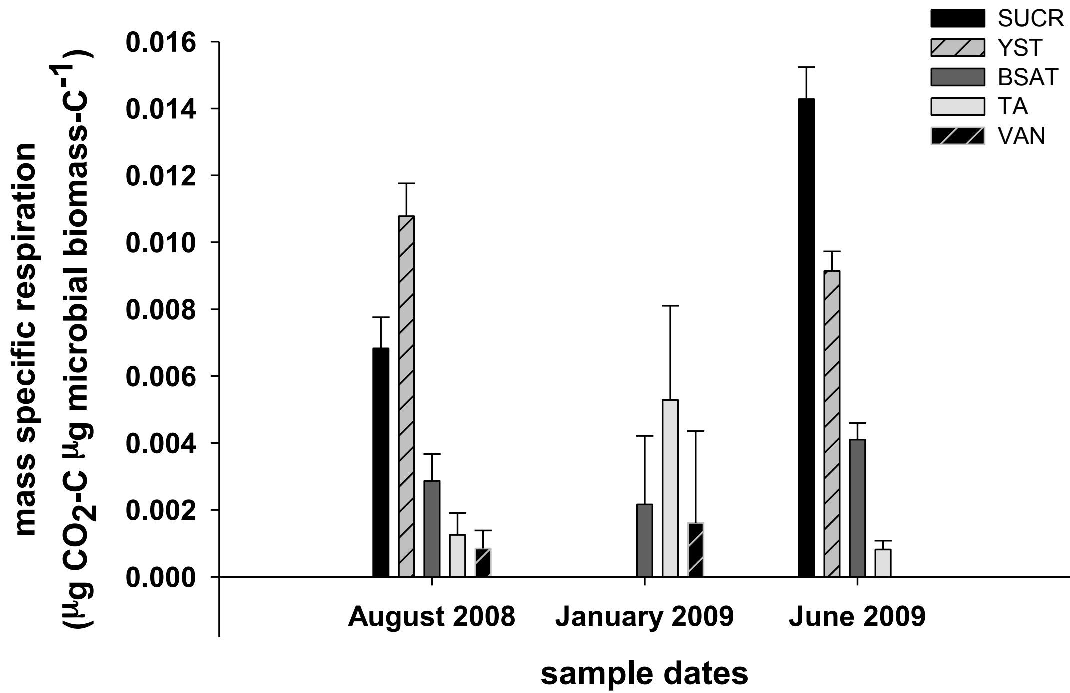

Figure 3.6 Mass-specific respiration (R_{mass}) based on biomass estimates from glucose addition and respiration from sucrose, yeast and BSAT respiration. R_{mass} in January 2009 could not be calculated using yeast and sucrose additions due to insufficient respiration, the same reason applies for the lack of R_{mass} calculation in June 2009 using vanillin.

4 ESTIMATING *IN SITU* ENZYME ACTIVITY USING CONTINUOUS FIELD SOIL MOISTURE AND TEMPERATURE DATA

4.1 ABSTRACT

Despite a long history of use as an indicator of microbial activity, potential enzyme activity measurements do not provide accurate estimates of *in situ* activity. The methods that are currently employed to assay enzyme activity rely upon highly artificial conditions that do not occur in the soil system, such as excess substrate, soil slurries and single temperature analyses. In particular, the use of soil slurries removes all diffusion constraints on substrates and enzymes that are inherent in the field. In addition, soil temperatures change drastically over the course of a day and year so that the use of single temperature assays provides a very static assessment. To address these limitations, I modified current enzyme assay methods to measure the moisture and temperature sensitivity of enzymes and combined the subsequent results with *in situ* soil moisture and temperature data to estimate *in situ* activity.

Soil samples were collected every two weeks during Fall 2009 from the Boston Area Climate Experiment to track enzyme dynamics as field temperature and moisture change. Samples were collected from the precipitation manipulation plots: ambient, +50% ambient (wet) and -50% ambient (drought). Temperature sensitivity of β-glucosidase was determined by performing the assay in slurry at four different temperatures (4, 15, 25 and 35˚C) for all collected samples. Moisture sensitivity was

determined by exposing soils to different moisture levels in the lab and adding substrate to the soil instead of slurry. Temperature sensitivity was calculated as Q_{10} and a linear regression was utilized for each field treatment at each sampling season to determine moisture sensitivity.

There was no change in temperature sensitivity between treatments or season. Moisture sensitivity varied significantly between the five sample dates and treatments. In almost every season, β-glucosidase activity in drought plots was more responsive to increases in soil moisture than activity in ambient and wet plots. Initially, *in situ* activity was estimated using the temperature and moisture sensitivities separately, always resulting in ambient plots having the highest activity followed by wet and then drought plots. The temperature-based model suggested that the drought plots had less activity despite having higher field temperatures and no difference in Q_{10}. The discrepancy in activity was due to a smaller enzyme pool under drought compared to other precipitation treatments, possibly because of reduced microbial biomass.

This was the first attempt at estimating activity using measured differences in moisture and temperature sensitivities. When combining the temperature and moisture sensitivity equations it became apparent that temperature was the primary control on enzyme activity fluctuations except when soil moisture was low. On the first sample date, soil moisture was very low and the activity predicted by temperature was hampered by soil moisture limitations. These results demonstrate that low soil moisture can limit *in situ* enzyme activities in soils.

4.2 INTRODUCTION

Current soil organic matter models are able to reproduce large-scale changes in carbon dynamics when systems are in a semi-steady state (Schimel et al. 1997; Parton et al 1998) without explicitly modeling microbial community dynamics (Andren & Balandreau, 1999). However, this is often not the case in highly variable environments, which may require more mechanistic models (Lawrence et al .2009). For example, microbial responses to disturbance can lead to large changes in nutrient cycling through altered microbial community and enzyme production (Fierer et al. 2003; Collins et al. 2008). A few studies have explicitly incorporated enzymes into models to better predict litter decomposition and microbial responses in dynamic systems (Moorhead & Sinsabaugh, 2000; Schimel & Weintraub, 2003; Lawrence et al. 2009). Enzymatic depolymerization of biopolymers is the rate limiting step in decomposition of unprotected soil organic matter (Schimel and Bennett, 2004; Allison et al. 2010) and it is important to investigate the controls on *in situ* enzymatic activity in order to fully understand how activities may be altered in the field due to environmental variability, such as altered temperature and precipitation regimes (Wallenstein & Weintraub, 2008).

Enzyme activity has been assayed in soils for over sixty years (Skujins, 1976) and used as a descriptor of soil quality, indicator of substrate use and nutrient cycling and to provide a mechanistic understanding of decomposition in natural and disturbed systems (Nannipieri, 1994; Dilly & Nannipieri, 1998; Bandick & Dick, 1999). There have been significant changes in the methodology used to assess activity but current methods still

only provide estimates of potential enzyme activity and do not quantify *in situ* activity (Drobnick, 1961; Skujins, 1976; Wallenstein & Weintraub, 2008). The three components of the method that hamper our ability to better estimate *in situ* activity are, (1) no diffusion limitations (2) single assay temperatures and (3) non-limiting substrate concentrations (Wallenstein & Weintraub, 2008; German et al. 2011).

Almost all contemporary enzyme assays are performed in slurry (Saiya-Cork et al. 2002) to ensure adequate homogenization of added substrates and consistent estimation of activity rates over time. However, most upland-soils, are rarely water saturated and even when saturated, substrates are not well mixed. The slurry decreases the substrate diffusion limitation observed in most soils resulting in higher activities than would be expected in the field. In addition, substrates are not homogenously distributed throughout soil (Ettema & Wardle, 2002) as in the slurry, again leading to an overestimation of activity. Diffusion constraints on substrate, enzyme or both can have a large impact on *in situ* activity, especially under drought conditions (Koch, 1990).

In early soil enzyme protocols, the temperatures used were very high (e.g. 40˚C), and outside of biologically relevant temperatures for most soils (Skujins, 1976). Current methods more often measure activity at temperatures closer to those experienced by soils in the field. However, enzyme activity is typically measured only at one temperature. Enzymatic reactions, like all other chemical reactions, are sensitive to temperature (Trasar-Cepeda et al. 2007). Field soil temperatures can change drastically over the course of a day and year, and the use of one assay temperature does not provide enough information on the sensitivity of soil enzyme activities and pools to

temperature (McClaugherty & Linkins, 1990). In addition, it is important to assess the temperature sensitivity of enzymes involved in the cycling of a diverse array of nutrients because they may have differential responses to temperature (Koch et al. 2007). Temperature sensitivity of enzyme reactions can be tempered by diffusion limitations in the field, resulting in a lack of enzyme temperature dependence (Davidson & Janssens, 2006), thus both temperature and moisture need to be considered together when trying to under field activity.

Not only is enzyme activity directly affected by moisture and temperature changes, but also indirectly through changes in microbial communities (Zogg et al. 1997; Sowerby et al. 2005). Microorganisms are sensitive to environmental conditions and can alter allocation of nutrients to different processes, such as enzyme production, in response to changes in their abiotic environment. Allocation of nutrients to enzyme production can vary based on microbial nutrient requirements and availability (Schimel et al. 2007; Griffiths et al. 2003). *In situ* enzyme activity is dependent on the production and turnover of enzymes which alter the enzyme pool size.

Enzyme production may decline under warming because enzymes become more efficient as temperatures rise, resulting in more products available for microbial assimilation without more enzymes, leading to a decrease in potential enzyme activity (Allison & Vitousek, 2005). However, soil warming can cause a decrease in soil moisture, potentially confounding direct temperature impacts on enzyme activity. Under drought conditions enzyme activity often declines because of reduced enzyme production (Sardans &Penuelas, 2005; Sardans & Penuelas, 2010). However, I measured greater

than expected activity under drought at BACE based on microbial biomass (Chapter 1). The stabilized enzyme activity under drought was attributed to reduced enzyme turnover, the other mechanism involved in changing enzyme pool size.

To address the mechanisms that might explain contrasting results from precipitation manipulation studies, I evaluated the independent and interactive effects of temperature and moisture at the Boston Area Climate Experiment. I hypothesized that soil moisture is the dominant control on enzyme activity and that as diffusion limitations are alleviated the amount of activity measured from existing enzymes will increase. β-glucosidase was chosen as the model enzyme because it is involved in the final step of cellulose breakdown and is produced by a wide variety of microorganisms. Enzyme assays were performed at different temperatures and soil moistures to assess changes in temperature and moisture sensitivity of enzyme activity in soils collected every two weeks during Fall 2009 from the Boston Area Climate Experiment (BACE) soil moisture manipulation plots. By utilizing the results from two different enzyme assay methods and field soil moisture and temperature data, I attempted to more closely approximate *in situ* activity than laboratory assays alone can allow.

4.3 METHODS

4.3.1 Study Site

Soils were obtained from the Boston-Area Climate Experiment (BACE), an old field ecosystem, located in Waltham, Massachusetts at the University of Massachusetts Agricultural Experiment Stations. Mean annual precipitation and temperature in nearby Boston, MA are 1054 mm yr^{-1} and 10.3˚C. Soils are classified as mesic Typic Dystrudepts

and the upper 30 cm consists of loam soils (45% sand, 46% silt, and 9% clay), with a pH 5.5. The site was previously an apple orchard, but was abandoned over 40 years ago. Current vegetation includes 38 primarily non-native grasses and forbs.

4.3.2 Field Experimental Design

The BACE exposes nine plots to one of three precipitation treatments. The three precipitation treatments are no change, plus 50% precipitation (wet), and minus 50% precipitation (drought) with three replicates for each treatment. Precipitation is controlled by clear partial roofs in the drought plots and additional precipitation is added after natural precipitation events in the wet plots. During the winter, drought plots are maintained, but additional water is not added to the wet plots. Soil moisture is measured weekly during the non-freezing months, usually beginning in April and ending in December, while soil temperature is monitored daily throughout the year. In the Fall of 2009 soils had been exposed to precipitation treatments for over two years

4.3.3 Soil Sampling and Pre-processing

Soils were collected every two weeks from August 2009 through October 2009 from precipitation-only plots. Two cores (5 cm diameter) were collected from each plot at 0-5 and 5-15cm depths. Soils were packaged on ice and shipped to the laboratory overnight, where the cores from each plot were 2mm sieved, rocks and roots removed, homogenized and frozen at -10˚C until analysis.

Subsamples from each plot were used for determination of water holding capacity. Water holding capacity was assessed after field moist soils were weighed and saturated with water for four hours. The water was drained and samples remained

covered for an additional twelve hours. Subsequently soils wetted to field capacity were weighed and then placed in a 60˚C drying oven for 48 hours and weighed again.

4.3.4 Enzyme Assays

Enzyme assays were performed on samples from all plots at each collection date, but using two different methods to assess temperature and moisture sensitivity. Temperature sensitivity of samples was assayed using a protocol modified from Sinsabaugh et al. (1992) which uses a slurry to reduce diffusion limitations. The moisture sensitivity assays were performed using soils from the field precipitation treatment plots with additional soil moisture manipulations in the laboratory.

The temperature sensitivity of Fall 2009 β-glucosidase was assessed by performing assays in deep (2 ml per well) 96-well plates at four temperatures: 4, 15, 25 and 35 ˚C. Two additional plates were used to create standard curves for each sample at 4 and 25˚C. The reference standard for β-glucosidase activity was 4-methylumbelliferone (MUB). The standard curve plates had a column for each soil sample and different concentrations in each of the wells of the MUB standards, 0, 2.5, 5, 10, 25, 50 and 100µM.

Soils were removed from the freezer and a 2.75g subsample was warmed to room temperature (~ 20˚C). The subsample was homogenized with 50mM sodium acetate (pH 5.5) for one minute in a Waring laboratory grade blender on high. Each column on the 96-deepwell plates corresponded to one sample. After homogenization, 800µL of suspension was aliquoted into each of the eight wells of one column on all six plates, one plate for each of the four assay temperatures and two for the standard curve

at 4 and 25˚C. Following the addition of twelve samples into their respective columns, the MUB-labeled substrate was added. Each substrate was added to one well in each column, so that all twelve samples received each of the six substrates once.

The plates were incubated for different lengths of time depending on incubation temperature, 1.5h at 35˚C, 3h at 25˚C, 6h at 15˚C and 23h at 4˚C. Different lengths of time were used to insure measurements were made when activity was linear. Following incubation, the plates were centrifuged for three minutes at 350g. Afterwards, 250µL of supernatant from each well was placed into the corresponding well on a 96-well black plate. Fluorescence was measured immediately following 5µL addition of NaOH to optimize fluorescence intensity. A Tecan Infinite M500 spectrofluorometer was used to measure fluorescence with wavelengths set at 365nm and 450nm for excitation and emission, respectively. The plates with the standards were used to calculate a linear standard curve and determine β-glucosidase activity for each sample as µmol h^{-1} g dry $soil^{-1}$and µmol h^{-1} g C^{-1}.

Moisture sensitivity of Fall 2009 β-glucosidase was assayed at 25˚C but at multiple moistures. Two subsamples, about 20g each, were taken for each plot. One subsample was kept at field moisture (hereafter called moist) while the other was allowed to dry overnight (hereafter called dried) at room temperatures, about 20˚C. The following day 2g of soil from each group, moist and dried, were weighted into different scintillation vials. Then 250µL of 591µM substrate was added along with between 100-400µL DI water to alter soil moisture among the moist and dried samples. Eight minutes after substrate addition, 31mL of 50mM sodium acetate (pH 5.5) was added and the

sample was vortexed for about five seconds. After vortexing, 800µL of slurry from each sample was added to three wells in a deepwell plate and centrifuged for three minutes at 350g. Following centrifugation, 250µL of each sample was transferred to a black 96-well plate and fluorescence was measured as previously described. Standard curves were also created using MUB at 0, 5, 25, 50 and 100µM. Preliminary trials indicated that soils dried down and then rewet to their original field moisture content behaved similarly to the field moist sample not dried.

4.3.5 Calculations & Statistics

The temperature sensitivity of β-glucosidase activity was assessed on samples incubated in the lab at 4, 15, 25 and 35˚C and calculated as Q_{10}:

$$Q_{10} = \frac{\text{activity at } T_2}{\text{activity at } T_1}^{\left(\frac{10}{T_2 - T_1}\right)}$$

where T_2 is the incubation temperature 10˚C greater than T_1. There were five dates resulting in 15 Q_{10} values, one for each field precipitation treatment. For the predictive model, Q_{10} values were interpolated between dates to obtain a Q_{10} for each day.

Moisture sensitivity was estimated for lab samples at each date by treatment using a linear regression. The slope and intercept were calculated from the activities measured at multiple lab soil moisture for samples from a single date and precipitation treatment. There was a significant difference in slope and intercept values by precipitation treatment at each date, so each precipitation treatment had its own slope and intercept estimation at each sampled date. Again, only five dates were assayed, so the slope and intercept were interpolated for days between sample dates for each field precipitation treatment for the model. In addition field soil moisture was measured

weekly, so interpolation was required to obtain soil moisture for each day between August 24, 2009 and October 22, 2009. PROC CORR was used to estimate the correlation coefficient and significance of the correlation between enzyme activity and soil moisture. PROC GLM was used to determine if there was a significant difference in moisture sensitivity slope and intercept between the treatments and dates (SAS Institute, Cary, NC).

Predicted *in situ* β-glucosidase activity was estimated first from soil temperature and moisture data individually. Using the Q_{10} values estimated from lab incubation and field soil temperature I estimated *in situ* activity using the following equation from Wallenstein et al. (2009):

$$in\ situ\ activity\ from\ field\ \ temperature = R_{25} * Q_{10}^{\left(\frac{t-25}{10}\right)}$$

where R is the β-glucosidase activity at 25˚C, Q_{10} is derived from the 25 and 35˚C assays, and t is the *in situ* temperature. Q_{10} did not significantly vary by treatment (PROC GLM, SAS Institute, Cary, NC), but instead of averaging over treatments I chose to use the estimated Q10s from each treatment. Soil moisture enzyme data was calculated using a linear regression, $y = mx + b$, with parameters estimated for each treatment at each. To predict *in situ* β-glucosidase activity using both field soil moisture and temperature I incorporated the equation used to estimate activity for those two variables individually. The linear equation used to estimate *in situ* activity by soil moisture replaces R_{25} in the equation used to estimate *in situ* activity

$$in\ situ\ activity\ from\ field\ temperature\ and\ moisture$$

$$= (m_{SM} * x_{SM} + b_{SM}) * Q_{10}^{\left(\frac{t-25}{10}\right)}$$

Where m and b are the slope and intercept, respectively, determined from soil moisture assays, x is the field soil moisture and t is the field soil temperature. Multiple comparisons ANOVA using PROC GLM were made for model results to determine if activities from each precipitation treatment averaged over all dates were significantly different based on model used (SAS Institute, Cary, NC).

4.4 RESULTS

Precipitation treatments altered soil moisture substantially, with soil moisture in drought treatments being on average 50% of ambient moisture at 0-10cm in 2009 (Figure 4.1a). There was no effect of additional water on the soil moisture of wet plots. Soil moisture increased from the end of August 2009 to the end of October 2009, while soil temperature declined by about 17˚C during that same time (Figure 4.1b). Drought plots tended to be slightly warmer than ambient or wet plots but the difference was minimal, about 0.25˚C higher on average.

At every sampling period, except October 22, 2009, β-glucosidase activity in drought plots was more sensitive to soil moisture than activity in ambient or wet plots, with slope values 2-5 times higher (Figure 4.2). Drought plots demonstrated a significant positive correlation between increasing soil moisture and enzyme activity for every sample date (Table 4.1). Ambient plots were significantly negatively correlated with increasing soil moisture on the first sample date but became positively correlated for the two October sample dates. Wet plots were significantly positively correlated

with soil moisture on two dates, September 10, 2009 and October 22, 2009. There were significant differences ($P<0.03$) in slope and intercept values between treatments at each date except September 25, 2009 when ambient and wet plots had similar responses.

The Q_{10} of enzyme activity in drought plots stayed between 2.7 and 3.3 throughout the sample period, except when temperatures began to drop around day 280, there was a subsequent decline in temperature sensitivity, to about 2.5 (Figure 4.3). Ambient-plot temperature sensitivity remained around 2.5 except in the middle of the sampling period, when it reached 4, which was the same time when the wet plot Q_{10} dropped from its average around 2.7 down to 2.5.

In situ β-glucosidase activity predicted by field temperature followed the same trend as temperature, with activity declining as field temperatures dropped from August to October (Figure 4.4a). Drought plots had the lowest predicted activity despite having on average slightly higher plot temperatures. *In situ* activity predicted from soil moisture tended to follow the field soil moisture curves, with lower activity as soil moisture declined (Figure 4.4b). But, there was a lag in the predicted activity moisture response in the ambient and drought plots, which experienced a dry down period around day 250, but enzyme activity did not decline until about day 270.

Using both field soil moisture and temperature to predict *in situ* activity resulted in estimated activities significantly different from the moisture model but similar to the temperature only model (Table 4.2). Activity averaged over the whole season was estimated to be highest in the moisture only model, followed by the temperature and

then the combined temperature-moisture model (Figure 4.5). In the drought plots predicted activity in the combined model initially followed the moisture only model and then shifted around day 250 to being most similar to the temperature only model. When estimating activity for the combined model in ambient and wet plots, both followed the temperature only model throughout the duration of the sampling.

4.5 DISCUSSION

Understanding soil enzyme dynamics can be quite difficult due to our limited understanding of enzyme production, residence time and turnover (Burns, 1982; Wallenstein & Weintraub, 2008). However, enzymes are an important component of the decomposition pathway and measures of their activity can provide insight into how nutrient cycling is affected by different factors (Sinsabaugh et al 1991; Caldwell, 2005). Here, I examined how enzyme activities were affected by both temperature and moisture because it is known that both of these variables affect activity (Trasar-Cepeda et al. 2007; Sowerby et al. 2005; Allison, 2005), but information about the magnitude of their effects in combination and in the field is limited (Sardans & Penuelas, 2008).

Assays of β-glucosidase activity in the laboratory at different soil moistures demonstrated a consistently strong positive effect of moisture on activity in the drought soils and occasionally in the ambient and wet soils. These assays were very short, meaning that any activity measured was from existing enzymes not new enzyme production. The large increase in activity with increased lab soil moisture, primarily in the drought plots, indicates an available pool of functional enzymes that are being released from constraints of substrate diffusion. The maintenance of an enzyme pool

during extreme drought at the beginning of the Fall could be due to either increased production (Harder & Dijhuizen, 1983) or reduced enzyme turnover. In a substrate-limited environment, whether due to diffusion constraints or low substrate quantity, the nutrient requirements to produce enzymes may exceed the amount gained via enzymatic reaction (Allison & Vitousek, 2005). Increased production seems unlikely in this scenario because of nutrient constraints, but a decline in enzyme turnover could account for the relatively large pool of enzymes available under dry conditions. As soils dry, enzymes may become adsorbed to clay and tannin materials rendering them less accessible to proteolytic breakdown but able to maintain reactivity (Ensminger & Gieseking, 1942; Kandeler, 1991). These results contradict Lawrence et al. (2009) who suggested that enzyme activity is insensitive to moisture because activity can continue in water films during drought. While activity can continue in water films under drought, the results of this study indicate that activity is very sensitive to soil moisture, particularly in dry plots. The steep increase in activity in the lab as diffusion limitations are eliminated indicates that soil moisture in the drought constrains the activity of the available enzyme pool. In ambient and wet plots, soil moisture stayed above 15% for the duration of the study and diffusion limitations may not have been imposed, thus predicted activity did not consistently show a strong positive correlation with increasing field moisture. In addition, field evidence from the BACE shows reduced soil respiration in the drought plots (Suessela et al. in review) indicating that despite available enzymes, enzyme activity and thus microbial respiration are moisture limited.

Enzyme assays conducted at multiple temperatures confirmed that temperature is a strong driver of enzyme activity, as has been previously observed (Trasar-Cepeda et al. 2007). Despite the positive response of enzymes to increasing lab temperature, there was no measurable change in the enzyme temperature sensitivity over the course of the season as others have measured (Wallenstein et al 2009), which was surprising since there was a 17˚C decline in soil temperature over the sample period. Changes in temperature sensitivity can be due to production of isoenzymes, enzymes with temperature optima different from the optima of the enzyme pool they are replacing (Wallenstein & Weintraub, 2008. The replacement of an enzyme pool with enzymes that had different optima may take longer than the 15 day time increment observed. Activities were always predicted to be lower in the drought plots than ambient treatment despite having similar field temperatures and temperature sensitivities, indicating that the pool might be smaller.

The activity averaged over all dates for each precipitation treatment was similar for activities predicted by temperature and temperature x moisture models indicating that temperature was a strong control on activity. However, when the data were separated into early and late Fall there was a strong effect of moisture on predicted activity in the drought plots. Drought plots in the first half of fall were extremely dry and the lack of moisture overwhelmed any enzyme temperature response, so that the moisture*temperature model followed the moisture model when moisture was below a certain threshold, about 7%. Once soil moisture went above 7%, temperature became the dominant control on activity in the drought plots, with activity following the

predicted temperature model. This response was most obvious after day 280 where soil moisture was rising in the drought plot, but temperature was low and predicted β-glucosidase activity was also low. The soil moisture control on activity was not seen in the ambient or wet plots because the moisture levels never reached the moisture limiting threshold in the field.

The activities predicted by the moisture and temperature models individually were vastly different. In this system, temperature is the dominant control on activity due to the lack of moisture limitation during the majority of the study period. The moisture model always predicted higher activity than the temperature model because it assumed that temperature was around 20˚C throughout the season (as do most estimates of enzyme activity). However, the temperature model overestimated activity when soils were very dry. The combination of the two models provided a more comprehensive view of soil enzyme dynamics, because the impact of both moisture and temperature can be large.

4.6 CONCLUSION

The three models developed here were the first attempts to estimate *in situ* enzyme activity using field soil moisture and temperature data. Current enzyme methodology provides information on potential activity, but more research needs to be performed to better understand how enzymes drive ecosystem processes (German et al. 2011). Temperature sensitivity of enzyme activity has received much attention as of late (McClaugherty & Linkins, 1990; Trasar-Cepeda et al. 2007; Wallenstein et al. 2009), but there is still a lack of understanding regarding their moisture sensitivity (Wallenstein

& Weintraub 2008). This research demonstrates that both moisture and temperature can be strong controls on enzyme activity, with temperature being the dominant control when soil moisture was not limiting. Since both of these variables influence enzyme activity predictions it is important to understand the threshold point and magnitude of their influence individually and in combination, such as the possibility of warming induced soil drying mitigating the positive influence of temperature on enzyme activity. This research provides a more complete understanding of *in situ* enzyme activity and thus the sensitivity of the depolymerization mechanism that transforms complex soil organic matter into a form that can be utilized by microorganisms.

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4.8 TABLES

Table 4.1 Correlation and p-values for the relationship of enzyme activity to soil moisture for each precipitation treatment at each ofthe sampling dates.

field treatment	date	R	*P*-value
drought	8/24/2009	0.67	<0.0001
	9/10/2009	0.24	0.08
	9/25/2009	0.46	0.0003
	10/6/2009	0.51	<0.0001
	10/22/2009	0.42	0.006

ambient	8/24/2009	-0.24	0.05
	9/10/2009	-0.16	0.23
	9/25/2009	0.11	0.43
	10/6/2009	0.51	<0.0001
	10/22/2009	0.65	<0.0001
wet	8/24/2009	0.26	0.07
	9/10/2009	0.39	0.01
	9/25/2009	0.01	0.95
	10/6/2009	0.03	0.79
	10/22/2009	0.29	0.02

Table 4.2 Average estimated *in situ* β-glucosidase activity over Fall 2009 by field treatment for each model. Letters indicate whether modeled averages are significantly different between models.

	moisture	temperature	moisture*temperature
drought	80.51 ±15.03 a	66.68 ±2.44 ab	33 ±6.19 b
ambient	205.91 ±1.38 a	96.9 ±1.29 b	90.23 ±2.35 b
wet	168.25 ±8.43 a	84.92 ±12.41 b	69.18 ±2.59 b

4.9 FIGURES

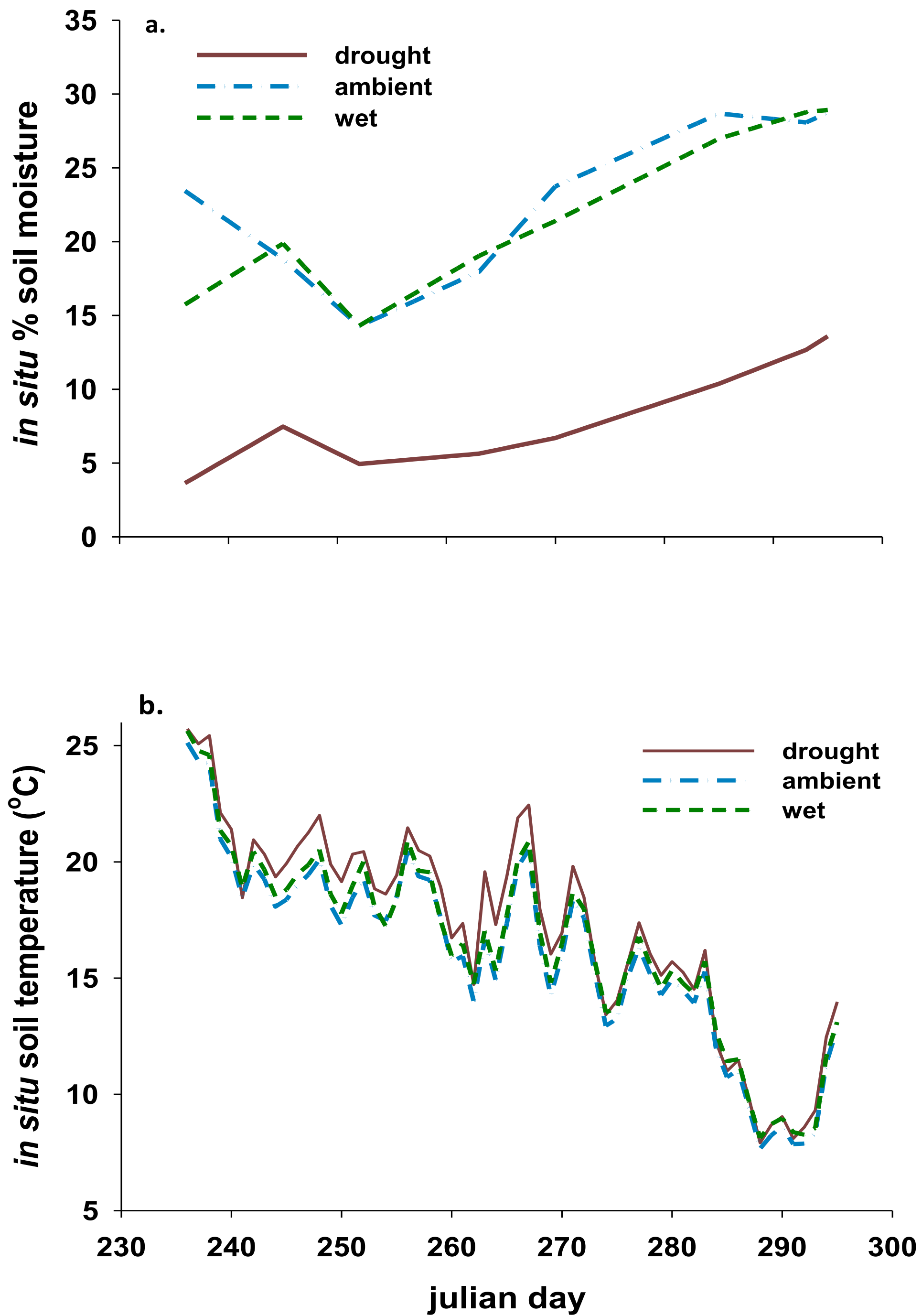
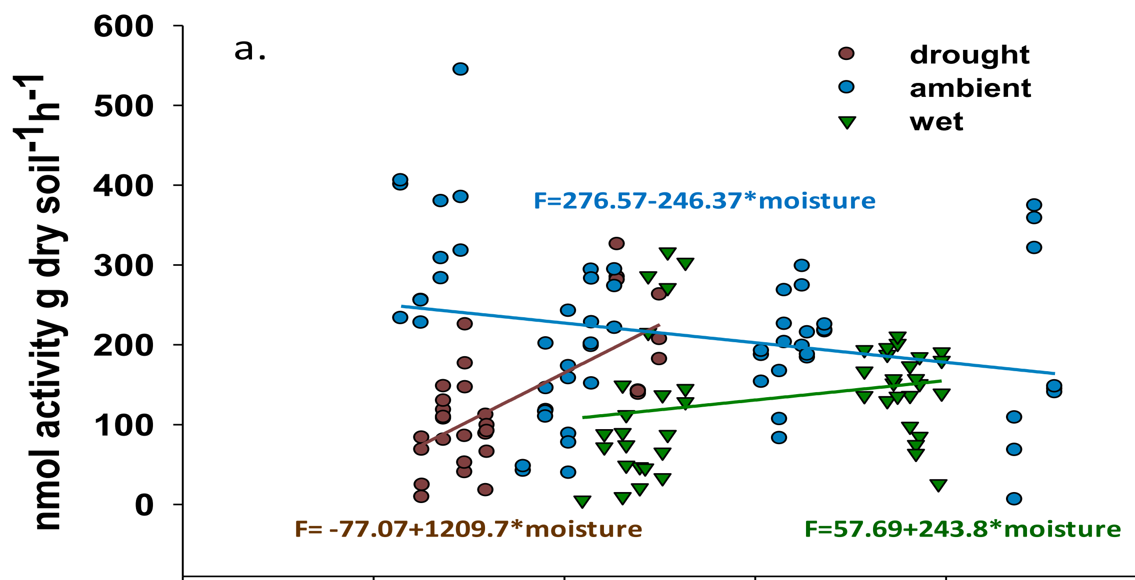
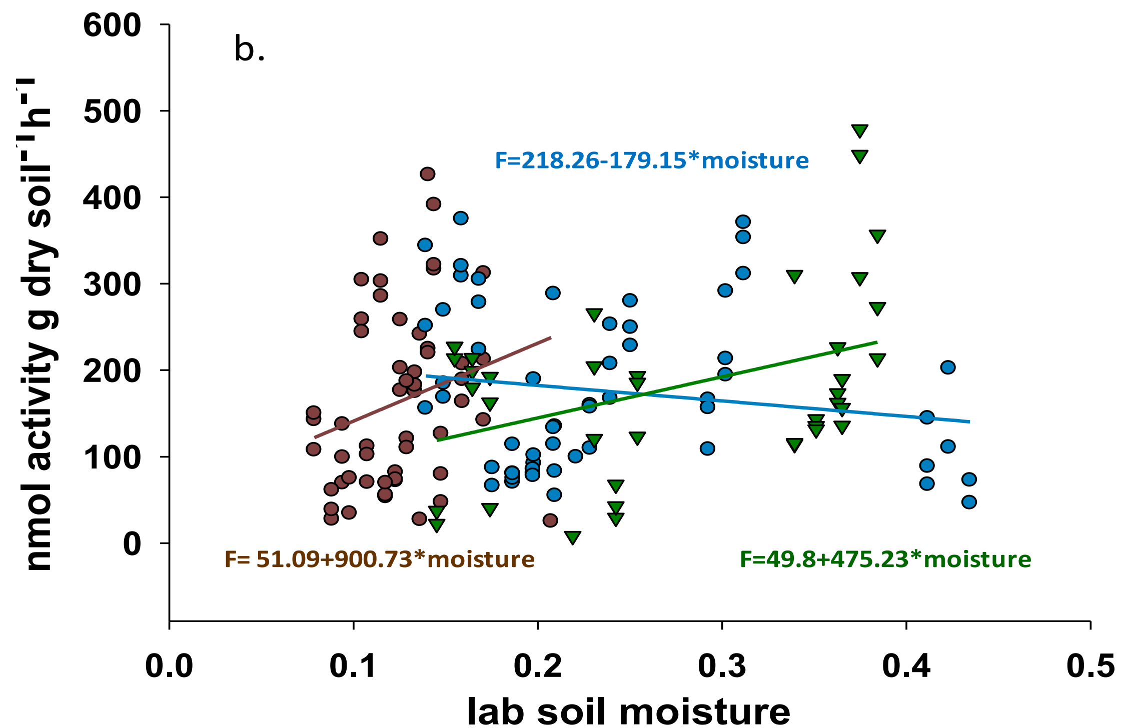
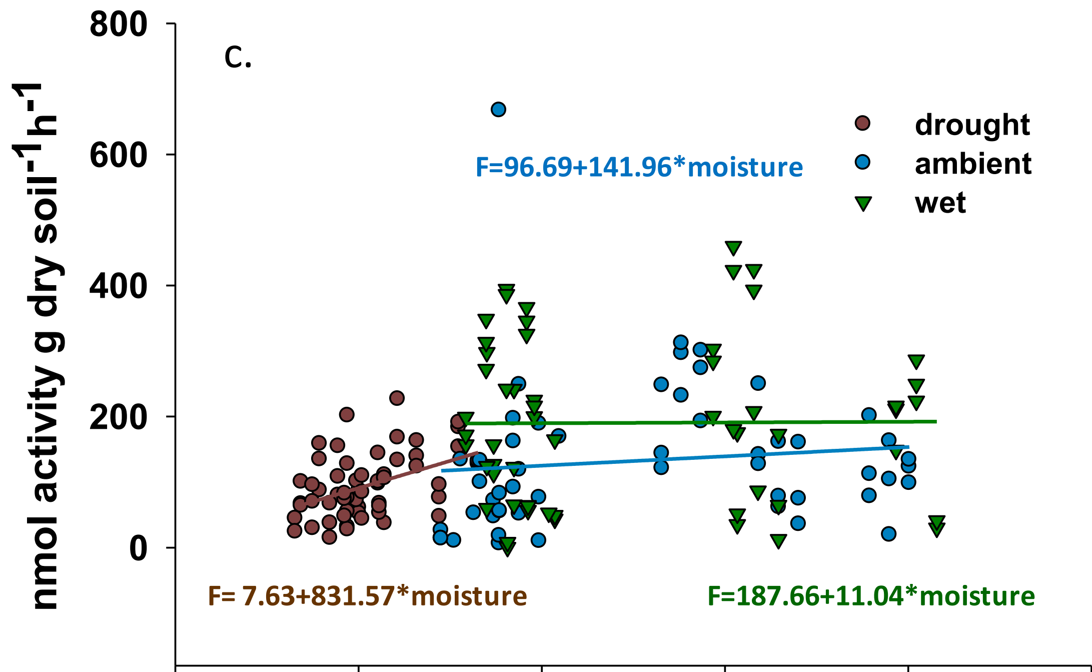
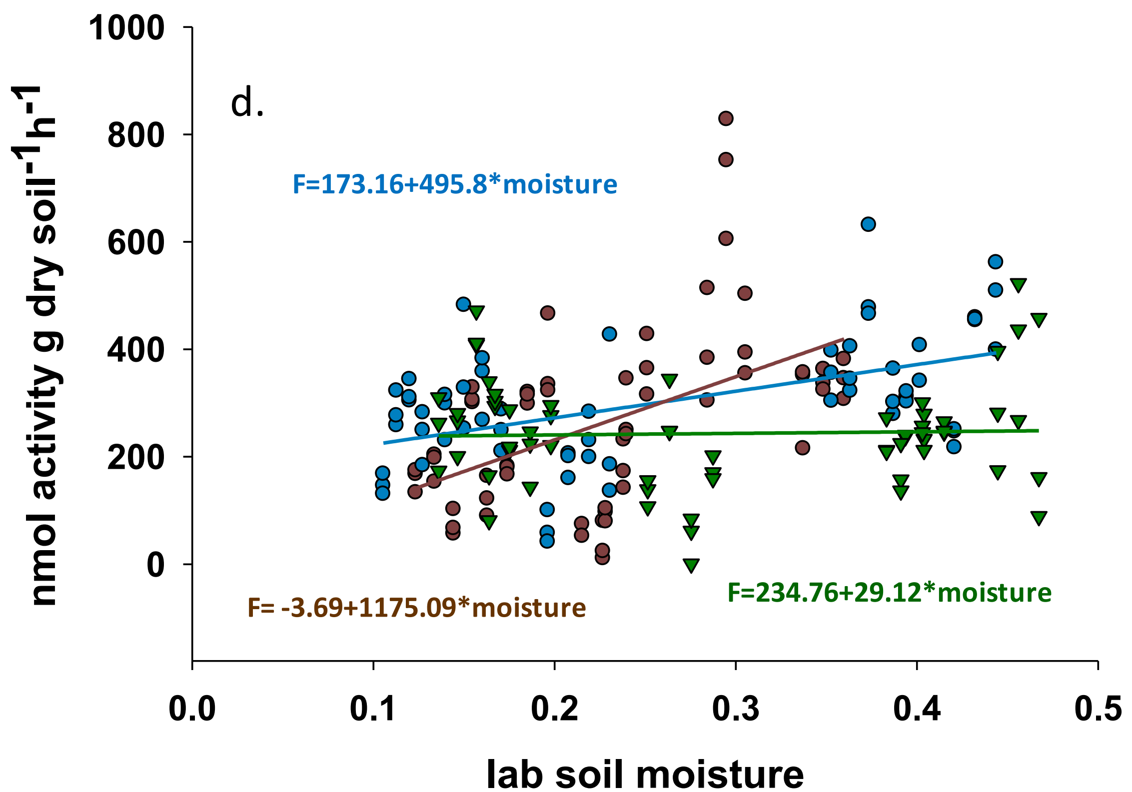


Figure 4.1. *In situ* (a) soil moisture and (b) temperature from August 2009 through October 2009 for precipitation manipulation plots.


a.
drought
ambient
wet
600
500
400
300
200
100
0
nmol activity g dry soil^{-1}h^{-1}
F=276.57-246.37*moisture
F= -77.07+1209.7*moisture
F=57.69+243.8*moisture


b.
600
500
400
300
200
100
0
nmol activity g dry soil^{-1}h^{-1}
F=218.26-179.15*moisture
F= 51.09+900.73*moisture
F=49.8+475.23*moisture
0.0
0.1
0.2
0.3
0.4
0.5
lab soil moisture


c.
800
600
400
200
0
nmol activity g dry soil^{-1}h^{-1}
drought
ambient
wet
F=96.69+141.96*moisture
F= 7.63+831.57*moisture
F=187.66+11.04*moisture


d.
1000
800
600
400
200
0
nmol activity g dry soil^{-1}h^{-1}
F=173.16+495.8*moisture
F= -3.69+1175.09*moisture
F=234.76+29.12*moisture
0.0
0.1
0.2
0.3
0.4
0.5
lab soil moisture

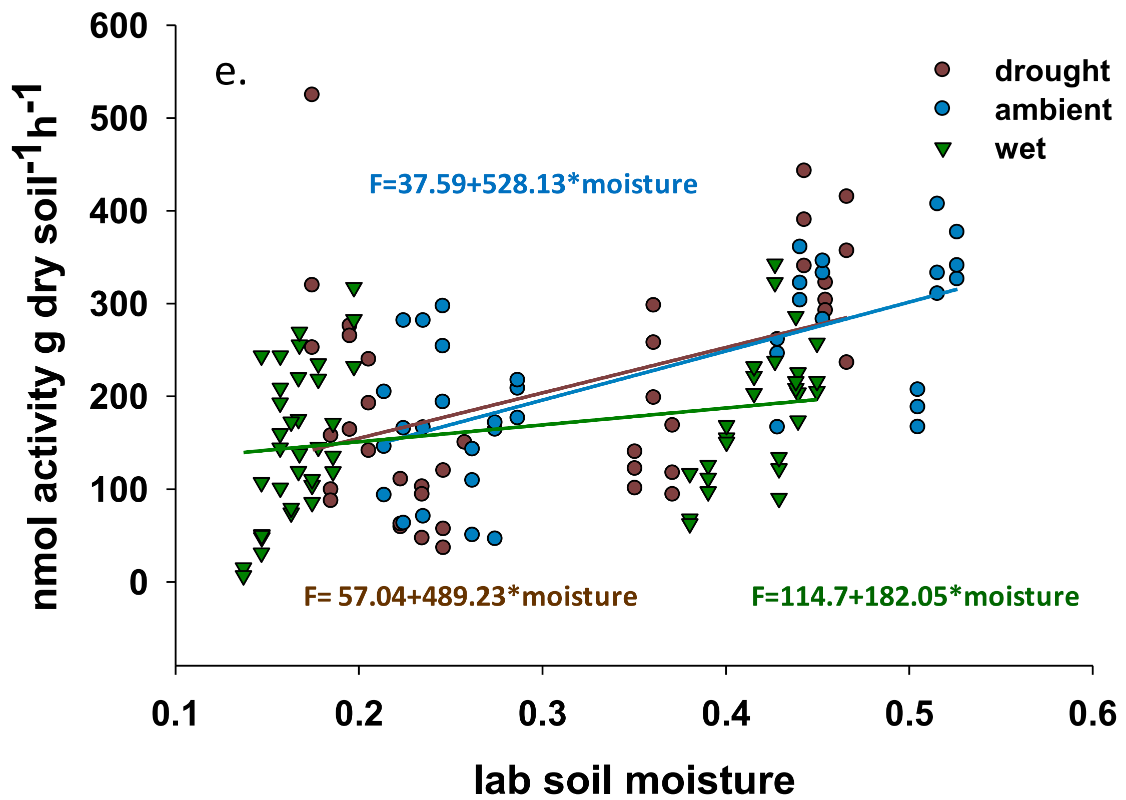


Figure 4.2 Potential β-glucosidase activity and respective linear regressions for each precipitation treatment at different lab induced soil moistures for each date, (a) August 24, 2009, (b) September 10, 2009, (c) September 25, 2009, (d) October 6, 2009 and (e) October 22, 2009.

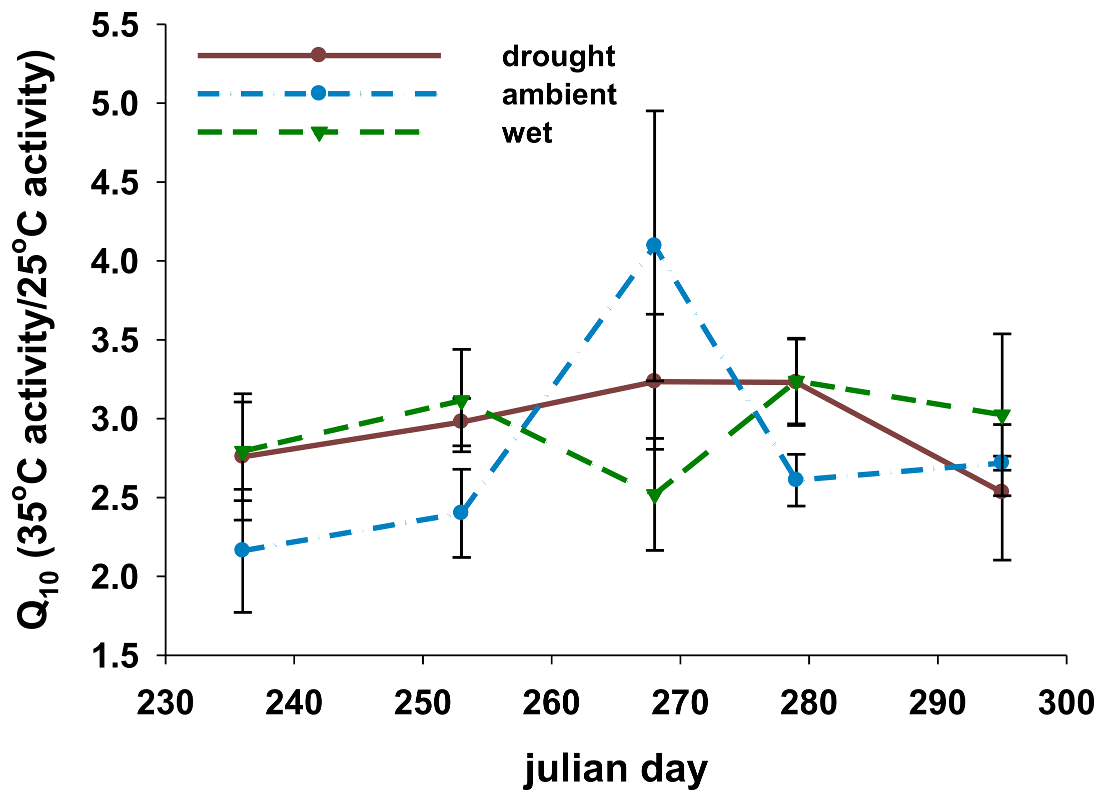


Figure 4.3. Temperature sensitivity of beta-glucosidase activity during Fall 2009 calculated from lab activity at 25˚C and 35˚C assays. Points are the average of Q_{10} collected from sample dates with error bars, n=3. The lines indicate interpolated values between sample points.

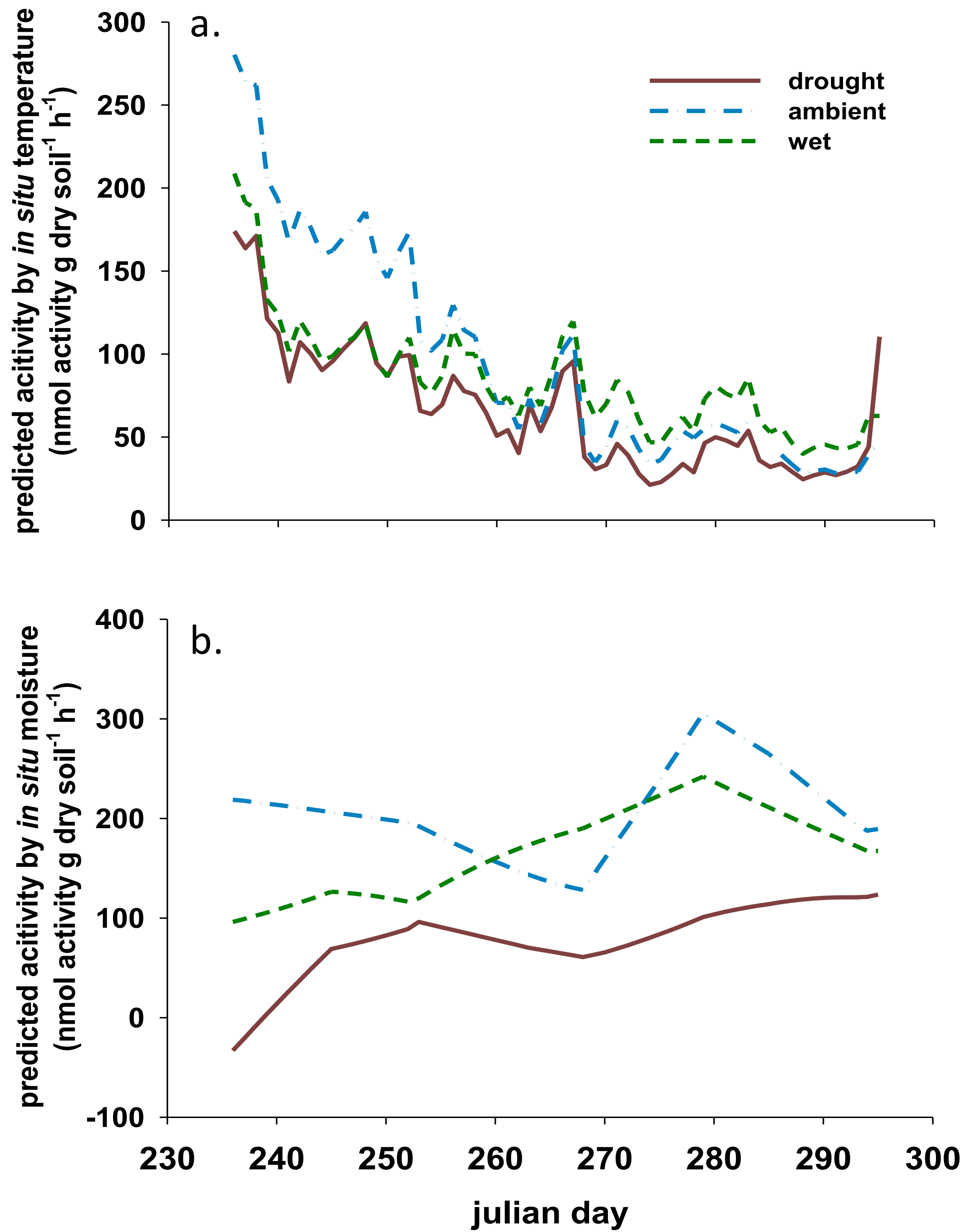


Figure 4.4. Predicted potential *in situ* β-glucosidase activity based on (a) field soil temperature and lab measured temperature sensitivity, (b) field soil moisture and lab measured moisture sensitivity.

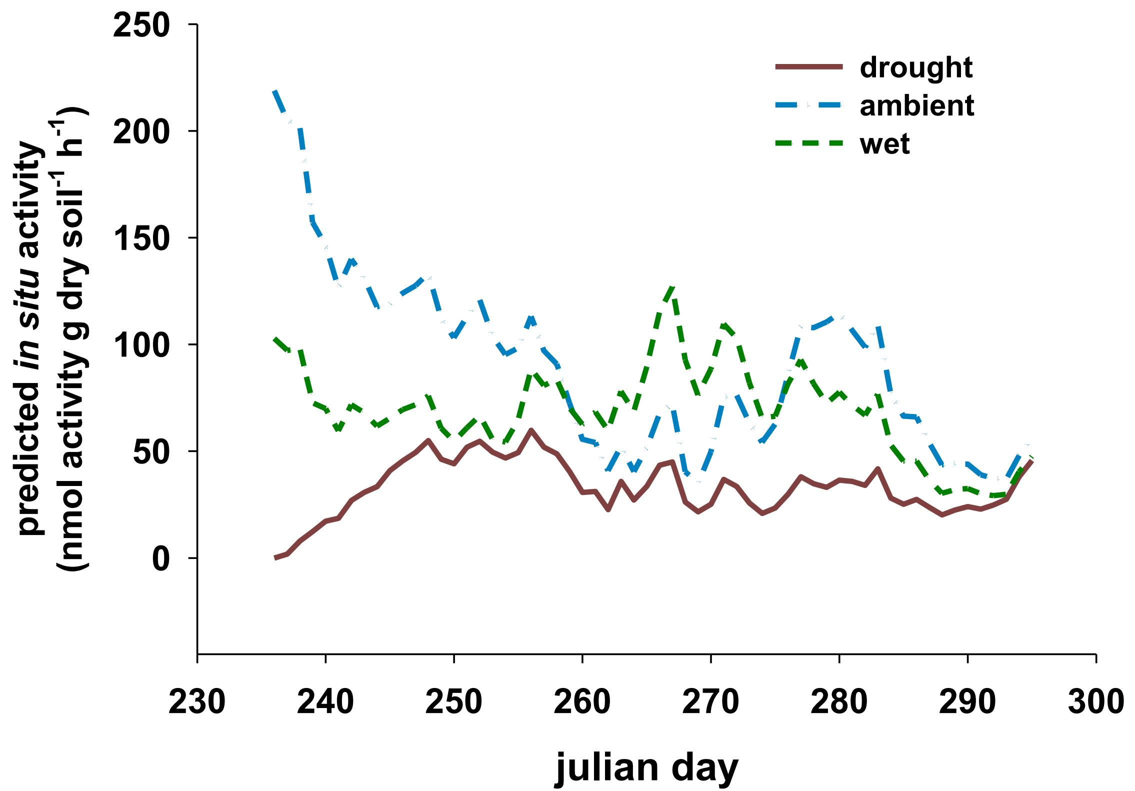


Figure 4.5. Predicted potential *in situ* β-glucosidase activity using temperature and moisture sensitivity equations together with field soil temperature and moisture for each precipitation manipulation.

5 SYNTHESIS

5.1 CONCLUSIONS

The research presented here examined soil microbial physiological responses to changes in temperature, precipitation and season in an old-field ecosystem at the Boston Area Climate Experiment. Microorganisms are the biological drivers of decomposition through production of enzymes involved in organic matter depolymerization, utilization of nutrients and production of CO_2 resulting in carbon loss from soils (Figure 5.1). Microorganisms have the ability to alter their metabolic pathways and carbon allocation to remain active (Dijkstra et al. 2011a, b, c). Carbon is allocated to growth, maintenance, respiration and enzyme production. I used measurements of enzyme activity and carbon utilization as indicators of change in microbial physiology. Potential enzyme activity is a function of the enzyme pool which is affected by both enzymatic production and turnover. In addition, measurements of enzyme temperature and moisture sensitivity were used to estimate *in situ* activity.

My findings suggest that enzymes may be protected against degradation under drought, resulting in a more stable enzyme pool and a decoupling of enzymes from the microbial biomass. In addition, by measuring moisture and temperature sensitivity I was able to estimate *in situ* activity and to determine the sensitivity of enzyme activity to changes in moisture and temperature. And finally, I found evidence for a shift in

carbon utilization profiles with climate and season. Together, these three results demonstrate how microorganisms can alter their physiology over short- and long-term climate manipulations, and provide a finer understanding of how decomposition processes may be affected by climate change.

5.1.1 Physiological responses to drought

The observation of enzyme pool stabilization under drought was different from the majority of studies involving enzyme-drought relationships where activity declined (Sardans & Penuelas 2005; Sardans & Penuelas 2010). Production and turnover of enzymes control the size of the enzyme pool and thus the rates of activity. The maintenance of the enzyme pool size despite a smaller microbial biomass under drought could have been the result of increased resource allocation towards enzyme production or reduced turnover. To maintain the stoichiometry of their biomass (driven by the fixed stoichiometry of cellular components), microbes produce enzymes targeting specific compounds that are rich in C, N or P (Sinsabaugh et al. 2008; Sinsabaugh et al. 2009). Nutrient limitation may have increased as soils dried, resulting in increased enzyme production to compensate for resource demand (Harder & Dijhuizen, 1983). However, this response would likely be short-lived as these same scarce resources are also required for enzyme production (Allison & Vitousek, 2005). The simplest explanation for a stable enzyme pool size with reduced microbial activity under drought is decreased enzyme turnover rate. As soils dry, enzymes may become stabilized on clay and organic residues and protecting them from proteolytic breakdown.

In areas where drought is uncommon, such as the northeast US, decreased turnover and resultant increased stability of enzyme pools could have large implications for nutrient cycling rates, especially if rates for enzymes involved with different nutrients become uncoupled. Despite a stable enzyme pool under drought, the *in situ* activity is likely to be low due to constraints of diffusion on substrates and products (Koch, 1990). Enzymatic depolymerization may be the rate limiting step in decomposition in systems with ample diffusion, however in drought affected areas decomposition rates may be governed primarily by substrate and product diffusion. Additionally, moisture sensitivity assays supported the hypothesis that diffusion is the rate limiting step under drought. High moisture sensitivity indicated that enzymes were available and capable of reacting but substrate depolymerization was limited due to diffusion limitations imposed by drought. These results in combination indicate a system that is poised to resume rapid nutrient cycling when diffusion limitations alleviated.

Drought alone did not result in a shift in substrate utilization profile, but the combination of drought and warming exacerbated already dry condition pushing the microorganisms into more moisture stressed conditions. Despite similar substrate availability, since root biomass was unchanged, the reduction in diffusion probably limited substrate accessibility. A reduction in substrate accessibility could have forced the microbial community to shift substrate utilization patterns or forced a shift in community species composition towards K-strategist microbes. Either of these two shifts could have resulted in the measured increase in tannin and vanillin utilization under the driest condition. The shift in utilization patterns in conjunction with an

increased utilization of complex compounds indicates an increased contribution of respiration from decomposition of phenolic compounds under extremely dry conditions. Based on these findings, it is important to focus not only on the total respiratory response of microbes to changing climate, but the functional capacity of the community and how substrate utilization is shifting. A shift in substrate utilization from labile to more resistant compounds could alter the rate of carbon cycling occurring and the current predictions of soil carbon storage.

5.1.2 Ecosystem Consequences

Current soil organic matter models are able to reproduce large scale changes in carbon dynamics when systems are in a semi-steady state (Schimel et al. 1998; Parton et al 1998), but this is an unlikely scenario with climate change. Precipitation regimes are likely to change with increasing temperatures, but the direction and magnitude of the change is uncertain. Measuring microbial physiology and its response to climate provides a more mechanistic understanding of the processes governing soil organic matter decomposition. In a rapidly changing system, it is imperative to understand the mechanisms underlying ecosystem-scale responses, such as net carbon storage. Our current body of knowledge regarding carbon utilization efficiency and allocation is limited because of the various ways in which microbes can change function.

The measurements made in this study involved fine-scale mechanisms that can have a large effect on ecosystem processes. By using field moisture and temperature data in conjunction with measures of enzyme moisture and temperature sensitivity data I've demonstrated how both climate variables control *in situ* activity. High sensitivity of

enzymes to moisture in combination with a stabilized enzyme pool under drought could result in a large release of low molecular weight nutrients when diffusion limitations are released. At the same time, carbon utilization profiles indicate increased utilization of complex compounds in dry, hot conditions. The combination of these results reveals an ecosystem with decreased concentrations of resistant compounds and the potential for rapid depolymerization of substrates. The predictions for precipitation regimes suggest that the frequency of rain events will decrease, but the intensity will increase. The results of this study suggest that, under intensified precipitation regime, this old-field ecosystem may experience pulses of rapid decomposition of simple and complex carbon compounds following precipitation events. Further study is needed to elucidate whether the patterns observed here are generalizable to other ecosystems that differ in climate, soils and vegetation.

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5.3 FIGURES

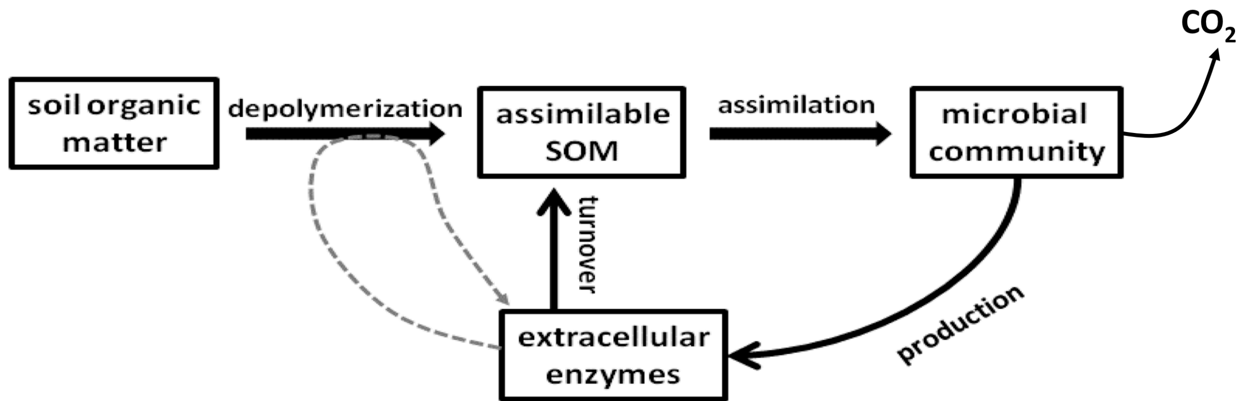

Figure 5.1 Microbial decomposition pathway (modified from Conant et al. in review)

APPENDIX I

Table A.1 Average potential enzyme activity and standard errors for main treatment plots at each date, 0-5cm depth, n=3.

Treatment	date	depth	β-glucosidase		cellobiohydrolase		xylosidase		phosphatase		N-acetyl glucoaminidase		leucine-amino peptidase	
ambient	Jun-08	0	319.40	81.63	120.84	26.51	72.72	18.64	653.17	131.45	144.66	42.98	196.20	27.79
	Aug-08	0	176.44	75.18	95.68	11.21	157.71	109.43	276.89	125.36	118.23	1.06	33.76	18.93
	Jan-09	0	819.62	221.19	264.95	88.00	144.21	49.96	1588.22	542.35	385.77	100.24	119.49	38.02
	Jun-09	0	225.62	27.44	82.37	18.55	56.94	9.42	416.25	63.44	116.56	21.83	95.80	54.33
drought	Jun-08	0	322.84	44.60	119.77	16.65	98.94	18.13	739.69	86.12	350.21	79.00	172.96	17.53
	Aug-08	0	399.99	176.29	173.54	84.16	152.44	58.43	578.42	384.33	300.80	119.31	69.65	13.56
	Jan-09	0	274.76	62.37	144.10	18.16	134.17	26.36	1083.14	157.70	239.91	25.31	134.16	37.60
	Jun-09	0	340.51	43.46	149.83	39.22	129.48	58.16	646.67	70.83	264.04	77.60	154.23	62.94
wet	Jun-08	0	347.40	89.49	137.92	51.80	79.19	11.73	666.49	101.60	235.84	55.04	168.94	26.41
	Aug-08	0	235.13	59.99	158.13	53.24	74.46	2.20	479.15	67.51	186.99	32.52	74.28	18.12
	Jan-09	0	1066.56	301.96	410.03	123.78	262.59	82.64	2351.46	565.83	520.93	152.08	140.26	40.45
	Jun-09	0	264.25	17.01	101.12	9.46	76.60	12.72	474.24	46.24	117.92	18.32	73.65	31.38
warm	Jun-08	0	308.56	92.43	118.53	30.46	60.71	10.37	617.20	127.88	101.32	26.37	173.86	32.77
	Aug-08	0	249.89	33.66	96.30	8.94	55.66	3.01	415.72	71.21	100.49	17.28	54.99	24.21

	Jan-09	0	500.56	142.67	178.00	46.75	84.20	18.11	973.38	181.35	219.04	59.75	53.25	7.29
	Jun-09	0	173.66	31.21	66.07	12.90	37.72	5.70	261.61	71.25	68.02	18.20	94.72	56.09
medium	Jun-08	0	410.14	138.31	171.45	56.71	88.93	27.21	714.94	184.02	204.30	62.48	184.14	44.84
	Aug-08	0	187.55	91.33	426.05	362.24	234.53	174.22	297.91	148.35	261.89	167.36	40.91	8.83
	Jan-09	0	799.89	203.90	308.13	83.87	164.43	37.77	1536.66	510.75	300.89	57.12	92.62	15.98
	Jun-09	0	222.66	28.76	98.59	10.61	67.50	11.92	410.61	29.96	87.09	10.05	75.71	21.23
hot	Jun-08	0	244.26	44.68	100.81	18.75	43.66	14.52	473.99	91.87	111.38	29.31	164.23	27.22
	Aug-08	0	338.98	133.42	177.96	64.69	170.19	106.26	1159.26	747.89	685.79	566.45	56.52	16.90
	Jan-09	0	686.66	315.05	263.70	124.60	113.68	45.34	1234.08	442.47	248.89	125.41	92.48	51.75
	Jun-09	0	233.45	31.51	95.08	13.43	45.16	8.52	371.38	47.98	105.85	34.46	68.61	27.47

Table A.2 Average potential enzyme activity and standard errors for main treatment plots at each date, 5-15cm depth, n=3.

Treatment	date	depth	β-glucosidase		cellobiohydrolase		xylosidase		phosphatase		N-acetyl glucoaminidase		leucine-amino peptidase	
ambient	Jun-08	15	1396.22	1207.94	621.96	560.48	321.99	290.15	3255.58	2773.91	690.16	644.67	132.22	14.50
	Aug-08	15	138.76	54.52	37.48	23.09	30.19	21.41	359.48	196.28	33.40	22.27	18.54	25.14
	Jan-09	15	166.32	23.28	54.90	12.15	30.78	9.90	435.37	52.77	41.74	14.67	19.91	4.17
	Jun-09	15	224.22	35.57	81.92	9.46	42.97	10.83	359.66	56.95	66.09	10.59	92.82	53.53
drought	Jun-08	15	159.02	41.59	58.07	14.38	33.98	6.19	442.11	79.29	64.72	10.32	91.51	1.35
	Aug-08	15	288.37	117.83	132.28	73.37	64.52	24.63	554.56	147.86	114.91	48.12	46.51	5.05
	Jan-09	15	343.65	180.39	93.24	30.05	51.47	11.35	506.44	132.24	107.29	46.61	34.13	23.27
	Jun-09	15	198.39	74.53	79.11	33.09	50.06	11.62	315.78	45.23	80.47	33.57	82.85	23.66
wet	Jun-08	15	235.55	64.32	84.86	28.72	42.51	9.13	518.06	108.93	92.86	31.56	103.58	16.30
	Aug-08	15	205.44	4.66	103.96	9.11	58.56	12.64	395.68	24.05	104.44	25.82	35.19	20.05
	Jan-09	15	138.03	8.23	52.51	5.59	28.41	2.57	426.31	25.07	42.25	3.53	19.34	3.23
	Jun-09	15	269.04	116.48	70.47	21.24	47.73	22.84	381.25	62.87	60.62	20.15	57.06	14.52
warm	Jun-08	15	253.02	51.45	98.52	21.97	55.32	18.06	561.42	108.38	95.31	41.30	114.89	23.25
	Aug-08	15	272.55	100.86	145.32	88.41	90.43	60.72	456.63	108.70	144.90	100.54	34.75	19.21

	39814	15	151.94	12.56	57.16	9.72	28.01	3.65	376.31	37.41	39.35	7.62	18.30	2.59
	Jun-09	15	172.39	23.52	63.25	10.13	28.82	7.18	299.50	40.46	34.53	5.31	86.39	50.69
medium	Jun-08	15	263.06	71.18	94.04	22.69	47.50	8.30	545.11	121.06	80.35	21.36	115.44	32.72
	Aug-08	15	92.48	34.65	18.29	16.92	10.98	13.98	131.09	59.70	37.48	36.45	-0.97	11.57
	Jan-09	15	170.78	22.44	62.47	11.36	30.85	6.91	419.74	52.18	46.96	10.20	22.03	2.41
	Jun-09	15	210.16	23.00	89.16	14.89	40.83	7.97	346.93	31.24	70.02	16.18	56.00	22.92
hot	Jun-08	15	150.57	11.53	60.75	5.29	26.31	3.55	386.34	38.68	30.78	6.88	101.95	12.20
	Aug-08	15	208.86	58.70	110.97	49.46	46.11	19.00	389.06	96.56	77.03	33.59	43.23	13.77
	Jan-09	15	241.69	43.13	94.17	15.97	42.47	11.70	529.46	89.87	60.93	21.16	33.53	6.07
	Jun-09	15	191.14	49.83	75.93	16.61	37.93	13.98	333.88	66.84	56.31	18.20	56.39	20.16

Table A.3 Average potential enzyme activity and standard errors for interaction treatment plots at each date, 0-5cm depth, n=3.

Treatment	**date**	**depth**	**β-glucosidase**		**cellobiohydrolase**		**xylosidase**		**phosphatase**		**N-acetyl glucoaminidase**		**leucine-amino peptidase**	
drought*warm	Jun-08	0	218.63	20.81	88.36	11.90	60.89	8.15	496.74	49.19	172.01	5.17	152.51	25.51
	Aug-08	0	121.86	60.19	88.26		151.39	58.68	225.49	128.74	180.57		47.76	15.70
	Jan-09	0	364.51	34.71	137.24	3.06	88.17	16.20	799.36	124.23	156.08	28.75	92.35	28.66
	Jun-09	0	291.26	47.76	125.30	31.49	86.31	25.27	495.67	98.57	179.90	51.29	168.15	51.76
drought*medium	Jun-08	0	282.86	90.58	110.25	30.40	79.67	27.88	571.85	144.81	188.45	57.61	157.24	13.09
	Aug-08	0	249.65	107.77	112.39	72.13	123.74	79.43	429.65	171.78	157.51	65.30	48.66	8.75
	Jan-09	0	549.08	253.62	209.89	104.32	113.57	20.06	998.37	343.54	244.05	75.46	85.28	20.93
	Jun-09	0	231.51	51.18	78.04	15.90	46.02	7.25	307.17	27.91	111.07	19.35	121.29	47.37
drought*hot	Jun-08	0	285.76	72.18	107.58	20.88	69.16	13.14	626.44	150.44	182.01	59.81	161.97	47.45
	Aug-08	0	1030.61	933.14	56.80	29.78	68.16	36.37	177.57	50.64	66.31	19.15	37.00	12.19
	Jan-09	0	272.54	13.10	98.07	2.40	56.15	6.28	542.87	35.91	118.97	10.82	41.72	8.65
	Jun-09	0	261.15	58.54	94.67	25.77	74.33	24.40	367.76	82.52	154.71	24.42	129.54	54.15
wet*warm	Jun-08	0	280.59	29.67	106.31	9.20	74.78	7.47	651.60	56.28	179.99	25.29	161.64	12.66
	Aug-08	0	278.06	23.30	251.90	73.24	146.70	45.72	505.58	56.26	334.59	103.39	51.25	12.25

	Jan-09	0	506.22	54.21	200.24	31.26	133.20	36.46	1227.02	175.90	211.72	32.34	71.80	9.66
	Jun-09	0	526.32	252.15	103.84	16.15	50.55	25.40	410.32	107.63	93.05	47.63	78.53	23.76
wet*medium	Jun-08	0	345.00	103.24	136.89	43.63	81.33	17.96	745.58	120.31	229.14	45.08	162.79	4.31
	Aug-08	0	168.52	75.57	172.89	40.63	122.73	40.56	273.00	129.81	247.69	99.77	83.90	8.87
	Jan-09	0	548.59	108.93	227.82	58.90	181.48	54.37	1736.24	611.56	391.36	133.28	105.68	40.26
	Jun-09	0	217.17	40.01	88.07	20.11	58.24	14.12	400.82	33.70	100.41	19.49	111.16	46.54
wet*hot	Jun-08	0	269.07	34.87	109.76	12.00	64.85	6.87	622.72	73.64	146.17	20.26	171.45	9.14
	Aug-08	0	198.17	53.64	65.62	44.20	69.87	33.41	302.93	119.98	77.25	38.20	55.88	13.57
	Jan-09	0	699.21	372.44	276.04	168.33	165.20	99.43	1673.19	850.99	309.75	171.30	60.37	44.33
	Jun-09	0	363.71	101.66	166.06	43.55	121.56	36.15	639.20	140.07	203.18	72.97	166.40	37.30

Table A.4 Average potential enzyme activity and standard errors for interaction treatment plots at each date, 5-15cmdepth, n=3.

Treatment	**date**	**depth**	**β-glucosidase**		**cellobiohydrolase**		**xylosidase**		**phosphatase**		**N-acetyl glucosaminidase**		**leucine-amino peptidase**	
drought*warm	Jun-08	15	142.69	10.96	62.70	8.37	30.16	1.31	365.59	33.07	53.55	6.22	86.71	16.51
	Aug-08	15	142.44	66.43	99.98	27.37	61.45	15.61	265.03	133.06	94.90	22.21	38.22	12.20
	Jan-09	15	335.58	179.59	79.59	48.02	52.46	16.31	318.28	110.98	44.25	10.10	42.55	12.19
	Jun-09	15	173.34	63.91	82.55	29.61	45.45	16.48	283.24	87.99	80.79	33.45	95.68	33.63
drought*med	Jun-08	15	158.94	51.57	53.96	19.07	26.67	4.99	325.34	69.58	50.12	16.11	108.72	12.02
	Aug-08	15	194.71	76.37	90.74	51.52	36.51	10.94	325.21	48.28	65.26	21.08	54.85	10.35
	Jan-09	15	148.70	26.81	54.68	16.41	26.35	3.36	360.00	13.28	32.15	1.64	40.74	17.53
	Jun-09	15	831.72	672.58	83.36	27.95	44.14	13.68	289.59	28.34	68.07	11.19	98.69	37.76
drought*hot	Jun-08	15	148.82	22.25	59.00	10.38	29.28	2.13	363.87	48.85	49.33	14.89	82.65	8.65
	Aug-08	15	181.82	31.58	81.88	28.29	39.61	2.87	329.91	37.53	62.05	3.58	27.71	16.47
	Jan-09	15	132.91	6.40	48.90	2.32	22.80	2.16	343.89	17.44	34.81	4.29	27.52	5.28
	Jun-09	15	153.07	39.12	57.85	23.80	36.68	11.80	216.38	50.32	52.34	23.39	94.41	40.98
wet*warm	Jun-08	15	160.97	24.33	49.62	5.20	28.99	3.00	413.87	40.45	43.07	12.22	102.22	6.52
	Aug-08	15	242.03	80.30	93.85	31.00	205.13	170.69	373.47	35.34	140.28	88.96	50.03	3.97

	Jan-09	15	154.14	26.71	60.21	12.90	32.72	7.24	470.89	42.24	45.82	10.34	19.91	4.67
	Jun-09	15	209.31	26.65	80.33	14.12	42.21	9.41	375.49	35.76	47.74	10.77	63.09	17.46
wet*med	Jun-08	15	213.66	71.35	69.38	18.33	33.79	7.70	508.14	119.74	69.93	23.91	119.54	13.27
	Aug-08	15	259.09	260.91	313.05	283.77	111.32	101.58	441.39	264.67	195.65	184.09	32.47	18.74
	Jan-09	15	154.88	30.68	61.60	18.76	27.27	10.66	466.51	87.19	47.08	18.18	14.44	4.41
	Jun-09	15	153.54	22.83	59.12	16.10	28.83	10.29	263.62	58.88	49.45	17.39	90.14	20.98
wet*hot	Jun-08	15	224.75	40.06	89.61	14.17	36.88	11.96	452.39	44.38	83.11	19.01	146.64	15.28
	Aug-08	15	732.65	573.45	242.31	179.79	93.78	75.01	2763.12	2506.75	156.26	127.13	34.36	20.52
	Jan-09	15	138.84	20.41	51.97	7.21	25.54	6.54	406.08	39.70	29.70	7.83	14.35	3.32
	Jun-09	15	196.18	37.67	72.22	9.28	55.47	14.87	345.36	51.67	71.28	21.15	74.19	13.66