EFFECTS OF NUTRIENTS, TEMPERATURE AND AERATION STATUS ON ENZYME KINETIC PROPERTIES OF SUBTROPICAL WETLAND SOILS

By

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2013

ACKNOWLEDGMENTS

This dissertation would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this research work. Last but not the least, my family and friends, for staying beside me always, especially during hard times. I dedicate this work completed to my family.

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EFFECTS OF NUTRIENTS, TEMPERATURE AND AERATION STATUS ON ENZYME KINETIC PROPERTIES OF SUBTROPICAL WETLAND SOILS

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August, 2013

ABSTRACT:
Chair: Kanika Sharma Inglett
Major: Soil and Water Science

Extracellular enzyme activity is a rate limiting step of soil organic matter decomposition. These enzyme activities are direct responses of dominant soil microbial communities to nutrient availability, nutrient limitations and the existing external environmental conditions in soils. Soil nutrient limitations also affect microbial biomass and have been shown alter relative abundances of microbial communities thereby, significantly influencing the production and activities of extracellular enzymes. Although there have been few studies investigating enzyme kinetics, most studies have focused on upland soils and there are not many studies on wetland soils. The overall goal of this study was to investigate how the nutrient limitation in soils affects the kinetic parameters of the extracellular enzymes and if these parameters exhibit differences in their temperature sensitivity to soil warming. In this laboratory study, V_{max} and K_m of six extracellular enzymes (phosphatase, bisphosphatase, ß-D-glucosidase, cellobiohydrolase, leucine aminopeptidase, N-Acetyl-ß-D glucosaminidase) was determined in soils from a subtropical wetland exhibiting an increasing gradient of phosphorus (P). Temperature sensitivity of these enzymes were also determined for the

six enzymes at each site to investigate the effect of nutrient gradient on the properties of enzymes. In wetland ecosystems, where the anaerobic conditions prevail, altering water levels can influence the oxygen levels in surface soils thereby affecting the enzyme activities. Therefore both these properties (kinetic and temperature sensitivity) were investigated under aerobic and anaerobic conditions.

Results from this study revealed that maximal activities of C and N enzymes were positively correlated with the soil P concentrations unlike the P enzyme activities. The substrate affinity of the six enzymes also showed significant differences. Significant differences in the temperature sensitivity (Q_{10}) of all enzymes were also observed. Observed differences in the both kinetic parameters and the Q_{10} values determined under both aerobic and anaerobic conditions suggest that the presence of oxygen either directly or indirectly effects the functional activity of the enzymes. Results from this study will help us understand the local adaptation of the soil C, N, P enzymes and may provide insight into how their response to altered external conditions (nutrients, temperature and presence of oxygen) may affect the enzyme products. Results of this study may also be used for modeling patterns of soil decomposition in wetland.

CHAPTER 1
INTRODUCTION

Function and Role of Soil Enzymes

Soil extracellular enzymes have long been recognized as the keys to releasing the energy and nutrients locked in the soil organic matter. It is estimated that global stock of carbon is primarily stored in the soil organic matter (SOM) in ecosystems such as peatlands, wetlands and permafrost (Davidson and Janssens 2006). This is primarily due to the prevailing anaerobic conditions in these ecosystems, under which microbial enzyme activities are either inhibited or severely impeded (Freeman et al 2000). Extracellular enzymes are produced by the soil microorganisms to acquire carbon and other nutrients from complex polymeric compounds by degrading them into simple monomers. These monomers are then taken up by the microorganisms into their cells and this facilitates the nutrient mineralization (nutrient cycling) in soils.

With the ever increasing studies focusing on soil enzymes in agricultural (Bolton, Elliott et al. 1985, Bergstrom, Monreal et al. 1998, Melero, Madejón et al. 2007) and ecological fields (Perucci 1992, Bandick and Dick 1999, Vepsäläinen et al. 2001) the role of soil enzymes in agricultural studies and ecosystem studies are well established. In the past, enzyme activities were used as descriptive parameters of an ecosystem and were measured to report their presence (Visser and Parkinson 1992, Wardle 1992) but now soil enzyme studies are deemed important for understanding soil mineralization processes, soil microbial function, rate of nutrient cycling and ecosystem responses due to global climate change (Sinsabaugh 1994, Waldrop et al. 2004, Sinsabaugh et al. 2005, Sinsabaugh, Lauber et al. 2008). Shi (2011) stated that; due to existence of different enzymes in the soil; significance and function of soil enzymes vary in any given

ecosystem. As for example activity of dehydrogenase is used as an indicator for total soil microbial activity and activity of lignocellulases is indicative of rate of SOM and litter decomposition. It has also been shown that enzyme function is conserved in different ecosystems and therefore can be used as an indicator for particular biogeochemical processes. Sinsabaugh et al (1992) developed a model where they showed that lignocellulose degrading enzymes such as endocellulases and ß-glucosidase are significantly correlated with the mass loss of birch sticks in different ecosystems such as upland, riparian and lotic system. The important functions of enzymes is the 'acquirement of nutrients'. Microbes have developed cost efficient strategies to explore different resources in the system depending upon their nutrient requirement. Allison and Vitousek (2005) stated that economic theories of microbial metabolism can predict the low enzyme production under conditions of abundant nutrients or when the substrates of enzyme reaction such as complex organic are limited. Another topic of interest where the study of enzymes has made significant contribution is the carbon sequestration. For example Carriro et al (2000) showed that low lignocellulose index (LCI) in dogwood litter increased nitrogen availability which caused the stimulation of both cellulases and phenol oxidase activity (Carreiro et al. 2000). Considering the wide range soil enzymes and their widespread distribution in the different ecosystems, makes it an important research area in several fields including ecology, forestry, agronomy and environmental sciences.

Soil Enzymes Importance in General

Enzymes are proteins that catalyze transformations of many chemical compounds in soils and do not get altered during the process. Most enzymes are distinguished by the adding a suffix 'ase' to the end of the compound they act upon. For

example ‘glucosidase’ acts upon the substrate glucoside. Enzymes in general belong to one of the six classes that are recognized by the International Enzyme Commission (http://www.chem.qmul.ac.uk/iubmb/enzyme/). These classes are oxidoreductase, transferase, hydrolase, lyase, isomerase and ligase. Most common soil enzymes fall one of the two classes 1. Oxidoreductase enzymes that catalyze the oxidation - reduction reactions in which oxygen and hydrogen are gained or lost and 2. Hydrolase enzymes which proceed with hydrolytic cleavage of bonds. The specific ability of specific enzymes to carry out a reaction using a wide or narrow range of substrates is called as the specificity of the enzyme that can be ‘broad range’ or narrow range’.

This large diversity of soil enzymes can regulate a wide range of biogeochemical reactions and therefore serve an important role in biogeochemical cycling of nutrients. For example production of microbial phosphatase enzyme is mostly induced under P-limitation conditions and the hydrolytic reaction causes the phosphate group from monoesters to be released for uptake by the microbes. Dehydrogenase enzymes are used as oxidative index of cell and therefore used as an indicator of soil microbial activity (Chu, Lin et al. 2007) and cellulases play an essential role in decomposition of soil organic matter and plant litter (Sinsabaugh 1994). Other common enzymes are amylase that plays a significant role in the breakdown of starch ß-glucosidase is involved in catalyzing the hydrolysis and degradation of various glucosidic substrates present in SOM. Chitinase or chitinolytic enzymes (like NAG) are key enzymes responsible for the degradation and hydrolysis of chitin. Commonly studied soil enzymes involved in P nutrient cycling are phosphatase and bisphosphatase. Important soil enzymes for C nutrient cycling are ligninase, cellulase, glucosidase and

xylosidases. For N-nutrient cycling important enzymes include leucine amino peptidase, N-acetyl glucosaminidase and chitinase. Measuring activities of all these enzymes can reveal the microbial functional diversity in different ecosystems. Generally soil enzymes are associated with different abiotic and biotic factors present in the soil such as soil aqueous phase, clay particles, viable cells and dead microbial cells. But the enzyme activities in soils represent the total effect of these individual component. Shakel and Freeman et al (2000) conducted a study where they demonstrated that manipulating quantity and quality of carbon supply in a constructed wetland can alter the microbial extracellular enzyme activities (Shackle, Freeman et al. 2000). Therefore enzyme activities are a direct expression of the microbial community to metabolic requirements and available nutrients.

Soil Enzyme Kinetic Parameters (Vmax and Km)

The enzyme function is more evident when Michaelis-Menten kinetics [maximal rate of velocity (Vmax) and half-saturation constant (Km)] of different enzymes are studied. As per the kinetic theory for a reaction to occur the substrates come together and they need activation energy to complete the reaction. Enzymes act as catalysts and lower the requirement for the activation energy that is needed to complete the reaction. For a catalyzed reaction in a system with constant amount of enzymes, as the substrate concentration increased from zero the kinetics first follow the first order kinetics. With increasing substrate concentrations the rate of initial velocity starts to decline till no change in the initial velocity occurs. The reaction then reaches the zero order reaction. The hypothesis followed for this in general theory of enzyme kinetics is that the enzyme and substrate form a complex to react and this complex is reversible. On this basis the

Michaelis-Menton equation was formulated. This equation makes few assumptions. 1) The initial rate of reaction is first order and it changes to zero order kinetics. 2) Enzyme and the substrate is bound reversibly during a reaction and the same enzyme can be used several times.3) the uncoupling of the enzyme and substrate complex to release the product is the rate limiting step for the enzyme reaction rate. The initial rate of reaction is reflective of that. Vmax is achieved when most of the free enzymes are bound in the enzyme-substrate complex. That is when the state of 'substrate saturation' is achieved. Therefore the enzyme kinetic parameters are studied by using the following Michaelis-Menten equation:

$$V = Vmax \frac{\lfloor S \rfloor}{(Km + [S])}$$

Where Vmax is the maximum rate of enzyme activity which is the highest enzyme activity for particular enzymes in a definite substrate concentration after which if substrate concentration increases; the velocity of enzyme reaction does not increase (Figure 1-1). Km is the Michaelis-Menten constant, S is the substrate concentration at any given time (Figure 1-1).

Lower Km values for enzymes generally higher affinity of that enzyme for a given substrate and can indicate higher efficiency of the enzymes. This value is easily determined using graphs that plot the reciprocal of the initial velocity of the enzyme-substrate reaction (on y axis) with the reciprocal of the substrate concentrations used in the reaction (x axis). Depending upon other environmental factors such as temperature, nutrient, water level condition, the enzyme kinetic properties, Vmax and Km may change. Most of the studies measure the Vmax because measurement of Km can be challenging due to uncertainty of relative contributions of artificial substrates relatively

over naturally occurring substrate under non saturating condition (Stone et al. 2012). Soil enzyme kinetics (V_{max} and K_m parameter) may change due to nutrient addition on that system. For instance if nutrient addition causes more microbial growth, then it may increase enzyme activities (V_{max}). Moreover previous studies have reported that long term N input results in a shift in microbial communities (Frey et al. 2004, Wallenstein et al. 2006, Allison et al. 2007, Allison and Martiny 2008, Nemergut et al. 2008) which then may produce different types of isoenzymes with altered active sites and those that exhibit different enzyme substrate affinity (Km) (Stone et al. 2012).

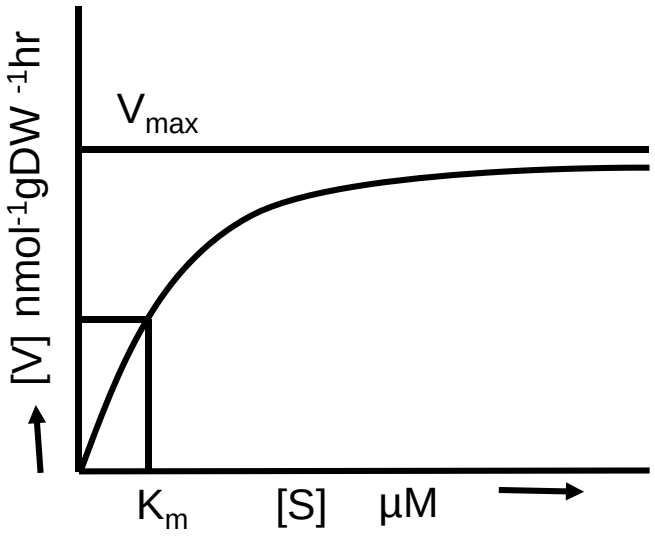


Figure 1-1. Enzyme activity as a function of substrate concentration

Factors Regulating Soil Enzyme Activities

Microbial enzyme production and activity is regulated by the soil nutrient content and availability of enzyme substrates and products. These are often considered as the rate limiting step for soil organic matter decomposition (Chróst and Rai, 1993). Soil enzymatic potential for hydrolyzing the labile components of soil organic matter is

correlated to substrate availability, soil pH and the stoichiometry of microbial nutrient demand. Soil extracellular enzymes released in the soil are sensitive to soil micro environmental conditions temperature, moisture, pH, etc. (Shackle et al. 2000). In wetland and aquatic systems only a small portion of large molecular weight compounds are easily available to the microbial communities (Benner et al, 1994; Chrost, 1991). These large macromolecules are then hydrolyzed by extracellular enzymes to low molecular weight compounds which then readily taken up by the microorganisms and utilized as a source of nutrients and energy source (Chrost, 1991). Like other ecological processes extracellular enzymes activitivities in an ecosystem can be regulated by different environmental variables including nutrient availability or limitations, temperature and aeration conditions (Stone et al. 2012; Sinsabaugh 1994). These three variable are discussed below.

Nutrients

Soil enzyme activities are direct responses of microbial communities present there and their response to nutrient requirements. Soil microbes and plants produce extracellular enzymes to mineralize the organically bound nutrients. Depending upon the nutrients availability, microbial biota may change their enzyme production to increase or decrease the enzyme pool in a system to maintain their supply of inorganic N, P and C (Olander and Vitousek 2000). Increased nutrients in wetland due to inflow from external point and non-point sources like the agricultural runoff has been shown to affect the biogeochemical processes, alter microbial community structure and stimulate growth of plants thereby affecting soil organic matter quality (Debosz et al. 1999; McLatchey,1998). Because the first step in organic matter decomposition is the

extracellular enzymatic degradation, the enzyme activities can reflect the changes in an ecosystem.

Determination of soil enzyme activities can therefore be important to understand the nutrient cycling in a given ecosystem (Kardol et al. 2010). Depending upon P and N availability; P, N and C mineralization rate may change. This was well demonstrated in a study by Penton and Newman (2007) where they reported that different N and P availability in wetland soils can change carbon mineralization processes (Penton and Newman 2007). Allison and Vitousek (2005) observed that P mineralization are most related to N availability (Allison and Vitousek 2005). Economic theory of microbial metabolism reflects the enzyme allocation pattern in soil which helps microbe to produce enzyme when simple nutrient are scarce and complex nutrient are abundant (Allison and Vitousek 2005). Not only does the simple and complex nature of resources but their combination effects enzyme activity (production and kinetics) but the activity is also dependent on the microbial demand and requirement of C and N for the enzyme synthesis. Therefore a sufficient nutrient supply is prerequisite for enzyme synthesis. Soil organic matter is the major reservoir of C and it also represents the nutrient source for plant (after microbial decomposition). It is generally accepted that fresh organic matter increases the decomposition rate by giving higher available energy to the microorganisms. But there is a competition between the microorganisms which use fresh organic matter to those which use polymerization of SOM (Fontaine, Mariotti et al. 2003). Relative concentrations of compounds of different qualities can further influence the SOM degradation as the assimilable resources may inhibit the decomposition of recalcitrant soil compounds. Based on allocation theory, higher C: P and N: P ratio in

SOM have been shown to initiate more immobilization of P by microorganisms whereas lower C: P and N: P initiate more P mineralization processes. Phosphorous is an important nutrient for microbial growth. Some studies on P limited systems have shown that P addition stimulate microbial biomass and soil enzyme activities (Debusk and Reddy 2005). On the other hand, there are studies that have observed no effect on P addition (Tate et al. 1991).

Effect of nutrient concentrations in soils can change the Vmax and Km responses. Stone et al (2012) observed that N fertilization of soils increased Vmax responses for hydrolytic enzymes. They have also observed that depending upon the nutrient availability or limitation microbes changed their both Vmax and Km parameters (Stone et al. 2012).

Temperature

In any chemical and biological reaction, the activity of enzyme is expected to double with the rise of 10°C temperature. The rate of enzyme activity increases until it reaches a high temperature after that it start to decrease its activity due to enzyme inactivation. This sensitivity of enzymes to increasing temperature is referred to as Q_{10} values. A Q_{10} value 2 reflects the factor by which the rate of enzyme activity changes with increase in 10° C of temperature. Several studies have focused on the temperature sensitivity of soil enzymes (Conant et al., 2011) in order to understand the differnces in the biodiverstiy of enzymes in a soil and to predict the changes in ecosystem functioning in response to soil warming and seasonal changes. Studies focusing on soils in upland ecosystems along latitudinal gradient have shown that cold adapted enzymes are more sensitive to temperature than warm adapted enzymes (Koch et al. 2007). The reason behind the temperature sensitivity of cold adapted protein is the loss

of its function with an increasing temperature. In response to changing temperature, enzyme function is more evident when Michaelis-Menten Kinetic parameters [maximal rate of velocity (Vmax) and half-saturation constant (Km)] of different enzyme and studied under different temperatures. Most of the studies concerned with temperature effect on Vmax rather than Km. Km is the substrate concentration at which haft-maximal velocity (Vmax) reached. If temperature increases then it will positively correlate with Vmax but up to certain limit. On arriving at the optimal temperature the activation energy increases and beyond which the enzyme substrate complex is destabilized. Decreased substrate affinity *i.e* higher Km value (Stone et al. 2012) with increasing temperatures can negate the positive effect of temperature on Vmax at low substrate concentration (Stone et al. 2012). Therefore, the absence of temperature response of enzymes can sometimes be due to opposite responses of the two kinetic parameters Km and Vmax (Davidson et al. 2006). On the other hand, substrate availability (substrate diffusion) can change in response to temperature and thus the temperature sensitivity of the whole reaction can be higher than that of each other individual process (Davidson et al. 2006).

There are two main aspects of temperature sensitivity of enzymes, which are 1) Thermal stability of enzymes within a certain range of temperature and 2) temperature sensitivity of enzyme active site (Wallenstein et al. 2011). Molecular structure of enzyme active site is weaker than the whole enzyme (Zou 2000) as a result the active site may denatures before the whole enzyme is denatured. Different enzymes may show different temperature sensitivity due to differences in their activation energy. Previous studies have reported the range of temperature sensitivity of soil enzymes (Olander and

Vitousek, 2000) and determination of this information has been used to predict the increases in soil respiration (CO_2) under increasing temperature (Stone et al. 2012). Studies have also shown that soil potential enzyme activities are less temperature sensitive than carbon mineralization i.e Q_{10} values<2 (Tabatabai, 1982). Differences in temperature sensitivities within different enzymes has also been reported. Koch et al (2007) showed a lower temperature sensitivity of aminopeptidase with respect to C processing enzymes (glucosidase, xylosidase and N-acetyl glucosaminidase) which suggest that temperature is an important factor in regulating the use of different substrate (Koch et al. 2007).

Aerobic and Anaerobic Condition

Soil moisture content is an important factor for SOM decomposition especially in the upland ecosystems because moisture can be a limiting factor for microbial growth and activity. Several studies have reported a moisture level optima for soil respiration and mineralization processes. Low water level can lower the intercellular water potential thus reduce the hydration and enzyme activity and can lower the substrate availability by limiting substrate diffusion in soils. But these factors are more relevant to upland soils. In wetland soils where the water level may fluctuate, soils can still remain saturated but the oxygen levels can vary. Therefore the prevalence of aerobic and anaerobic conditions commonly occur in wetland systems.

Although some studies reported differences in synthesis of hydrolase enzymes under aerobic and anaerobic conditions, in steady state of aerobic or anaerobic condition extracellular enzymes do not have any significant differences in enzyme activity (Goel et al. 1998). For instance anaerobic or aerobic conditions did not appear to effect the activity of alkaline phosphatase and acid phosphatase unlike the protease

activity which was found to be 40-75% higher under anaerobic condition than aerobic conditions (Goel et al. 1998). Water logging is important factor in regulating wetland biogeochemistry. Lowering water table may also exhibit properties that have resemblance of another ecosystem such as lowering of methane and dissolve organic carbon release and increased release of stored C as CO_2 and increase release of inorganic nutrients (Freeman et al. 1997). Recent concern about climate change can reduce water logging condition frequently which may change wetland biogeochemistry under that condition (Manabe and Wetherald 1986. As mentioned before, the Michaelis-Menten kinetics parameters of the enzymes can exhibit a more complete picture of how the biogeochemical reactions will respond to soil warming and temperature change then just measuring one parameter (V_{max}). Several upland studies have shown the measurement of both kinetic parameters to estimate the response of an activity to temperature increase, not many studies have addressed this in the wetland ecosystems. In the past, studies have determined the V_{max} of some soil enzymes, they have never been measured in conjunction with the K_m values. Because different enzymes behave differently under water saturated conditions where anaerobic conditions exist and the drained conditions where the aerobic conditions exist it is important to study the effect of increasing temperature on wetland soil enzymes under both aerobic and anaerobic conditions.

Conceptual model

Conceptual model (provided below, Figure 1-2) shows a summary of major factors that can affect the enzyme activities in a given system. Overall general enzymatic activity in soils is regulated factors that fall under two broad categories that are substrate related and site related (Sinsabaugh, 1994). Site related factors are

moisture and nutrient availability (or limitation). These factors can directly influence the microbial biomass growth or relative abundance of microbial groups which ultimately affect the enzyme activity (V_{max} and K_m) and via enzyme production (enzyme pool). On the other hand, substrate related factors are substrate concentrations, substrate diffusion and substrate stability in soils. Any changes in these factors can affect either one or both factors of enzyme kinetics. Both site and substrate related factors may also be affected by temperature. Temperature may indirectly effect the enzyme activity by stimulating the microbial biomass or by increasing the available substrate or directly affect enzyme activity by changing its activation energy. Therefore, the nutrient availability/limitation and temperature effects on enzyme kinetics are critical to understand the first step of SOM decomposition in a given system.

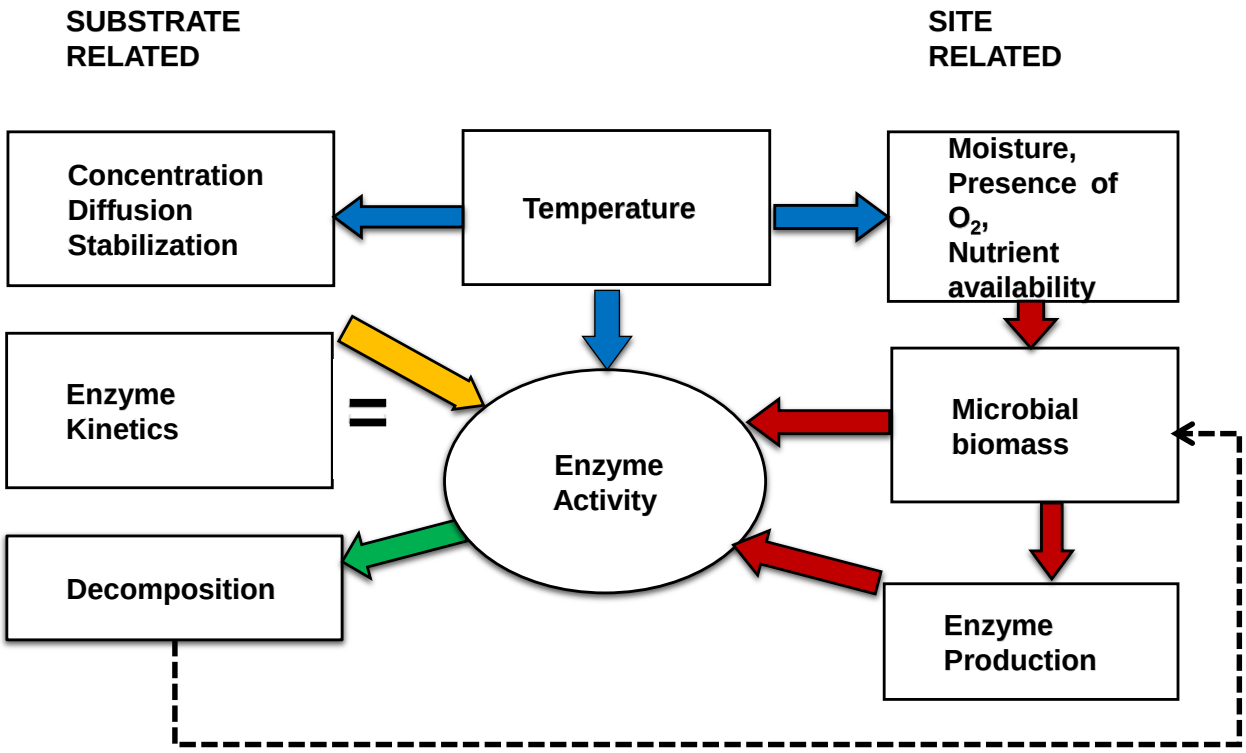


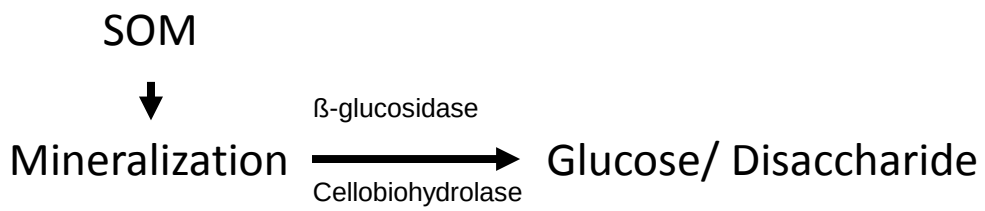
Figure1-2. Conceptual diagram of enzymatic factors which regulate enzyme kinetics.

Commonly studied Soil Enzymes

Acquisition of nutrients is one of the major functions of soil microorganisms that produce extracellular enzymes in soils. These enzymes act upon the detrital pool associated with soil organic matter and maintain the nutrient cycle in the soil. The extracellular enzymes commonly studied in soils belong to those involved in C, N and P cycling in soils. Examples of some of these enzymes are described below.

Carbon Processing Enzymes

Most important carbon processing enzymes in the soils are ß-glucosidases and cellobiohydrolase.



Both these enzymes are hydrolytic enzymes. There is another category of oxidoreductases (phenol oxidase and peroxidase) that are also studied in soils. ß-glucosidase (BGLU) is important enzyme in microbial decomposition processes which breakdown cellulose to glucose which acts upon two glucose molecule linked by ß1-> 4 bonds. This enzyme is included in the category of glucosidases that hydrolyze disaccharides. ß-glucosidase is the most prominent soil enzymes among all the glucosidase enzymes and it responds more quickly than other soil variables. Therefore it can be used as an indicator for biological changes (Bandick and Dick 1999). Previous studies have shown that after 2 years of cover crop initiation ß-glucosidase activity increased significantly whereas total carbon content remain same in the soil (Dick 1994). Figure 1-3a shows the reaction process of ß-glucosidase acting upon cellulose to produce glucose monomers. Herrandez et al (2003) observed highest ß-glucosidase (collected from Yeast extract) activity in between pH 4 to 5 and a temperature range of 40 -50°C (Hernández, Espinosa et al. 2003). Moreover they also observed a drop in 25% activity under anaerobic conditions. Another important C enzyme is Cellobiohydrolase (CBH). This enzyme catalyzes the degradation of cellulose chain by binding to the terminal end and cleaving off cellobiose units from the end of cellulose polymer. Glucosidase then hydrolyze those cellobiose to glucose.

Phosphorous Processing Enzymes

The two common enzymes that are generally studies in soils are the monoesterases that include the acid and alkaline phosphatases and the diesterases that include the bis phosphatases. Phosphatase (PHO) catalyze the hydrolysis of esters and anhydrides of phosphoric acid. Acid phosphatase are most active in soils that have lower pH values and the alkaline phosphatases are active in alkaline soils (Figure 1-3. c). Phosphatase is correlated with soil P stress and plant growth and has been shown to play an important role in soil fertility (Dick et al. 1992). Low soil P leads to adaptation of plant having a transcription activity that is biased towards acid phosphatase which tends to increase high P stress (Tarafdar and Jungk 1987, Haran et al. 2000). Phosphomonoesterases are expected to be higher in a system which has higher quantity of organic carbon. Soil pH also plays a significant role in the synthesis, release and stability of this enzyme. Alkaline phosphatase activity is used as an indicator of P limitation (Wojewodzic, Kyle et al. 2011) and its activity has been shown to increase by a factor of 1.67 for every 10°C temperature increase (Wojewodzic, Kyle et al. 2011). So when alkaline phosphatase activity is used as a P indicator; the temperature of the system and assay should be considered. Soil P is generally negatively correlated to the activity of alkaline phosphatase.

Phosphodiesterase (bisphosphatase, BPHO) is an enzyme which breaks phosphodiesterbonds (Figure 1-3 b). Phosphodiesterase activity was first detected by Ishii and Hayano (1974). They discovered it by using systhetic substrate which was later found in various plants and microoganisma and soils (Browman and Tabatabai 1978, Margesin and Schinner 1994).

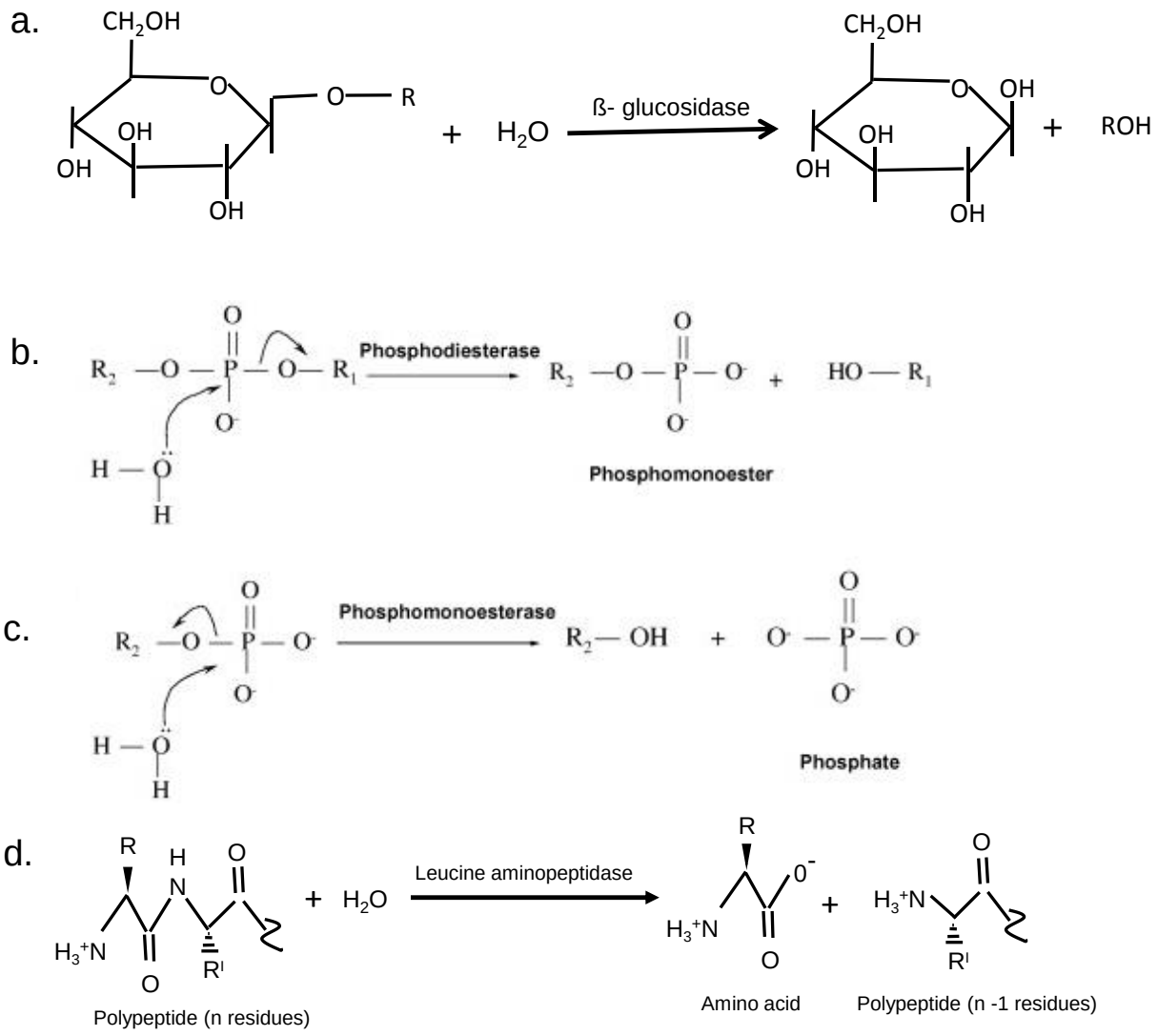


Figure 1-3. Enzyme substrate reaction and their release product

Nirogen Processing Enzymes

Two important nitrogen processing enzymes are leucine amino peptidase (LAP) and N-acetyl glucosaminidase (NAG). Aminopeptidases mainly target N-terminal residues of protein and peptides (Figure 1-3 d). These enzymes have a high pH and temperature optima. N-acetyl glucosamine hydrolyzes *N*-acetyl-β-D-glucosamine

residues from the terminal non-reducing ends of chitin oligosaccharides. This enzyme has optimum pH 5.5 and temperature 63°C (Parham and Deng 2000). Previous studies showed that soil nitrogen mineralization in North Central zone of United States are significantly correlated to N-acetyl glucosamine activity and arylamidase (Dodor and Tabatabai 2007). Alisson et al in (2008) also observed significantly lower activity of protein and chitin degrading enzyme activity in presence of nitrogen fertilization (Allison, Czimczik et al. 2008).

In soil inverse relationship between phosphorous and phosphatase enzymes are more common than a relationship between available nitrogen and nitrogen processing enzymes (Allison and Vitousek 2005). The reason behind this is the association of nitrogen tightly with soil organic carbon. As a result mineral nitrogen can be produce as a byproduct of carbon decomposition. So the nitrogen mineralization will not be a negative relation with soil available nitrogen.

Research Goal

The overall goal of this study was to understand how the importance of nutrient limitation on the activity (kinetic parameters) and the inherent property (temperature sensitivity) of the extracellular C, N, and P-acquiring enzymes in subtropical wetland soils under different aeration status. In other words this study aimed to generate a complete assessment for the effectiveness of microbial enzymatic response which can act as an indicators of different ecological perturbation such as temperature, different water table scenario and nutrient variation. This information will be helpful to clearly understand the microbial mediate decomposition of OM and different nutrient cycling.

This study can provide an insight, how biogeochemical processes create a global pattern in ecological stoichiometry and organic matter storage.

Specific Objectives

The research goal was achieved by identifying the specific objectives for this study which are as follows

1. Determine the Michaelis-Menten kinetic parameters of C, N, and P acquiring enzymes along a nutrient gradient in subtropical wetland soils and compare the parameters of the same enzyme across the nutrient gradient and all enzymes within each specific site.
2. Determine and compare the temperature sensitivities of the C, N, and P acquiring enzymes within each site and for the same enzyme across the nutrient gradient.
3. Determine the effect of aeration status (aerobic versus anaerobic) on the above mentioned parameters.

Site Description

Everglades water conservation area (WCA 2A) is located in South Florida and is a part of the Florida Everglades that is maintained by the South Florida Management District. The Everglades covers approximately 9,000km^2 (approximately 1.5 million acres) and is the largest subtropical wetland in the United States. The movement of water in an aquatic ecosystems is a fundamental construct of ecosystem structure and function. Water flows are closely linked to water levels, and their alterations have caused environmental damage. The Everglades has its identity about the slow

movement of water across the vast, low gradient, wetland landscape. External nutrient loading by the agricultural outflows into the wetland has led to eutrophication near the inflow regions where the indigenous habitat and the oligotrophic ecosystem has been replaced by a P enriched eutrophic system with altered vegetation and soil biogeochemistry. However the ecology of this system is still maintained and the oligotrophic region exists further away from the inflows thereby creating an impressive P gradient in the WCA-2A area. Samples for the current study were collected from five different areas along through the transect with different P concentrations in peat soil of WCA-2A. The sampling sites were in five zones that vary according to vegetation and P concentration. F1 is the impacted site, F4 is moderate impacted site and U3 is unimpacted site (Figure 1-4). Whereas F4 and U3 both have slough and ridge both vegetation types. The ridge and slough are one of several major habitat types in Everglades, which is generally consist of dense saw grass ridges with an adjacent open slough. The ridge and slough systems makes the deeper central portion of the Everglade basis. The saw grass ridge represent the topographically higher and shorter hydro period than adjacent slough. Bernhoard Ce et al mentioned in their studies that pollen data indicate that saw grass ridges and sloughs have been vegetationally distinct since the initiation of Everglade wetlands.

Microbial community responses have been studied in Everglades WCA-2A in impacted, intermediate and unimpacted site. Studies also revealed that different microbial biomass (C, N, and P) number in impacted and intermediate site compared to unimpacted site (Table 1-1). We also provided the studied enzyme activity on Everglades Water Conservation area and different wetland and lakes all over the

country (Table 1-2). Soil enzyme activities on decomposition aspect have been studied several times. Here so far to some extent we want to study microbial enzymatic response in different P concentration, different temperature and also in different water table.

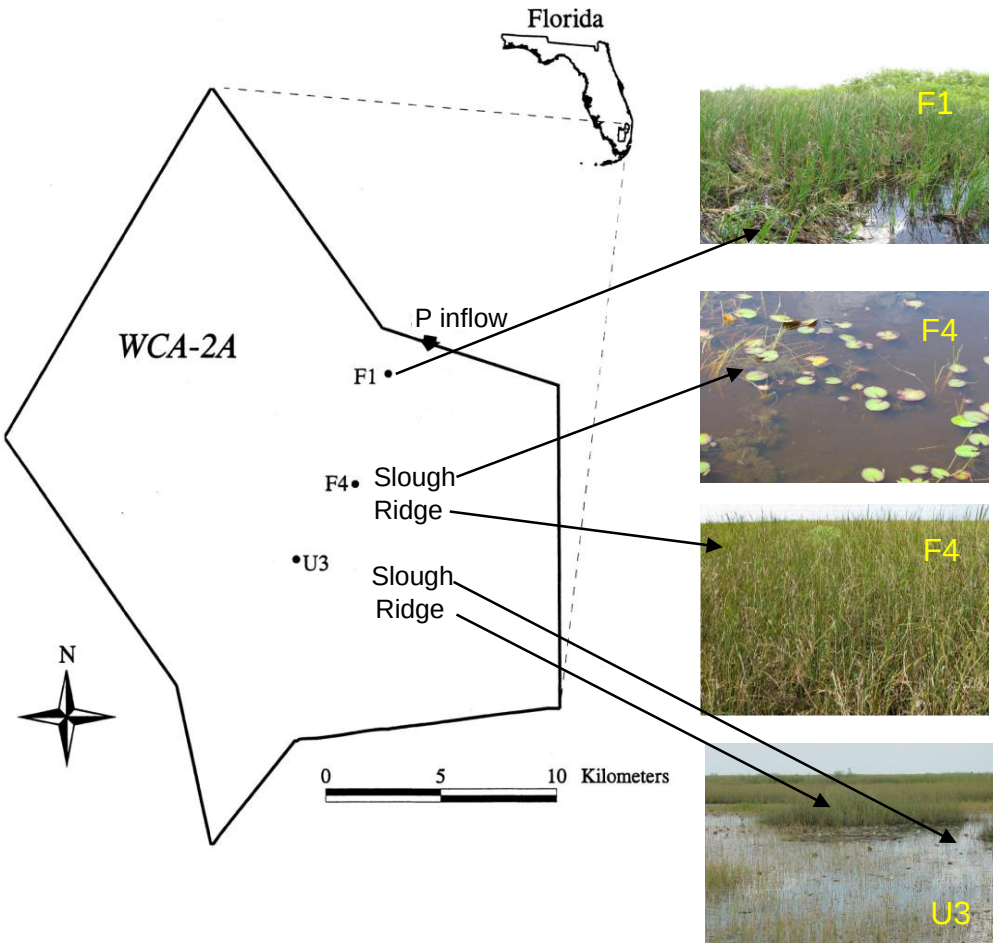


Figure 1-4. Location of sampling sites in WCA-2A. The F1 is the high Phosphorous zone dominated by *Typha*, F4-S is moderate Phosphorous zone dominated by *Nymphea* and F4-R dominated by *Cladium*, U3-S is the pristine zone dominated by *Nymphea* and U3-R by *Cladium*

Hypotheses

This study was conducted in this wetland region where in the enzymes response to the P-gradient was investigated. Loading of P in historically low P freshwater wetland Everglades, over 40 years affected the nitrogen to phosphorous and carbon to phosphorous ratio. Understanding response of microbial enzyme kinetics to in situ P concentration and temperature is important for predicting the future release of soil C to the atmosphere. Change in enzyme kinetics (Vmax and Km) in response to temperature change has been previously reported in several upland systems (Stone et al. 2012). This temperature response may vary with in situ nutrient concentrations. To our knowledge there were no other studies that have focused on temperature sensitivities of enzyme kinetics in wetland soils under nutrient (Phosphorous) gradient in Everglade Water Conservation Area 2A. Based on literature reviews we hypothesized the following:

Hypothesis 1: Based on the fact that nutrient limitation and availability is correlated with soil enzyme activity we hypothesized that as per the allocation theory higher Vmax (maximal reaction velocity) and low Km (higher substrate affinity) for C and N processing enzymes will exist in sites with high P concentration (Figure 1-5). Inversely, due to the P limitation in sites with low P, the microorganisms will allocate their resources towards the P acquiring enzymes thereby increasing the Vmax and lowering the Km of P enzymes in low P sites (Figure 1-6).

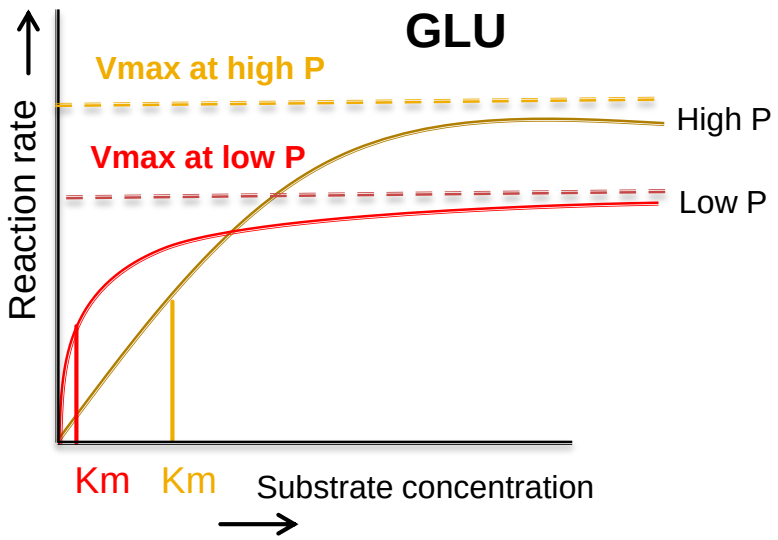


Figure 1-5. Expected enzyme kinetics in GLU

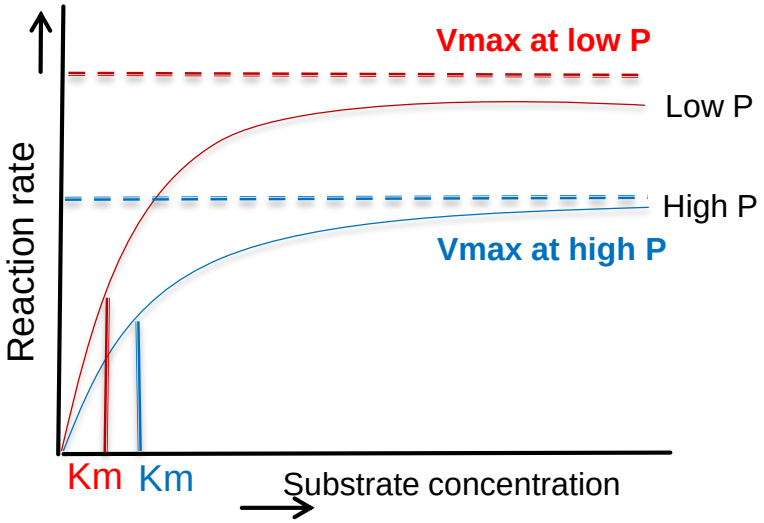


Figure 1-6. Expected enzyme kinetics in PHO

Hypothesis 2 Enzymatic activity is also affected by temperature changes. Based on previous studies it is known that temperature increase will increase enzyme activity (Vmax) and will lower enzyme substrate affinity (Km) (Stone et al. 2012). Because nutrient availability also plays an important role in enzyme kinetics, in this study we expected to find an interactive effect of temperature and nutrients on the microbial

enzyme kinetic parameters. According to biochemical theory (Davidson et al. 2006) and based on previous studies on digestive enzymes (Somero 1978, Somero 2004) we hypothesized that Vmax and Km would increase with temperature but the temperature sensitivity of each enzyme will be different under different in situ nutrient concentrations. Moreover we hypothesized that nutrient concentration difference will change Q_{10} values of same enzymes along the sites (Figure 1-7)

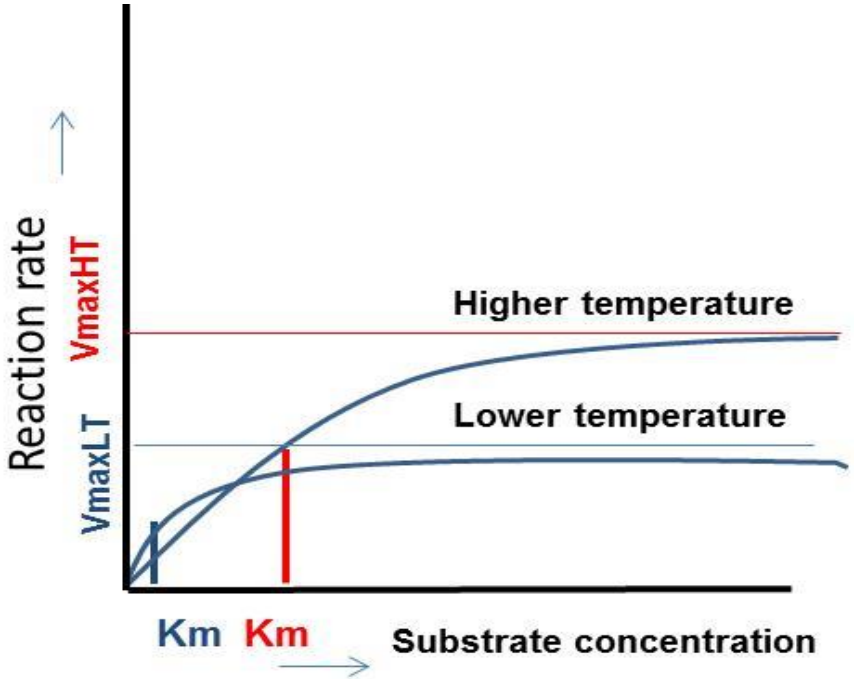


Figure 1-7. Expected enzyme kinetics under high and low temperature

Hypothesis 3: The aeration status was not expected to change the microbial enzyme activities because the enzymes investigated were mainly the hydrolytic enzymes.

Table 1-1. Previous biogeochemical information of the research site

Biogeochemical parameters	F1	F4	U3
TP(mg kg^{-1})	1355	707	305
TC(g kg^{-1})	419	377	358
TN(g kg^{-1})	25.1	24.7	26.8
MBP(mg kg^{-1})	159	237	73
MBC(g kg^{-1})	7.5	12	9
MBN(mg kg^{-1})	1019	1709	897
C:N	17:1	17:1	14:1

(Corstanje et al. 2007)

Table 1-2. Range of values observed for different enzyme activities in different wetland soils and lake sediments in different seasons

Enzymes	Depth	Enzyme activity	Units	Month	Site	Reference
PHO	0-10cm	1.8	mg $g^{-1}h^{-1}$(P-nitophenol)	Feb	Everglades	Wright *et.al.,*2001
PHO	0-10cm	9.9	mg $g^{-1}h^{-1}$(P-nitophenol)	May	Everglades	Wright *et.al.,* 2001
PHO	0-10cm	2.3	mg $g^{-1}h^{-1}$(P-nitophenol)	Aug	Everglades	Wright *et.al.,*2001
PHO	0-10cm	3.8	mg $g^{-1}h^{-1}$(P-nitophenol)	Mar	Everglades	Wright *et.al.,*2001
BGLU	0-10cm	0.08	µmols $^{-1}$g AFDM $^{-1}$h		WCA-2A-enriched	Penton *et.al.,*2007
BGLU	0-10cm	0.05	µmols $^{-1}$g AFDM $^{-1}$h		WCA-2A-Reference site	Penton *et.al.,*2007
LAP	0-10cm	1.95	µmols $^{-1}$g AFDM $^{-1}$h		WCA-2A-Enriched site	Penton *et.al.,*2007
LAP	0-10cm	2.16	µmols $^{-1}$g AFDM $^{-1}$h		WCA-2A-Reference site	Penton *et.al.,*2007
PHO	0-10cm	1.35	µmols $^{-1}$g AFDM $^{-1}$h		WCA-2A-Enriched site	Penton *et.al.,*2007
PHO	0-10cm	1.14	µmols $^{-1}$g AFDM $^{-1}$h		WCA-2A-Reference site	Penton *et.al.,*2007
BGLU	0-10cm	0.39	µmols $^{-1}$g AFDM $^{-1}$h		WCA-3A-enriched	Penton *et.al.,*2007
BGLU	0-10cm	0.21	µmols $^{-1}$g AFDM $^{-1}$h		WCA-3A-Reference site	Penton *et.al.,*2007
LAP	0-10cm	2.87	µmols $^{-1}$g AFDM $^{-1}$h		WCA-3A-Enriched site	Penton *et.al.,*2007
LAP	0-10cm	2.31	µmols $^{-1}$g AFDM $^{-1}$h		WCA-3A-Reference site	Penton *et.al.,*2007

PHO	0-10cm	0.80	µmols $^{-1}$g AFDM $^{-1}$h	WCA-3A-Enriched site	Penton *et.al.,*2007
PHO	0-10cm	2.18	µmols $^{-1}$g AFDM $^{-1}$h	WCA-3A-Reference site	Penton *et.al.,*2007
CBH	0-15cm	140	mg $g^{-1}h^{-1}$	EVA(Forest)	Ye, R., *et.al.,*2009
CBH	0-15cm	51	mg $g^{-1}h^{-1}$	EVA(sugarcane)	Ye, R., *et.al.,*2009
CBH	0-15cm	191	mg $g^{-1}h^{-1}$	EVA(Turf)	Ye, R., *et.al.,*2009
CBH	0-15cm	271	mg $g^{-1}h^{-1}$	EVA(Pasture)	Ye, R., *et.al.,*2009
LAP	0-15cm	1.42	mg $g^{-1}h^{-1}$	EVA(Forest)	Ye, R., *et.al.,*2009
LAP	0-15cm	1.96	mg $g^{-1}h^{-1}$	EVA(sugarcane)	Ye, R., *et.al.,*2009
LAP	0-15cm	3.64	mg $g^{-1}h^{-1}$	EVA(Turf)	Ye, R., *et.al.,*2009
LAP	0-15cm	2.58	mg $g^{-1}h^{-1}$	EVA(Pasture)	Ye, R., *et.al.,*2009
BGLU	0-10cm	581	nmol g $C^{-1}h^{-1}$	Lake superior	Hill, B.H., *et.al.,*2006
BGLU	0-10cm	632	nmol g $C^{-1}h^{-1}$	Lake Huron	Hill, B.H., *et.al.,*2006
BGLU	0-10cm	1262	nmol g $C^{-1}h^{-1}$	Lake Michigan	Hill, B.H., *et.al.,*2006
BGLU	0-10cm	610	nmol g $C^{-1}h^{-1}$	Lake Erie	Hill, B.H., *et.al.,*2006
LAP	0-10cm	185	nmol g $C^{-1}h^{-1}$	Lake superior	Hill, B.H., *et.al.,*2006
LAP	0-10cm	8225	nmol g $C^{-1}h^{-1}$	Lake Huron	Hill, B.H., *et.al.,*2006

LAP	0-10cm	904	nmol g $C^{-1}h^{-1}$	Lake Michigan	Hill, B.H., *et.al.,*2006
LAP	0-10cm	3428	nmol g $C^{-1}h^{-1}$	Lake Erie	Hill, B.H., *et.al.,*2006
LAP	0-10cm	901	nmol g $C^{-1}h^{-1}$	Lake Ontario	Hill, B.H., *et.al.,*2006
PHO	0-10cm	1143	nmol g $C^{-1}h^{-1}$	Lake superior	Hill, B.H., *et.al.,*2006
PHO	0-10cm	3202	nmol g $C^{-1}h^{-1}$	Lake Huron	Hill, B.H., *et.al.,*2006
PHO	0-10cm	1940	nmol g $C^{-1}h^{-1}$	Lake Michigan	Hill, B.H., *et.al.,*2006
PHO	0-10cm	3304	nmol g $C^{-1}h^{-1}$	Lake Erie	Hill, B.H., *et.al.,*2006
PHO	0-10cm	1028	nmol g $C^{-1}h^{-1}$	Lake Ontario	Hill, B.H., *et.al.,*2006
NAG	0-10cm	185	nmol g $C^{-1}h^{-1}$	Lake superior	Hill, B.H., *et.al.,*2006
NAG	0-10cm	223	nmol g $C^{-1}h^{-1}$	Lake Huron	Hill, B.H., *et.al.,*2006
NAG	0-10cm	221	nmol g $C^{-1}h^{-1}$	Lake Michigan	Hill, B.H., *et.al.,*2006
NAG	0-10cm	461	nmol g $C^{-1}h^{-1}$	Lake Erie	Hill, B.H., *et.al.,*2006
NAG	0-10cm	144	nmol g $C^{-1}h^{-1}$	Lake Ontario	Hill, B.H., *et.al.,*2006

CHAPTER 2
METHODS

Site Description and Soil Sampling

For this study, soils were collected from Florida Everglades Water Conservation Area (WCA-2A) that covers an area of 54700 hectares (Figure 2-1). Soils in WCA- 2A region are classified as histosols (Table 2-1). The Everglades are naturally a low P freshwater system, however inflow of waters with high P concentrations (>100 μg P L^{-1}) from the adjoining Everglades Agricultural Area (EEA) for 40 years has resulted in a significant P increase in the system. Phosphorus loading to the system has resulted in a nutrient gradient in the system with high levels of Phosphorous (1305±91 mg $^{-1}$ kg) in soils and water column near the inflow region. Dominant stands of *Typha domigenesis* (commonly called Cattails) are also indicators for altered vegetation pattern. The interiors regions of the WCA-2A area represent the native Everglades with low P (510±23 mg $^{-1}$ kg in U3-Ridge and 196±22 mg $^{-1}$ kg in U3-S) concentrations and are characterized by the ridge and slough ecosystems (Figure 2-1). Vegetation dominating the ridge areas is *Cladium jamaicense* commonly called sawgrass and the open sloughs are primarily dominated by *Nymphea odorata*. The region between the low P pristine interior and the high P regions near the inflow represents the transition zone with moderate P (807±45 mg $^{-1}$ kg in F4-Ridge and 492±66 mg $^{-1}$ kg in F4-S) concentrations in soils and are characterized by the ecosystems of ridge and slough (Figure 2-1). Sampling sites selected for my study were along a previously constructed transect within the WCA-2A area such as, 1) F1- P-impacted, 2) F4- Ridge, transition zone 3) F4- Slough, transition zone and 4) U3- Ridge, Interior pristine zone and 5) U3-Slough Interior pristine zone .

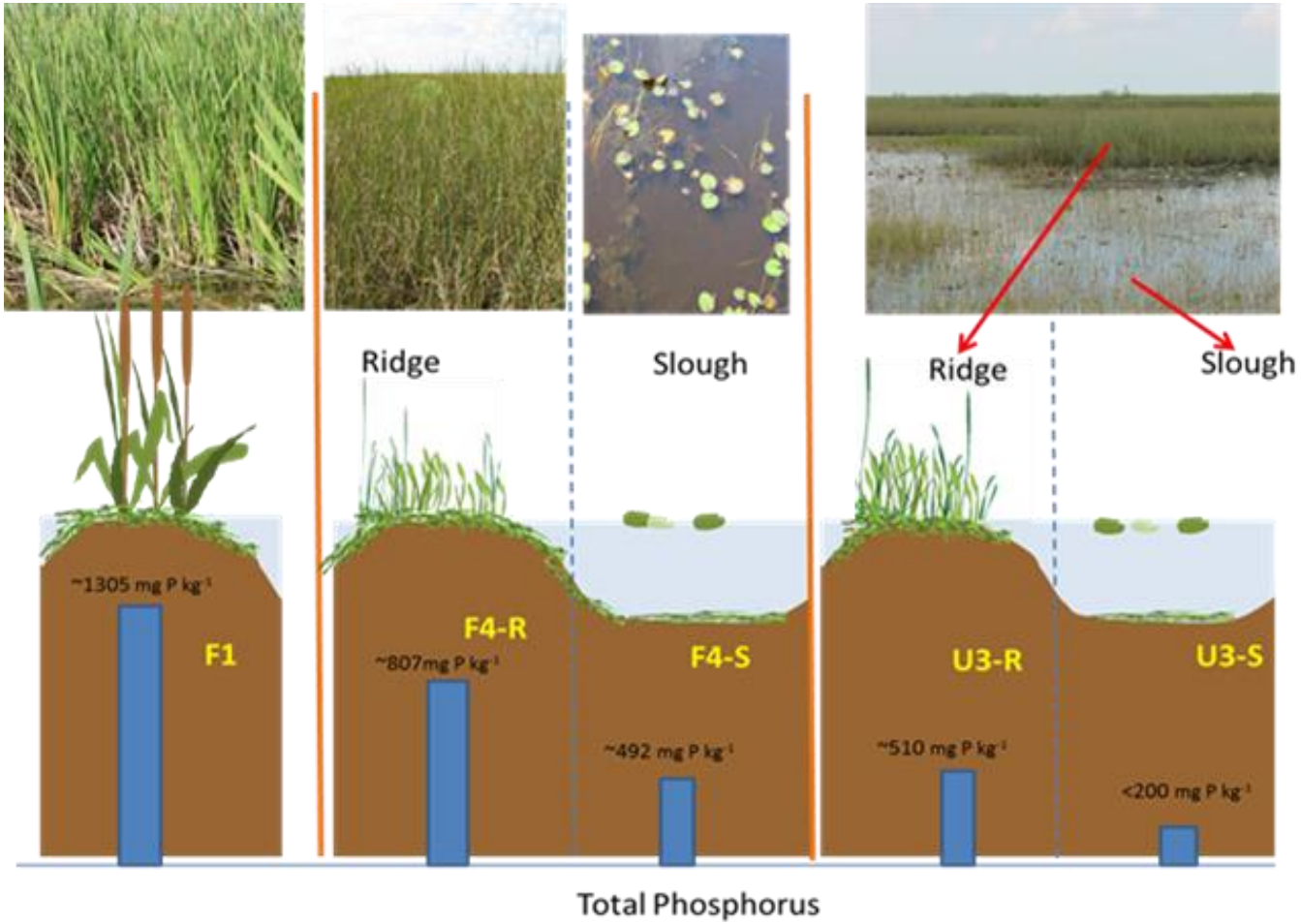


Figure 2-1. . Schematic of the sampling sites in WCA-2A region of the Fl Everglades. The F1 is the high-P region dominated by *Typha*, F4, a moderate-P region is characterized by the ridge and slough ecosystems and U3 is a low-P pristine regions which is also characterized by the ridge and slough ecosystems. In both region F4 and U3, the ridge ecosystem is dominated by *Cladium* spp and the slough region is dominated by Nymphaea spp

Table 2-1. Geographic coordinates and general soil information for the five sampling sites used in this study.

Location	Coordinate	Soil depth (cm)	Soil temperature	Dominant Vegetation
F1	26.35N,80.35W	(0-10)	15°C	*Typha domigenesis*
F4-S	26.31N,80.38W	(0-10)	17°C	*Nymphea odorata*
F4-R	26.31N,80.38W	(0-10)	15°C	*Cladium jamaicense*
U3-S	26.28N,80.40W	(0-10)	20°C	*Nymphea odorata*
U3-R	26.28N,80.40W	(0-10)	16°C	*Cladium jamaicense*

Soil Biogeochemical Parameters

At each sampling site, three replicate soil cores (10cm depth) were collected on 13th March 2012 and individually placed in airtight plastic bags. Soil samples were transferred on ice to the (Wetland Biogeochemistry laboratory in Soil and Water Science department at the University of Florida) and stored at 20°C temperature. Soil samples were homogenized in the laboratory to remove the roots and shells. All the soil samples were kept at same (20°C) temperature to closely mimic field temperature upon the time of collection. For each sample approximately 50g soil samples were dried at 70°C for 3 days to determine the soil moisture content. Values for soil pH were measured using 1:2

soil to deionized water (Thomas, G.W. 1996. Soil pH and soil acidity. p. 475–490). A subsample of dried soils was ground into fine powder by using a mortar pestle and analyzed for total C and N content using a Thermo Flash EA 1112 elemental analyzer (CE Elantech, Inc.). Soil total P was analyzed by auto analyzer AA-2 by acidic (HCL) ash extraction (Andersen, 1976) which is done by combustion of soils at 550°C. Soil total organic matter was determined by loss of ignition at 550°C (Wright et al. 2008).

Soil microbial biomass carbon (MBC) was determined by a chloroform fumigation extraction procedure using 0.5 M K_2SO_4 for extraction (White and Reddy, 2001). We have presented the second season data for MBC as we compared the data with previous study which appeared to be same with our study MBC number (Corstanje, Reddy et al. 2007). The extracted Dissolve Organic Carbon (DOC) was determined on a Shimadzu total organic carbon analyzer (TOC-5050A). Extraction efficiency factor was used as 0.35. Same 0.5 M K_2SO_4 extract used for determining MBN after digestion of the samples and analyzed in auto analyzer AA-2. Microbial biomass phosphorus (MBP) was similarly determined by fumigation extraction, using 25 mL 0.5 M $NaHCO_3$ extractant. The extraction efficiency for MBN was used as 0.42.The difference in TP (Total Phosphorous) between the fumigated and non-fumigated sample considered as MBP (Ivanoff et al., 1998).

Extracellular Enzyme Activity Assays

Soil microorganisms govern the rate of organic matter decomposition by enzymatic breakdown of polymeric compounds. Here activities of extracellular enzymes involved in soil C,N,P cycling were determined using fluorometric assays previously described by Inglett et al (2011) and German et al (2011).Table 2-2 describes the six extracellular enzymes, their substrates and the released products: 1) β-1,4, Glucosidase

(BGLU, Sigma M-5650), 2) Cellobiohydrolase (CBH, Sigma M-5530), 3) β-1, 4-N-Acetyl-glucosaminidase (NAG, Sigma M-5504), 4) Leucine amino peptidase (LAP, Sigma L-2800), 5) Phosphatase (PHO, Sigma M-5800), 6) Bis- phosphatase (BPHO, Sigma B-3500). All fluorimetric assays were carried out in 96 microwell titer black microplates. For each sample, soil homogenate was prepared by homogenizing 2g fresh weight soil in 100ml of sterile water (pH 7) (Table 2-1). We used sterile water for homogenizing the soil instead of buffer; to provide almost field condition to the soil microbes. From well stirred slurries, aliquot of 100µL was pipetted into microtiter plates. To each of the sample wells, 150uL of the fluorometric substrate solution was added and the plate incubated for a 2-4 hours. Reference standard wells were prepared with 100uL of standard and 150uL of DDI water. Replicate microplates were incubated at each of the four temperatures 15°C, 20°C, 25°C and 30°C for 2-3 hrs. Soil free negative controls were set up to account for any background fluorescence in substrate or in dilution matrix. Quench standard curves were determined with soil homogenates to account for any interference of fluorescence by soil particles. For these to achieve; we added 150 uL soil homogenate which was combined with 100 uL of standard (7-amino-4-methylcoumarin for LAP, 4-methylumbelliferone for BGLU, NAG, BPHO and PHO). Quench curves were generated, to know how much quench potential soil samples have. Briefly, we prepared four row of well in microtitrate plate with 150µl of autoclaved DDI water and 100µl of standard substrate. Whereas the bottom four row (from E to H) in the microtitrate plate were treated with 150µl of respective soil solution and 100µl of standard substrate. To calculate quench we used the ratio of these two reaction slope. Negative controls were prepared using 150µl of soil solution with100µl of water; to

account for the fluorescence substrate were produced by soil enzymes after adding the respective synthetic substrate. Fluorescence readings from controls were used to account for fluorescence not generated from the substrate-enzyme reaction (German, Weintraub et al. 2011).

For determining the kinetic parameters Vmax and Km enzyme activities within each soil sample was assessed with a range of substrate concentration (Table 2.2). Following the specified time of incubation the enzyme reaction was stopped by the addition of 10 µl of 0.5 M NaOH in every well, following which fluorescence in each sample was measured on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA). Fluorescence was measured at 365 nm excitation and 460 nm emission.

Table 2-2. Extracellular enzymes, their function, their substrates and range of substrate concentrations used in this study

Enzymes	General function (Release)	Substrate (synthetic)*	E.C. number	Substrate (µM)
Phosphatase(PHO)	Hydrolyzes phosphoric ester bonds to mineralize organic P into phosphate.	4-MUB- phosphate	3.1.3.1	10-400
Phosphodiesterase (BPHO)	phosphate monoester	Bis-(4-MUB)-phosphate	3.1.4.1	10-400
ß-D-glucosidase (BGLU)	Enables hydrolysis of 1, 4-linked β-D-glucose residues from compounds, cellobiose and a short-chain cellulose oligomer to release glucose.	4-MUB-ß-D-glycopyranoside	3.2.1.21	10-400
Cellobiohydrolase (CBH)	Disaccharides	4-MUB ß-D-cellobiopyranoside	3.2.1.91	10-400
Leucineamino peptidase (LAP)	Enables hydrolysis of the peptide bonds adjacent to free amino groups, heavily targeting leucine while also breaking down other amino acid amines and methyl esters.	L-leucine hydrochloride	3.4.11.1	10-400
N-Acetyl-ß-D glucosaminidase (NAG)	Facilitates hydrolysis of acetyl-β-D-glucosaminide residues with 1, 4-β linkages in chitin and chitin-derived oligomers.	N-4-MUB ß-D glucosaminide	3.2.1.52	10-400

*Fluorescent dye 4-methylumbelliferone is abbreviated as MUB

Calculations for Enzyme Kinetics

Enzyme kinetic parameters, the maximal velocity, Vmax and the substrate concentrate (Km) where reaction will reach half Vmax were determined for each enzyme at each of the four temperatures by using different concentration of enzyme substrates and was calculated using the Michaelis-Menten equation:

$$V = Vmax \frac{[S]}{(Km + [S])}$$

Where V is the reaction velocity and is dependent on the enzyme concentrations. [S] is the substrate concentration, Km is the substrate concentration at half maximal velocity which also indicates the substrate affinity and Vmax is the maximum potential rate of enzyme activity. Vmax and Km values were determined using a nonlinear regression and were transformed in natural log.

Temperature Sensitivity

Temperature sensitivity of each enzyme was determined by using the Q_{10} equation. Where regression coefficient (slope and intercept) were plotted in a linear relationship and the slope represent the temperature sensitivity and was calculated by the following equation (Stone et al. 2012).

$$Q10 = exp^{(slope*10)}$$

Statistical Analysis

Enzyme activity (Vmax) and Enzyme efficiency (Km)

Enzyme activities were calculated as nmol of substrate converted per gram of dry weight soils per hour ($nmol^{-1}\, g^{-1}$ h). Prior to statistical analyses, enzyme analyses were

transformed as natural logs to satisfy assumptions of normality and homogeneity of variance.

All statistical analyses were performed using JMP software v.8© (SAS Institute Inc., Cary, NC) for both response variables such as Vmax and Km. Comparisons of means for significant effect of temperature and nutrient gradient for Vmax and Km were determined by Tukey HSD tests. REML (Restricted Maximum Likelihood) was used to make inferences on the model procedures, which is the recommended option in JMP. *P* value for each model fit, of the F-test for the fixed effects (main and interaction effects) was reported. Results of the mean separation (Tukey multiple comparison procedure) for the main interaction effects have also been reported. Enzyme activity responses to site (nutrient gradient), temperature and their interaction under both aerobic and anaerobic condition were calculated using a statistical model which is described below. Although impact of site (nutrient) were presented as in actual Vmax and Km numbers, a split plot statistical model with site as the whole plot factor (5 levels) and temperature as the split plot factor (with four levels) was used to analyze the results. The whole plot treatment was arranged in a completely randomized design, with three replicates. The equation of the split plot statistical model is:

$$Y_{ijk} = \mu + \alpha_i + \epsilon_{ij} + ß_k + (\alpha ß)_{ik} + \delta_{ijk}$$

Where,

Y_{ijk} = is the enzyme responses (value) recorded on the ith replicate of the ith site under treatment level k,

μ = overall mean,

α_i = main effect of the ith site,

ϵ_{ij} = whole plot errors with ϵ_{ij} $\underset{\sim}{iid}$ $N(0, \sigma_{\epsilon}^2)$,

$ß_k$ = main effect of the kth temperature

$(\alpha ß)_{ik}$ = interaction between the ith site and kth temperature

δ_{ijk} = split plot error with $\delta ijk \underset{\sim}{iid} N(0, \sigma_{\delta}^2)$,

Mean plot for the main effects and the interaction as well as the results of the Tukey HSD tests are presented in chapter 3 (result figures). Significantly different means are assigned different letters and their magnitude is arranged in an alphabetical order. These plots enabled the identification of the treatments that reach the highest mean responses (enzyme activity and efficiency levels).

Temperature sensitivity:

Differences in temperature sensitivities of the six separate enzymes across the five sampling were determined by obtaining significant differences between multiple means by standard least square test to analyze every enzyme separately with site interaction ($p<0.05$) using Tukey HSD test. Q_{10} values were calculated for both aerobic and anaerobic conditions separately. Statistical model used was where site acts as the whole plot factor for each enzyme separately. The linear model for the analysis was done by the equation presented below,

$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$

Where,

Y_{ij} = is the enzyme responses (value) recorded on the ith replicate of the ith site,

μ = overall mean,

α_i = main effect of the ith site,

ϵ_{ij} = whole plot errors with $€ij \underset{\sim}{iid} N(0, \sigma_{\epsilon}^2)$,

In order to explore the associations between soil variables (nutrients, microbial biomass number, and their ratios) and enzyme activities or temperature sensitivity (Q_{10}) values of enzymes, treatment correlation coefficients were determined. These associations were separately conducted for aerobic and anaerobic treatments. Moreover significant difference of temperature sensitivity between two conditions were also determined. For the enzyme activities averaged values of Vmax and Km (for all 4 temperatures) were determined for each enzyme and each site.

CHAPTER 3
RESULTS AND DISCUSSIONS

Results

Soil Biogeochemical Parameters

Overall analysis of all the measured biogeochemical parameters revealed significant differences among the sites. There was a significant gradient of total P in soils along the five sites selected for this study. Within the F4 and the U3 sites there were significant differences observed between the soils from the ridge and slough regions. Details of soil biogeochemical parameters are presented below.

Soil temperature was measured in the surface soils (0-10 cm) during the time of collection and it ranged from 15°C to 20°C (Table 2-1 in chapter 2). Soil pH values were near neutral ranging from 6.2 to 7.8 (Table 3-1) but were not significantly different between the sites. Although the sites were all flooded, after decanting the overlying water, the soil moisture was determined and it ranged from 87 to 92% within the five sites. Ridge soils appeared to have higher moisture retained in them when compared to the sloughs soils. Soil organic matter (determined as LOI) was higher (above 90%) in soils from F1 site and ridge sites (F4 and U3) relative to the slough soils (70%) (Table 3-1).

Table 3-1. Physico-chemical parameters for soil samples collected from five sites in the WCA-2A region of the Florida Everglades.

	F1	F4-S	F4-R	U3-S	U3-R
pH	6.9(0.2)	7.8(0.02)	6.7(0.4)	6.9(0.2)	6.2(0.1)
Moisture %	92(0.3)	87(1)	92(0.2)	89(2.1)	92(0.7)
LOI %	96(0.8)	73(8.3)	93(1.2)	69(14)	92(1.4)

S, Slough; R, ridge; LOI- Loss On Ignition

Total C in soils ranged from 306±16 g kg^{-1} in F4-S to 449±4 g kg^{-1} in F-1 sites which was 1.4 time lower in F4-S than F1. Among all sites only F4-S had significantly lower carbon content than F1 and all other sites did not vary significantly in total carbon content (Table 3-2). Soil total nitrogen content in the five sites ranged from 23±1 g kg^{-1} to 35±5 g kg^{-1} with lowest TN in F4-S and highest in U3-R. Total nitrogen in soils did not appear to be significantly different (Table 3-2). Soil total phosphorous concentrations varied significantly among the sites. Soil P concentration was the highest in F1-site (1305±91 mg kg^{-1}) followed by F4-R, F4-S, U3-R and U3-S. Ridge soils (F1, F4 and U3) appeared to follow a significant phosphorous gradient following high P concentration in F1 soil, observed which was high in F1, moderate in F4-ridge (807±45) and lowest in U3-R (510±23). Similar gradient was also observed in F1 and slough soils higher P concentration F4-S (492±66) than U3-S (196±22) (Table 3-2). Soil P concentration at F4-S soils were similar to U3-R soils. Soil P concentration at F4-R were 1.7 times lower than F1 and U3-R soils were 2.5 times lower than F1 soils. A 2.5 times higher P concentration of P in F4-S than U3-S was also noticed.

Soil microbial biomass carbon (MBC) was determined in soils that were collected at another sampling period (September 2012) to get approximate trends of MBC across the sites. Soil total microbial biomass carbon (MBC) ranged from 2 g kg^{-1} in U3-S to 7.9 g kg^{-1} in F4-S. Among the 5 sites significantly lower concentration of MBC was observed in F4-R and in U3-S (Table 3-2). Soil microbial biomass nitrogen (MBN) ranged from 186±80 mg kg^{-1} in U3-S to1547±12 mg kg^{-1} in F1 site. Soil MBN values were similar in all sites but soils of U3-S sites showed significantly lower MBN (Table 3-2). MBN values in soils in U3-S soils were 8 times lower than those in F1 soils. Moreover soil MBP at all

sites average at all sites with exception of U3-S where observed values were significantly lower.

On comparing the soil nutrient ratios; no significant differences were observed in TC: TN ratios among the five sites; however the TC: TP ratio in U3-S soils were significantly higher than other soils. Soils TN: TP ratios were ranged from 25±1.6 in F1 soils to 158±13 in U3-S soils (Table 3-2). Microbial biomass ratios MBC: MBN and MBN: MBP did not vary significantly among the sites. Significant difference were observed in MBC: MBP ratios which ranges from 8±1 in F4-R to 31±2 in U3-S soils (Table 3-2).

Table 3-2. Nutrients, microbial biomass and their ratios across the sites.

	Units	F1	F4-S	F4-R	U3-S	U3-R
TC	gkg^{-1}	449(4)^{a}	306(16)^{b}	423(2)^{ab}	366(64)^{ab}	403(10)^{ab}
TN	gkg^{-1}	32(0)^{a}	23(1)^{a}	29(2)^{a}	30(5)^{a}	35(5)^{a}
TP	$mg\ kg^{-1}$	1305(91)^{a}	492(66)^{c}	807(45)^{b}	196(22)^{d}	510(23)^{c}
MBC†	gkg^{-1}	7.2(0.2)^{a}	7.9(0.4)^{a}	2.7(0.3)^{bc}	2.0(0.4)^{c}	5.3(1.1)^{ab}
MBN	$mgkg^{-1}$	1547(12)^{a}	930(203)^{ab}	737(239)^{ab}	186(80)^{b}	739(18)^{ab}
MBP	$mgkg^{-1}$	273(46)^{a}	246(16)^{a}	333(14)^{a}	70(13)^{b}	352(33)^{a}
TC:TN		14(0.1)^{a}	13(0.1)^{a}	15(0.8)^{a}	12(1)^{a}	12(1.5)^{a}
TC:TP		348(24)^{b}	636(51)^{b}	528(28)^{b}	1856(243)^{a}	794(42)^{b}
TN:TP		25(1.6)^{c}	48(3.5)^{bc}	36(1.3)^{c}	158(13)^{a}	69(7.9)^{b}
MBC:MBN		5(0.2)^{a}	7(4.2)^{a}	5(1.5)^{a}	19(11)^{a}	7(1.5)^{a}
MBC:MBP		28(4.9)^{ab}	23(11.5)^{a}	8(1)^{c}	31(2)^{a}	15(3)^{bc}
MBN:MBP		5(0.7)^{a}	4(0.9)^{a}	2(0.6)^{a}	1(0)^{a}	2(0)^{a}

† MBC is presented as second season data. Significant difference is presented as superscript for nutrients, microbial biomass and their ratios.

Vmax of the extracellular enzymes across the sites

The maximal enzyme velocities of all enzymes were significantly different among the five sites with the exception of bisphosphatase under aerobic condition. Observed differences between the ridge and slough soils within the same region further indicated the enzyme diversity between the two regions. Overall, among the six enzymes, phosphatase activity (Vmax) was observed to be the highest under both aerobic and anaerobic conditions. Furthermore under both conditions activities of BGLU, PHO and LAP appeared to be higher than those observed for CBH, BPHO and NAG respectively.

Carbon processing enzyme Vmax

Carbon enzyme activities varied significantly across the five sites (BGLU, P = 0.0024; CBH, P = 0.0047) with higher Vmax values for BGLU activity observed in high P site, F1 followed by those in F4-R soils. Low P soils showed significantly lower activities of BGLU (Figure 3-1) under aerobic condition. Results indicate significantly higher activity for glucosidase (BGLU) Vmax (1957 ±175 $nmol^{-1}g\ dw^{-1}h$) in F1 site when compared with U3-S site (413± 117 $nmol^{-1}g\ dw^{-1}h$) and F4-S site (189±58) under aerobic condition. Although results showed significantly lower activity in F4 site for glycosidase (BGLU) from F1 and U3 sites respectively. Average values of activity of BGLU in ridge soils were comparatively higher than average slough soils activity. Moreover we observed that within F4 soils, ridge soils enzyme activity were significantly higher than slough soils.

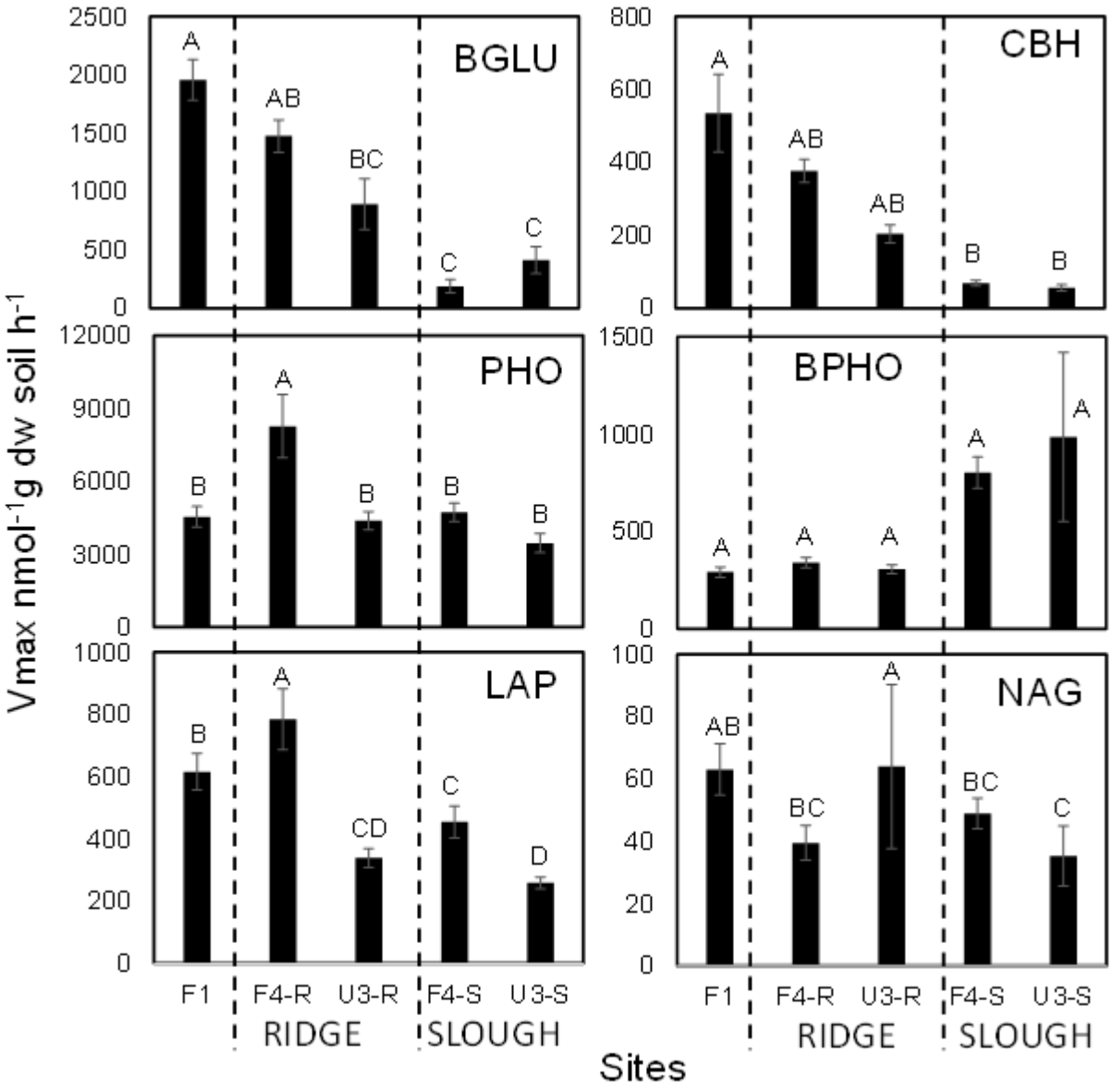


Figure 3-1. Mean plot for Vmax activity of BGLU, CBH, PHO, BPHO, LAP and NAG under aerobic condition are presented across the site levels. The standard errors of the means are calculated using the empirical variance estimates across the sites.

CBH also showed a significantly higher activity in F1 site compared to low nutrient site (Figure 3-1). Moreover similarly of BGLU we observed similar results for cellobiohydrolase with V_{max} higher in F1 (535±107 $nmol^{-1}g$ dw $soil^{-1}h$) site as compared with U3-S site (56± 8 $nmol^{-1}g$ dw $soil^{-1}h$) and F4-S site (69±8). Incontrast of BGLU we did not observed any significant difference in CBH activity between the ridge and slough soil. Although average ridge soils activity were higher than slough soils.

Similarly of aerobic condition, we observed significantly higher activity in F1 site for carbon processing enzyme BGLU than low nutrient site U3-S (Figure 3-2) under anaerobic condition (Figure 3-2). Although we observed a different result in F4-S under anaerobic condition than F4-S under aerobic condition. Under anaerobic condition the glucosidase activity ranges from 2040 ± 154 $nmol^{-1}g$ dw $soil^{-1}h$ in F1 to 222 ±51 $nmol^{-1}g$ dw $soil^{-1}h$ in U3-S. Under anaerobic condition there were no significant difference between ridge and slough soils.

Moreover we also observed similar results for CBH under anaerobic condition which showed a significantly higher activity in high nutrient (F1) site than low nutrient (F4-S and U3-S) sites (Figure 3-2). CBH activity ranges from 538 ± 81 $nmol^{-1}g$ dw $soil^{-1}h$ in F1 to 46 ±7 $nmol^{-1}g$ dw $soil^{-1}h$ in U3-S. CBH under anaerobic condition did not appeared to be significantly different between ridge and slough soils. Although the average activity of two ridge soils appeared to be higher than average activity of two slough soils.

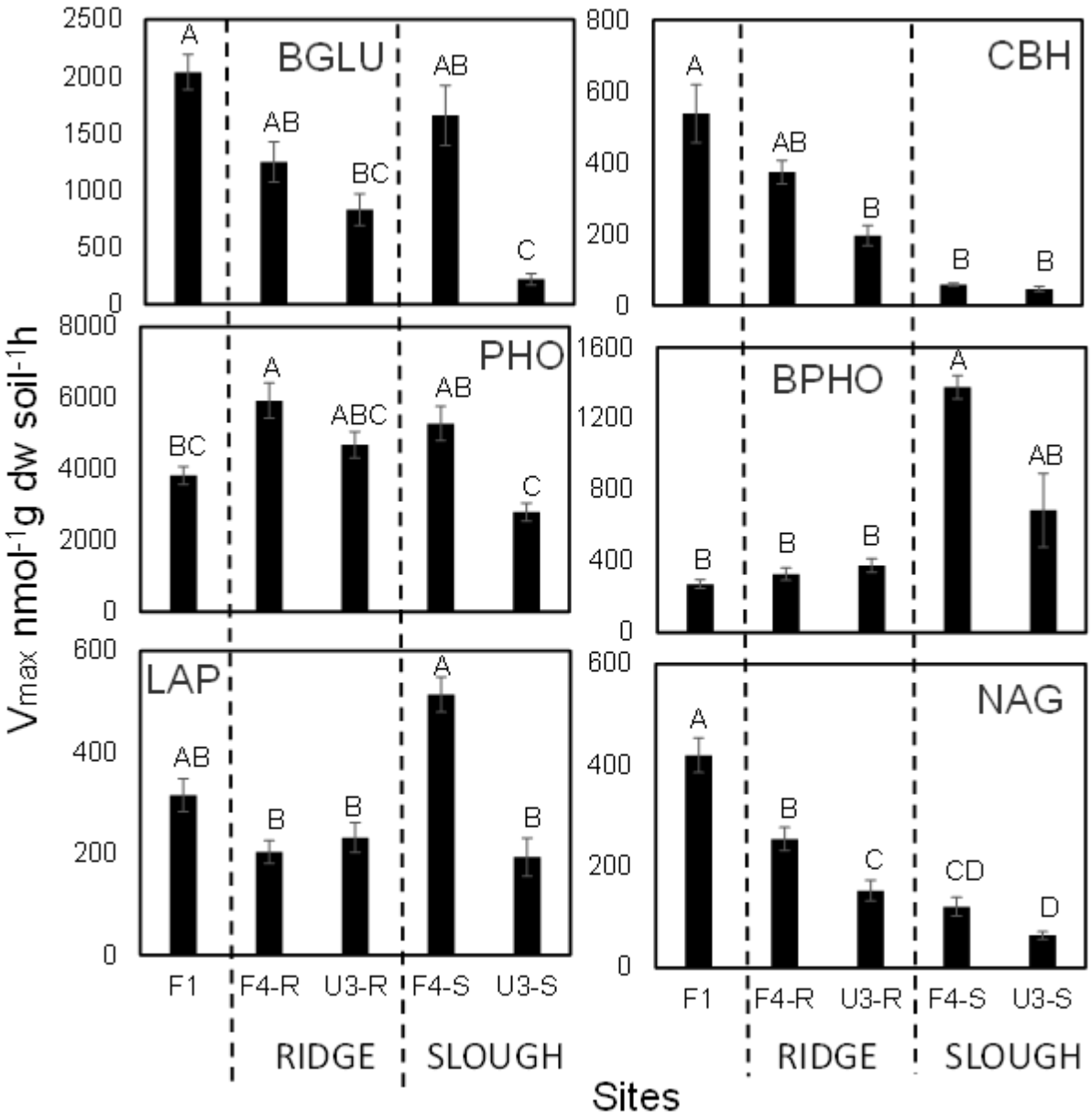


Figure 3-2. Mean plot for Vmax activity of BGLU, CBH, PHO, BPHO, LAP and NAG under anaerobic condition are presented across the site levels. The standard errors of the means are calculated using the empirical variance estimates across the sites.

P Processing Enzymes Activity (Vmax)

Among the two P processing enzymes we found significantly higher enzyme activity for phosphatase in F4-R (8276 ±1308 $nmol^{-1}g$ dw $soil^{-1}h$) than other sites under aerobic condition (Figure 3-1). P processing enzyme (PHO) showed a significant site effect (P = 0.0003). Phosphatase activity under aerobic condition ranges from 8276 ± 1308 $nmol^{-1}g$ dw $soil^{-1}h$ in F4-R to 2797 ±37 in U3-S $nmol^{-1}g$ dw $soil^{-1}h$. We observed a significant difference in enzyme activity between ridge and slough soil only under F4 site.

Any significant difference in bisphosphatase (BPHO) activity was not observed among the sites under aerobic condition (Figure 3-1). Highest bisphosphatase activity noticed in U3-S (985±434 $nmol^{-1}g$ dw $soil^{-1}h$) and lowest in F1 (290±26 $nmol^{-1}g$ dw $soil^{-1}h$) in F1 under aerobic condition. BPHO did not showed any site effect under aerobic condition.

Similarly of aerobic condition PHO showed higher activity in F4-R than U3-S under anaerobic condition (Figure 3-2). Site effects for PHO under anaerobic condition was P = 0.02. Phosphatase activity under anaerobic condition ranges from 5925 ±34 $nmol^{-1}g$ dw $soil^{-1}h$ in F4-R to 3461 ± 391 in U3-S $nmol^{-1}g$ dw $soil^{-1}h$.

Interestingly higher BHO activity was observed in F4-S site with respect to other sites (Figure 3-2). Under anaerobic condition it was ranges from 1377±35 $nmol^{-1}g$ dw $soil^{-1}h$ in F4-S to 271 ±33 $nmol^{-1}g$ dw $soil^{-1}h$ in F1. Bisphosphatase (BPHO) showed a significant site difference only under anaerobic condition (P = 0.017). Interestingly we observed an average higher activity in slough soils than ridge soils.

Nitrogen Enzymes Activity (Vmax)

Similar kind of results were observed for both nitrogen enzymes activity under both condition. Both the nitrogen processing enzymes showed a significant site effects. Among the two nitrogen processing enzymes the site effect for leucine aminopeptidase is $P < 0.0001$ under aerobic conditions. Whereas N-acetyl glucosamine showed a significant site effect under both conditions ($P = 0.0012$ aerobic, $P < 0.0001$ anaerobic condition).

Highest LAP activity was observed under F4-R site. Moreover both in F1 and F4-R sites showed a significantly higher activity for LAP than slough soils (low nutrient) under aerobic condition. Within the F4 site we observed significant difference between ridge and slough soil. Moreover we observed significant differences in activity within the two slough soils. Leucine amino peptidase showed enzyme activity (Vmax) which ranges from 616 ±59 $nmol^{-1}g\ dw^{-1}h$ in F1 and 785 ±98 $nmol^{-1}g\ dw^{-1}h$ in F4-R to 259 ±19 $nmol^{-1}g\ dw^{-1}h$ in U3-S site under aerobic condition.

Higher enzyme activity for N-acetyl glucosamine was noticed in U3-R site under aerobic condition (Figure 3-1). NAG did not show any significant difference within two slough soil although we noticed significant difference in NAG activity within the two ridge soils. N-acetyl glucosamine activity under aerobic condition ranges from 73 ± 26 in U3-R to 35±10 $nmol^{-1}g\ dw^{-1}h$ in U3-S. Moreover U3-S showed a significantly lower activity for NAG than F1 and U3-R soils.

In contrast of aerobic condition we observed a highest LAP activity in F4-S soil under anaerobic condition (Figure 3-2). We also observed significantly higher activity in F4-S soil than U3-S soil whereas there were not any significant difference between two

ridge soils. Although there was no significant difference within the two U3 soils but we noticed significantly higher activity F4-S soil than F4-R soil. Under anaerobic condition it ranges from 514 ± 18 $nmol^{-1}g\ dw^{-1}h$ in F4-S to 193 ± 7 $nmol^{-1}g\ dw^{-1}h$ in U3-S site.

Under anaerobic condition NAG showed a significant nutrient gradient effect (Figure 3-2). High nutrient sites showed a significant difference in enzyme activity than low nutrient sites (Figure 3-2). Moreover we noticed significantly higher activity in F4-R than U3-R soils. Under anaerobic condition it ranges from 420 ± 34 in F1 to 64 ± 7 $nmol^{-1}g\ dw^{-1}h$ in U3-S.

Aerobic to Anaerobic Enzyme Activity (Vmax) Ratio

Higher than 1 ratios of aerobic to anaerobic Vmax values for all enzymes at all 5 sites indicated that all enzymes with the exception of NAG which showed higher or equal maximal enzyme activity under aerobic conditions (Figure 3-3). No observed differences in the ratios within the C-acquiring enzymes suggested that there was no site effect. For P acquiring enzymes, the F4-S was significantly lower than the F4-R (PHO and BPHO) and the U3-S (for BPHO). In F4-R significantly higher ratios for LAP Vmax was observed than the other sites. Whereas in NAG both F4-S and U3-S showed relatively higher ratios than the F4-R and the F1 sites.

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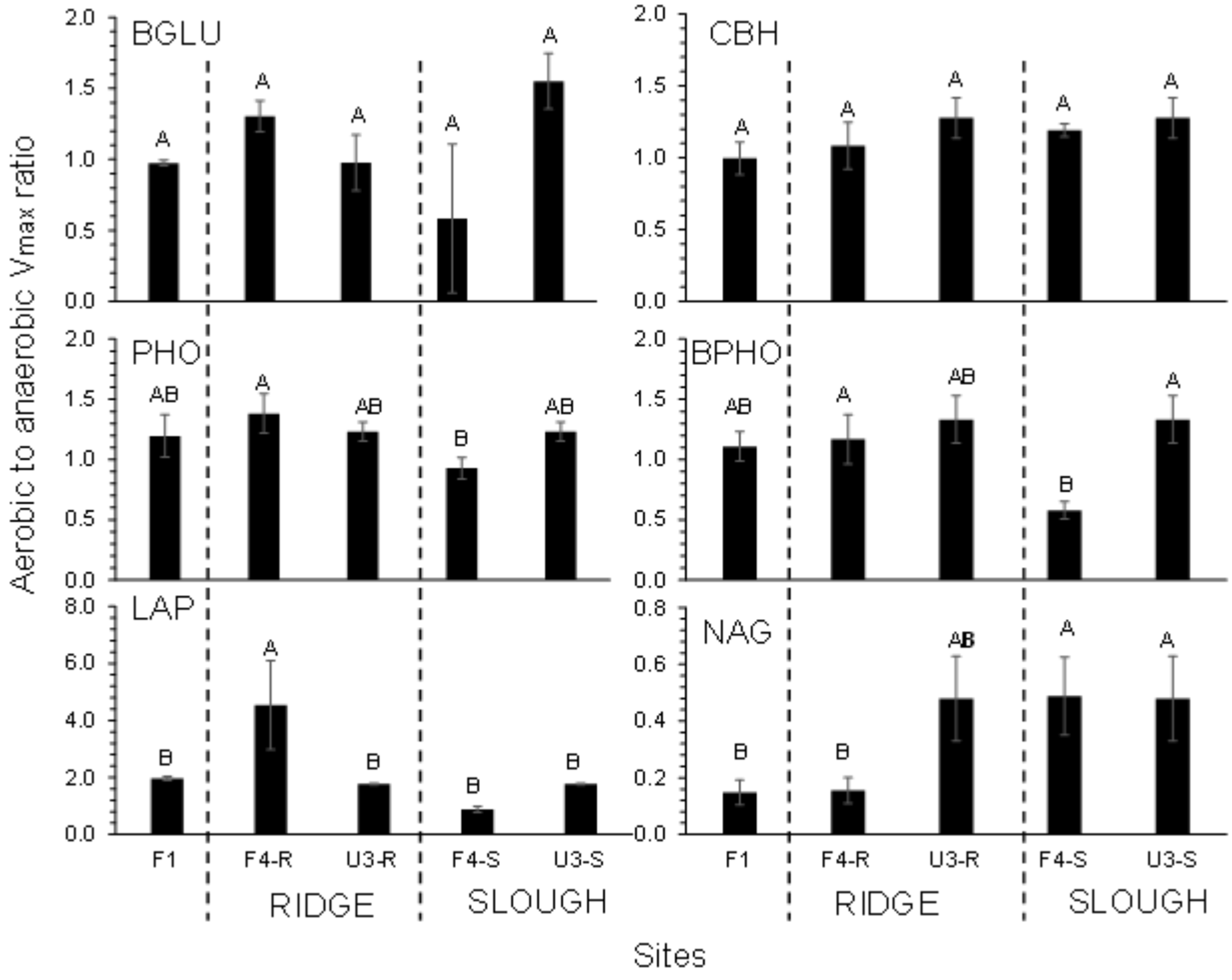

Figure 3-3. Ratio of maximal enzyme activities as measured under aerobic and anaerobic conditions for six extracellular enzymes in 5 sites along the P gradient in the Florida Everglades soil are presented. Bars represent the mean of samples (n= 12) with standard errors.

Correlation of Carbon Enzymes Activity (Vmax) with Soil Biogeochemical Parameters

All the enzymes showed some significant correlation with most of the soil biogeochemical parameter with the exception of NAG under aerobic condition (Table 3-3). Specifically carbon processing enzymes appeared to be more correlated with the soil biogeochemical parameters.

Carbon Processing Enzymes

A significant correlation between the enzyme activities (Vmax) for glucosidase (P<0.0001) and cellobiohydrolase (P<0.0001) with phosphorous concentration was observed under both aerobic and anaerobic condition (Table 3-3 and 3-4). Interestingly we also observed significant positive correlation of both carbon processing enzyme activity (Vmax) with soil total carbon, C: N ratio, MBN and LOI (Table 3-3) under aerobic condition but we did not observed any significant correlation with TN. Moreover we observed positive correlation of BGLU with MBP under both aerobic and anaerobic condition and soil total carbon and MBN with CBH under anaerobic condition. Whereas we have found significantly negative correlation between the carbon processing enzymes activity (Vmax) with soil total C: P and N: P ratio under both condition (Table 3-3 and 3-4). Although we did not observed any significant correlation of CBH with MBP under aerobic condition and BGLU with TC under anaerobic condition (Table 3-3 and 3-4). Interestingly we have observed a significant correlation between both carbon processing enzymes under both aerobic (P <0.0001) and anaerobic (P < 0.0001) condition.

Table 3-3. P value of correlation between enzyme activity (Vmax) and soil biogeochemical parameters under aerobic condition.

	BGLU	CBH	PHO	BPHO	LAP	NAG
TP	<0.0001	<0.0001	0.134	0.0167†	<0.0001	0.50
TC	<0.0001	<0.0001	0.273	<0.001	0.044	0.38
TN	0.0691	0.117	0.4506	0.0002	0.507	0.5
C:P	0.0004†	<0.0001†	0.0174†	0.283	<0.0001†	0.26
C:N	<0.0001	0.0067	0.0038	0.1048	<0.0001	0.6
N:P	<0.0001†	<0.0001†	0.009†	0.16	<0.0001†	0.25
MBP	0.0056	0.322	0.0159	0.0044†	0.014	0.3
MBN	0.0016	0.0006	0.94	0.0206†	0.064	0.2
LOI	<0.0001	<0.0001	0.117	<0.0001†	0.026	0.1

† Negative correlation.

Phosphorous Processing Enzymes

Phosphatase showed a negative correlation with C: P and N: P ratio under both condition (Table 3-3 and 3-4). We also observed a positive correlation of phosphatase with MBP under both the conditions (Table 3-3 and 3-4). Moreover bisphosphatase showed a negative correlation with soil total phosphorous, MBP and LOI respectively under both condition (Table 3-3 and 3-4). We did not observed any significant correlation between the both phosphorous processing enzymes under both conditions.

Table 3-4. P value of correlation between enzyme activity (Vmax) and soil's biogeochemical parameters under anaerobic condition.

	BGLU	CBH	PHO	BPHO	LAP	NAG
TP	<0.0001	<0.0001	0.25	0.0021†	0.54	<0.0001
TC	0.65	<0.0001	0.65†	<0.0001†	<0.0001†	<0.0001
TN	0.17†	0.13	0.38†	<0.0001†	0.0003†	0.22
C:P	<0.0001†	<0.0001†	0.0008†	0.60†	<0.0023†	<0.0001†
C:N	0.0023	0.0054	0.098	0.09†	0.86†	0.0020
N:P	<0.0001†	<0.0001†	0.0001†	0.901	0.0048	<0.0001†
MBP	0.0283	0.0002	0.0159	0.0087†	0.24	0.142
MBN	<0.0001	0.0002	0.12	0.16†	0.109	<0.0001
LOI	0.03	<0.0001	0.24	<0.0001†	0.005	<0.0001

† Negative correlation.

Nitrogen Processing Enzymes

We observed significant positive correlation of leucine amino peptidase with soil TP, TC, C: N, MBP, MBN and LOI and negative correlation with C: P and N: P under aerobic condition (Table 3-3). Whereas we observed negative correlation of LAP with TC, TN and C: P ratio and positive correlation with N: P and LOI under anaerobic condition (Table 3-4). Although we did not find any significant correlation of N-acetyl glucosamine with phosphorous gradient and other biogeochemical parameters (Table 3-3) under aerobic condition but we observed a positive correlation of NAG with TP, TC, C: N, MBN and LOI under anaerobic condition whereas we observed negative correlation of NAG with C: P and N: P under the same condition respectively (Table 3-

4). None of the condition showed a significant correlation between both nitrogen processing enzymes.

Kinetics Parameter (Km) Across the Sites

In contrast of Vmax no significant differences in Km were observed along the sites. Moreover we noticed some differences in Km between the two conditions

No significant difference in enzyme substrate affinity (Km) with the sites for both the carbon processing enzymes under aerobic condition were observed (Figure 3-4). Results showed different enzyme efficiency for glucosidase (BGLU) Km which ranges from (16 ± 2 $nmol^{-1}g\ dw^{-1}h$) in U3-R to (26 ± 4 $nmol^{-1}g\ dw^{-1}h$) in U3-S under aerobic condition.

Whereas opposite result was observed for cellobiohydrolase (CBH) as it showed higher Km in U3-R (26 ± 4 $nmol^{-1}g\ dw^{-1}h$) and lower Km in U3-S (8 ± 2 $nmol^{-1}g\ dw^{-1}h$) under aerobic condition. Moreover it appeared that slough soils has lower activity than ridge soil.

In contrast of aerobic condition we observed a significant difference in enzyme substrate affinity (Km) for glucosidase (BGLU) between F1 and U3-R under anaerobic condition (Figure3-5). Furthermore there was no observed significant difference in Km between the ridge and slough soils. Under anaerobic condition enzyme substrate affinity (Km) for glucosidase ranged from (17 ± 2 $nmol^{-1}g\ dw^{-1}h$) in U3-R to (33 ± 3 $nmol^{-1}g\ dw^{-1}h$) in F1 under anaerobic condition.

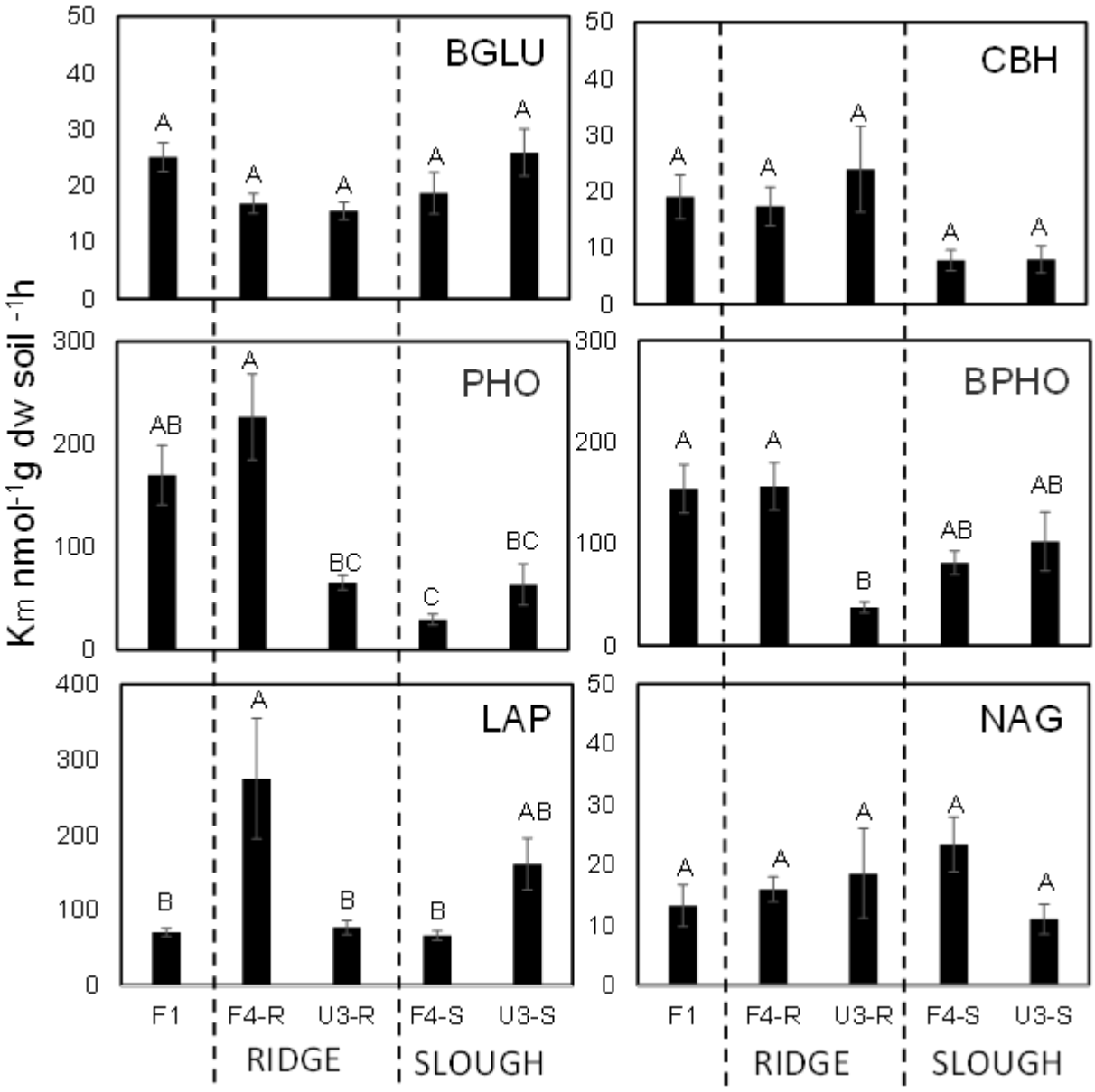

Figure 3-4. Mean plot for Km of BGLU, CBH, PHO, BPHO, LAP and NAG under aerobic condition are presented across the site levels. The standard errors of the means are calculated using the empirical variance estimates across the sites.

There were no significant difference in enzyme substrate affinity for cellobiohydrolase (CBH) under anaerobic condition. Cellobiohydrolase (CBH) enzyme efficiency (Km) ranged from (9 ± 3 $nmol^{-1}g\ dw^{-1}h$) in F4-S to (32 ± 11 $nmol^{-1}g\ dw^{-1}h$) in U3-S under anaerobic condition.

P Enzymes Efficiency (Km)

We have observed lower phosphorous processing enzyme efficiency (high Km) in high nutrient site (F1) and F4-R under aerobic condition. Among the two phosphorous processing enzymes substrate affinity (Km) the site effect for phosphatase under aerobic condition was significant $P = 0.0023$. Whereas we have noticed significantly higher enzyme substrate affinity (low Km) in low nutrient sites (Figure 3-4). Results indicate significantly higher Km for phosphatase (226 ±42 $nmol^{-1}g\ dw^{-1}h$) in F4-R and (170 ± 29 $nmol^{-1}g\ dw^{-1}h$) in F1 site when compared with U3-S site (63± 120 $nmol^{-1}g\ dw^{-1}h$) and F4-S site (29 ±5 $nmol^{-1}g\ dw^{-1}h$) under aerobic condition. Average ridge soils enzyme Km appeared to be higher than slough soils.

Similarly of phosphatase we also observed significantly lower efficiency (high Km) for bisphosphatase (BPHO) under aerobic condition. Moreover for BPHO it ranges from 156± 28 $nmol^{-1}g\ dw^{-1}h$ in F4 R and 154± 24 $nmol^{-1}g\ dw^{-1}h$ in F1 to 38 ± 6 $nmol^{-1}g\ dw^{-1}h$ in U3 R. BPHO also showed a significant site effect ($P = 0.0033$).

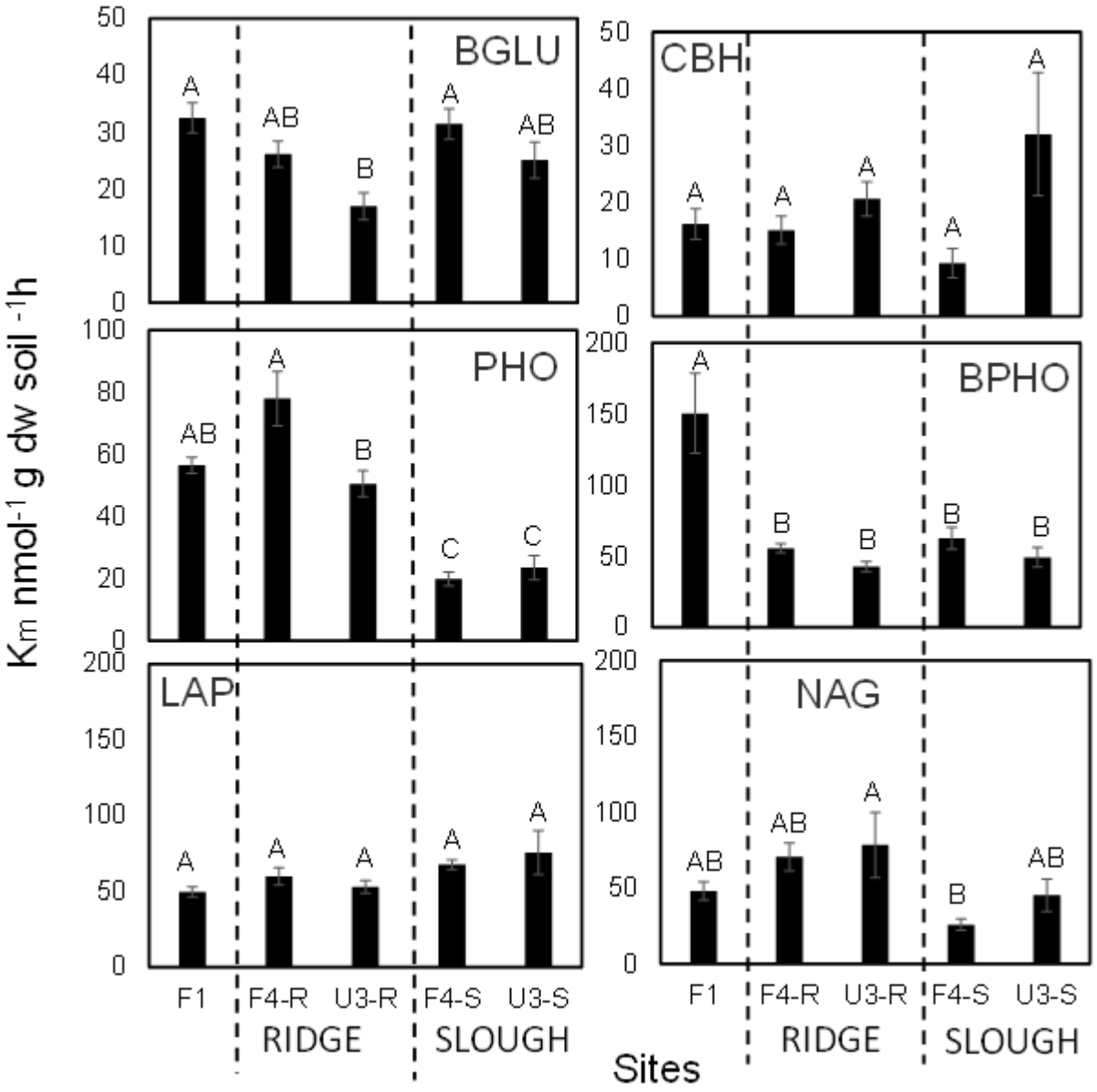

Figure 3-5. Mean plot for Km of BGLU, CBH, PHO, BPHO, LAP and NAG under anaerobic condition are presented across the site levels. The standard errors of the means are calculated using the empirical variance estimates across the sites.

Under anaerobic condition we also observed significantly higher Km value in F4-R site than low nutrient sites. PHO activity under anaerobic condition ranges from (78 ± 9 $nmol^{-1}g\ dw^{-1}h$) in F4-R to 24 ± 9 $nmol^{-1}g\ dw^{-1}h$ in U3-S. Under anaerobic condition phosphatase also showed significant site effect (P <0.0001).

In contrast to PHO we did not observed any significant difference in enzyme substrate affinity for bisphosphatase (BPHO) under anaerobic condition. For BPHO it ranged from (150 ±28 $nmol^{-1}g\ dw^{-1}h$) in F1 to 43 ± 3 $nmol^{-1}g\ dw^{-1}h$ in U3-R respectively.

N Enzymes Efficiency (Km)

We have found different results for both nitrogen enzymes activity. We observed significant site effect of LAP (P = 0.0003) under aerobic condition and NAG (P < 0.0001) under anaerobic condition

We have observed significantly low Km for leucine amino peptidase (LAP) in F1, U3-R and F4-S site (Figure 3-4) under aerobic condition. Leucine amino peptidase enzyme substrate affinity (Km) ranges from (66 ± 7 $nmol^{-1}g\ dw^{-1}h$) F4-S to in (275 ± 80$nmol^{-1}g\ dw^{-1}h$) F4-R under aerobic condition. In contrast of aerobic LAP enzyme efficiency we did not noticed any significant difference in Km for N-acetyl glucosamine among all the sites. N-acetyl glucosamine activity ranges from 11 ± 2 in U3-S to 23 ± 4 in F4-S under aerobic condition. But we did not observed any significant site effect in N-acetyl glucosamine under aerobic condition.

In contrary to aerobic condition we observed an opposite results under anaerobic condition (Figure 3-5); where there was no significant difference for LAP Km along the sites. Under anaerobic condition it ranged from 50 ± 3 $nmol^{-1}g\ dw^{-1}h$ in F1 to 76 ± 15 $nmol^{-1}g\ dw^{-1}h$ in U3-S.

In contrast of aerobic condition NAG showed a significantly lower activity in F4-S than any other sites. Under anaerobic condition it ranged from 26±4nmol^{-1}g dw^{-1}h in F4-S to 78±21 nmol^{-1}g dw^{-1}h in U3-R.

Correlation of Efficiency (Km) with Soil Biogeochemical Parameters

Among the all six enzymes only PHO Km showed significant correlation with all biogeochemical parameters under anaerobic condition (Table3-6). N processing enzymes and BGLU didn't showed any correlation with any biogeochemical parameters under aerobic condition (Table 3-5).

Carbon Processing Enzymes

No significant correlation between the enzyme efficiency (Km) for glucosidase and cellobiohydrolase with soil total phosphorous concentration were observed (Table 3-5) under aerobic condition. Interestingly we observed a significant correlation of soil total phosphorous with BGLU under anaerobic condition (Table 3-6). Moreover we did not observed any significant correlation of both carbon processing enzyme efficiency (Km) with soil total carbon, C: N ratio, C: P ratio, N: P ratio, MBP, MBN and LOI (Table 3-5) under aerobic condition. Although cellobiohydrolase barely showed a significant positive correlation with soil total nitrogen. Whereas under anaerobic condition we observed a significant negative correlation between CBH and TC, C: N, MBP, MBN and LOI respectively. We did not observed any significant correlation between the two carbon processing enzyme efficiency under aerobic condition. Similarly of aerobic condition both carbon processing enzymes efficiency did not showed any significant correlation between them under anaerobic condition.

Table 3-5. P value of correlation between enzyme efficiency (Km) and soil biogeochemical parameters under aerobic condition.

	BGLU	CBH	PHO	BPHO	LAP	NAG
TP	0.5	0.16	0.0003	0.012	0.75	0.94
TC	0.07	0.20	0.0001	0.74	0.175	0.63
TN	0.17	0.046	0.277	0.23	0.96	0.93
C:P	0.11	0.1	0.023†	0.10	0.32	0.3
C:N	0.97	0.21	0.0006	0.06	0.07	0.69
N:P	0.13	0.21	0.0115†	0.09	0.54	0.36
MBP	0.13	0.13	0.042	0.51	0.83	0.67
MBN	0.25	0.9	0.63	0.98	0.4	0.59
LOI	0.56	0.13	0.0017	0.79	0.36	0.97

† Negative correlation

Phosphorous Processing Enzymes

A significant positive correlation between the enzyme efficiency (Km) for phosphatase and bisphosphatase with phosphorous concentration were observed under both aerobic and anaerobic condition (Table 3-5 and 3-6). Furthermore we also observed significant positive correlation of phosphatase enzyme efficiency (Km) with soil total carbon under both condition (Table 3-5 and 3-6), C: N ratio, MBP and LOI (Table 3-6) under aerobic condition. Whereas we have found significantly negative correlation between the phosphatase enzymes efficiency (Km) with soil total C: P and N: P ratio (Table 3-6) under aerobic condition and TN, C: N, MBP, MBN and LOI under anaerobic condition (Table 3-6). In contrast we did not observed any significant correlation with those for bisphosphatase under aerobic condition but we do observed a significant correlation of BPHO with C: P, N: P and MBN under anaerobic condition

(Table 3-6). However we did not observed any significant correlation between the both P-processing enzymes Km under any condition.

Table 3-6. P value of correlation between enzyme efficiency (Km) and soil biogeochemical parameters under anaerobic condition.

	BGLU	CBH	PHO	BPHO	LAP	NAG
TP	0.037	0.12	<0.0001	<0.0001	0.0232	0.85
TC	0.86†	0.037†	<0.0001	0.33	<0.0001†	0.30
TN	0.11†	0.208†	0.0022†	0.88†	<0.0001†	0.14
C:P	0.09†	0.886	0.0018†	0.0139†	0.68	0.49†
C:N	0.59	<0.0001†	0.0049	0.26	0.05†	0.61†
N:P	0.11†	0.06	0.0001†	0.0087†	0.18	0.68
MBP	0.11	0.017†	<0.0001	0.46†	0.0017†	0.0446
MBN	0.14	0.0067†	0.0285	0.0091	0.022	0.17
LOI	0.67	0.0001†	<0.0001	0.32	<0.0001	0.39

† Negative correlation

Nitrogen Processing Enzymes

We did not observed any significant correlation with leucine amino peptidase and N-acetyl glucosaminidase with any of the biological parameters (Table 3-5) under aerobic condition but we observed a significant correlation of LAP with TC, TN, MBP, MBN and LOI under anaerobic condition (Table 3-6). Interestingly NAG showed a positive correlation with MBP under anaerobic condition (Table 3-6). Both the N-

processing enzymes did not showed any significant correlation between them under both condition.

Aerobic to Anaerobic Enzyme Substrate Affinity (Km)

Ratios of aerobic to anaerobic Km values for all enzymes at all 5 sites indicated that all enzymes with the exception of NAG showed equal or higher maximal enzyme affinity under anaerobic conditions (Figure 3-6). No observed differences in the ratios within substrate affinities of the C-acquiring enzymes suggest that there is no site effect. For P acquiring enzymes, the substrate affinity was higher under anaerobic conditions when compared to the aerobic conditions as indicated by the higher than 1 ratio of the aerobic to anaerobic Km values. There was a significant site effect in the Km of BPHO with F4 ridge showing higher substrate affinities in enzymes under anaerobic conditions. In F4-R the higher ratios for LAP Km was significantly different from those observed in the F1, and F4S. Lower ratios in NAG enzyme indicated higher substrate affinities under aerobic conditions. There did not appear to be any site effect on the Km values of this enzyme.

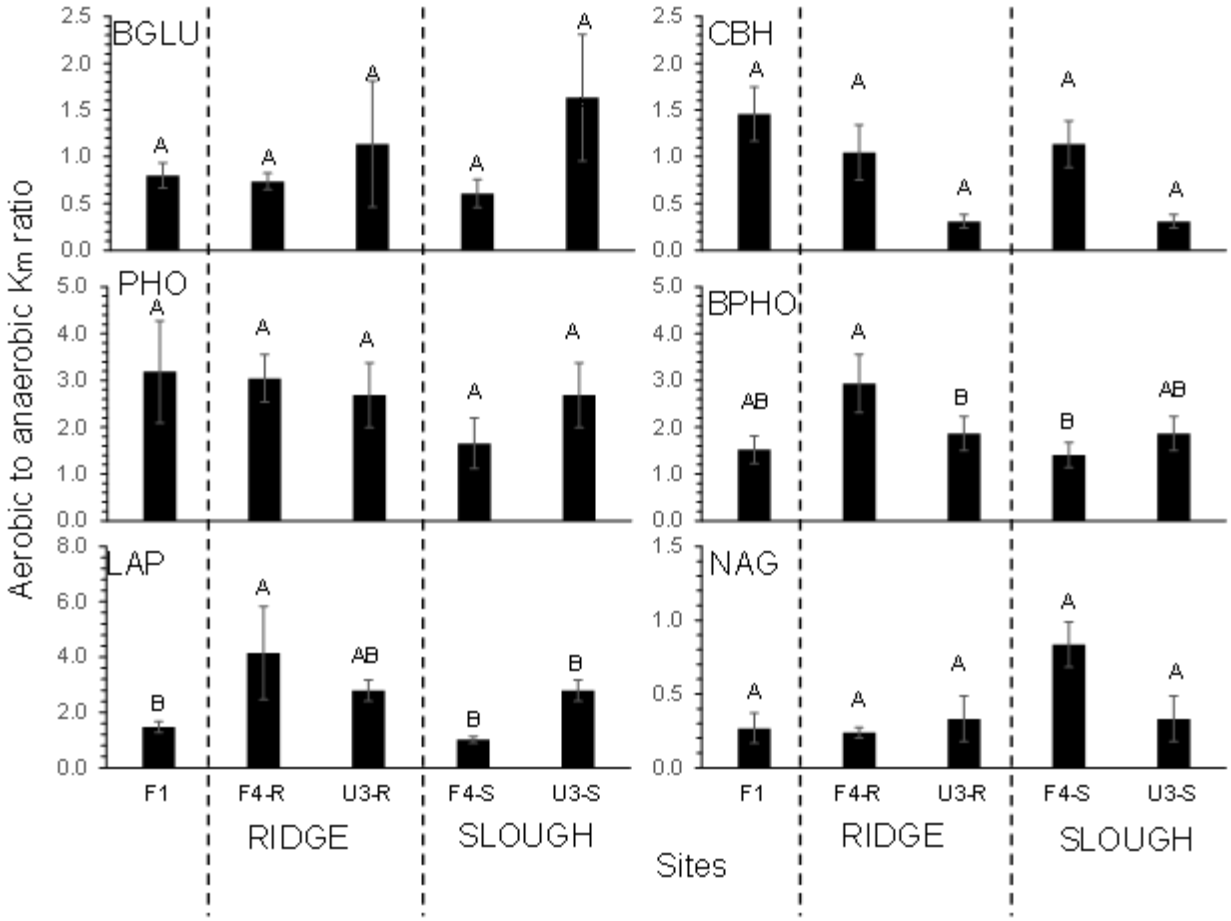

Figure 3-6. Ratio of enzyme efficiency (Km) as measured under aerobic and anaerobic conditions for six extracellular enzymes in 5 sites along the P gradient in the Florida Everglades soil are presented. Bars represent the mean of samples (n= 12) with standard errors.

Temperature Response of Enzyme Activity (Vmax)

The Vmax values increased significantly with temperature for all enzymes under both conditions (Figure 3-7 and 3-8) with an exception of bisphosphatase under aerobic condition (Figure 3-7).The magnitude of temperature response of Vmax varied across enzymes, ranging from -0.008 $°C^{-1}$ in bisphosphatase (F4-R) to 0.099 $°C^{-1}$ in glucosidase (U3-R) which corresponds to Q_{10} values of 0.9 to 2.7 respectively respectably (Table 3-7) under aerobic condition. Whereas under anaerobic condition the magnitude of temperature response of Vmax also varied across enzymes, ranging from 0.011 $°C^{-1}$ in CBH (F4-S) to 0.067 $°C^{-1}$ in NAG (U3-S) which corresponds to Q_{10} values of 1.12 to 1.95 respectably (Table 3-8). In glucosidase under aerobic condition temperature effects on Vmax are significantly high in higher temperature [30°C ^{a}], medium in 25°C ^{b} and lower temperature did not show any significant difference between temperature (20°C^{bc}, 15°C^{c}) for Vmax. Glucosidase did not show any interaction between site and temperature under aerobic condition; meaning the temperature effects are similar along the sites (Figure.3-7). On the contrary we observed a significant site and temperature interaction under anaerobic condition (Figure 3-8). For cellobiohydrolase we noticed significant difference between higher and lower temperatures under both condition. However cellobiohydrolase showed an interaction of temperature along the sites (P = 0.0258 for aerobic and P= 0.0076) under both conditions.

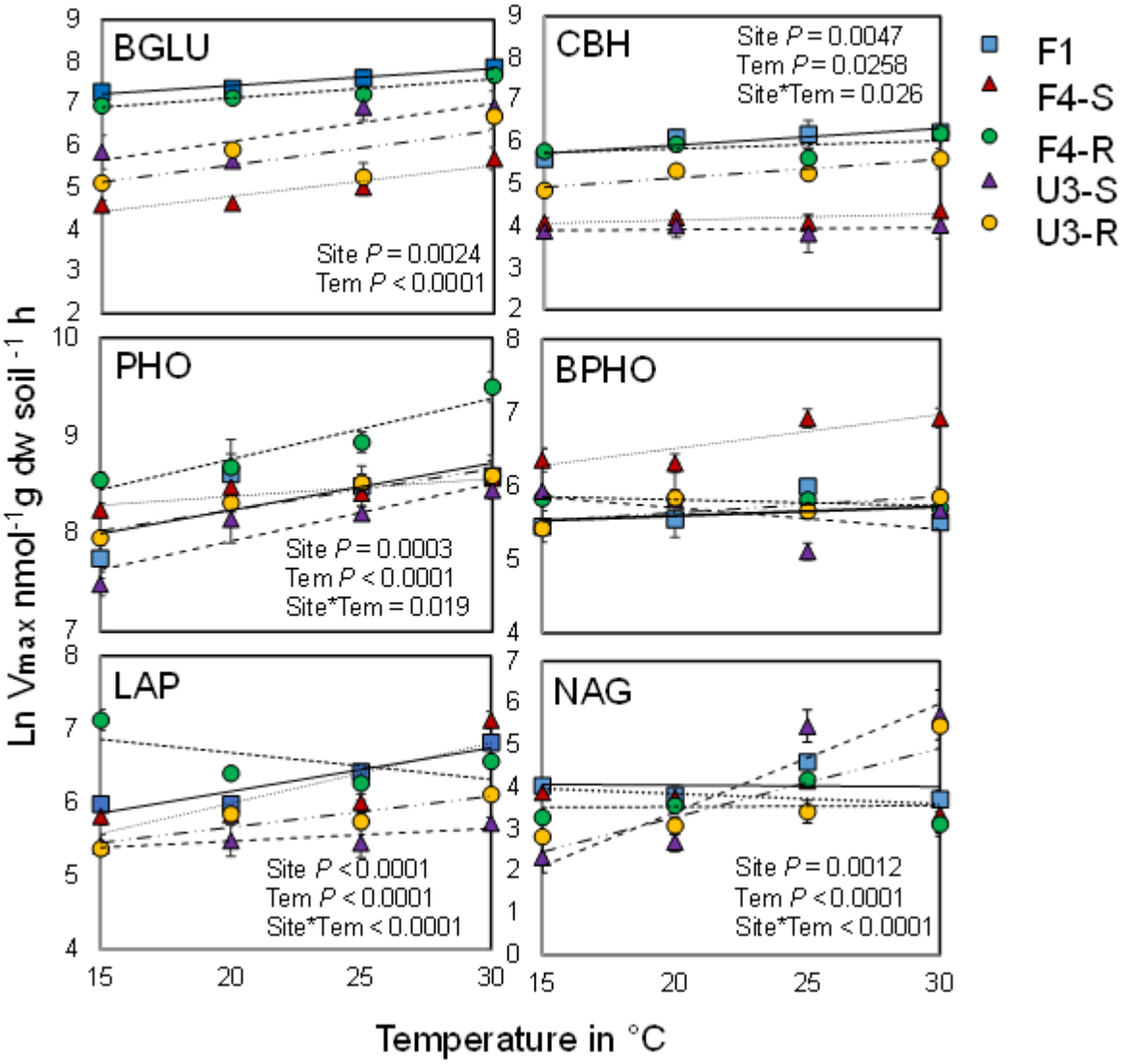

Figure 3-7. Temperature response under aerobic condition for Vmax ß- glycosidase, cellobiohydrolase, phosphatase, bisphosphatase Leucine amino peptidase and N-acetyl glucosaminidase along the temperature change. Vmax is expressed as nmol g^{-1} dw soil h^{-1}.Symbols represent the mean (± SE) Vmax for three replicate at a given temperature. Regression lines are shown for each site. Effects significant at P < 0.05 were considered from 2 factor with 2 way interaction in a linear mixed model (split plot design) where site as whole plot factor and temperature as split plot factor.

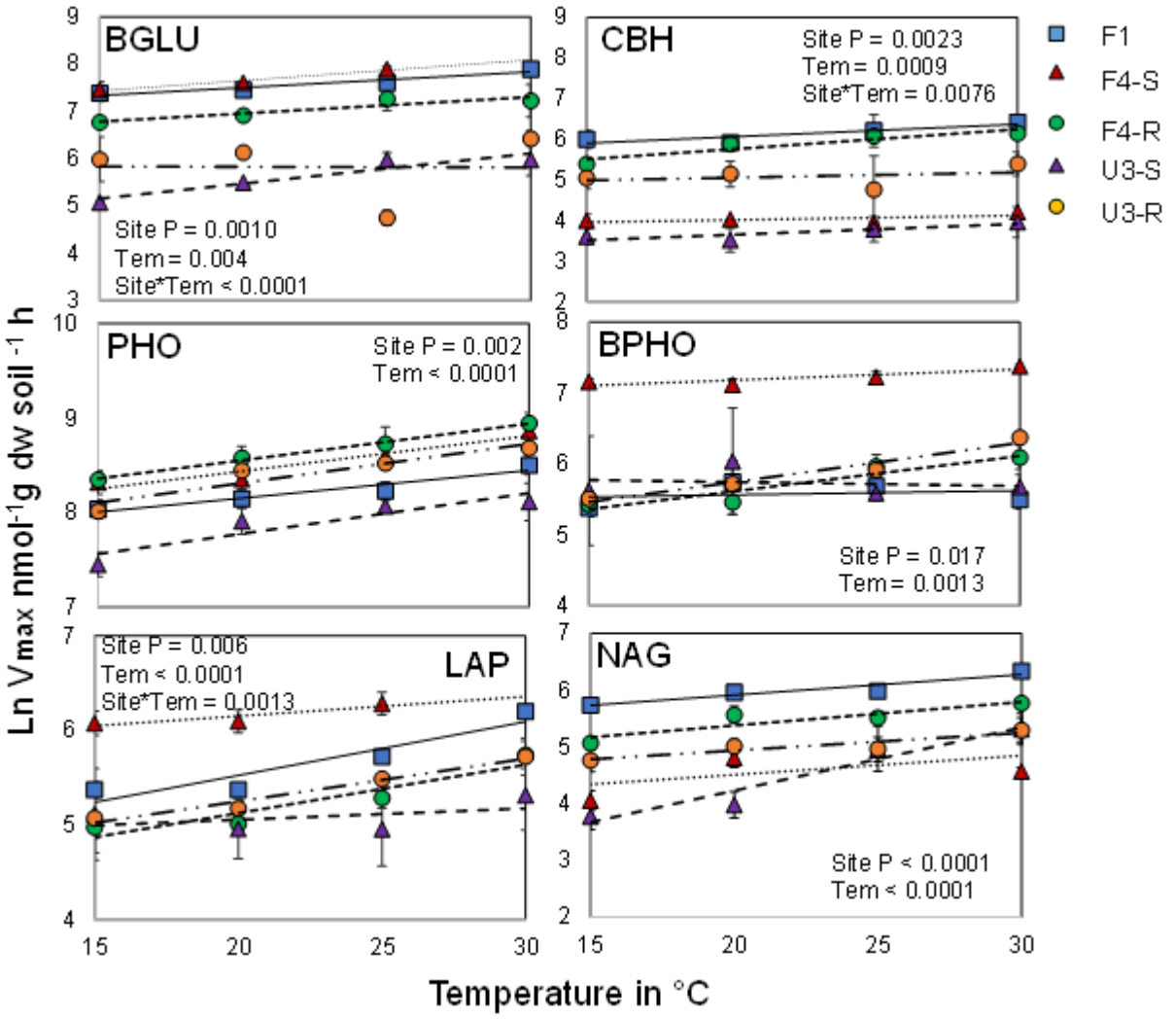

Figure 3-8. Temperature response under anaerobic condition for Vmax ß- glycosidase, cellobiohydrolase, phosphatase, bisphosphatase Leucine amino peptidase and N-acetyl glucosaminidase along the temperature change. Vmax is expressed as nmol g^{-1} dw soil h^{-1}.Symbols represent the mean (± SE) Vmax for three replicate at a given temperature. Regression lines are shown for each site. Effects significant at $P < 0.05$ were considered from 2 factor with 2 way interaction in a linear mixed model (split plot design) where site as whole plot factor and temperature as split plot factor.

Phosphatase and glycosidase showed similar kind of temperature effects with a range of temperature from 15°C to 30°C. Although it showed a significant temperature and site interaction (*P*=0.0190) only under aerobic condition (Figure 3-7).

Between the two nitrogen processing enzymes, only Leucine amino peptidase showed a significant temperature and site interaction (Figure 3-7 and 3-8) under both condition but N-acetylglucosamine showed significant site and temperature interaction only under aerobic condition (Figure 3-7). In leucine amino peptidase we found significant difference in Vmax between high [30°C[a]] and lower temperature under aerobic condition. But N-acetyl glucosamine highest enzyme activity was noticed in 30°C [a] intermediate in 25°C [b] and lowest in two lower temperatures under aerobic condition. On the other hand under anaerobic condition both the N-processing enzymes showed significantly lower enzyme activity under lower temperature than the higher temperatures.

Table 3-7. Regression slopes for (Vmax) in nmol g^{-1} dw soil h^{-1} as a function of temperature (n = 20 for each enzyme) under aerobic condition.

Enzyme	Site	Slope	± SE	Q_{10}
BGLU	F_1	0.045	0.011	1.6
	F_4-S	0.056	0.009	1.8
	F_4-R	0.045	0.011	1.6
	U_3-S	0.060	0.054	1.8
	U_3-R	0.099	0.099	2.7
CBH	F_1	0.043	0.012	1.6
	F_4-S	0.018	0.010	1.2
	F_4-R	0.027	0.001	1.3
	U_3-S	0.005	0.001	1.0
	U_3-R	0.044	0.014	1.6
PHO	F_1	0.048	0.011	1.6
	F_4-S	0.018	0.008	1.2
	F_4-R	0.040	0.007	1.5
	U_3-S	0.059	0.000	1.8
	U_3-R	0.042	0.014	1.5
BPHO	F_1	0.013	0.011	1.1
	F_4-S	0.046	0.015	1.6
	F_4-R	-0.008	0.013	0.9
	U_3-S	0.005	0.020	1.1
	U_3-R	0.022	0.006	1.3
LAP	F_1	0.054	0.002	1.7
	F_4-S	0.049	0.006	1.6
	F_4-R	0.053	0.027	1.7
	U_3-S	0.049	0.004	1.6
	U_3-R	0.042	0.011	1.5
NAG	F_1	0.020	0.028	1.2
	F_4-S	0.026	0.014	1.3
	F_4-R	0.090	0.006	2.4
	U_3-S	0.044	0.000	1.5
	U_3-R	0.019	0.012	1.2

Table 3-8. Regression slopes for (Vmax) in nmol g^{-1} dw soil h^{-1} as a function of temperature (n = 20 for each enzyme) under anaerobic condition.

Enzyme	Site	Slope	± SE	Q_{10}
BGLU	F_1	0.034	0.007	1.4
	F_4-S	0.044	0.014	1.6
	F_4-R	0.035	0.017	1.4
	U_3-S	0.063	0.016	1.9
	U_3-R	0.034	0.011	1.4
CBH	F_1	0.041	0.003	1.5
	F_4-S	0.011	0.010	1.1
	F_4-R	0.053	0.001	1.7
	U_3-S	0.041	0.003	1.5
	U_3-R	0.036	0.004	1.4
PHO	F_1	0.029	0.003	1.3
	F_4-S	0.037	0.003	1.5
	F_4-R	0.039	0.003	1.5
	U_3-S	0.042	0.002	1.5
	U_3-R	0.042	0.005	1.5
BPHO	F_1	0.021	0.004	1.2
	F_4-S	0.015	0.007	1.5
	F_4-R	0.043	0.009	1.5
	U_3-S	0.043	0.011	1.5
	U_3-R	0.049	0.008	1.6
LAP	F_1	0.054	0.004	1.7
	F_4-S	0.025	0.002	1.3
	F_4-R	0.051	0.010	1.7
	U_3-S	0.017	0.006	1.2
	U_3-R	0.045	0.004	1.6
NAG	F_1	0.036	0.015	1.4
	F_4-S	0.054	0.008	1.7
	F_4-R	0.044	0.001	1.6
	U_3-S	0.067	0.025	1.9
	U_3-R	0.014	0.031	1.1

Temperature Sensitivity of Vmax

Temperature sensitivity of a reaction is often represented as a Q_{10} value. Higher Q_{10} values represent higher temperature sensitivity. Q_{10} values higher than 1 represent positive relationship with temperature and Q_{10} values lower than 1 represent a negative relationship with temperature. Q_{10} values of the maximal enzyme activities varied under aerobic and anaerobic conditions (Table 3-9 and 3-10).

Aerobic

All the enzyme showed a positive relation with temperature which is $Q_{10} > 1$ but only for bisphoaphatase in F4-R it is lower than 1(Q_{10}=0.9) under aerobic condition (Table 3-9). In glucosidase we have noticed significant site effect in Q_{10} (*P* = 0.0032) but the other enzymes did not showed a significant site effect on temperature sensitivity under aerobic condition. We have noticed some different effect of temperature sensitivity when comparing between slough and slough, ridge and ridge or between slough and ridge under different condition. While comparing the temperature sensitivity under aerobic condition between the two ridge soils we have noticed significant difference in temperature sensitivity only in glucosidase and in N-acetyl glucosaminidase. In glucosidase higher temperature sensitivity in U3-R than F4-R (*P* = 0.0111) but in NAG the temperature sensitivity is high in F4-R than U3-R (*P* =0.0134) under aerobic condition observed. In F4 site we have noticed only in N-acetyl glucosamine there is a significant difference in temperature sensitivity between F4-R[a] and F4-S[b] (*P* = 0.0065) under aerobic condition. In U3 site only glucosidase showed a difference in temperature sensitivity which is high in U3-R than U3-S (*P* = 0.0114) under aerobic condition. Similarly in phosphatase we have noticed a significant difference

between F4-S and U3-S (*P*=0.0030) which is high in U3-S than F4-S under aerobic condition.

Anaerobic

Under anaerobic condition we observed a significant site effect for CBH (*P* = 0.021), LAP (*P* = 0.001) and NAG (*P* = 0.016) respectively. In contrast of aerobic condition, in BGLU and NAG we did not observed any significant difference between two ridge soils under anaerobic condition. Moreover we did not observed any difference in temperature sensitivity for CBH and BPHO but under anaerobic condition CBH and BPHO showed a significantly higher activity in F4-R than F4-S. Moreover LAP showed significantly higher Vmax temperature sensitivity in U3-R than U3-S under anaerobic condition.

Temperature Response of Enzyme Efficiency (Km)

In contrast of Vmax we did not observed any positive temperature response of Km for most of the sites. Among the six enzymes we have studied only phosphatase and both nitrogen processing enzymes [leucine amino peptidase (*P* < 0.0001) and N-acetyl glucosaminidase (*P* = 0.0414)] showed a significant temperature effect only under aerobic condition (Figure 3-9). Other enzymes did not showed a significant difference between the temperature changes under both condition. Moreover we have found a significant interaction of temperatures and site for phosphatase (*P* = 0.0411) and leucine amino peptidase (*P* < 0.0001). The magnitude of temperature response of Km varied across enzymes, ranging from -0.148 $°C^{-1}$ in Leucine amino peptidase (F4-S) to 0.073 $°C^{-1}$ in cellobiohydrolase (F1) which correspond to Q_{10} values of 0.23 to 2.08 (Table 3-9) under aerobic condition. Under anaerobic condition it ranges from -0.106 $°C^{-1}$ in BPHO (F1) to 0.052 $°C^{-1}$ in PHO (U3-S) which correspond to Q_{10} values of 0.35 to

1.67 (Table 3-10). We have found that other than F1 and F4-S site phosphatase showed a positive effect on temperature sensitivity (Table 3-9) under aerobic condition. However other than F4-R in LAP both nitrogen processing enzyme showed a significant negative temperature sensitivity. Although other enzyme did not show a significant temperature effect but majority of sites in other enzymes showed a negative temperature effect on Km (Table 3-9). Among the six enzymes, BGLU showed positive temperature effect on Km in all the sites under anaerobic condition (Table 3-1).

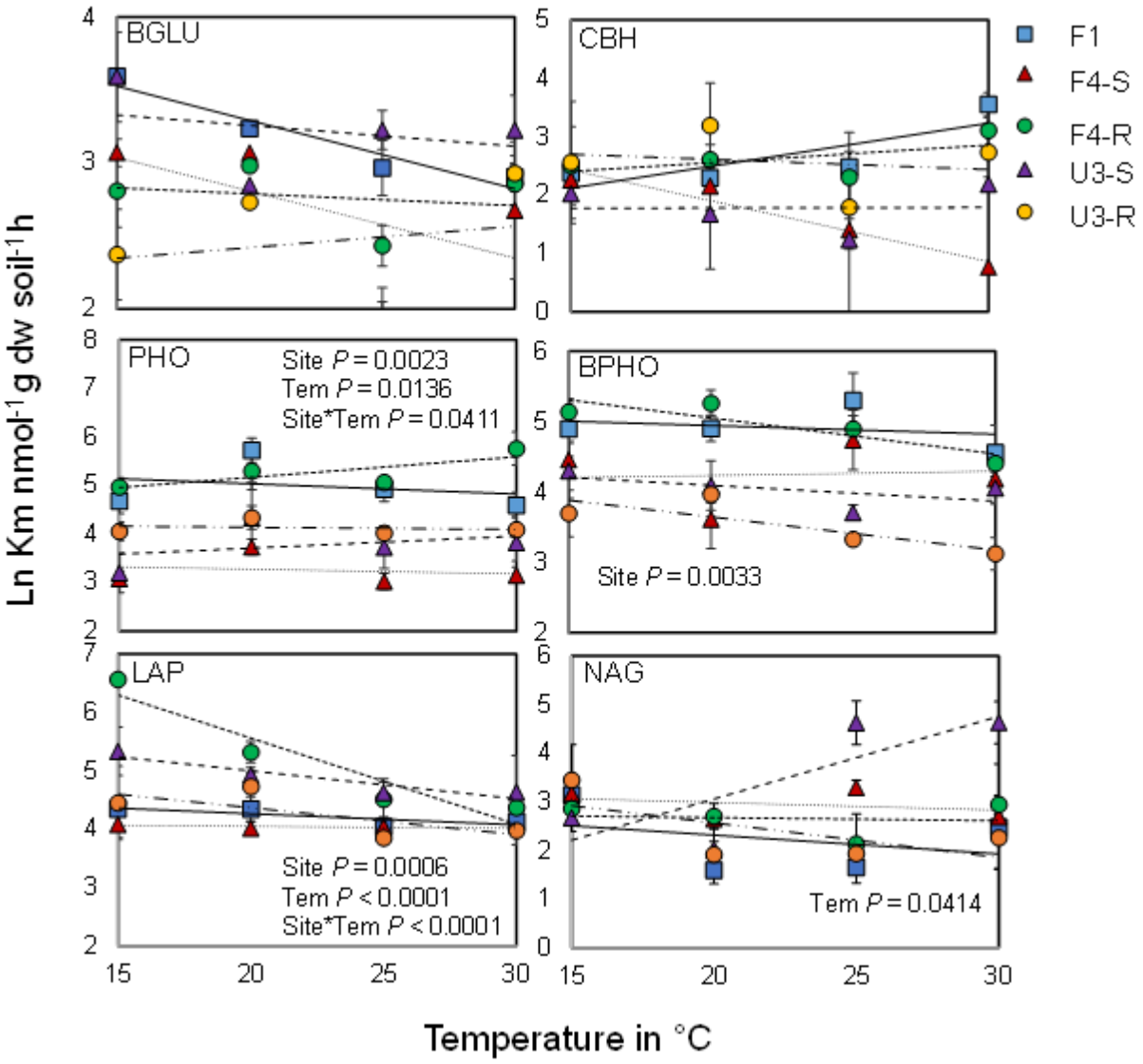

Figure 3-9. Temperature response for Km ß- glycosidase, cellobiohydrolase, phosphatase, bisphosphatase Leucine amino peptidase and N-acetyl glucosaminidase along the temperature change. Vmax is expressed as nmol g^{-1} dw soil h^{-1}.Symbols represent the mean (± SE) Vmax for three replicate at a given temperature. Regression lines are shown for each site. Effects significant at P < 0.05 were considered from 2 factor with 2 way interaction in a linear mixed model (split plot design) where site as whole plot factor and temperature as split plot factor.

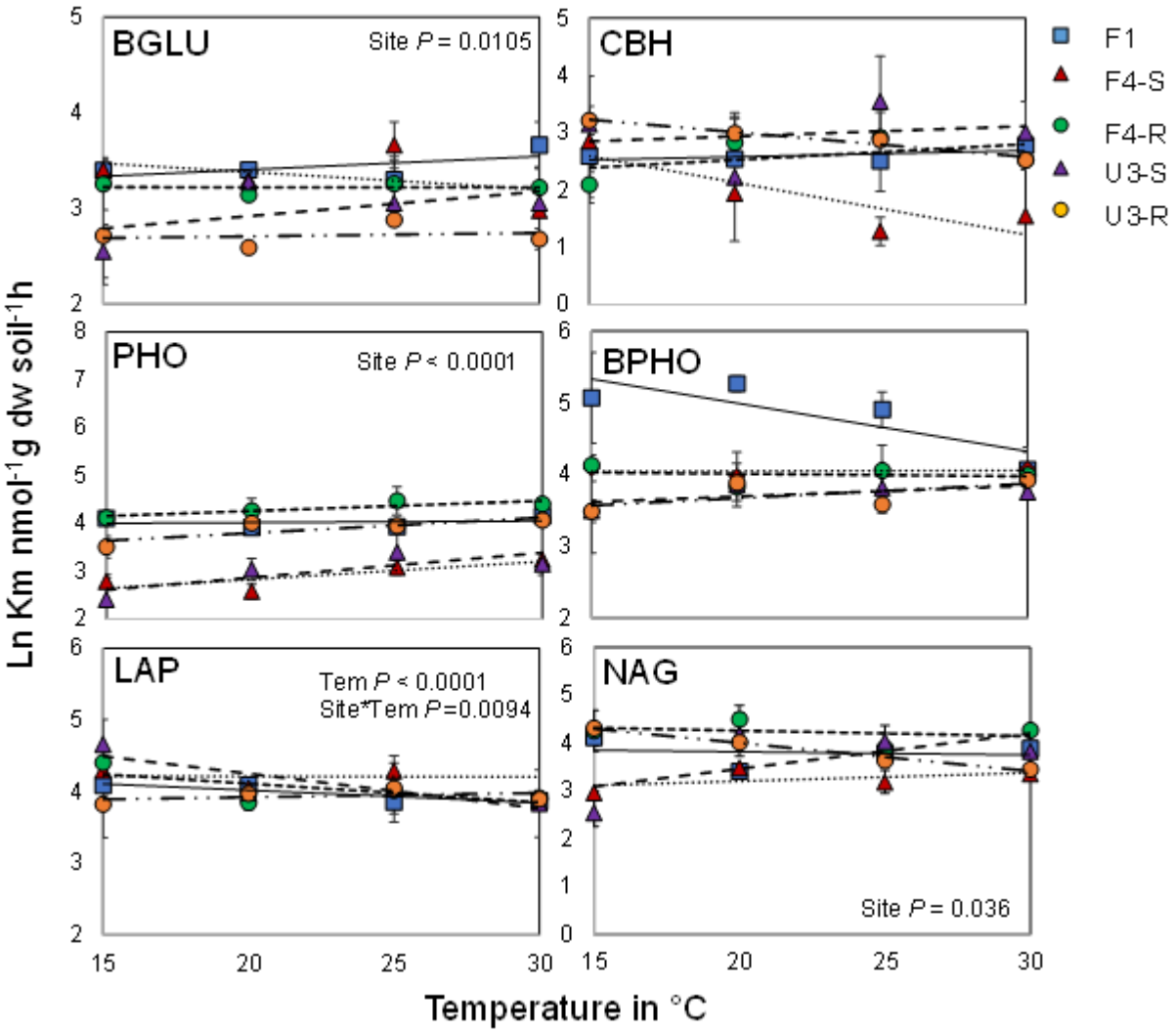

Figure 3-10. Temperature response for Km ß- glycosidase, cellobiohydrolase, phosphatase, bisphosphatase Leucine amino peptidase and N-acetyl glucosaminidase along the temperature change. Vmax is expressed as nmol g^{-1} dw soil h^{-1}.Symbols represent the mean (± SE) Vmax for three replicate at a given temperature. Regression lines are shown for each site. Effects significant at $P < 0.05$ were considered from 2 factor with 2 way interaction in a linear mixed model (split plot design) where site as whole plot factor and temperature as split plot factor.

Table 3-9. Regression slopes for (Km) under aerobic condition in nmol $^{-1}$g dw h^{-1} as a function of temperature (n = 20 for each enzyme).

Enzyme	Site	Slope	± SE	Q_{10}	
BGLU	F_1	-0.047	0.004	0.63	
	F_4-S	-0.065	0.024		0.52
	F_4-R	-0.018	0.008	0.83	
	U_3-S	-0.009	0.031	0.91	
	U_3-R	-0.046	0.051	0.63	
CBH	F_1	0.073	0.011	2.08	
	F_4-S	-0.105	0.065	0.35	
	F_4-R	0.063	0.007	1.88	
	U_3-S	-0.051	0.056	0.60	
	U_3-R	-0.006	0.021	0.95	
PHO	F_1	-0.034	0.015	0.71	
	F_4-S	-0.002	0.013	0.98	
	F_4-R	0.044	0.027	1.56	
	U_3-S	0.041	0.016	1.50	
	U_3-R	0.025	0.002	1.20	
BPHO	F_1	0.015	0.042	1.16	
	F_4-S	0.006	0.012	1.06	
	F_4-R	-0.051	0.008	0.6	
	U_3-S	0.008	0.035	1.08	
	U_3-R	-0.047	0.017	0.62	
LAP	F_1	-0.020	0.006	0.82	
	F_4-S	0.023	0.016	1.25	
	F_4-R	-0.148	0.008	0.23	
	U_3-S	-0.048	0.024	0.62	
	U_3-R	-0.046	0.022	0.63	
NAG	F_1	-0.012	0.023	0.88	
	F_4-S	-0.054	0.057	0.58	
	F_4-R	-0.027	0.016	0.76	
	U_3-S	-0.084	0.020	0.43	
	U_3-R	-0.082	0.035	0.44	

Table 3-10. Regression slopes for (Km) under anaerobic condition in nmol $^{-1}$g dw h^{-1} as a function of temperature (n = 20 for each enzymes).

Enzyme	Site	Slope	± SE	Q_{10}
BGLU	F_1	0.015	0.005	1.16
	F_4-S	0.026	0.015	1.3
	F_4-R	0.018	0.024	1.2
	U_3-S	0.021	0.028	1.23
	U_3-R	0.004	0.041	1.04
CBH	F_1	-0.032	0.005	0.73
	F_4-S	-0.091	0.031	0.40
	F_4-R	0.027	0.009	1.31
	U_3-S	-0.024	0.013	0.78
	U_3-R	-0.074	0.028	0.48
PHO	F_1	0.003	0.005	1.03
	F_4-S	0.037	0.003	1.45
	F_4-R	0.021	0.002	1.24
	U_3-S	0.052	0.025	1.67
	U_3-R	0.032	0.013	1.38
BPHO	F_1	-0.106	0.038	0.35
	F_4-S	-0.023	0.002	0.79
	F_4-R	-0.011	0.004	0.90
	U_3-S	0.024	0.035	1.28
	U_3-R	0.020	0.005	1.22
LAP	F_1	-0.010	0.004	0.90
	F_4-S	-0.002	0.010	0.98
	F_4-R	-0.026	0.005	0.77
	U_3-S	-0.048	0.013	0.62
	U_3-R	0.006	0.003	1.06
NAG	F_1	-0.006	0.026	0.94
	F_4-S	0.019	0.023	1.21
	F_4-R	-0.028	0.017	0.75
	U_3-S	-0.015	0.017	0.86
	U_3-R	-0.058	0.023	0.56

Temperature Sensitivity of Enzyme Efficiency (Km)

Aerobic

We observed a significant difference in temperature sensitivity along the all sites only in cellobiohydrolase (*P*=0.0006) and leucine amino peptidase (*P* = 0.0030). Similarly of Vmax we have noticed some different effect of temperature sensitivity while comparing between slough and slough, ridge and ridge or between slough and ridge soils. But we did not noticed any significant difference of temperature sensitivity between F4-S and U3-S in all enzymes. Although we have noticed significant difference in temperature sensitivity in two ridge soil in cellobiohydrolase (*P* = 0.0246) and leucine amino peptidase (*P* = 0.0438) under aerobic condition. But they have showed an opposite result; for example cellobiohydrolase F4-R have showed a higher temperature sensitivity than U3-R and in Leucine amino peptidase showed a higher temperature sensitivity in U3-R than F4-R under aerobic condition.

In F4 site we observed difference in temperature sensitivity for cellobiohydrolase (*P* = 0.0023), bisphosphatase (*P* = 0.0325) and leucine aminopeptidase (*P* = 0.0054). For leucine aminopeptidase and bisphosphatase the temperature sensitivity was higher in F4-S than F4-R but for cellobiohydrolase it was high in F4-R than F4-S.

However we did not find any significant difference in temperature sensitivity between U3-S and U3-R for all enzymes.

Anaerobic

Under anaerobic condition we observed significantly higher (*P* = 0.0276) temperature sensitivity for BPHO (*P* = 0.0276) and LAP (*P* = 0.0031) in U3-R than F4-R. However while comparing between two sloughs soils we did not find any significant difference for any enzymes. Moreover we have observed significant difference in

temperature sensitivity for CBH and PHO in F4 site ridge and slough. CBH (P = 0.0088) showed significantly higher temperature sensitivity in F4-R than F4-S whereas we noticed opposite result for PHO (P = 0.0078). Comparing between ridge and slough soil in U3 site we found only LAP show significantly (P = 0.0101) higher temperature sensitivity in U3-R than U3-S soil.

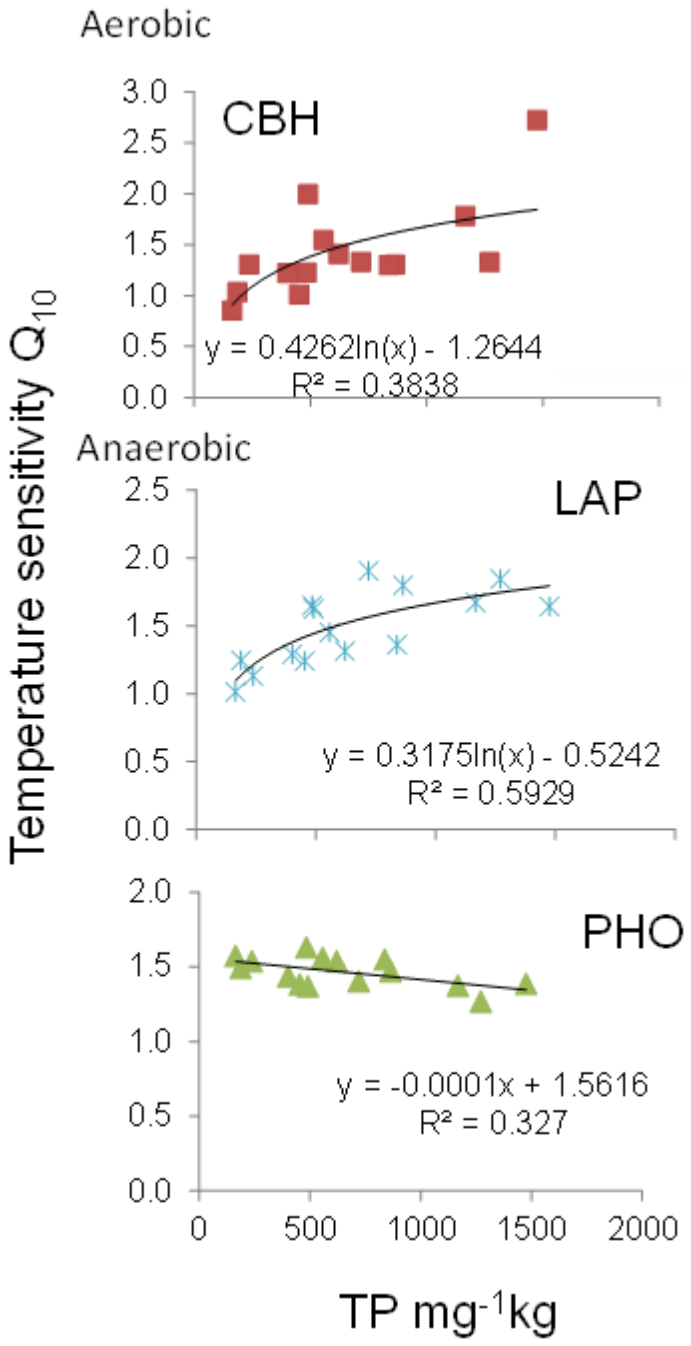

Figure 3-11.Correlations of soil phosphorus concentrations and temperature sensitivities of maximal enzyme velocity under aerobic and anaerobic conditions.

Correlation of Q_{10} of Kinetic Parameters (Vmax and Km) with Soil Biogeochemical Parameters

While correlating the various soil biogeochemical parameters in soils with the kinetic parameters measured under both aerobic and anaerobic conditions we found that several factors were positively correlated with the enzyme Vmax. Such as we have noticed that only BGLU showed some positive correlation with TN under aerobic condition whereas we observed some correlation of Vmax for CBH with TC, PHO with N: P and LAP with TP, TC, MBP and LOI under anaerobic condition respectively. Some of these factors were also found to be negatively correlated (as for example BPHO with TC and TN under aerobic condition and PHO with TP and C: N, LAP with N: P under anaerobic condition). Concentrations of soil P displayed a stronger association with enzyme activities as compared to the total soil C and N. Microbial biomass parameters did not appear to correlate with the enzyme kinetic parameters.

Km Q_{10} also observed some correlation with soil biogeochemical parameters. Such as we observed significant positive correlation of CBH with TP, TC, and C: N and NAG with TP under aerobic condition whereas we noticed positive correlation of CBH with C: N and BPHO with C: P and N: P under anaerobic condition.

We also observed negative correlation of CBH Q_{10} with C: P and N: P and NAG Q_{10} with C: P and N: P under aerobic condition. Under anaerobic condition PHO Q_{10} with TP, TC and C: N, BPHO with TP and LAP with C: P and N: P observed respectively.

Correlations of Q_{10} values of Vmax for each enzyme with soil P revealed a significant positive logarithmic relationship ($P = 0.06$) for CBH. This observed correlation was absent under anaerobic enzyme assay. Under anaerobic assay conditions, soil P

was positively correlated to Q_{10} N acquiring (LAP) enzyme activity (P = 0.01) and negatively correlated to P-acquiring enzyme (PHO) enzyme activity (P = 0.04) (Figure 3-11). These correlations were not present under aerobic assay conditions. Q_{10} values of the enzyme efficiency also varied under aerobic and anaerobic conditions (Figure 3-12). Correlations of the Q_{10} values with the soil P revealed a significant positive logarithmic relationship (P = 0.005) for CBH and a positively linear relationship (P = 0.009) for NAG under aerobic conditions. Significant negative linear relationships between Q_{10} of Km and soil P were also observed for PHO (P = 0.02) and BPHO (P = 0.03) under anaerobic conditions.

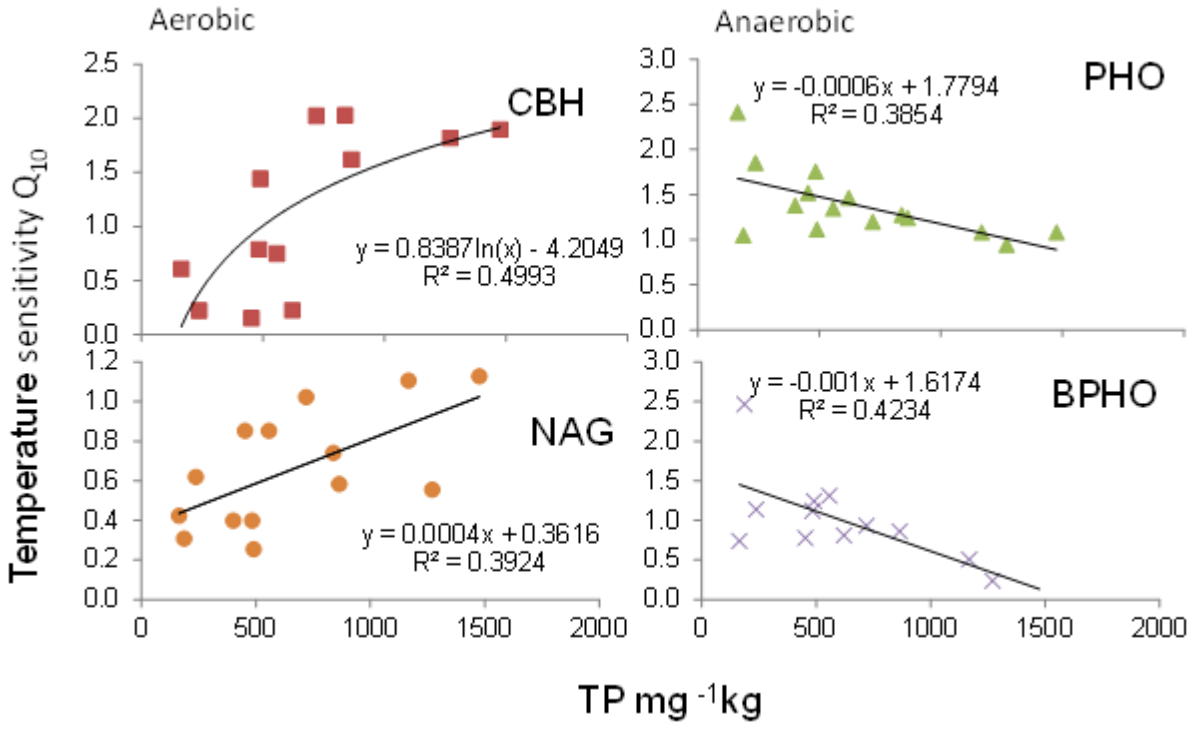

Figure 3-12. Correlations of soil phosphorus concentrations and temperature sensitivities of enzyme efficiency under aerobic and anaerobic conditions.

Discussion

Biogeochemical Parameters

In this study we investigated soil enzyme kinetic parameters, namely maximal reaction velocity (Vmax) and Michaelis Menton constant (Km) for six extracellular enzymes in response to P gradient in wetland soils. The five sites chosen for this study were situated on transect that represents a soil P gradient and it extends from the high P exterior region of WCA- 2A to a low P interior region. Analysis of soil biogeochemical parameters and their ratios revealed that five sites significantly differed in their concentration of total P and total C with P concentration being most notable. Ratios of total C: total N, total C: total P and total N: total P have been used in the past to indicate nutrient limitation in soils (Cleveland and Liptzin 2007). In our study total C: total P and total N: total P ratio suggested that U3-S soils were significantly P limited compared to other site. Ranges of nutrient concentration in these soils are similar to those reported in other studies (Reddy, DeBusk et al. 1993). Although we do not have the microbial biomass data from the soil samples collected in March 2013, microbial biomass numbers from another set of samples collected in august indicated higher microbial biomass in eutrophic high P sites and significantly low MBC values in the U3 sites (Table 3-2). Previous studies on this site have also revealed that nutrient enrichment can change soil substrate quality which ultimately can change soil microbial function thereby affecting the microbially mediated rates of SOM decomposition (DeBusk and Reddy 1998). Amador and Jones (1997) reported that substrate quality may be the limiting factor when the nutrient ratios of total C to total P are1200:1 or higher. They also observed significantly suppressed microbial respiration due to high C: P value. Moreover in support of their observation we also noticed carbon to phosphorous ratio in

U3-S site is 1856:1 (Table 3-2) which indicated the importance of available substrate. Previous studies by Vyzamal and Richardson, (1995) showed higher N: P ratio in Florida Everglades periphyton created P limitation for periphyton growth (Vymazal and Richardson 1995). In our study site significantly higher N: P ratio in U3-S than other sites also suggested P limitation. Moreover Cleveland et al (2007) stated that soil microbial biomass ratio is 60:7:1 and lower N: P ratio will indicate higher P demand. In our nutrient limited site the MBN: MBP ratio are very low (Table 3-2) which indicate higher P demand on that sites (Cleveland and Liptzin 2007). As a result we observed lower microbial activity on nutrient limited site.

Nutrient Enrichment and Limitation on Enzyme Kinetics

Carbon Enzymes

According to our hypothesis we expected lower values of Vmax for carbon processing enzymes in P limited site than P impacted site. We expected lower activity of carbon processing enzyme in low nutrient site because of two reasons as 1) lower carbon demands by microbes on that site 2) lower microbial biomass will produce less amount of enzymes. Moreover we expected same or low Km for P limited site as P impacted site. Observed differences in both carbon enzymes across the five sites indicated increased C enzymes in the high P regions. Significant site (BGLU $P = 0.0024$ and CBH $P = 0.0047$) effects on Vmax values for both carbon enzyme supported our hypothesis. In support of our hypothesis we also observed nutrient limitation effect on BGLU and CBH in nutrient limited site (U3-S) under aerobic condition which showed significantly lower Vmax than P impacted site. This may be explained by the allocation theory that explains that under P limitation conditions microorganisms channel all the resources and energy towards producing P acquiring enzymes. In soils with high soil P

concentrations demand on C and N was expected to be higher and the increased Vmax values in the F1 and F4-R sites supported the hypothesis. Much lower CBH Vmax value for CBH enzymes can be explained by the fact that the enzymes that degrade cellulose were much lower in the U3 sites because of the lack of cellulosic substrate material. Moreover lower microbial biomass in P limited site also supported lower Vmax activity in P limited site (Table 3-2). Significant positive correlation of BGLU (P<0.0001) and CBH (P<0.0001) with TP suggested that nutrient P played an important role in carbon enzymes activity. In support of our observed results we have found that both the enzymes are negativity correlated with C: P ratio which suggested that when there will be more P demand that is higher C: P value then there will be low carbon enzyme activity. Previous study also reported negative correlation of BGLU with C: P ratio (Penton and Newman 2007). So significantly higher C: P ratio in P limited site suggested the lower carbon enzyme activity on that site. As we mentioned earlier in oligotrophic wetlands such as Everglades; high C: P ratio of organic matter due to phosphorous limitation in U3 influences microbial activities which then influence decomposition process. Decomposition of organic matter is directly related to soil total nutrients concentration (Nye 1960). As a result microbial enzymatic activities are directly dependent to nutrient availability. Stevenson (1986) reported that as C: P ratio increases that is > 300 in soil then it will decrease the decomposition processes (mineralization) and there will be net immobilization. In our research site C: P ratio in F1 site is 348(±24) whereas in U3-S it is 1856(±243) which indicate higher immobilization process on U3 sites.

Importance of nutrient demand by microbes varies among ecosystems depending upon the relative nutrient supply (Fölster and Khanna 1997). Microorganisms which grow in low nutrient condition try to scavenge more nutrients from the surrounding environment. Which create net immobilization of growth limiting nutrients. We have found that high concentration of P in F1 site created microbial carbon limitation, resulting increase in carbon enzymes activity on that site under both aerobic and anaerobic condition (Figure 3-1 and 3-2). Previously, Penton and Newman 2007 observed highest BGLU enzyme activity in P enriched site at 100 µM concentration, which supports our findings. In addition, we explored the enzyme kinetics at varying concentration levels (50 µM to 400 µM).

Moreover previous study by Khalid, Gamble and Patrick in (1978) reported that when soil O_2 concentration and redox potential decline then it would create a direct impact on C, N, Fe and P form and mobility (Khalid Jr et al. 1978). Furthermore Silver and Keller in (1999) reported in their study in forest ecosystem that availability of P are significantly correlated with SOM under well aerated soil, where inorganic P bind strongly with iron oxide and hydroxide. Not only that they also observed that due to lower O_2 concentration under anaerobic condition there will be more available P in the soils (Silver et al. 1999). In our study anaerobic condition may create a lower C: P ratio in F4-S soil than U3-S soil due to higher availability of P which may be the reason of higher activity on BGLU in F4-S site than U3-S site under anaerobic condition.

According to our hypothesis we did not observed any significant difference enzyme substrate affinity except BGLU under anaerobic condition.

So our first hypothesis that limitation of phosphorous decrease carbon processing enzymes activities in P limited site and P enriched site will increase activity of carbon enzymes under both conditions was supported by our results. Which emphasize that nutrient ratio play a key role in an ecosystem nutrient cycling. Moreover lower oxygen availability or higher oxygen availability may sometime influence that process.

Nitrogen Enzymes

According to our hypothesis we expected lower nitrogen enzyme activity in P limited site and higher in P impacted site mainly of two reasons 1) as N is an important constituent for enzyme structure, its demand would be higher where the microbial biomass is high and the lower microbial biomass would reduce its need for nitrogen; as a result we expected lower Vmax of N processing enzymes in P limited site. 2) Higher P demand would reduce lower N enzyme activity in P limited site. We also expected low or similar Km in P limited site like P impacted site.

We observed significant site effect for both nitrogen processing enzymes under both condition. Although we did not show any significant P gradient effect on nitrogen processing enzymes but it appeared that average activity of LAP and NAG were lower than P impacted and ridge soils. As we did not observed any significant difference in TN along the site; so significant differences in P may create differences in N-processing enzymes activity along the site. Moreover leucine amino peptidase showed a significant correlation with TP under aerobic condition. Similarly NAG showed a significant correlation with TP under anaerobic condition. Which indicate that presence or absence of oxygen may create different effects on both N-processing enzymes under different conditions. Miao and DeBusk (1999) stated that 1064 -1774 mg $^{-1}$kg of P can result in

nitrogen limitation in a site. Our observed TP in F1 site created nitrogen limitation. Whereas P limited site did not appeared to be N limited as a result we observed lower nitrogen enzyme activity in P limited site. Previous studies reported that under N limitation when the N: P supply is low as 16 or less and by P when the N: P ratio is higher than that (Güsewell 2004, GÜSewell and Freeman 2005). Both the nitrogen processing enzymes showed a similar higher activity in F1 and F4 site than U3-S site; which may be due to significantly lower ratio of TP:TN in F1 (25±1.6) and F4-R site (36±1.3) than U3-S site (153±13). Rejmankova and Sirova (2007) stated that they have observed a higher leucine amino peptidase activity in P enriched soil in Belize marsh wetland (Central America) (Rejmánková and Sirová 2007) because of higher investment of N acquiring enzymes due to P limitation. Moreover they also reported significant correlation of microbial biomass which they measured as PFLA with decomposition process mediated by enzymes (Carbon and nitrogen acquiring enzymes) which were high in P enriched site. Similarly we observed the microbial biomass nitrogen number were significantly high in F1 site (P enriched) than U3 (P limited) site. Rejmankova and Sirova (2007) also reported higher gram negative bacteria (monosaturated fatty acid), gram positive bacteria (branched fatty acid) and fungal biomarker in P enriched site (Rejmánková and Sirová 2007) which indicate higher enzyme activity in P enriched site. Similarly we have found higher leucine-aminopeptidase activity in F1 and F4-R site (Figure 3-1) under aerobic condition and in F1 and F4-S under anaerobic condition (3-2). Whereas N-acetyl-glucosaminidase showed higher activity in F1 under both condition (3-1 and 3-2) which is the main fungal cell wall degrading enzymes. If Phosphorous is not limiting, it may be energetically efficient for microbes to produce

enzyme that mainly target carbon and nitrogen (Olander and Vitousek 2000) whereas carbon on that system mainly recycled by BGLU and CBH. As we mentioned earlier that anaerobic condition may create higher available phosphorous; so may be this cause the higher NAG activity under anaerobic condition as presence of higher P trigger NAG activity. Higher activity of NAG over LAP under anaerobic condition was observed by Nagata et al (1989) in renal tubular system where they observed that NAG activity was higher in 0% oxygen than 95% oxygen level group (Nagata et al. 1989). We can relate our result with higher NAG activity under anaerobic condition. They also mentioned that LAP activity will not change significantly with anaerobic condition (Nagata et al. 1989) which may be the reason of lower LAP activity under anaerobic condition than aerobic condition in our study. Moreover lower activity of LAP under anaerobic condition and NAG under aerobic condition did not showed a significant correlation with TP in our case.

There is a loading of P in Everglades over 40 years and this elevated P affected the nitrogen to phosphorous ratio (Table 3-2) (White and Reddy 2003). Previous study also reported that higher P increases N mineralization (Inglett, Reddy et al. 2007). Although any significant correlation between two N-processing enzymes was not found for our results but results indicate that both the carbon enzyme are significantly correlated ($P < 0.0001$) with each other. Moreover we have found significant correlation ($P < 0.0001$) with soil total phosphorous and ß-glucosidase and cellobiohydrolase enzymes which proved the dependence of each enzyme activity with another and with P concentration in soil, as a result this emphasizes on more carbon requirement on high nutrient site. We have noticed higher BGLU activity over LAP which showed a contrary

to what Rejmankova and Sirova (2007) got in their study (Rejmánková and Sirová 2007).The reason behind this may be the presence of larger amount of carbon containing polymer from plants over protein nitrogen were present in our study site.

The results of the studies showed the consistent support for the hypothesis regarding carbon and nitrogen processing enzymes that Vmax value will increase significantly with enhanced phosphorous concentration in the high nutrient (F1) site due to carbon and nitrogen limitation on that site (Figure 3-1 and 3-2). We have collected the soil samples between (0-10) cm which has more microbial biomass than deeper depth and were expected strongest microbial responses than (10-20)cm Sihi and Inglett et al (2013) unpublished data. Importantly in F1 site (high nutrient site) we noticed more enzyme activity for carbon and nitrogen processing enzymes which indicate more carbon and nitrogen limitation on that site and microbe are investing more energy to producing carbon and nitrogen enzyme on that site. Higher carbon enzyme activity than nitrogen processing enzyme emphasize more carbon demand for microbes on that site. Vmax value of all carbon and nitrogen processing increased significantly ($P < 0.05$) with P gradient. Moreover the highest site effect was noticed in high nutrient site (F1 and F4-R) for all of these carbon and nitrogen processing enzymes.

According to our hypothesis we observed same enzyme substrate affinity for NAG under aerobic condition and LAP under anaerobic condition. Although some differences in efficiency of LAP under aerobic condition and NAG under anaerobic condition may indicate the presence of different isoenzymes. The organisms which produce those isoenzymes may be facilitated by presence or absence of O_2. We did not observed any differences in enzyme substrate affinity along the sites which indicate that

enzyme efficiency did not changed with nutrient limitation but actual number of enzymes production and enzyme activity rate increased under P impacted site. Also we did not expected to change in enzyme substrate affinity under anaerobic condition but we observed some differences in Km along the sites in LAP which is low in U3-R than all other sites.

P processing Enzymes

According to our hypothesis we would expected higher Vmax for phosphorous processing enzymes in P limited site than P impacted site as well as we expected lower activity of phosphorous processing enzymes in high nutrient site because of two reasons as 1) According to previous study (Wright and Reddy 2001) higher phosphorous demands by microbes on low nutrient site due to P limitation would increase Vmax value in low nutrient site 2) As microbial biomass are lower in number in P limited site; we expected low substrate affinity (Low Km) enzymes in P limited site.

We observed significant site effect for both Phosphatase (P = 0.0003 aerobic and P = 0.002 anaerobic) and bisphosphatase under anaerobic condition (P = 0.017). Although we did not observed higher activity of phosphatase in low nutrient sited as we hypothesized under both conditions. Moreover we observed negative correlation of phosphatase with C: P ratio and positive correlation with MBP under both aerobic and anaerobic condition. These result suggest that both high C: P ratio and MBP may influencing reduced phosphatase activity in P limited site. Similarly Freeman et al (1997) observed that lower biological activity reduce the demand of phosphate; allowed phosphate to accumulate (Freeman et al. 1997). As a result they did not observed higher phosphatase activity in P limited condition. Although our Km value for Phosphatase under both condition suggest that P limitation created higher substrate

affinity (Low Km) in P limited site which we hypothesized. Biogeochemical correlation phosphatase Km also showed positive correlation of phosphatase with TP and MBP which indicate that high P and higher MBP will increase Km value (lower substrate affinity of that enzymes).

Although we did not find any significant difference in BPHO activity between low nutrient site and high nutrient site but the trend showed that BPHO activity was higher in low P site (Figure 3-1). Whereas under anaerobic condition we observed higher activity in low nutrient site that high nutrient site. Moreover significant negative correlation of BPHO with TP and MBP under both condition (Table 3-3 and 3-4) suggest that higher P concentration will lower the BPHO activity. Observed significantly lower BPHO substrate affinity in low P site supported our hypothesis. The significant correlation of BPHO Km with TP under both condition also strengthen our observed results.

Temperature Sensitivity of Soil enzyme Kinetic Parameters Across a P-gradient

Our objective was to determine temperature sensitivity of soil enzyme kinetics parameters across the sites. Temperature increase not only effects the microbial biomass and enzyme production (increasing enzyme pool) but can also stimulate the enzyme-substrate reaction rate. This study was designed to study only the latter i.e. changes in the enzyme substrate reaction rate because the soil were pre incubated at the experimental temperature for no more than 24 hours and the reaction was assayed at the specified temperature. Overall the observed significant correlations of the Q_{10} values with the soil P concentrations for some select enzymes suggested that the possibility of different isozymes being present in soils with different nutrient concentrations.

Temperature Response of Potential Enzyme Activity (Vmax)

According to our hypothesis we expected that 1) increasing temperature would increase the enzyme activities under both aerobic and anaerobic conditions equally. 2) Q_{10} values of the six enzymes would vary within each site. 3) The Q_{10} values of the same enzyme would vary across the sites.

Consistent with our hypothesis we observed significant positive temperature responses of all enzymes under both (aerobic and anaerobic) conditions only with the exception of bisphosphatase under aerobic condition. Although we observed that the magnitude of temperature response were different for different enzymes in different sites. Previous studies also observed positive temperature sensitivity of Vmax (German, Marcelo et al. 2012, Stone, Weiss et al. 2012). We observed different temperature sensitivity for different enzymes in each site as temperature response were different for different enzymes. Wallenstein et al (2011) stated that temperature sensitivity of enzyme can be can be varied by two aspect such as 1) temperature can change the thermal stability of a specific enzyme and 2) temperature may be responsible for change of flexibility and rigidity of enzyme active site (Wallenstein et al. 2011). So all these reasons can change enzyme substrate reaction rate. As a result we observed different temperature sensitivity for different enzymes. In soils different enzyme has different structure and soils also have different isoenzymes which may be the reason of having different temperature sensitivity of different enzymes in each site and different temperature sensitivity of same enzyme in different sites.

We observed that BGLU has significant positive temperature response (Figure 3-7 and 3-8) under both condition. Q_{10} value revealed that BGLU has average temperature sensitivity around 1.9 which ranged from 1.6 to 2.7 (Table 3-7). The

observed highest temperature sensitivity in U3-R site may be due to nutrient limitation on that site. Moreover we observed a negation correlation BGLU Vmax Q_{10} with TP. Although it was not significant but the trend indicate that P limitation may increase Vmax temperature sensitivity of BGLU. Furthermore the CBH we also observed highest temperature sensitivity in U3-R and F1 which indicate that substrate quality and nutrient limitation both can affect Vmax temperature sensitivity. Moreover the higher temperature sensitivity of CBH Vmax in ridge soils than slough soil indicate that vegetation plays a significant role during enzymes temperature response. Fierer et al (2005) showed that in litter decomposition process; more labile carbon substrate had lower temperature sensitivity than structurally complex carbon substrate (Fierer, Craine et al. 2005). Being observed higher temperature sensitivity in BGLU than CBH may be due to labile organic matter in (0-10) cm soil which provide enough substrate to BGLU. Rungruangsak et al (1998) observed different temperature sensitivity for trypsin isoenzymes in marine system (Rungruangsak-Torrissen, Pringle et al. 1998). Moreover Christian et al 1995 reported different temperature sensitivity of BGLU and LAP due to presence of isoenzymes in marine system (Christian and Karl 1995)This result suggest that different isoenzymes can have different temperature sensitivity. In wetlands we expected to the presence of isoenzymes produce by different organisms.

Phosphatase showed average temperature sensitivity around 1.5 which ranges from 1.2 to 1.8. Whereas in in bisphosphatase the average temperature sensitivity around 1.2 which ranged from 0.9 to 1.6. As we noticed that in carbon processing enzymes that temperature response along the site was different under anaerobic condition but P processing enzymes did not show that trend which indicate that

substrate quality difference and P concentration difference has an effect on carbon enzymes but not in P processing enzymes under anaerobic condition.

Leucine aminopeptidase showed overall same temperature sensitivity along the sites which indicate nutrient differences does not have any effect on Vmax temperature sensitivity of LAP. NAG Vmax temperature sensitivity were similar across the sites but the significant difference between F4-R and U3-R remain unclear. Several studies have found that C acquiring enzymes have higher temperature sensitivities that the N-acquiring enzymes (Wallennstein et al 2009, 2012; stone et al 2012). These finding have important implications especially under soil warming conditions. This may lead to increasing N limitation in soils with respect to the thereby affecting the production of the C acquiring enzymes.

Significant positive correlation of CBH under aerobic condition and LAP under anaerobic condition Vmax Q_{10} with TP suggest that nutrient limitation has significant effect on those enzymes (Figure 3-11). Negative correlation of phosphatase Vmax Q_{10} with TP suggest in low P concentration PHO temperature sensitivity (Vmax) will increase.

Comparing the anaerobic temperature sensitivity of Vmax we observed that overall all the enzymes showed average temperature sensitivity which range from 1.4 to 1.5 which indicate that under anaerobic condition temperature response may not change significantly. Although our results are important to predict nutrient cycle in wetland under temperature change but it has some limitation. Short term temperature manipulation may be ignored by global climate change (Stone, Weiss et al. 2012). If

microorganisms are able to adapt with the temperature change then the temperature sensitivity of those enzymes will be lower than what we have reported.

Temperature response of potential enzyme substrate affinity (Km)

According to our hypothesis we did not find that all the enzymes are positively temperature sensitive under both the condition. In our study we noticed positive temperature response of Km in phosphatase and both nitrogen processing enzymes. The negative correlation of both P processing enzyme substrate affinity (Km) with TP suggest that in low P concentration K of both P processing enzymes will be less sensitive to temperature. Moreover we observed that Km value were low in P limited site for both P processing enzymes. So lower temperature sensitivity of Km on that site will helps microbes to recycle P very quickly. Although previous studied have reported temperature sensitivity of Km but those sites were N limited (Stone et al. 2012) but in our case it has P gradient which makes the difference. More over Stone *et.al* studied only all the carbon processing enzymes (Stone et al. 2012). Interestingly anaerobic condition for enzymes in most of the sites created greater positive response of Km with temperature. To our knowledge, no other studies have measured Km temperature sensitivity of soil enzymes in Everglades. Our study is to report temperature sensitivity on Everglades soil. Since soils also contain heterogeneous mixture of organic substrate (Steven D. Allison 2006), it is not surprising that soil Km temperature sensitivity will be different for different enzymes in different sites.

Finding that Km respond positively to temperature for phosphatase and nitrogen processing enzymes is important because higher Km values could offset increases in Vmax with increasing temperatures at low substrate concentration (Davidson et al.

2006). Moreover more positive temperature sensitivity under anaerobic condition negate the positive effect of temperature for V_{max} under that condition.

CHAPTER 4
SUMMARY AND CONCLUSIONS

Soil microorganisms play a key role in nutrient cycling and organic matter decomposition processes because of their ability to produce extracellular enzymes.

Microbial extracellular enzymes activity release nutrients and energy stored in soil organic matter by depolymerizing the complex polymeric substances and produce simple monomers that are then taken up by a wide range of microorganisms in soils including those that do not have the ability to produce extracellular enzymes. The activity of soil enzymes have been studied from many perspectives including their structural and kinetic parameters, their ability to be used as indicators of nutrient limitation/availability and their ecological significance in nutrient cycling. Although the importance of rates of enzymatic degradation of organic matter.

Recently another factor that has stimulated the interest in this field is the recognition of enzymatic processes as a critical variable to assess the soil decomposition under soil warming conditions. This study aimed to generate a complete assessment for the effectiveness of microbial enzymatic response which would act as an indicator of different ecological perturbations such as temperature, aeration condition and nutrient variation. Results from this study would help predict the qualitative responses of wetland soils with different nutrient concentrations to soil warming under flooded and drained conditions. Data from this study can be used in models that are being developed for predicting the rates of SOM degradation

Although results from this study are based on a manipulative laboratory study, the results have revealed some interesting patterns of differences in the C, N, and P enzyme activity responses to the P gradient in soils and in their temperature gradients.

In the support of microbial allocation theory, we have found that phosphorous inflow increases the carbon and nitrogen Vmax i.e. phosphorous inflow may stimulate the microbial community to produce more C and N accruing enzymes. Our study also address that phosphorous inflow may stimulate microbial community to produce a lower substrate affinity (high Km) in P enriched site. Different enzyme exhibited different patterns of enzyme activity with a difference between ridge and slough. Results seems to appear that nitrogen processing enzymes dose not differ between ridge and slough soil but ridge soil showed a higher activity in carbon processing enzyme which may facilities more carbon mineralization in ridge than slough. However slough soil appeared to be more efficient for phosphorous processing enzymes which may create more P mineralization in slough soil on P limited site.

Our result showed an interaction of site with temperature in some of the enzymes (CBH, PHO, LAP and NAG). So our result emphasize the importance of nutrient (Phosphorous) difference (site effect) and temperature interaction to predict enzymatic decomposition pattern. We have observed positive response of Vmax with temperature however we did not find any positive response of Km for different enzymes in most of the sites which emphasize that P gradient may have different effect on enzyme kinetics which is different under different condition. Result showed a different temperature sensitivity for Vmax and Km. Result also emphasize that Vmax is more temperature sensitive than Km. Observed positive temperature response on phosphatase and nitrogen processing enzymes suggest that higher Km value can offset the positive temperature response of those enzymes. Moreover our result indicated some differences temperature sensitivity between ridge and slough soils for some enzymes.

It is found that aerobic condition facilitate enzyme activity (Vmax) for all enzymes except NAG which has <1 aerobic to anaerobic Vmax ratio. However our result emphasize the higher enzyme efficiency (low Km) under anaerobic condition (>1 aerobic to anaerobic ratio) except NAG. Our results suggest that higher temperature sensitivity for Km under anaerobic condition than aerobic condition can negate the positive effect of temperature on Vmax under anaerobic condition.

Results of this study suggest the possibility of different isozymes at different sites with different nutrient concentrations.

Also this study is the first to document the effect of the p gradient on not only the Vmax but the Km parameters of the extracellular enzymes that are important for the C, N and p cycling in the wetland soils. The temperature sensitivity of the enzymes has been studied inly in the upland systems and this is the first study to document the Q_{10} of enzymes from a wetland soil and to compare them under aerobic and anaerobic conditions. Although this study has raised several questions, the results of the study can provide some basic values for the wetland soil decomposition models. One of the future study that is warranted is the evaluation of the seasonal changes in the enzyme kinetic patterns along the nutrient gradient and to relate the enzyme activities to the carbon quality parameters.

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BIOGRAPHICAL SKETCH

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