INFLUENCES OF NUTRIENT LOADING, VEGETATIVE HABITATS AND SIMULATED DROUGHT ON MICROBIAL ENZYME ACTIVITITES IN THE EVERGLADES

By

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Wetlands frequently function as a long term carbon (C) sink as organic matter accumulates in response to reduced decomposition rates. In general, enzyme catalyzed reactions are considered the rate-limiting step in organic matter degradation. Nutrient status, vegetative community and hydrological regimes significantly influence microbial enzyme activities. This thesis exams the effects of nutrients, vegetative communities and water level drawdown on enzymes involved in C, nitrogen (N), phosphorus (P) and lignin mineralization within four hydrologically distinct areas of the Florida Everglades.

Examining enzyme activities along distinct phosphorus gradients, this study demonstrates that P enriched sites exhibit lower microbially perceived N and P limitations on C mineralization in addition to enhanced cellulose decomposition rates. Phosphorus loading resulted in a decreased microbial mobilization of resources for P mineralization which resulted in a greater energetic allocation for C mineralization. Nitrogen appears to become less limiting to C mineralization in the enriched sites within

Everglades National Park. A simple two component model, incorporating total phosphorus and the relationship between the enzymes involved in C and P mineralization accounted for 62% of the variability in cellulose decomposition rates.

Vegetative composition was found to significantly alter the allocation of enzymatic resources due to varying substrate complexities. Carbon mineralization in the open water was significantly less influences by lignin than the sawgrass habitats. An index relating hydrolytic and oxidative enzymes, the Enzyme Index of Carbon Quality, was significantly greater in the open water habitats. This enzymatic index suggests more favorable C mineralization conditions within the open water communities and provides insight into the development of the slough-sawgrass ridge topography of the Everglades.

Laboratory water level drawdown was found to effect the microbial allocation of enzymatic resources in intact Everglades soil cores. Peat dominated sediments exhibited elevated enzyme activities and a larger response to lower water levels over time. Enzymatic N mineralization increased significantly in the surficial soil horizons as a response to water level drawdown. The results indicate that extended periods of low water may result in enhanced C and N mineralization of Everglades sediments.

The results of these studies indicate that microbial enzyme activities are responsive to changes in nutrient conditions, vegetation and water levels. The extrapolation of these enzyme activities to potential decomposition based on perceived C qualities connects the microbial condition to ecosystem level processes. Changes to the Everglades ecosystem as a result of management practices can thus be initially recognized through alterations in the highly responsive microbial component that functions in nutrient cycling and serves as the basis for the trophic food chain.

CHAPTER 1
INTRODUCTION

Background

Wetlands often have decreased O_2 availability as a result of decreased O_2 diffusion rates through water, resulting in decreased overall supply (Ponnanmperuma, 1972). Additionally, increased O_2 demand, due to generally high carbon (C) availability, results in overall decreases in decomposition rates in wetlands (Reddy and D'Angelo, 1994). An important and significant role of wetlands in the global ecosystem is C sequestration. The accumulation of organic matter and thus the role in peat development and global C sequestration may be affected by changes in ambient nutrient conditions (Wetzel, 1991; Newman et al., 2003), vegetative composition (DeBusk and Reddy, 1998; Berg, 2000; Fioretto et al., 2000; Kourtev et al., 2002a), and differing hydrological regimes (Volk, 1973; Elliott et al., 1984; DeBusk, 1996). The mineralization of organic matter by the resident heterotrophic microbial communities within a system plays a crucial role in the cycling of C and exerts an influence on the overall energy flow within a system (Elliot et al., 1984). The activity of the heterotrophic bacteria and fungi whose activity is related to the biochemical structure, nutrient content, and quantity of available substrates produces enzymes that are often considered the rate limiting steps in decomposition (Chróst and Rai, 1993).

The synthesis and activities of the enzymes involved in organic nutrient mineralization are most regulated by the induction of macrophytic and macromolecular substrates within soils (Burns, 1986; Nausch et al., 1998) as well as the intrinsic factors

of plant litter (Linkins et al., 1990a; Carreiro et al., 2000; Kourtev et al., 2002a). Lignin content has been specifically correlated with decomposition rates (Meentemeyer, 1978; Mellilo et al., 1989), especially when nitrogen (N) is readily available (Mellilo et al., 1982; Taylor et al., 1989). Nitrogen content, presented as C:N or lignin:N are also often used as predictors of litter decomposition rates (Mellilo et al., 1982; Taylor et al., 1989; Sinsabaugh et al., 1993; DeBusk and Reddy, 1998; Carreiro et al., 2000) and may influence the decomposition of large molecular weight organic matter (Sinsabaugh et al., 1993; Carreiro et al., 2000). Phosphorus (P) availability has also been shown to control decomposition rates in generally P-limited systems (Newman et al., 2001).

Decomposition of organic matter is a community level process that involves specific interactions within a microbial consortium (Sinsabaugh et al., 1991). The advantage of enzyme assays is that they present specific information on one process in a complex community. The microbial degradation of particulate organic matter, such as plant litter, has been shown to be most influenced by the enzymes involved in lignocellulose degradation, P cycling, and N cycling (Sinsabaugh et al., 1991; Sinsabaugh and Moorhead, 1996), which are often considered the rate limiting steps in degradation (Chróst and Rai, 1993). Strong relationships have been established between lignocellulose-degrading enzymes and litter mass loss rates among varying litter qualities (Sinsabaugh et al, 1992a).

Enzyme activities have the potential to affect all major wetland functions where decomposition is low. Peat accumulation is dependent upon a lower rate enzymatic activity resulting in C storage and inorganic nutrients remain sequestered within the poorly degraded peat matrix when decomposition is low. This impairment of nutrient

cycling by the microbial consortia causes inorganic nutrients to accumulate and to be retained within the wetland (Freeman et al., 1996). However, the interpretation and relation of individual enzyme activities to higher trophic processes and observations is often a difficult and tedious process that frequently results in vague relationships to soil and microbial parameters in the system (Marsden and Gray, 1986).

A current strategy involves a resource allocation rationale in interpreting enzyme activities and the MARCIE (Microbial Allocation of Resources Among Community Indicator Enzymes) model for exposing linkages between individual enzymes (Sinsabaugh and Moorhead, 1994; Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1996, 2002). The model indicates that the expression of enzymes is tied to environmental nutrient availabilities and that the distribution of enzyme activities can be interpreted as a resource allocation strategy (Sinsabaugh et al., 2002). Underlying this concept is the relationship between lignocellulose degradation and environmental N and P concentrations. Components of this model have been linked to bacterial productivity, microbial biomass, and particulate organic carbon turnover times (Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997). Specifically, the Enzyme Index of Carbon Quality (EICQ), which relates the activities of hydrolytic enzymes to those involved in lignin degradation, has been shown to decrease as decomposition proceeds, which is reflective of lower substrate quality (Kourtev et al., 2002b). This index has also been correlated with microbial biomass, productivity and negatively correlated with particulate organic carbon (POC) turnover time (Sinsabaugh and Findlay, 1995). Furthermore, enzymes are divided into functional units based on C, N and P mineralization (Ecell, En, Ep) and lignin degradation (Eox). Enzyme ratios using these compartmentalized units

such as Ecell/Ep and Ecell/En, which reflect apparent P and N control on C mineralization, respectively, have been correlated with bacterial productivity (Sinsabaugh and Findlay, 1995; Sinsabaugh and Moorhead, 1994).

Statement of the Problem

The Florida Everglades was once a vast peat wetland that encompassed 10,000 km^2 and stretched from Lake Okeechobee southward to the shores of Florida Bay. In the late 1800's and early 1900's large areas of the original landscape was altered for agricultural and urban development. Water Conservation Areas (WCAs) were created as a result of compartmentalization by dikes, levees, and water control structures. The remnant Everglades consists mainly of four managed, hydrologically distinct areas: Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR), WCA-2, WCA-3, and Everglades National Park (ENP). Intensive agricultural development in the northern periphery has resulted in the establishment of P and, to a lesser extent, N gradients within these four hydrologic components of the oligotrophic Everglades. As a consequence, shifts in macrophyte species composition (Davis, 1943, 1991; Vaithiyanaithan and Richardson, 1999), increases in net primary production (NPP) (Davis, 1991) and peat accumulation (Reddy et al., 1993), loss or taxonomic shifts of native periphyton assemblages (McCormick and O'Dell, 1996; McCormick et al., 1996), increases in microbial activity and biomass (White and Reddy, 2000), and other ecological changes have been observed in Everglades areas receiving nutrient inputs from canal water with total P (TP) concentrations as much as 10 to 30 fold higher than background conditions (McCormick et al., 1996).

Altered surface flow as a result of compartmentalization and hydrological management also affected the C cycling within the Everglades. The net area covered by

the deeper sloughs or open water habitats has diminished, being replaced by shallower, monotypic sawgrass stands. This affected the natural landscape of the Everglades which was dominated by a slough, slough-ridge topography. The development and maintenance of this topographical relief is not well understood and has been attributed to particulate transport and differential decomposition.

Water level fluctuations as a consequence of natural seasonal variations or hydrologic management have the potential to drastically affect the ability of a wetland system, such as the Everglades, to sequester C. Previous studies have shown increases in CO_2 flux with lowered water table depths (Volk, 1973) and the highest total C flux under drained conditions in Everglades peat sediments (DeBusk, 1996). The mineralization of soil organic N sources has been found to be greater in aerobic than anaerobic conditions (Reddy and Patrick, 1984; McLatchey and Reddy, 1998), to increase after the drying of wet soils (Cabrera 1993, Bridgham et al., 1998), and specifically, to be 2-3 times greater (White, 1999; Venterlink et al., 2002) in drained wetland soils. The impact of water fluctuations thus has a potentially large role in the nutrient cycling and retention of the Everglades.

Objectives and Scope of Research

The overall goal of this study was to determine the influences of different vegetative habitats, nutrient loading and water table drawdown on microbial enzyme activities in the Everglades. Chapters 2 through 5 address more specific objectives:

1.) Develop an appropriate enzyme experimental method for performing assays in wetland systems.

2.) Determine the effects of vegetative habitats on microbial enzyme activities in Water Conservation Area 3A of the Everglades.

2.) Determine the relationships between nutrient conditions and microbial enzyme activities among all four hydrologic units of the Everglades.

3.) Determine the validity of different enzyme models in predicting potential decomposition among different litter qualities and in varying nutrient conditions.

4.) Determine the effects of a simulated drought on enzyme activities in WCA-3A and ENP-Taylor Slough (ENP-TS) cores.

The overall hypotheses for this study were: (i) Deeper water slough habitats exhibit accelerated potential decomposition due to substrate quality differences; (ii) Nutrient loading generally enhances microbial enzyme activities; and (iii) Decreased water levels result in an enhancement of enzyme activities, suggesting greater potential decomposition based on perceived soil quality.

CHAPTER 2
EXPERIMENTAL CONSIDERATIONS IN DETERMINING OPTIMUM ENZYME ASSAY CONDITIONS IN WETLAND SYSTEMS

Introduction

Fluorogenic substrates have been widely adopted in investigations of enzyme activities in a variety of systems such as lakes, grasslands, wetlands, streams, groundwater and oceans (Chróst, 1989; Freeman et al., 1995; Miettinen et al., 1996; Sinsabaugh and Foreman, 2001; Debosz et al., 1999; Mayr et al., 1999, Shackle et al., 2000; Wittmann et al., 2000; Burns and Ryder, 2001; Newman et al., 2003; Saiya-Cork et al., 2002; Wittmann et al., 2004). These compounds have become popular due to their production of fluorescent compounds, which exhibit less interference by highly colored phenolic compounds than colorimetric substrates (Sinsabaugh et al., 1991; Freeman et al., 1995). Methylumbelliferyl (MUF) and amidomethylcoumarin (AMC) substrates are among the most widely adopted fluorogenic substrates.

Fundamentally different methodological approaches have been adopted in the literature. The most recent approaches include the use of microtiter plates (Marx et al., 2001). Standardized approaches use substrate saturating conditions, standard temperature, and an assay pH that maximizes the fluorescence of the fluorochrome. Enzymes have differing optimum pH levels which have influenced the use of different pH conditions between enzymes (Parham and Deng, 2000). The environmental approach utilizes substrate concentrations similar to the local environment with assay temperature and pH approximating field conditions. A compromise between these approaches is to

use substrate saturating conditions with assay temperature and pH approximating field conditions. However, temperatures as high as 45 °C have been used (Sinsabaugh et al., 1991). Sample alkalinization prior to fluorescence measurement is also often performed to maximize the fluorescence intensity (Freeman et al., 1995).

Enzyme activity is generally more resolvable with substrate saturation and the resultant generally linear function of enzyme activity allows for shorter incubation times and less error. Strong correlations in the literature between potential enzyme activities and nutrient conditions or correlated processes allow enzyme activities to reflect the magnitude or process relationships among samples or between systems (Cembella et al., 1984; Jansson et al., 1988; Foreman et al., 1998).

The methodological heterogeneity of enzyme assays is a reflection of ecological diversity and localized microbial community structures among studies. For example, substrate incubation times in the literature range from 10 minutes to 3 days (Miettinen et al., 1996; Foreman et al., 1998; Debosz et al., 1999; Burns and Ryder, 2001; Kourtev et al., 2002b). Assay incubation times are generally based on the ability to measure within the linear portion of the time curve while limiting the opportunity for microbial growth (Freeman et al., 1995, 1996). Reported MUF and AMC substrate concentrations are also highly variable between studies and ranges from 1 to 500 μM in soil and water column enzyme studies (Chróst, 1989; Sinsabaugh et al., 1997; Debosz et al., 1999; Burns and Ryder, 2001; Shackle et al., 2000; Sinsabaugh and Foreman, 2001; Saiya-Cork et al., 2002; Wittmann et al., 2004). An emphasis of setting substrate concentrations at saturating conditions has been documented in several studies (Chróst, 1989; Sinsabaugh and Findlay, 1995; Burns and Ryder, 2001).

The majority of enzyme studies are performed on soil, water, and litter samples. However, the use of enzymes in wetland systems is comparatively rare. The combination of soil and water matrices introduces additional issues in the development of enzyme assays. Fluorescence interference, due to the rather large accumulation of organic matter in various stages of decay in wetland systems as well as the accumulation of phenolic compounds can play a major role in enzyme activity determination. Therefore it is essential to calculate a quench adjustment for each sample by placing standards in sample solutions and relate the fluoresences to the standard curve so that samples can be accurately compared (Freeman et al., 1996; Marx et al., 2001). However, this does not eliminate other issues that can introduce problems between samples. The presence of inhibitors, alternative reaction paths and competing substrates can also affect the results of an assay (Sinsabaugh et al., 1991).

Enzyme activity is usually expressed as the net change in fluorescence from an initial to a final measurement over time to determine the substrate conversion rate. Laboratory replicate errors can be quite large in soil enzyme assays due to difference in particle distribution as well as sample heterogeneity on a minute scale, even in homogenized samples. What is not clear from the literature is the problem associated with relying on these endpoint values. The use of net enzyme activity changes without observing changes in fluorescence over smaller increments of the total incubation time can result in larger replicate errors. The use of automated spectrofluorimeters allows the investigator to observe the enzyme kinetics over time and thus identify any replicates from analysis that produce both outlying net activity and erratic kinetics over time, thus reducing replicate error and increasing precision.

The goal of this study was to develop an enzyme assay protocol for Everglades soils to be utilized in further investigations of enzyme activities and their relation to nutrient regimes. The objectives of this study were to address the effects of (1) varying substrate concentrations; (2) incubation times; and (3) the use of multiple time point measurements in determining optimum enzyme assay conditions.

Materials and Methods

Soil cores were collected in Water Conservation Area 2A, a 447 km^2 impounded wetland in the northern Everglades that has received agricultural drainage for over 30 years (Davis, 1991). Three soil cores were collected at nutrient enriched (F1) and reference (U3) transect sites along a distinct P gradient. Soil sampling was accomplished using a 5 cm diameter piston type corer in the spring of 2001. The benthic layer or flocculent detrital layer was separated from the remaining soil to be utilized in this experiment. This layer, composed of active algal remains and plant components in various stages of decay, was chosen due to greater microbial activity and variability between sites. The samples were stored on ice until transfer to the laboratory. Large roots and rocks were removed. The samples were homogenized for 5 min using a handheld Biospec Biohomogenizer™ in a 500 mL beaker. Approximately 10 g wet weight was transferred to a second 250 mL beaker and 100 mL deionized H_2O was added. The suspension was homogenized for an additional 5 min and 1 mL was transferred to another 250 mL beaker to which 99 mL of DI H_2O was added. The suspension was mixed and 50 mL of the suspension was transferred to a 50 mL Eppendorf centrifuge tube. This suspension was used for the assays and refrigerated until use. Previous studies have shown that freezing generally caused increased enzyme activities due to the disruption of enzyme complexes and cell lysis as compared to

refrigeration and was thus avoided (Sinsabaugh and Linkins, 1989; Sinsabaugh et al., 1991). Biostatic agents were not used in this study as their functions differ among samples and have been found to repress the activity of some enzymes (Sinsabaugh et al., 1991).

Four enzymes were investigated utilizing the following substrates: MUF-β-D glucoside (Sigma M3633), MUF-cellobioside (Sigma M6018), MUF-phosphate (Sigma M8168) and L-leucine amidomethylcoumarin (Sigma L2145). These substrates were used for the determination of β-glucosidase, cellobiohydrolase, phosphatase, and leucine aminopeptidase activities, respectively.

Enzyme substrate solutions were prepared in 10 mM Tris-HCl, pH 8.5 in varying concentration ranges. Buffered conditions stabilize the pH dependent intensity of the fluorescent product (Chróst and Krambeck, 1986). Enzyme assays were performed using a Cytofluor 600™ (Perseptive Biosystems, Inc. Framingham, MA) automated spectrofluorometer at with Kineticalc™ software at 360 nm excitation and 460 nm emission. Samples were analyzed in Corning ® 48-well microplates. 360 µL Tris-HCl pH 8.5 was added to each sample well followed by 400 µL soil suspension and 40 µL of the enzyme substrate. Triplicate replicates of each suspension were used.

The spectrofluorimeter was set to 25° C, to shake for 3 sec before each reading, and for end-point analysis. Sensitivity was set at 95, which refers to the sensitivity of the fluorimeter with a maximum value of 100. The plate was inserted and the initial (Ti) fluorescence was recorded. A reading was performed every 10 minutes for the duration of the incubation. A similar detailed spectrofluorimeter method used 1 minute intervals

between readings (Marx et al., 2001). A final fluorescence (Tf) was recorded by setting the fluorimeter to end point analysis at the end of the run.

Various amounts of substrates were added to the samples to establish enzyme saturation (Chróst, 1991). Incubations were carried out at 5, 10, 30, 60, 125, 250, and 500 μM for MUF-cellobioside; 5, 10, 20, 30, 50, 125, 250, and 500 μM for MUF-phosphate; 5, 10, 20, 30, 50, 70, 100, 250, 500 μM for MUF-glucoside and 5, 10, 20, 50, 100, 300, 400, 800, and 1600 μM for L-Leucine amidomethylcoumarin. These substrate concentrations reflect in-well measurements as a consequence of dilution and are similar to the range of substrate concentrations used in another methodological study (Marx et al, 2001). In most cases fluorescence readings were taken over 2 hours. After evaluating the kinetic curves, the initial fluorescence (Ti) was subtracted from the final fluorescence (Tf) across incubation time to determine the potential enzyme activity. The largest difference in fluorescence (Tf-Ti) should yield the best resolution of activity for a given site. Alkalinization of the samples prior to fluorescence measurement was found unnecessary as all sample fluoresences were resolvable due to the high sensitivity of the spectrofluorimeter. Further conversion of potential enzyme activity to μmols MUF or AMC released g^{-1} AFDM h^{-1} was not necessary to meet the goals of this study.

All figures represent averaged data with standard errors. Data from selected substrate concentrations are presented in figures to represent the extremes associated with each enzyme.

Results

Cellobiohydrolase

The effect of substrate concentration and incubation time on the potential activity of cellobiohydrolase was investigated at the enriched and reference sites. Tf-Ti values are simply referred to as "activity" for the purposes of this study.

The response of the highest MUF-cellobioside concentration (500μM) over incubation time varied between the enriched (F1) and reference (U3) sites (Figures 2-1a & 2-1b). Mean activities over the incubation period were 302 and -240 (unitless) with standard errors 179% and 48% of the mean for the F1 and U3 sites, respectively. Both sites exhibited erratic readings with initial reductions in activity that was reflected in the large replicate errors. F1 responded differently than U3 with net increases in activity after 120 mins.

Positive activity changes were exhibited at the lowest MUF-cellobioside concentration of 5 μM at both sites with mean activities of 223 and 295 and standard errors 20% and 17% of the mean at the F1 and U3 sites, respectively. These activities were considered low and reflect an insufficient quantity of substrate to obtain near saturating conditions. This low resolution in the difference between Tf and Ti values occurred in the range of 5 to 30 μM.

The behavior of cellobiohydrolase was found to be most linear at a substrate concentration of 60 μM. This concentration reflected the greatest activity among the sites before a large decrease in activity at 125 μM (Figure 2-1c). Mean activities of 673 and 503 with standard errors 9% and 15% of the mean occurred for the F1 and U3 sites, respectively. Due to linear increases in activity, extended incubation times were not

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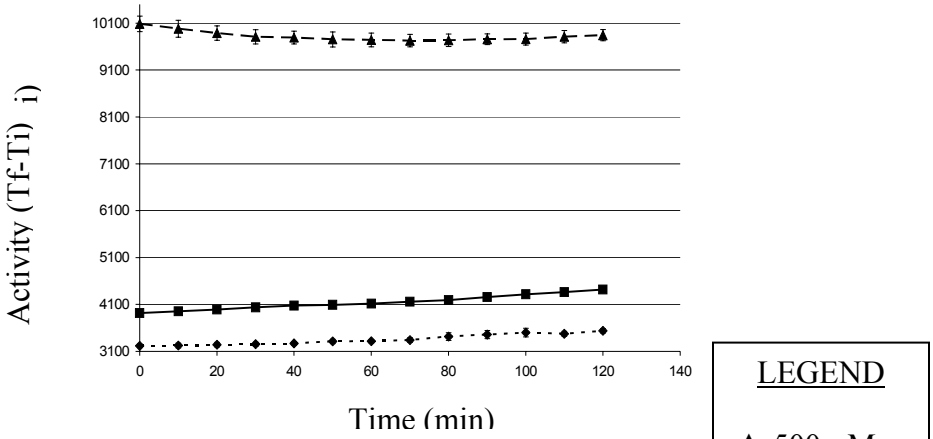

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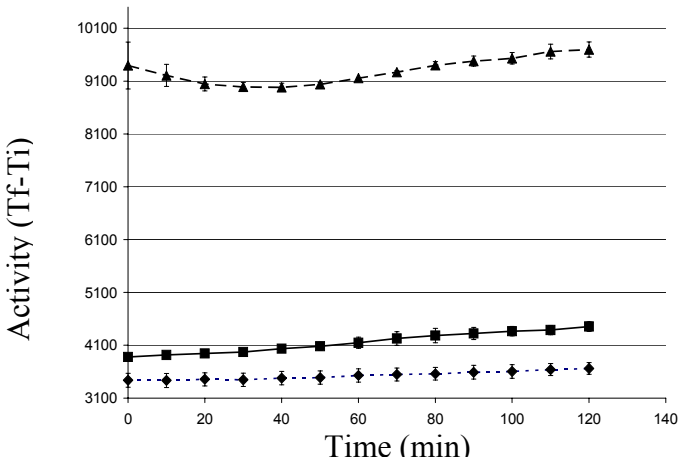

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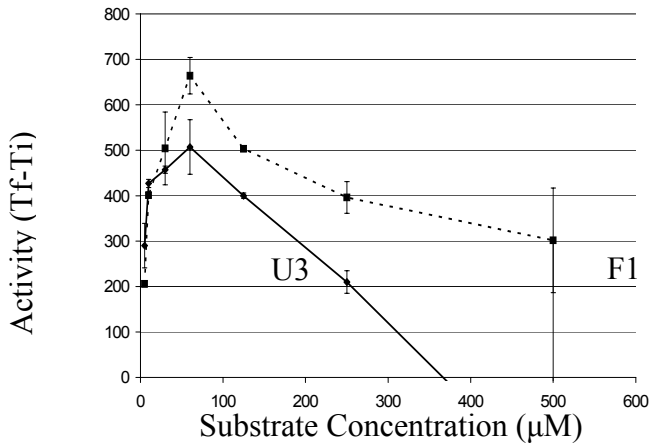
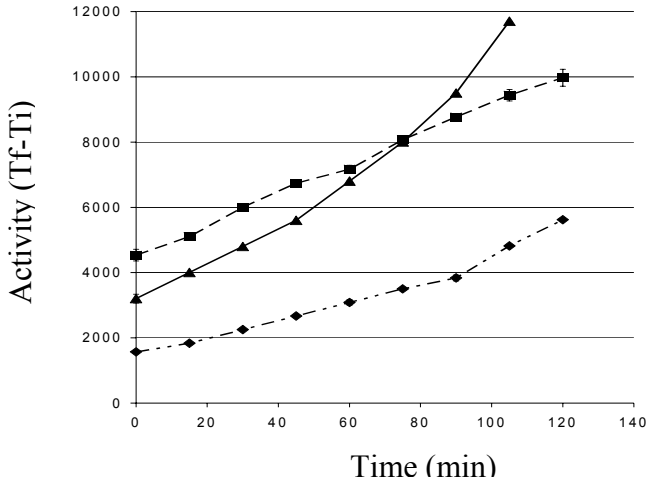


Figure 2-1. Effects of (a) time on net activities in F1 sediments, (b) time on net activities in U3 sediments and (c) varying MUF-cellobioside substrate concentrations on net activities.

a.)



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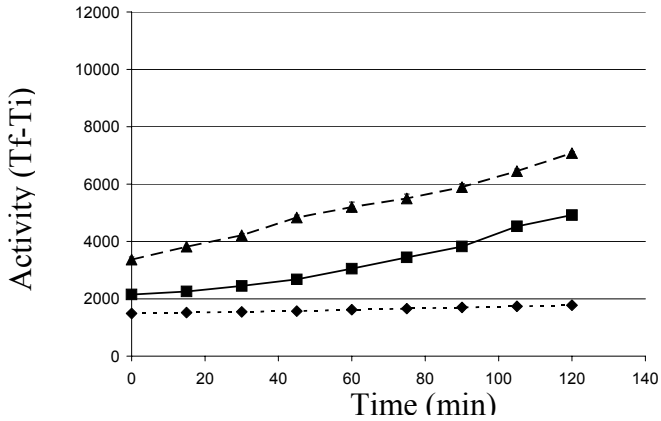

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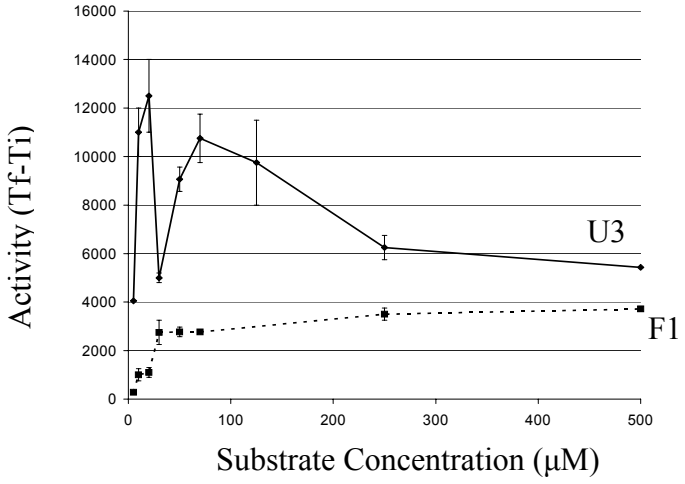


Figure 2-2. Effects of (a) time on net activities in F1 sediments, (b) time on net activities in U3 sediments and (c) varying MUF-phosphate substrate concentrations on net activities.

considered important at 60 μM as substrate saturating conditions were maintained throughout the duration of the run. However, at higher substrate concentrations, such as 500 μM, incubation time does appear to influence the determination of activity (Fig 2-1a & 2-1b). At 500 μM, incubation times exceeding approximately 80 minutes for F1 and 120 minutes for U3 would be necessary in order to resolve positive activities at both sites.

Phosphatase

Mean activities for F1 and U3 sites were 3717 and 5433 (unitless) with standard errors 2.5% and 5.0% of the mean at 500 μM, respectively (Figures 2-2a & 2-2b). Relative differences between sites substantially decreased at concentrations at or above 250 μM. In contrast to the MUF-cellobioside assay, high concentrations did not result in erratic behavior over the incubation period but rather remained linear (Figures 2-2a & 2-2b). The lowest concentration of 5 μM exhibited very linear relationships over time in all replicates in both nutrient conditions. Mean activity values were 287 and 4050 with standard errors 6.5% and 1.2% of the mean for the F1 and U3 sites, respectively. The low activity at the F1 site reflected the need for a higher substrate concentration in order to counteract potential interferences in future experimental runs where microbial phosphatase production may be lower.

The most appropriate concentration for the purpose of future studies was determined to be in the 50 μM range (Figure 2-2c), before a substantial decrease in activity occurred in the U3 sample. Linear responses occurred in both the F1 and U3 samples over the length of the incubation period with mean activity values of 2770 and 9067 with standard errors 2.5% and 2.9% of the mean, respectively. These values reflect the highest activity value while still minimizing laboratory error. Incubation time at 50 μM does not appear to be an important consideration since linear relationships were

exhibited in both samples. This concentration was 8 times lower than another wetland study that examined phosphatase linearity in order to establish optimum substrate concentrations (Kang and Freeman, 1999).

β-glucosidase

The highest MUF-glucoside concentration of 500 μM yielded Tf-Ti activity differences with average values of 565 and 989 in F1 and U3 samples respectively, with laboratory replicate standard errors of 1.8% and 9.3% of the mean (Figures 2-3a & 2-3b). Quasi-linear graphs were produced with the highest activity values occurring at the U3 site. 5 μM average activity values were 267 and 88 with standard errors representing 7.6% and 50.8% of the mean for the F1 and U3 sites, respectively. The relationship of enzyme activity between sites shifted from the general trend at approximately 200 μM and continued until approximately 300 μM (Figure 2-3c). This illustrates the profound effect that substrate concentrations can have on formulating conclusions.

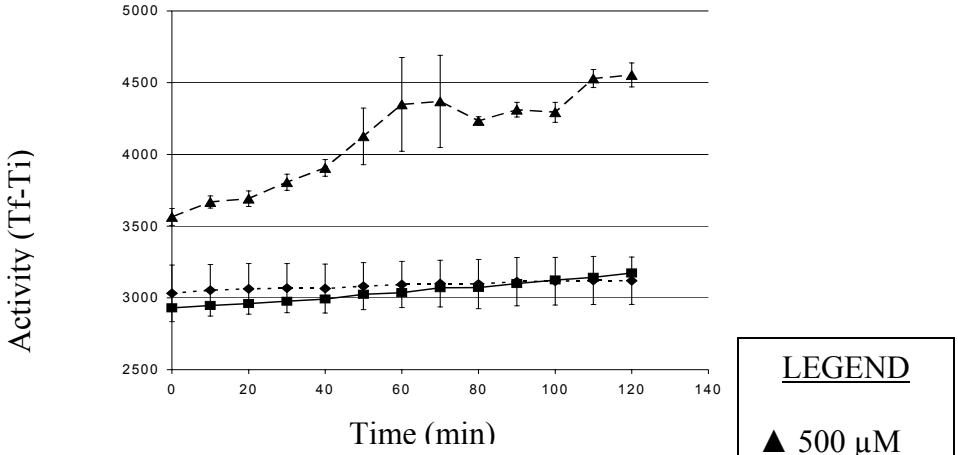
The most consistent substrate concentration in terms of net activity, linearity and error was identified at 100 μM. These runs yielded mean activities of 416 and 248 with standard errors of 8.1% and 4.8% of the mean for the F1 and U3 sites, respectively.

There was a linear response of enzyme activity over time with no apparent effect of increasing incubation time on mean activities or differences among the sites.

Leucine Aminopeptidase

Leucine aminopeptidase exhibited typical activity responses to increasing substrate concentrations (Figure 2-4c). Peak activity was around 800 μM and decreased in response to a higher or lower concentration. As substrate concentration was decreased from 800 μM, the differences between the two sites and the replicate errors also

a.)



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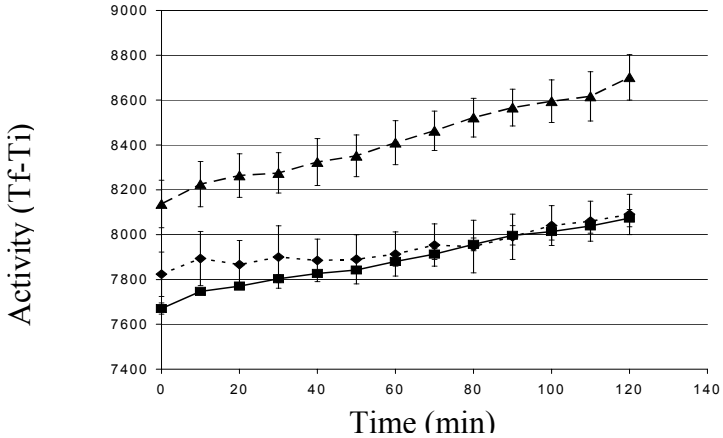

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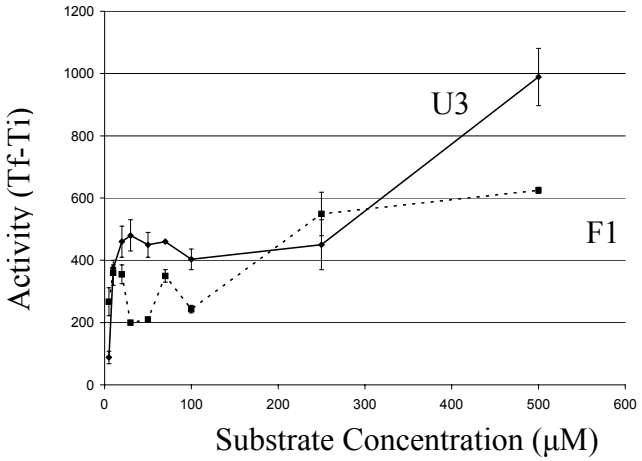


Figure 2-3. Effects of (a) time on net activities in F1 sediments, (b) time on net activities in U3 sediments and (c) varying MUF-glucoside substrate concentrations on net activities.

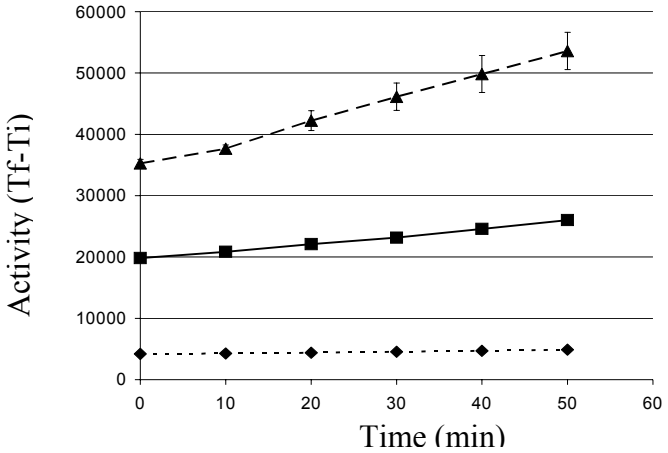
decreased. At 1600 μM the mean activities were 11167 and 18833 with standard errors of 16.5% and 13.4% of the mean for the F1 and U3 sites, respectively.

The 300 μM runs exhibited mean activities of 6197 and 11866 with standard errors of 1.0% and 1.0% of the mean for the F1 and U3 sites, respectively. Linear graphs at 300 μM were the most consistent, showing no apparent influence of incubation time on site differences or individual activities (Figs 2-4a & 2-4b). Mean activity values for the 10 μM runs were 417 and 710 with standard errors representing 5.9% and 7.8% of the mean. This concentration represented the lowest activity difference between samples among the range of substrate concentrations.

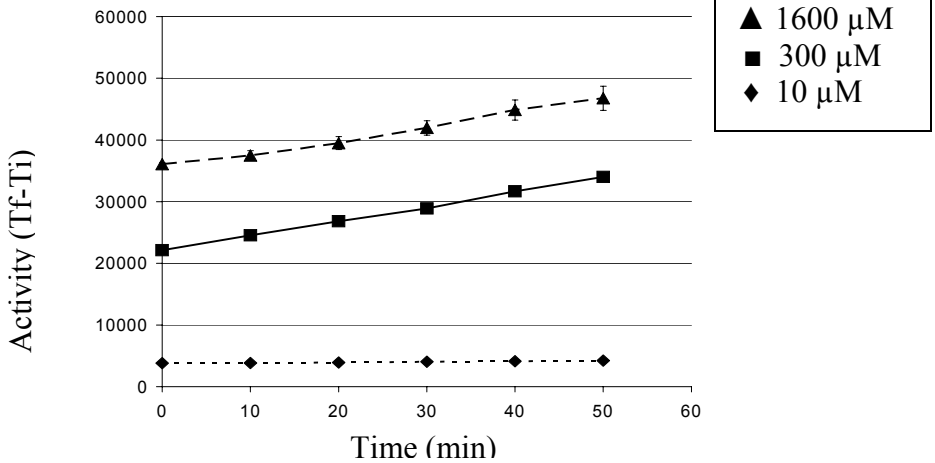
Discussion

These results demonstrate the need for the investigation of incubation time and substrate concentration effects to ensure the validity of data in future experiments. Enzyme activity is extremely sensitive to changes in environmental conditions, thus it is necessary to construct the assays such that enzyme efficiency is maintained through a range of conditions that may be encountered. The enzymes in this study were responsive to changes in nutrient concentrations. These responses were evident in different relationships to changes in substrate concentration and incubation time. In some cases, different contrasts between the enriched and reference samples were observed as the substrate concentration was altered. This may lead the investigator to assume inverse relationships between samples, which are not necessarily reflected in the majority of other substrate concentrations. Therefore, the most contrasting conditions must be assayed in order to develop the most consistent substrate concentration and incubation time in relation to changes in sample properties.

a.)



b.)



c.)

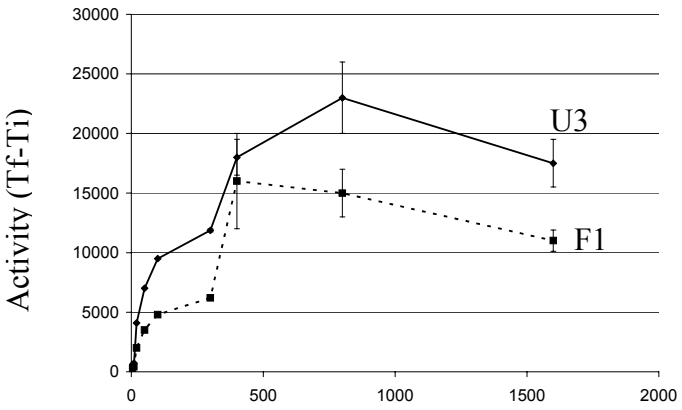


Figure 2-4. Effects of (a) time on net activities in F1 sediments, (b) time on net activities in U3 sediments and (c) varying L-Leucine amidomethylcoumarin substrate concentrations on net activities.

The use of the curves produced over the total incubation period is necessary to observe enzyme behavior over time. While the endpoint based activity (Tf-Ti) produced as a result of incubation may indicate a net change in substrate converted, there is often erratic conversion behavior over time. Without the use of these curves, the data would be considered valid since there would be no reason to discard any values that appear to reflect net activity. The resultant experimental protocol for future studies discards any non-linear runs from further analysis when the activity reflects outlying values.

In determining optimum substrate concentrations, it is generally evident that very high and low concentrations tended to produce erratic changes in activity over time and lower differences between sites. The high concentration response can be attributed to intermolecular quenching in which fluorescence is masked by the presence of other fluorescent and non-fluorescent molecules. Therefore, there can be a false decrease or a lesser increase in substrate conversion as measured by the fluorimeter. At low concentrations, saturation kinetics did not appear to be satisfied, thereby resulting in a lower perceived reaction rates. This is due to the lack of adequate substrate that fails to retain a constant reaction velocity. The appropriate MUF and AMC substrate concentrations that were determined in this study fall within the range of 40 to 200 μM documented in other studies of water column and soil enzymes (Burns and Ryder, 2001; Shackle et al., 2000; Sinsabaugh and Foreman, 2001; Saiya-Cork et al., 2002).

Incubation time was shown to influence the resolution between the two study sites. In most cases, incubation times less than 30 minutes result in a decrease in resolution. In addition, substrate conversion is generally erratic with large errors between replicates. An incubation time of 45 minutes is deemed suitable for capturing activity as

well as enabling a rapid turnover of samples in future studies. This lies within the ranges observed in other studies (Kourtev et al., 2002b; Foreman et al, 1998; Burns and Ryder, 2001). Short incubation times minimize any changes that may occur in the microbial community (Hoppe, 1993). It should be noted that longer incubation times are acceptable if enzyme dynamics or environmental factors warrant. Such incubations may be performed in order to resolve very low enzyme activities or overcome phenolic quenching in heavily lignified or tannic samples. However, in order to investigate the temporal function of the enzymes over the incubation period, the samples must remain in the automated spectrofluorimeter, thus delaying the processing of other samples. Therefore, the most expeditious 45-minute incubation is preferred to decrease sample storage time and increase efficiency.

Conclusions

These results demonstrate the need for the investigation of incubation time and substrate concentration effects to ensure the validity of data in future experiments. Further, it is prudent that enzyme kinetics be incorporated into the experimental methodology due to the differences among substrate concentration responses over time that were observed in this study. This process can be done expeditiously using a microplate approach to enzyme assays and analyzing with an automated spectrofluorimeter, compared to older cuvette based measurements. It is also important to analyze a range of concentrations in the most widely contrasting environmental conditions within the study area in order to determine the most appropriate substrate concentration. However, the final determination of the warranted incubation conditions will be dependent on the specific goals of the study, sample matrix, number of assays, the desire for precision versus accuracy, and available analytical equipment.

CHAPTER 3
EFFECTS OF HABITAT DIFFERENTIATION ON MICROBIAL ENZYME ACTIVITIES IN THE EVERGLADES

Introduction

Organic matter accumulation within wetlands is a consequence of the balance between net primary production (NPP) and microbial heterotrophic metabolism. Microbial decomposers play a crucial role in carbon (C) cycling and are responsible for driving the C energy flow up the detrital food chain. The mineralization of organic nutrients by the microbial community exerts an appreciable influence on energy flow by regulating nutrient availability for further decomposition and primary production (Elliot et al., 1984). Mineralization of plant matter is governed by the chemical and physical properties of available substrates such as lignin, nitrogen (N), and phosphorus (P) (DeBusk and Reddy, 1998; Berg, 2000; Fioretto et al., 2000; Kourtev et al., 2002a) as well as other environmental and physiochemical influences. Therefore, changes in these parameters associated with different litter types and nutrient conditions have the potential to alter peat accumulation rates and potentially topography over time.

The microbial degradation of particulate organic matter (POM), such as plant litter, has been shown to be most influenced by the enzymes involved in lignocellulose degradation, P cycling and N cycling (Sinsabaugh et al., 1991; Sinsabaugh and Moorhead, 1994), which are often considered the rate limiting steps in degradation (Chróst and Rai, 1993). Due to the sometimes complex macrophytic structure, a large quantity of diverse enzymes may be necessary to complete degradation (Eriksson and

Wood, 1985; Ljungdahl and Eriksson, 1985). The synthesis and activity of enzymes may be most regulated through the induction by macrophytic and macromolecular substrates present in the soils (Burns, 1986; Nausch et al., 1998), with strong relationships established between lignocellulose-degrading enzymes and litter mass loss rates among various litter qualities (Sinsabaugh et al., 1992a). Consequently, soils beneath different plant species have been shown to support microbial communities that differ in both structure and function (Degens and Harris, 1997; Grayston et al., 2001; Kourtev et al., 2002a; Kourtev et al., 2003) and can affect sediment nutrients through plant uptake and rhizosphere characteristics (Templer et al., 1998). Litter breakdown rates and enzyme activities have been shown to vary among species and have been attributed to intrinsic factors of the leaves (Linkins et al., 1990a & 1990b; Carreiro et al., 2000; Kourtev et al., 2002b) such as specific chemical composition (Linkins et al., 1990a & 1990b). For example, litter N content, presented as C:N or lignin:N are often used as predictors of decomposition rates (Melillo and Aber., 1982; Sinsabaugh et al., 1993; DeBusk and Reddy, 1998; Carreiro et al., 2000).

Microbial degradation by-products and exogeneous nutrients may also influence microbial activity. For example, polyphenols, the by-products of lignin degradation, have the potential to inhibit enzyme complexes. These compounds can, in certain appropriate environmental conditions, become the dominant regulatory mechanisms involved in microbial respiration (McClaughtery and Linkins, 1990; Wetzel, 1993). The availability of nitrogen and other nutrients has been shown to control the early phases of decay, when mass loss is less than 30% (Taylor et al., 1989). Conversely, the addition of N has been shown to retard the decomposition of large molecular weight organic matter

by the repression of phenol oxidase (Sinsabaugh et al., 1993; Carreiro et al., 2000). As decomposition proceeds, nutrient availability becomes progressively less important and lignin content controls litter decay rates (Taylor et al., 1989; Berg, 2000). As a consequence of these complex interactions, the interpretation of individual enzyme activities as simple responses to environmental substrate concentrations may not serve to adequately predict the actual microbial community dynamics.

A current strategy for predicting microbial degradation rates involves the use of a resource allocation rationale and the MARCIE (Microbial Allocation of Resources Among Community Indicator Enzymes) model for exposing linkages between individual enzymes (Sinsabaugh and Moorhead, 1994; Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997, 2002). The model is based on the premise that enzyme mediated decomposition of complex molecules are the rate-limiting step in C mineralization. It relates bacterioplankton production or litterbag mass loss through a first order model that includes C, N, and P allocation. It further indicates that the expression of enzymes is tied to environmental nutrient availabilities and that the distribution of enzyme activities can be interpreted as a resource allocation strategy (Sinsabaugh et al., 2002).

Related to the MARCIE model, the Enzyme Index of Carbon Quality (EICQ) is a relative index of the normalized activities of the hydrolytic enzymes to oxidative or lignin degrading enzymes. EICQ has been shown to be correlated with microbial biomass ($r=0.71$), productivity ($r=.80$) and negatively correlated with particulate organic carbon (POC) turnover time ($r=-0.99$) (Sinsabaugh and Findlay, 1995). EICQ values and other enzyme ratios reflect microbial community responses to the perceived abundance of nutrients as well as lignin and thus respond to changes in substrate and environmental

nutrient conditions in terms of microbial resource allocation dynamics. MARCIE model components, such as Ecell/Ep and Ecell/En, which reflect apparent phosphorus and nitrogen control on C mineralization, have also been correlated with bacterial productivity (Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997). An underlying concept is that lignocellulose degradation by extracellular enzymes is tied to environmental N and P concentrations. Since only a certain amount of metabolic energy can be utilized in the production of enzymes, an abundant expression of one enzyme resulting from a lack of directly utilizable substrate will result in less energy available for the production of other enzymes.

In organic matter dominated wetlands, such as the Everglades, the microbial mineralization of organic matter is a key process involved in the initial development, accumulation, and maintenance of the peat profile. Differential decomposition processes over time may result in the development of topographic features related to local biogeochemical characteristics which are, in turn, influenced by larger scale ecosystem processes. Specifically, the Everglades were historically dominated by a slough-ridge landscape interspersed with tree islands. This landscape consisted of dense sawgrass ridges with soil surfaces 2 to 3 feet higher than the adjacent, deeper sloughs (Baldwin and Hawker, 1915). However, after over 50 years of compartmentalization and altered surface flow, the area covered by the deeper sloughs is diminishing, being replaced by shallower, monotypic sawgrass (Cladium jamaicense crantz) stands. Changes in overall productivity have accompanied shifts in vegetative communities, which have the potential to alter the nutrient storage capability of the system (Davis, 1991).

Differences between the habitats are primarily observed in plant litter chemistry, characteristics, and localized nutrient conditions. For example, approximately 13-fold greater root P accumulation rates in cattail over sawgrass have been documented (Lorenzen et al., 2001) as well as greater P accumulation in cattail in high P conditions (Davis, 1991; Koch and Reddy, 1992; Chiang et al., 2000). Conversely, sawgrass root phosphatase activities were also found to be significantly greater than cattail in low P conditions (Kuhn et al., 2002). In a 4 year P dosing study in the Everglades, tissue P accounted for approximately 5% of the added P in sawgrass while less than 2% was recovered from plant tissue at the slough site due to the disappearance of the native community (Chiang et al., 2000). Localized dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) bioavailability to a microbial community has also been shown to differ between cattail and spikerush (Eleocharis spp.) stands in a coastal humic lake (Stepanauskas et al., 2000). These differences in nutrient conditions and plant characteristics ultimately influence the efficiency and composition of the resident heterotrophic microbial decomposers, which should be exhibited as changes in enzyme activities.

The main objective of this study was to determine the effects of vegetative habitat types and nutrient loading on apparent enzyme activity and apply the results to varying enzyme models to account for differences in potential decomposition among the three dominant habitat types (sawgrass, cattail, and open water). By investigating these relationships, one possible factor in the development of the topographical relief of the Everglades may be more fully understood.

Materials and Methods

Study Sites

The field study sites for this project were within Water Conservation Area 3A (WCA-3A) located in Broward County, FL. WCA-3A is a freshwater marsh incompletely impounded by a combination of levees and canals and covers an area of 2,339 km^2. The area is a mosaic of sawgrass stands and open water areas or sloughs interspersed with tree islands. The result of over 40 years of agricultural runoff into the Everglades has been the establishment of a P gradient originating in the northern regions of the Everglades at points downstream of discharges. Cattail (Typha spp.) has invaded into areas previously dominated by sawgrass (Davis, 1991; DeBusk et al., 1994; DeBusk et al., 2001).

Six sites were selected for sampling, representing P enriched and slightly P enriched (designated reference) cattail, sawgrass, and open water sites. Enriched sites were designated ECS, ESS, EOS, ECB, ESB, and EOB where the first letter is designated E=enriched, the second; C=cattail, S=sawgrass, O=open water, and the third; S=soil layer, and B=benthic layer. Reference P sites followed the same nomenclature with RCS, RSS, ROS, RCB, RSB, and ROB where R=reference site. GPS coordinates were 26˚04.900’ 80˚49.520’ for the enriched site and 26˚02.410’ 80˚48.870’ for the reference site. Sites were located near established transects utilized by the South Florida Water Management District for water quality monitoring. Dense cattail, sparse sawgrass, and open water areas were present at the P enriched sites, located approximately 1.22 km from the canal inflow. Sparse cattail, stands of sawgrass, and open water or slough communities consisting of water lily (Nymphaea), spikerush and periphyton constituted

the reference (slightly impacted) site, located approximately 5.83 km from the canal inflow.

Sampling

Soil cores were obtained utilizing a 5 cm stainless steel piston type corer with butyrate inserts on September 9th, 2001. Cores were collected in triplicate at each habitat-site combination. The coring procedure involved pushing the coring apparatus through the soil layer to a depth of approximately 30 cm. A serrated metal knife was used to cut around the perimeter of the tube to sever large roots and other plant matter. The core was extruded and the benthic matter, defined as the unconsolidated or pourable core fraction, was separated from the soil layer. The soil section was extruded to a depth of 10 cm and both the benthic and soil layers were stored in plastic bags on ice for transport to the laboratory.

Soil Preparation

Soil sample analysis began within 24 hours after field collection. Each layer, corresponding to the 0 to –10 cm soil or benthic, was prepared separately. 10 g sub-samples were placed in pre-weighed aluminum pans for dry mass (DM) and ash free dry mass (AFDM) determination. Dry mass was determined by incubating the pans in a drying oven for 36 hours at 105˚ C. Ash weight determination involved ashing the samples at 500˚ C for 2 hours and AFDM was calculated by subtracting the ash weight from the dry weight measurements.

Samples were transferred to a 500 mL beaker and large objects, such as rocks, were discarded. The samples were homogenized for 10 minutes with a Biospec Biohomogenizer™, resulting in a soil slurry. 10 g of the slurry was added to 100 mL DI H_2O and homogenized for an additional 5 minutes. 10 mL of the suspension was added

to 90 mL of DI H_2O and subsequently transferred to a 100 mL centrifuge tube. The suspensions were refrigerated until use (Sinsabaugh et al., 1991).

Enzyme Analysis

Hydrolytic enzyme activity was determined using methylumbelliferyl (MUF) and aminomethylcoumarin (AMC) substrates. Substrate concentrations were optimized at saturating conditions. The activities of β-glucosidase (BGL), cellobiohydrolase (CBH), phosphatase (PHO), leucine aminopeptidase (LEU), phenol oxidase (PHE) and peroxidase (PER) were assayed using MUF-β-D-glucoside (Sigma M3633), MUF-cellobioside (Sigma M6018), MUF-phosphate (Sigma M8168), L-Leucine amidomethylcoumarin (Sigma L2145), L-3,4-dihydroxyphenylalanine (DOPA), and DOPA + H_2O_2 as substrates, respectively.

MUF and AMC substrate conversion was measured using a Cytofluor 600™ automated spectrofluorimeter (PerSeptive Biosystems, Inc., Framingham, MA) with Kineticalc™ software at 360 nm excitation and 460 nm emission at 20º C. Assays were performed using Corning ® 48-well culture plates in which 400 µL of sample, 360 µL of 10 mM Tris-HCl pH 8.5, and 40 µL of substrate were added. Stock substrate concentrations were 2000 µM for MUF-β-D glucoside, 1000 µM for MUF-phosphate, 1200 µM for MUF-cellobioside, and 6000 µM for L-Leucine aminomethylcoumarin resulting in well concentrations of 100, 50, 60 and 300 µM, respectively. Each sample analysis was performed in quadruplicate. Initial and final fluorescence measurements, as well as measurements every five minutes, were taken during the 1 hour incubation. Graphs produced from the readings taken every five minutes were analyzed to ensure that linear kinetics were being observed. Final apparent enzyme activities were calculated as µmoles substrate released g^{-1} AFDM h^{-1}.

Phenol oxidase activity was determined by adding 2.0 mL soil suspension to 2.0 mL 10 mM L-DOPA (L-dihydroxphenylalanine) in 10 mM Tris-HCL pH 8.5 in 10 mL Eppendorf™ centrifuge tubes. Peroxidase activity was determined by adding 2.0 mL soil suspension to 2.0 mL 10 mM L-DOPA with 200 µL 0.3% H_2O_2. The solutions were vortexed for 30 seconds and placed on a shaker plate in a light-proof box for 45 minutes. The solutions were then centrifuged at 3000 rpm for 30 seconds and 500 µL supernatant was extracted and quadruplicates were placed in a Corning™ 48-well culture plate. Controls consisting of 250 µL DI H_2O and 250 µL 10 mM L-DOPA solution were added to the remaining wells. Absorbance was read at 360/460 nm excitation/emission on the spectrofluorimeter. Quenching was performed using the soil suspensions with a total volume of 500 µL in each well.

Sample nutrient analysis was performed by DB Labs, Rockledge, FL. Total phosphorus (TP) (EPA 365.2), total nitrogen (TN) (MVP), total organic carbon (TOC) (MVP), calcium (Ca)(SW7140), magnesium (Mg)(SW7450), and lignin (AOAC 973.18) analysis was performed using standard methods on homogenized samples.

Models

Extracellular enzymes can be grouped into four categories: Ecell (BGL and CBH), En (LEU), Ep (PHO), and Eox (PHE and PER); allowing the enzymes to be separated into those involved in C, N, and P mineralization as well as lignin degradation, respectively. While there are many enzymes in each category, it is assumed that the activity of one, or more preferably a few enzymes in each group are sufficiently correlated such that the activity of a few can act as indicators for the entire group.

Enzyme activities are averaged if there is more than one enzyme in the group (Sinsabaugh et al., 1997).

Potential decomposition models were constructed from enzymic data using normalized enzyme activity across both the benthic and soil layers. This was done in order to compare different enzymes at the same scale so that the more active enzymes do not heavily weight the calculations of model components. The Enzymic Index of Carbon Quality (EICQ) compares the activities of oxidative to hydrolytic enzymes (Sinsabaugh and Findlay, 1995). In addition, several other indices were formulated from the data. Ecell/En reflects apparent N control over C mineralization, Ecell/Ep is a relative measure indicating P control over C mineralization, and Ecell/Eox reflects apparent lignin control over C mineralization.

To improve normality and heteroscedescity, data were log transformed before statistical analysis using ©SAS version 8 statistical software (SAS, 1999). A split-plot model for the data was adopted for subsequent ANOVA analysis using PROC GLM in SAS. The whole plot corresponded to the sites in reference to the nutrient gradient while the subplots referred to the specific vegetative communities within the whole plots. Multiple comparisons were made between habitats, sites, and habitat-site combinations. Other analyses were performed using the Statistical Package for the Social Sciences (™ SPSS) version 11.0.0. All regressions and significant differences are significant at the $p<.05$ level unless otherwise noted.

Results

Nutrient Composition

Average nutrient concentrations for the benthic and soil layers with standard errors are presented in Table 3-1. Benthic TP values were significantly different among sites

and all habitats in the reference plots while soil TP varied among individual sites. Benthic TP concentrations were generally higher than soil TP values.

Total nitrogen values were significantly different among individual sites while only the sawgrass was affected by location on the gradient in the benthic layer. Soil TN values were significantly different among individual sites and between the enriched and reference open water and cattail habitats. TN concentrations were higher in the enriched habitats with the exception of the sawgrass soil and benthic samples. TN was correlated with TP in the benthic layer (Table 3-3).

Benthic TOC values were significantly different among sites, although the effect of the nutrient gradient was only significant at the open water site. Soil TOC was significantly different among sites with the cattail ($p<0.0001$) and open water affected by location on the gradient. There was no clear relationship between TOC concentrations in the benthic and soil layers. Benthic TOC was significantly correlated with TP and TN, while soil TOC was correlated with TP (Tables 3-3 and 3-4).

Carbon to nitrogen (C:N) ratios exhibited differences based on habitat type in both the soil and benthic layers, range from 11.4 to 16.2, and were significantly correlated with PHO and LEU in the soil layer. The open water habitat exhibited the lowest C:N ratios among habitats with the largest difference occurring between the open water and sawgrass habitats. There was no clear relationship between depth and C:N ratios.

Lignin data were only available for the soil layer with a small sample size (n=6). Lignin content increased from the reference to the enriched sites and was the highest in the sawgrass habitat. Lignin ranged from 6% at the RCS site to 49% at the ESS site.

Enzyme Activities

All of the enzyme assays, with the exception of PER, yielded detectable activities. PER activity was not detectable in the majority of samples. Standard errors of hydrolytic enzyme activity were generally lower than the oxidative enzymes, ranging from 4% to 46% of the mean. Laboratory replicate error was generally less than field replicate variability.

Benthic ß-glucosidase (BGL) activity did not vary significantly between habitats or along the gradient (Table 3-2). Though not significant, the cattail and open water communities generally exhibited higher activities in the enriched sites. Benthic BGL was significantly correlated with PHO, CBH, and LEU, while soil layer BGL was only weakly correlated with LEU (Tables 3-3 & 3-4). BGL activities were consistently higher in the benthic layer, ranging from approximately 2 to 40 times the activity in the soil layer, however, benthic and soil layer BGL activities were not significantly correlated to one another. Soil layer BGL was significantly correlated with TN but did not exhibit significant differences between habitats or along the gradient.

Phosphatase activities were significantly lower than those reported in Everglades periphyton mats (Newman et al., 2001). While soil PHO significantly ($p<0.0001$) increased with distance from the inflow, there were no significant differences among habitats. This increase in PHO with distance from the inflow has been documented in other Everglades areas (Wright and Reddy, 2001b) as well as decreases in PHO with P loading in periphyton (Newman et al., 2001). Benthic PHO was significantly correlated with BGL, CBH, and LEU while soil layer PHO was negatively correlated with LEU and PHE. Benthic PHO activities were greater than in the soil, ranging from approximately 2 to 100 times the activities of the soil layer with the only significant change occurring

Table 3-1. Mean chemical properties of the soil and benthic layers. Units are expressed as g kg^{-1}, lignin content expressed as % by mass with corresponding standard errors. TN= total nitrogen, TP=total phosphorus, TOC=total organic carbon, C:N=ratio of TOC to TN. Ben=Benthic layer, Soil=Soil 0 to -10 cm layer. N/A represents lack of replication due to insufficient sample. ND=not determined.

		Site	TN	TP	TOC	C:N	Lignin
Ben	Enr	Cat	36.9 ± 0.5	0.9 ± 0.0	445.5 ± 3.5	12.1 ± 0.3	ND
		Saw	28.7 ± 1.1	0.7 ± 0.1	439.7 ± 16.1	15.3 ± 0.2	ND
		Open	37.0 ± 0.3	1.7 ± 0.1	435.3 ± 2.6	11.8 ± 0.0	ND
Ben	Ref	Cat	6.0 ± 0.7	0.1 ±0.0	702.3 ± 5.5	11.8 ± 0.5	ND
		Saw	28.9 ± 1.1	0.5 ± 0.1	403.0 ± 3.0	14.5 ± 0.2	ND
		Open	22.7 ± 0.2	0.3 ± 0.0	262.3 ± 15.1	11.5 ± 0.6	ND
Soil	Enr	Cat	32.4 ± 0.2	1.3 ± 0.5	408.0 ± NA	12.6 ± 0.8	41.7 ± NA
		Saw	28.1 ± 1.0	0.9 ± 0.1	454.7 ± 21.9	16.2 ± 0.5	49.4 ± NA
		Open	38.0 ± 0.4	1.0 ± 0.1	438.5 ± 2.5	11.5 ± 0.1	39.2 ± NA
Soil	Ref	Cat	28.4 ± 1.0	1.1 ± 0.1	377.3 ± 18.2	13.3 ± 0.2	5.6 ± NA
		Saw	29.0 ± 5.1	0.5 ± 0.1	394.0 ± 12.5	13.9 ± 1.5	32.6 ± NA
		Open	30.2 ± 1.9	0.3 ± 0.0	343.0 ± 28.0	11.4 ± 0.2	29.5 ± NA

between the enriched and reference cattail habitats. This relationship has been documented in other aquatic systems in Florida (Newman and Reddy, 1992; Wright and Reddy, 2001b). Benthic PHO was correlated to the nutrient parameters TN, TOC, and TP, while soil PHO was negatively correlated with TOC and TP.

Neither benthic or soil CBH activity were significantly different between habitats or along the gradient. As with BGL, there were no trends evident among the habitats or any consistent relationship with the gradient. Benthic CBH was significantly correlated with BGL, PHO, and LEU. There were no significant correlations among enzymes in the soil layer (Table 3-3). Benthic CBH was correlated to the nutrient parameters TN and TP. No significant correlations between CBH and nutrient parameters were observed in the soil layer.

Cattail and open water benthic LEU was significantly higher at the enriched sites. Open water and sawgrass soil layer LEU was significantly higher at the enriched sites. Sawgrass LEU was lower ($p<0.05$) than the other habitats at the enriched sites. Benthic LEU was significantly correlated with BGL, PHO, and CBH (Table 3-3), while soil layer LEU was correlated with PHO and PHE (Table 3-4). Benthic LEU was higher than soil layer activities in the enriched sites, however this relationship was mixed at the reference sites. Benthic LEU was negatively correlated to the nutrient parameters TN, TOC, and TP while the soil LEU was negatively correlated with TOC and TP.

Phenol oxidase did not vary significantly between habitats or along the gradient. Standard errors were greater in the PHE assays than the hydrolytic assays, consistent with higher variability reported by Sinsabaugh (personal communication). Activities were

Table 3-2. Mean enzyme activities of the soil and benthic layers. Data reflects means with corresponding standard errors. ND=not determined. Units for ß-glucosidase (BGL), cellobiohydrolase (CBH), phosphatase (PHO), and leucine aminopeptidase (LEU) are μmoles substrate released g^{-1} AFDM h^{-1}. Units for PHE and PER are μmoles DICQ released g^{-1} AFDM h^{-1}.

Site	BGL	CBH	PHO	LEU	PHE	PER
ECB	0.080±0.031	0.072±0.022	7.34±3.77	4.54±1.75	124.5±52.3	ND
ESB	0.023±0.002	0.030±0.004	2.56±0.35	1.69±0.44	45.9±6.3	ND
EOB	0.080± 0.023	0.054±0.013	6.22±2.20	5.06±1.48	37.2±10.0	ND
RCB	0.044±0.018	0.019±0.009	1.87±1.10	0.87±0.47	75.9±11.8	ND
RSB	0.044±0.013	0.030±0.008	4.28±0.88	1.96±0.50	60.2±35.5	ND
ROB	0.025±0.010	0.034±0.019	3.49±2.22	2.42±0.97	14.9±14.9	ND
ECS	0.015±0.001	0.038±0.002	0.13±0.017	1.17±0.16	55.5±7.1	0.12±0.02
ESS	0.010±0.001	0.025±0.005	0.062±0.009	0.77±0.16	35.1±8.4	0.16±0.03
EOS	0.016±0.004	0.026±0.005	0.11±0.017	1.11±0.19	18.4±9.7	0.07±0.04
RCS	0.001±0.001	0.032±0.004	0.81±0.12	1.03±0.10	59.3±7.3	ND
RSS	0.018±0.008	0.034±0.011	0.76± 0.14	2.09±0.37	98.1±4.5	ND
ROS	0.012±0.002	0.021 ±0.004	1.54±0.13	2.25±0.01	78.8±14.2	ND

Table 3-3. Correlation coefficients for benthic enzyme and nutrient data. Correlations significant at $p<0.05$ unless otherwise noted. *=$p<0.0001$, NS=not significant. BGL= β-glucosidase, PHO= phosphatase, CBH= cellobiohydrolase, LEU= leucine aminopeptidase, PHE=phenol oxidase, TN=total nitrogen and TOC=total organic carbon.

	BGL	PHO	CBH	LEU	PHE	TN	TOC
PHO	0.73						
CBH	0.83*	0.89*					
LEU	0.71	0.94*	0.92*				
PHE	NS	NS	NS	NS			
TN	NS	0.60	0.45	-0.65	NS		
TOC	NS	0.54	NS	-0.64	NS	-0.99*	
TP	NS	0.56	0.46	-0.63	NS	0.89*	0.87*

generally higher at the reference habitats in the soil layer and were within the ranges of reported activities in the Everglades (Wright and Reddy, 2001b) and other systems (Sinsabaugh and Linkins, 1990; Sinsabaugh et al., 1992b). Another study found no significant changes in phenol oxidase activity along the gradient in WCA2A in the Everglades (Wright and Reddy, 2001). The positive correlation of soil PHE with LEU suggests that a greater proportion of N may be in recalcitrant form. Soil PHE was also negatively correlated with TOC. There was not a significant trend of decreasing phenol oxidase activity with depth.

Overall normalized hydrolytic (ET_{hyd}) and hydrolytic plus oxidative (ET_{hyd+ox}) enzyme activities in the benthic layer exhibited mixed relationships with the gradient and among habitats (Table 3-5). ET_{hyd} values were within the range reported in a previous study of a riverine system (Sinsabaugh et al., 1997). ET_{hyd} and ET_{hyd+ox} in both the cattail and open water habitats were significantly greater at the enriched sites. However, there were no consistently significant differences between habitats. The differences between the ET_{hyd} and ET_{hyd+ox} values demonstrate the contribution of apparent lignin degradation to the overall enzyme activity. The reference open water benthic layer (ROB) exhibited the smallest contribution of oxidases to the overall enzyme activity (7%) while the reference sawgrass habitat soil layer (RSS) showed the greatest (37%). The contribution of normalized enzyme activities are illustrated in radar plots for the benthic and soil layers (Figure 3-1) for visualizing the relationships presented for the calculated MARCIE model components. It is interesting to note that the shapes of the radar plots for each layer are similar in both the enriched and reference sites. However, energy allocation does appear to be different in the soil versus benthic layers.

Table 3-4. Correlation coefficients for soil enzyme and nutrient data. Regressions significant at $p<0.05$ unless otherwise noted. *=$p<0.0001$, NS=not significant. BGL= β-glucosidase, PHO= phosphatase, CBH= cellobiohydrolase, LEU= leucine aminopeptidase, PHE=phenol oxidase, TN=total nitrogen and TOC=total organic carbon.

	BGL	PHO	CBH	LEU	PHE	TN	TOC
PHO	NS						
CBH	NS	NS					
LEU	NS	-0.71	NS				
PHE	NS	0.71	NS	0.64			
TN	0.60	NS	NS	NS	NS		
TOC	NS	-0.79	NS	-0.50	-0.65	NS	
TP	NS	-0.54	NS	-0.65	NS	NS	0.57

Table 3-5. Overall benthic and soil normalized total enzyme activities. Total activities are expressed as the sum of the hydrolytic (BGL, CBH, PHO, LEU) and oxidative (PHE) enzymes (ET_{hyd+ox}) as well as the hydrolytic enzymes only (ET_{hyd}) with standard errors. Enr=enriched site, Ref= reference site. Values are unitless.

			ET (hyd)	ET (hyd+ox)
Benthic	Enr	Cat	2.27 ± 0.88	2.82 ± 1.11
		Saw	0.81 ± 0.11	1.01 ± 0.13
		Open	2.10 ± 0.60	2.27 ± 0.56
Benthic	Ref	Cat	0.71 ± 0.34	1.05 ± 0.30
		Saw	1.11 ± 0.28	1.38 ± 0.29
		Open	0.94 ± 0.35	1.01 ± 0.39
Soil	Enr	Cat	0.59 ± 0.02	0.84 ± 0.06
		Saw	0.38 ± 0.06	0.54 ± 0.03
		Open	0.49 ± 0.09	0.57 ± 0.08
Soil	Ref	Cat	0.51 ± 0.05	0.77 ± 0.07
		Saw	0.75 ± 0.20	1.18 ± 0.22
		Open	0.65 ± 0.05	1.00 ± 0.10

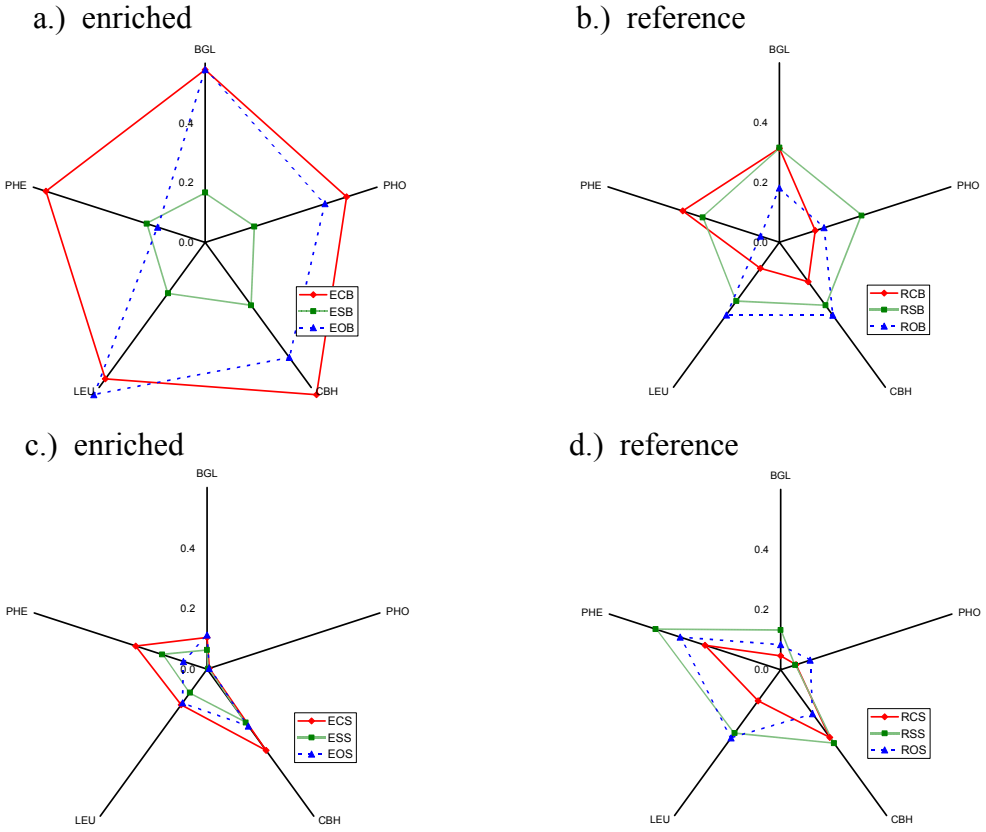


Figure 3-1. Radar Plots of benthic (a & b) and soil (c & d) layer average normalized enzyme activities for the enriched and reference habitats showing the basis for MARCIE model components.

MARCIE Model Components

Ecell/Ep values reflect the apparent P control on C mineralization based on resource allocation (Table 3-6). Higher Ecell/Ep values indicate decreased production of PHO in relation to the hydrolytic enzymes involved in C mineralization. Therefore, higher Ecell/Ep values suggest decreased control of P on C mineralization. Benthic and soil Ecell/Ep were significantly higher at all the enriched habitats. The enriched benthic layer did not show any significant differences among the habitats. However, all of the habitats exhibited significantly different benthic Ecell/Ep values at the reference sites

with the open water habitats having the lowest values. Significantly greater values were found in the soil, compared to the benthic, among the enriched habitats. Benthic Ecell/Ep was negatively correlated with TN and TOC while soil Ecell/Ep was positively correlated with TOC and TP (Table 3-7).

Ecell/En values reflect apparent N control on C mineralization. Higher Ecell/En values, like Ecell/Ep, indicate decreased LEU production in relation to C mineralization, which in turn suggests lesser control of N on C mineralization. Ecell/En values were within the high range of values reported in a study of a riverine system (Sinsabaugh et al., 1997). The enriched site benthic and soil layers exhibited no significant differences among the habitats. However, at the reference site, in both layers, all of the habitats were significantly different than one another with the lowest Ecell/En associated with the open water habitat. Benthic Ecell/En was negatively correlated with TN, TOC, and TP while soil values were positively correlated with TOC and TP.

The relative degrees of apparent N and P influences on C mineralization can be compared simultaneously in order to further differentiate the sites (Figures 3-2 & 3-3). The reference benthic layer sites generally lie along a line from the open water habitats (low N, low P) to the cattail habitats (high N, high P). The grouping of these habitats is more distinct than that of the enriched habitats, which are generally in the two high P quadrants, with varying relative N levels.

In comparison, the soil layer relationships are more distinctive in relation to the gradient, although not as clear as the benthic layer in terms of habitat grouping. Enriched sites are found in the upper portion of the high N, high P category while the majority of

Table 3-6. Enzyme ratios calculated with normalized apparent enzyme activity means and associated standard errors. Values expressed are unitless. Ecell/Eox is the apparent lignin influence on C mineralization, Ecell/Ep and Ecell/En are the apparent phosphorus and nitrogen influences on C mineralization and EICQ is the Enzyme Index of Carbon Quality. ND reflects a lack of replication due to zero oxidative enzyme activities in the denominator, thus reflecting conservatively low estimates.

Site	Ecell/ Eox	Ecell/Ep	Ecell/En	EICQ
ECB	1.18 ± 0.11	1.43 ± 0.26	1.11 ± 0.20	5.35 ± 0.54
ESB	1.06 ± 0.06	1.26 ± 0.10	1.16 ± 0.28	4.99 ± 0.41
EOB	4.47 ± 2.48	1.33 ± 0.13	0.86 ± 0.13	19.02 ± 10.48
RCB	0.81 ± 0.41	2.38 ± 0.41	2.52 ± 0.30	3.42 ± 1.32
RSB	0.58 ± 0.15	0.97 ± 0.19	1.16 ± 0.09	3.25 ± 0.33
ROB	2.24 ± ND	0.57 ± 0.03	0.71 ± 0.10	7.98 ± ND
ECS	0.91 ± 0.09	26.19 ± 2.69	1.57 ± 0.25	3.44 ± 0.20
ESS	1.10 ± 0.46	33.81 ± 2.02	1.55 ± 0.26	4.00 ± 1.24
EOS	1.24 ± 0.11	22.60 ± 2.06	1.25 ± 0.04	4.50 ± 0.27
RCS	0.63 ± 0.08	3.03 ± 0.12	1.29 ± 0.16	2.95 ± 0.19
RSS	0.48 ± 0.15	4.04 ± 0.62	0.79 ± 0.13	2.68 ± 0.39
ROS	0.39 ± 0.06	1.28 ± 0.22	0.47 ± 0.07	2.92 ± 0.23

Table 3-7. Correlation coefficients for soil and benthic layer enzyme ratio components and nutrient data. Regressions significant at $P<0.05$ unless otherwise noted. NS=not significant. Ecell/Ep and Ecell/En are the apparent phosphorus and nitrogen influences on C mineralization and EICQ is the Enzyme Index of Carbon Quality. TN= total nitrogen, TOC=total organic carbon and TP=total phosphorus.

	Benthic				Soil		
	TN	TOC	TP		TN	TOC	TP
Ecell/En	-0.77	-0.70	-0.60	Ecell/En	NS	0.63	0.87
Ecell/Ep	-0.56	-0.51	NS	Ecell/Ep	NS	0.81	0.62
Ecell/Eox	NS	NS	0.57	Ecell/Eox	NS	0.68	0.56
EICQ	0.47	NS	0.62	EICQ	0.51	NS	NS

reference sites are found in the low N, high P quadrant in the soil layer. The reference habitat soil layers exhibit similarities to the corresponding benthic layers, with apparent grouping, especially in terms of the open water habitat. The reference open water habitat is again found in the lowest section of the low N, high P quadrant with the cattail habitats generally grouped in a higher N area than the other habitats. The differences between the benthic and soil layers are probably due to lower microbial activities in the soil layer, which results in a lower demand for N and P as well as shifts in community composition due to decreased carbon quality with depth.

Ecell/Eox values reflect the apparent lignin control on C mineralization. Higher Ecell/Eox values indicate decreased PHE production, which is a reflection of apparent lignin content, suggesting lesser lignin control on C mineralization. The reference open water habitat benthic Ecell/Eox was significantly higher than the other habitats while the enriched open water was higher ($p<0.05$) than the sawgrass. These differences were not found in the soil layers. The enriched sites generally exhibited higher values than the reference, with only 3 of 6 comparisons in both layers significantly different. Ecell/Eox was correlated with TP in the benthic layer in addition to TOC and TP in the soil layer.

By combining the average Ecell/Ep, Ecell/En, and Ecell/Eox values for each habitat, the general trends for each habitat concerning P, N, and lignin influences on C mineralization can be constructed (Figure 3-4). Comparing the enriched to reference benthic sites, the largest apparent shifts in N and P dynamics occurs in the cattail habitats while the smallest shift occurs in the sawgrass sites. The open water habitats exhibited

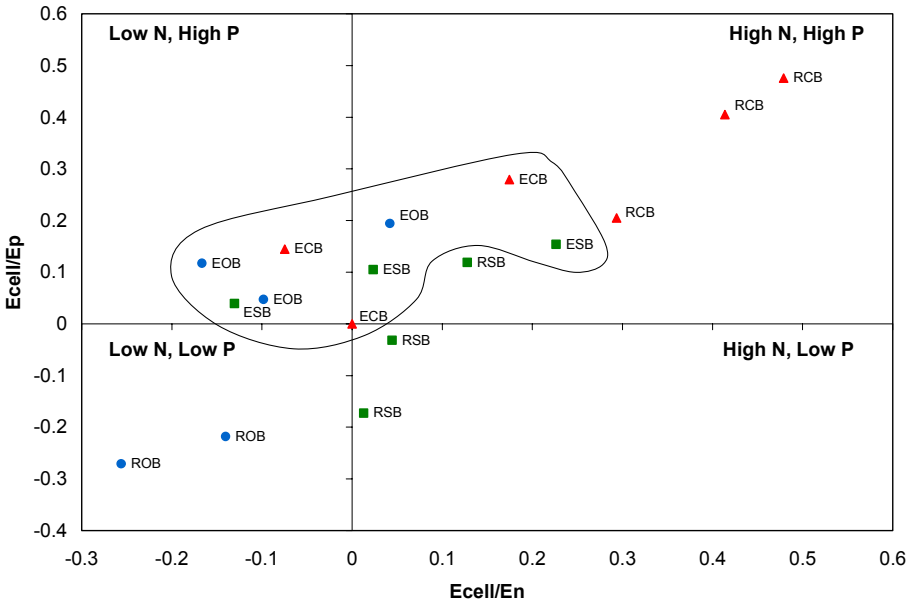


Figure 3-2. Benthic microbial N and P resource allocation in terms of C mineralization. The Y-axis represents apparent P limitation while the X-axis represents apparent N limitation on C mineralization. Values are log-transformed for comparison. N and P conditions reflect microbially perceived concentrations.

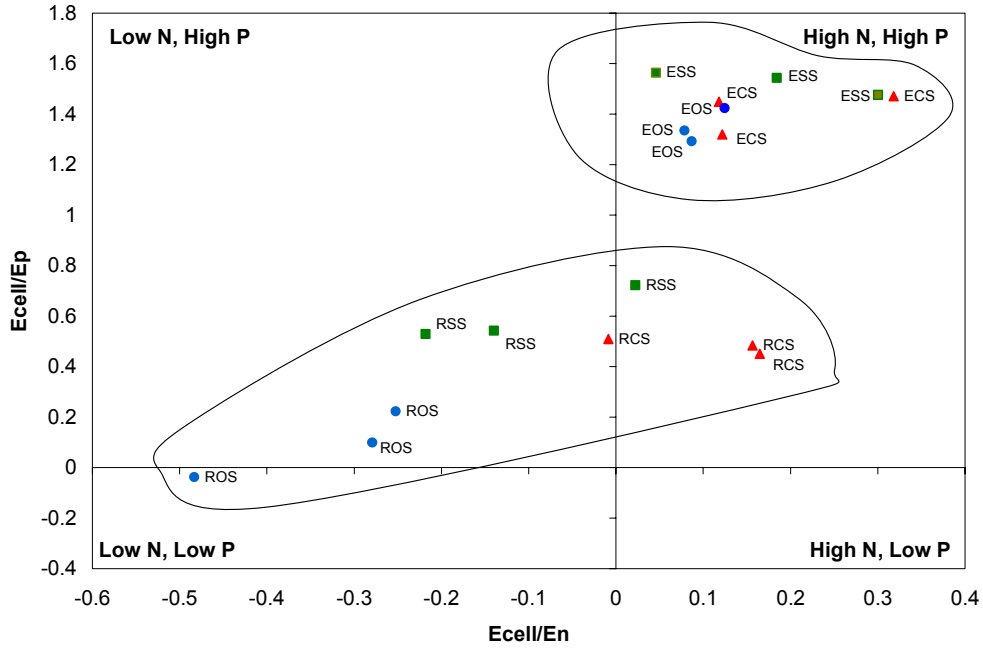


Figure 3-3. Soil microbial N and P resource allocation in terms of C mineralization. The Y-axis represents apparent P limitation while the X-axis represents apparent N limitation on C mineralization. Values are log-transformed for comparison. N and P conditions reflect microbially perceived concentrations.

the least amount of lignin influence in both the enriched and reference sites in the benthic layer, which are accompanied by the greatest amount of apparent N limitation with the exception of the ROS site. The reference open water site also exhibited the greatest N and P limitation in relation to C mineralization in both layers. The distinct grouping in the soil layer reflects primarily changes in P among the enriched habitats and changes in N among the reference habitats (Figure 3-4). However, this relationship is not evident in the benthic layer.

Enzyme Index of Carbon Quality (EICQ) values did not vary in a significantly consistent fashion along the gradient. However, open water habitat EICQ values were significantly higher in the benthic layers at both the enriched and reference sites, indicating greater perceived C quality. EICQ was only weakly correlated with the benthic nutrient parameters TN and TP as well as soil TN.

Discussion

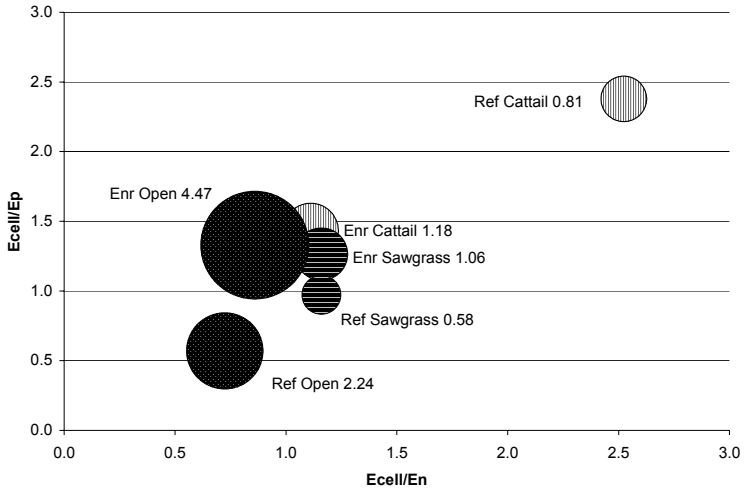
The use of enzyme comparisons, such as Ecell/Ep and Ecell/En, are based on the premise that the use of energy to produce certain extracellular enzymes reduces the net energy available for the expression of other enzymes. The relationships between certain enzyme groups are unique and serve to convey information that is specifically related to perceived environmental conditions. The MARCIE model components, originally developed as a relationship to mass loss rates (Sinsabaugh and Moorhead, 1994 & 1996), may be used as a tool for determining microbial productivity and perceived nutrient limitations (Jackson et al., 1995; Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997). The use of these models as predictive components in this paper is due to the lack of corresponding decomposition data provided by litter bags or other means. However,

relationships with published microbial community characteristics and activities are referenced to provide a strong basis for the use of these models.

The model components Ecell/En and Ecell/Ep generally predicted lower N and P limitation on C mineralization at the enriched habitats. This is concurrent with decreases in nutrient limitations that have been documented in other enriched Everglades regions (McCormick et al., 1996). Ecell/Ep values spanned an almost 20-fold greater range than Ecell/En. This relationship is consistent with 10-fold greater ranges observed in an aquatic study (Sinsabaugh et al., 1997) and is due largely to greater phosphatase variability along the primarily P gradient. Additionally, Ecell/Ep was as much as 4-fold lower than values reported in an aquatic study (Sinsabaugh et al., 1997), reflecting the low P nature of this system. Positive relationships between Ecell/En and Ecell/Ep and microbial productivity have also been observed (Sinsabaugh et al., 1997). The greater potential productivity at the enriched habitats is further supported by generally higher Ecell/Eox values at these sites, reflecting lower apparent lignin influence on C mineralization. The shift in the microbial response along the gradient may reflect changes in microbial community composition that have been observed in enriched sites in the Everglades (DeBusk and Reddy, 1998), with subsequent increased C mineralization rates.

Changes in the apparent quality of the litter with nutrient concentrations such as decreased C:N and C:P ratios and (Shaver and Melillo, 1984; Craft and Richardson, 1995; Bridgham et al., 1996; DeBusk and Reddy, 1998) have the potential to influence microbial activity which may be reflected by shifts in Ecell/Eox, Ecell/En, and Ecell/Ep along the gradient. These changes has been observed in shifts of sawgrass C:N:P ratios

a.)



b.)

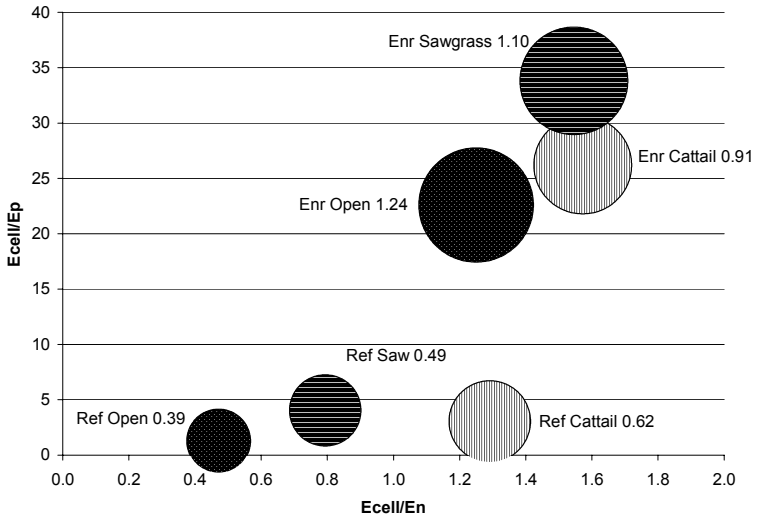


Figure 3-4. Bubble graph comparison of (a) benthic and (b) soil enzyme ratio components Ecell/En, Ecell/Ep, and Ecell/Eox, representing apparent nitrogen, phosphorus, and lignin control on carbon mineralization, respectively. Bubble size represents Ecell/Eox. Ref=reference site, Enr= enriched site, open=open water habitats. Note the change in scale between the two layers.

between enriched and reference sites in WCA-2A in the Everglades with values of 2520:63:1 and 360:9:1 in the enriched and reference sites, respectively, which were correlated with higher C turnover rates (Koch and Reddy, 1992). Additionally, the general increases in TP, TN, and TOC of both the benthic and soil layers at the enriched habitats appears to support greater the higher Ecell/Ep and Ecell/En values.

The supposition of greater productivity at the enriched habitats is also supported by larger EICQ values, which are generally limited to the more active benthic layer. Greater EICQ values in the benthic layer are synonymous with early stages of decomposition, which have been shown to have a higher carbon quality (Sinsabaugh and Linkins, 1990; Sinsabaugh and Moorhead, 1994; Sinsabaugh and Findlay, 1995). The positive relationships between EICQ and productivity (r=0.80), microbial biomass (r=0.71) as well as the negative relationship with POC turnover time (r=-0.99) (Sinsabaugh and Findlay, 1995) therefore also suggest greater C mineralization at the enriched sites. Additionally, this relationship between EICQ and microbial biomass is supported in the Everglades by significantly higher microbial biomass C (MBC) and microbial biomass P (MBP) observed within the benthic layer along a P gradient in WCA-2A (Wright and Reddy, 2001b), greater soil respiration in higher P conditions (DeBusk, 1996), as well as faster decomposition in a P dosing study in WCA-1A (Newman et al., 2001). High levels of productivity (Sinsabaugh et al., 1997) have also been associated with more eutrophic conditions. This relationship is dependent on a sufficient carbon flow that includes saccharides and amino acids (Sinsabaugh et al., 1997). Increases of TOC, general increases in primary production, and lower apparent N limitation on C mineralization at the enriched habitats further supports these relationships. Therefore, the consequence of

these elevated model parameters is predicted to be increased decomposition at the enriched habitats. However, increases in net primary production, resulting in greater C flow, may exceed any increase in decomposition, resulting in a net accumulation of organic matter (Davis, 1991).

Ecell/En and Ecell/Ep values did not predict more favorable microbial conditions in the open water habitat. Rather, this habitat was generally the most limiting in terms of N and P on C mineralization. This may be a consequence of algal competition for N and P resources due to the more prolific periphyton community in the open water habitats. This contradiction in prediction between the N and P models with Ecell/Eox and EICQ may be due to varying shifts in the type of microbial community responses to different environmental changes. The nutrient gradient is most related to changes in N and P and thus it would be expected that these nutrients would be the main driving force behind microbial changes when habitat types remain constant. Conversely, changes in vegetative types would be expected to influence the actual degradability of the C source by the microbial community, due to varying shielding effects on nutrients by refractory compounds within the plant structures. In fact, the mineralization of organic C was most affected by C quality with total P concentration and the lignocellulose index (LCI) accounting for 91% of the variability in aerobic C mineralization of plant litter along the WCA-2A gradient (DeBusk and Reddy, 1998). Thus, different enzyme components would be involved in regulating the productivity between these two types of changes. These contrasts in enzyme components most related to productivity have been documented with the BGL and PHO correlation with bacterial production in nutrient enriched and unenriched mesocosms, respectively (Chróst and Rai, 1993). This suggests

that the coupling and de-coupling of enzymes to production may be dependent on localized biogeochemical properties.

The components Ecell/Eox and EICQ predicted that the open water habitats were likely to have higher potential decomposition rates than the other two vegetative habitats, especially in the benthic layer. EICQ has been shown to be most affected by litter quality, independent of specific site characteristics in a litter bag study (Kourtev, 2002b). The lower apparent lignin influence on C mineralization at these habitats is most certainly related to changes in substrate structure and composition. Generally lower C:N ratios also predict a more favorable substrate quality within the open water habitats. This increase in potential decomposition over time would be expected to develop a lower elevation in relation to the sawgrass habitat, in particular. Additionally, this increase is coupled with a lower C input due to lower primary productivity in the form of vegetative turnover. Conversely, the lower potential degradation at the sawgrass habitats, coupled to a much greater C input, would be expected to exceed the metabolic capability of the resident microbial community, resulting the accumulation of organic matter. The combination of these factors support field observations of lower elevation open water habitats and higher elevation sawgrass stands.

Conclusions

Lower apparent lignin influence on C mineralization, lower C:N values, and higher EICQ values all predict that the open water habitat benthic layers are likely to have greater substrate qualities than cattail and sawgrass habitats with higher potential decomposition. When coupled to a lower C input, the results support field observations of lower elevations within the open water habitats. Therefore, this study

points to the potentially significant role that differential decomposition has on the development of the Everglades landscape.

The limited range of enzymes used in this study, while allowing for the expeditious processing of samples, may not accurately reflect the full array of mineralization pathways. The use of a greater range, possibly utilizing exo- and endocelullases, chitinase, and other enzymes will allow for a greater enzymatic resolution. Additionally, the use of litter decomposition bags in this type of study would allow the effects of substrate concentration on enzyme activities to be extrapolated to actual decomposition rates.

CHAPTER 4
NUTRIENT LOADING EFFECTS ON BIOGEOCHEMICAL AND MICROBIAL ENZYME DYNAMICS IN THE EVERGLADES

Introduction

The accumulation of organic carbon within wetlands is the result of the balance between net primary production (NPP) and microbial heterotrophic metabolism. Microbial decomposers play a crucial role in carbon (C) cycling and are responsible for driving the C energy flow up the detrital food chain. The mineralization of organic nutrients by the microbial community exerts an appreciable influence on energy flow by regulating nutrient availability for further decomposition and primary productivity (Elliot et al., 1984). Although most of the organic C in aquatic and marsh systems is processed and recycled entirely by the microbial community without entering the higher order food webs (Wetzel, 1984), alterations in microbial decomposition associated with nutrient loading have the potential to drastically affect the higher trophic levels of the system. The relationships between the heterotrophic microbial community and other effects of nutrient loading may serve to further facilitate the understanding of ecosystem component linkages within wetlands. Due to their contribution to the ability of the soil to degrade organic matter, enzyme activities may be especially significant in determining changes in environmental conditions and through the subsequent effects on the resident microbial community (Frankenberger and Dick, 1983; Dick, 1997).

The decomposition of plant organic matter is largely regulated by the synthesis and activities of extracellular enzymes produced by microbial communities, which operate at

the biochemical level (Sinsabaugh and Moorhead, 1994), and are induced by the presence of macromolecular and macrophytic substrates within soils (Nausch et al., 1998). Due to the complex structure of macrophytic tissue, a large quantity of diverse enzymes may be necessary to complete degradation (Eriksson and Wood, 1985; Ljungdahl and Eriksson, 1985). The mineralization of plant matter is governed by the chemical and physical properties of available substrates such as lignin, nitrogen (N), and phosphorus (P) availability (DeBusk and Reddy, 1998; Berg, 2000; Fioretto et al., 2000; Kourtev et al., 2002a & b) as well as other environmental and physicochemical influences.

The nature of the many interactions that occur between extracellular enzymes and inducing and repressing environmental components are not well understood and may play a significant role in regulating enzyme activity. Some examples of potentially significant interactions include the polyphenolic inhibition of enzyme complexes as a consequence of lignin degradation. These compounds can, in certain appropriate environmental conditions, become the dominant regulatory mechanisms involved in microbial respiration (McClaughtery and Linkins, 1990; Wetzel, 1993). The addition of N has been shown to retard the decomposition of large molecular weight organic matter by the repression of phenol oxidase (Eriksson et al., 1990; Blanchette 1991; Sinsabaugh et al., 1993; Hammel, 1997; Carreiro et al., 2000) and UV photolysis has been shown to enhance enzyme activities by relieving the inhibition by humic compounds (Wetzel et al., 1995; Boavida and Wetzel, 1998; Wetzel, 2000). As a consequence of these complex interactions, the interpretation of individual enzyme activities as simple responses to environmental substrate concentrations may not serve to adequately predict the actual microbial community response.

Enzyme activities have the potential to reflect changes in nutrient cycling as a result of changes in water and soil quality (Wetzel, 1991). For example, in a P-limited system the activities of phosphatases may be a dominant regulatory mechanism controlling microbial productivity. Phosphatase regenerates inorganic P through the hydrolysis of organic P to inorganic P (Wetzel, 1991). It is repressed by P enrichment and dissolved reactive phosphorus (DRP) (Jansson et al., 1988; Chróst, 1991; Newman et al., 2003), and has been recommended as a parameter to assess P impact in the Everglades (Newman et al., 2003). Phosphatase is one example of a suite of enzymes involved in organic matter degradation and nutrient cycling that can be affected by nutrient loading (Wetzel, 1991; Newman and Reddy, 1993; Marx et al., 2001).

Interpreting and relating individual enzyme activities to higher order trophic processes and observations is often a difficult and tedious process and are often not linked to soil and microbial parameters in the field (Marsden and Gray, 1986). A current strategy involves the use of a resource allocation rationale and the MARCIE (Microbial Allocation of Resources Among Community Indicator Enzymes) model for exposing linkages between individual enzymes (Sinsabaugh and Moorhead, 1994; Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997, 2002). The model is based on the enzyme mediated decomposition of complex molecules being the rate-limiting step in decomposition. It further indicates that the expression of enzymes is tied to environmental nutrient availabilities and that the distribution of enzyme activities can be interpreted as a resource allocation strategy (Sinsabaugh et al., 2002). An underlying concept is that lignocellulose degradation by extracellular enzymes is tied to environmental N and P concentrations. For example, model components such as

Ecell/Ep and Ecell/En, which reflect apparent P and N control on C mineralization, respectively, have been correlated with bacterial productivity (Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997). Since a certain amount of metabolic energy can be utilized in the production of enzymes, an abundant expression of one enzyme resulting from a lack of directly utilizable substrate will result in less energy available for the production of other enzymes.

The objectives of this experiment were to (1) determine the effects of nutrient loading on microbial enzyme activities in the four hydrologic units of the Everglades, (2) investigate differences in microbial responses to nutrient impacts among the areas, and (3) establish a potential decomposition model relating enzyme activities to cotton strip decomposition rates (CRR). In addition, determinations of possible interactions between site specific biogeochemical parameters and microbial enzyme activities were investigated.

Materials and Methods

Site Description

The Florida Everglades is an oligotrophic system with reference surface water total phosphorus (TP) levels averaging less than 10 $\mu g\ L^{-1}$ throughout the interior of the marsh (McCormick and O'Dell, 1996; McCormick et al., 2000). The result of decades of nutrient loading from the Everglades Agricultural Area (EAA) has been the establishment of P and, to a lesser extent, N gradients within the four hydrologic compartments of the remnant Everglades. Consequently, shifts in macrophyte species composition (Davis, 1943, 1991; Vaithiyanaithan and Richardson, 1999), increases in NPP (Davis, 1991) and peat accumulation (Reddy et al., 1993), loss or taxonomic shifts of native periphyton assemblages (McCormick and O'Dell, 1996), increases in microbial activity and biomass

(White and Reddy, 2000), and other ecological changes have been observed in Everglades areas receiving nutrient inputs from canal waters with TP concentrations as much as 10-30 fold higher than background (McCormick et al., 1996).

Field study sites were located in transects situated along nutrient gradients in four distinct hydrologic units of the Everglades: Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR), Water Conservation Area 2A (WCA-2A), Water Conservation Area 3A (WCA-3A), and Taylor Slough within Everglades National Park (ENP-TS).

Loxahatchee National Wildlife Refuge (LNWR) is the northernmost of the hydrologic areas in the Everglades, completely impounded by levees and canals, and encompasses 566 km^2. The phosphorus gradient in this area exhibits a steep decline with surface water and soil TP levels decreasing to reference levels within 2.2 km of the L-7 canal (SFWMD, 2003). LNWR hydrology is primarily rainfall driven (54%) and is, unlike the rest of the Everglades, an acidic soft water system. Sites were chosen on a transect located along a nutrient gradient extending from the L-7 canal and represented enriched (X1), transitional (X2), and reference (X4) P conditions which are designated as the ENR, TRANS, and REF sites, respectively. The average surface water TP concentrations were between 7.2 and 12.3 $\mu g\ L^{-1}$ between 1996 and 2001 for the reference site (SFWMD, 2003).

Water Conservation Area 2A is a 442 km^2 area located in the northern Everglades, completely enclosed by canals and levees, and is the most studied among the regions. The primary source of water and nutrient loading to the area are the S-10 structures that transfer water from agricultural areas and LNWR via the Hillsboro Canal. Nitrogen and phosphorus gradients exist in the water column and periphyton tissue

(McCormick and O'Dell, 1996). Soil P concentrations have ranged from approximately 400 to 1600 mg kg^{-1} in the reference and enriched areas, respectively (Reddy et al., 1993; DeBusk et al., 1994). Three sites were sampled, designated F1, F4, and U3. These sites are referred to as ENR, TRANS, and REF for the enriched, transitional and reference nutrient sites, respectively. The enriched site is characterized by robust stands of Typha domingensis Pers. (cattail) and a floating surface layer of Lemnaceae (duckweed). The surface waters contain a large quantity of particulate organic matter that is composed of plant matter in various stages of decay. The reference site consists of Eleocharis spp. (spike rush), Nymphae (water lily), Utricularia spp. (blatterwort) and benthic and floating periphyton mats surrounded by stands of Cladium jamaicense Crantz (sawgrass), with little suspended organic matter. Water depth was, on average, shallower at the reference site with clear surface water and particulate peat intermixed in some areas with a thin carbonate layer underlying the surficial periphyton mats.

Water Conservation Area 3A (WCA-3A) encompasses 2,012 km^2 and is predominantly a vast sawgrass marsh interspersed with sloughs, tree islands, and wet prairies. It is the only area not completely enclosed by levees. The highest annual mean surface water TP levels in the inflow and interior marsh were 67.3 and 20.3 μg L^{-1} for 1978-2000 (Newman et al., 2002). The extent of downstream enrichment is similar to that of LNWR and extends approximately 3 km from the inflow (Newman et al., 2002). Sampling sites were located along a SFWMD water quality monitoring transect located at 0.5E, 1.5W, and Nmeso for the enriched, transitional, and reference sites, respectively. The reference site consisted mostly of water lilies, spikerush, and periphyton.

Everglades National Park (ENP) is a 5,569 km^2 wetland consisting primarily of marl forming wet prairies, sawgrass stands, freshwater sloughs, and mangrove stands at the southern periphery. Marl forming prairies are characterized by the formation of calcitic mud, especially in the southern regions. Surface water flow into Taylor Slough originates at the S-332 structure at the southeastern side of the park. The overlying water column at the enriched site contains less suspended organic matter, resulting in a clearer profile when compared to the other enriched sites. Lower net primary productivity (NPP) at the enriched site is manifested in decreased litterfall, as compared to the other hydrologic units. Vegetative changes relating to nutrient input occur in a relatively short distance from the canal inflow and is generally limited to the deeper slough region, less than 50 meters in width. The reference site is characterized by dense periphyton and epiphyton accompanied by spikerush. Sampling sites were located along a SFWMD water quality monitoring transect designated as 0.5W, 1.5E, and Smeso for the enriched, transitional, and reference sites, respectively (Newman et al., 2002).

Sampling

Soil cores were obtained using a 10 cm thin-walled stainless steel corer on 12/14/2001, 5/18/2002, and 10/14/2002. Cores were collected in triplicate at each site. The coring procedure involved pushing the coring apparatus through the soil layer to a depth of approximately 30 cm. During insertion, a serrated metal knife was used to cut around the perimeter of the corer to sever large roots and other plant matter. The top of the core was sealed with a plastic cap allowing the core to be excavated from the soil whole. The core was extruded and the benthic matter, defined as the unconsolidated or pourable core fraction, was separated from the soil layer. The soil section was extruded

to a depth of 10 cm and both the benthic matter and soil sections were stored in plastic bags on ice for transport to the laboratory.

Soil Preparation

Soil sample analysis began within 24 hours of field collection. Each layer, corresponding to the 0 to –10 cm soil and benthic layers, were prepared separately. 10 g sub-samples were placed in pre-weighed and pre-ashed aluminum pans for dry mass (DM) and ash free dry mass (AFDM) determination. Dry mass was determined by incubating the pans in a drying oven for 36 hours at 105˚ C. Ash weight determination involved ashing the samples at 500˚ C for 2 hours.

Samples were transferred to a 500 mL beaker and large objects, such as snail shells and rocks, were discarded. The samples were homogenized for 10 minutes with a Biospec Biohomogenizer™, resulting in a soil or benthic slurry. 10 g of the slurry was diluted to a concentration of 10^{-3} with deionized water and homogenized for an additional 5 minutes. The resulting suspension was transferred to a 100 mL centrifuge tube and refrigerated until use (Sinsabaugh et al., 1991). Enzyme analysis began within 6 hours of sample preparation.

Enzyme Analysis

Hydrolytic enzyme activity was determined using methylumbelliferyl (MUF) and amidomethylcoumarin (AMC) substrates. Substrate concentrations were optimized at saturating conditions. The activities of β-glucosidase (BGL), phosphatase (PHO), leucine aminopeptidase (LEU), phenol oxidase (PHE) and peroxidase (PER) were assayed using MUF-β-D-glucoside (Sigma M3633), MUF-phosphate (Sigma M8168), L-Leucine amidomethylcoumarin (Sigma L2145), L-3,4-dihydroxyphenylalanine (DOPA), and DOPA + H_2O_2 as substrates, respectively.

MUF and AMC substrate enzymatic analysis was measured using a Cytofluor 600™ (PerSeptive Biosystems, Inc., Framingham, MA) automated spectrofluorimeter with Kineticalc™ software at 360 nm excitation and 460 nm emission at 20º C. Assays were performed using Corning ® 48-well culture plates in which 400 µL of sample, 360 µL of 10 mM Tris-HCl pH 8.5, and 40 µL of substrate was added. Stock substrate concentrations were 2000 µM for MUF-β-D glucoside, 1000 µM for MUF-phosphate, and 6000 µM for L-Leucine aminomethylcoumarin resulting in well concentrations of 100 µM, 50 µM, and 300 µM, respectively. Each sample run was performed in quadruplicate. Initial and final fluorescence measurements as well as measurements every five minutes were taken during the 1 hour incubation. Changes in fluorescence were determined by subtracting the initial from the final fluorescence. Graphs produced from the readings taken every five minutes were analyzed to ensure that linear kinetics was being observed. Concentrations of MUF and AMC released were calculated by the application of standard curves to the initial and final fluoresences. The absolute difference in concentrations yielded the substrate released during the incubation period.

The effects of quenching on MUF and AMC substrates were determined in order to account for fluorescence blocking or absorption effects caused by coloring, particle suspension, humic matter, self-quenching, or other inhibitions in the suspensions. MUF and AMC standards were placed in each sample suspension to determine the quench percentage of each matrix. The final and initial fluoresences were then converted using the appropriate quench percentage.

Phenol oxidase and peroxidase analysis was performed by adding 2.0 mL soil suspension to 2.0 mL 10 mM L-dihydroxyphenylalanine (L-DOPA) dissolved in 10 mM

Tris-HCl pH 8.5 in 10 mL Eppendorf™ centrifuge tubes. The solutions were vortexed for 30 seconds and placed on a shaker plate in a light-proof box for 45 minutes. The solutions were then centrifuged at 3000 rpm for 30 seconds, 500 µL supernatant was then extracted and placed in quadruplicate in a Corning™ 48-well culture plate. Controls consisting of 250 µL DI H_2O and 250 µL 10 mM L-DOPA solution were added to the remaining wells.

Sample nutrient analyses was performed by DB Labs, Rockledge, FL. Total phosphorus (TP) (EPA 365.2), total nitrogen (TN) (MVP), total organic carbon (TOC) (MVP), calcium (Ca) (SW7140), and magnesium (Mg) (SW7450), and lignin (AOAC 973.18) analysis was performed using standard methods on homogenized samples.

Potential enzyme activities are expressed in µmoles MUF released g^{-1} AFDM h^{-1} for glucosidase (BGL) and phosphatase (PHO), µmoles AMC released g^{-1} AFDM h^{-1} for leucine aminopeptidase (LEU), and µmoles DICQ released g^{-1} AFDM h^{-1} for phenol oxidase (PHE) and peroxidase (PER).

Cotton Strips

Cotton strips (Shirley Institute, Manchester, England) were prepared by cutting 12 cm wide strips and securing them in duplicate to stainless steel wire frames (6mm thick) with staples and waterproof tape (Newman et al., 2001). The assemblies were deployed within 10 meters of the soil coring sites at a depth of at least 15 cm below the soil surface for a period of 2 weeks at all sampling sites, corresponding within a week to soil coring events. Retrieval involved measuring the distance from the top of the frame to the soil layer using visual methods to determine the surface of the soil. The depth of the benthic layer was also recorded. Control strips on wire frames were quickly deployed in duplicate and immediately retrieved. The retrieved strips were then washed in ambient

water to remove excess detrital matter. The cotton strips were removed from the wire frame and cut into 2 cm wide strips 10 cm below the soil surface mark. 2 cm wide strips were also cut in the corresponding benthic layer. The strips were washed in DI H_2O and allowed to soak for 10 minutes to ensure saturation prior to stretching. A tensiometer (Chatillon TCD-200) with a digital force gauge (DFIS 200, Chatillon, Greensboro, North Carolina, USA) was used to determine the remaining tensile strength of the cotton strips. This value was subtracted from the control strip reading from the corresponding area. The loss of tensile strength over time is expressed as a rate constant. Since the loss of tensile strength is analogous to first-order decay, the calculation of the rate constant requires linearization of the curve. Cotton Rottenness Rate (CRR) was calculated as (Hill et al., 1985): $CRR= \{[((yo-y)/y)^{1/3})] / \#\text{ of days deployed}\} * 365$, where yo is the tensile strength of the control strips and y is the tensile strength of the test strips at each 2 cm increment.

Models

Extracellular enzymes were grouped into four categories: Ecell (BGL), En (LEU), Ep (PHO), and Eox (PHE and PER). This grouping allows the enzymes to be grouped into those involved in C, N, and P mineralization as well as lignin degradation, respectively. Enzyme activities are normalized on a scale of 0-1 to eliminate the weighting effects of the more active enzymes. Enzyme ratios were formulated to reflect the premise of resource allocation and were based on assumptions derived from the MARCIE (Microbial Allocation of Resources Among Community Indicator Enzymes) model (Sinsabaugh and Moorhead, 1994, 1996; Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997, 2002). The model is based on the premise that the enzyme mediated decomposition of complex molecules is the rate limiting step in C

mineralization, indicating that the expression of enzymes are tied to environmental nutrient availabilities and that the distribution of enzyme activities can be interpreted as a resource allocation strategy (Sinsabaugh et al., 2002). MARCIE model components, such as Ecell/Ep and Ecell/En, which reflect apparent P and N control on C mineralization, respectively, have been correlated with bacterial productivity (Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997). These ratios are based on the underlying concept that lignocellulose degradation by extracellular enzymes is tied to environmental N and P concentrations. Since only a certain amount of energy is available for enzyme production, an abundant expression of one enzyme resulting from the lack of directly utilizable substrate will result in less energy available for the production of other enzymes. Ecell/En is the ratio calculated by: BGL/LEU, which reflects apparent N control over potential cellulose decomposition. Ecell/Ep are relative measures indicating P control over potential cellulose decomposition and is calculated by: BGL/PHO. Ecell/Eox reflects apparent lignin control over potential cellulose decomposition and is calculated by: BGL/(average(PER+PHE)).

The Enzyme Index of Carbon Quality (EICQ) is a relative index of the normalized activities of the hydrolytic enzymes to the oxidative or lignin degrading enzymes. EICQ has been correlated with microbial biomass ($r=0.71$), productivity ($r=0.80$), and negatively correlated with particulate organic carbon (POC) turnover time ($r=-0.99$) (Sinsabaugh and Findlay, 1995).

Statistics

Data was statistically analyzed with © SAS v.8 statistical software (SAS, 1999) using mixed model repeated measures to determine significant differences ($p<0.05$) between sampling periods, soil depths, hydrologic units, and sites. The benthic and soil

layers were analyzed independently. Due to significant AREA*SITE*SAMPLING PERIOD interactions, contrast statements were used to differentiate among specific sites. Data was log-transformed to improve normality and heteroscedescity. Regressions were performed using SYSTAT® 10.2 (SYSTAT, 2002) for individual time periods. Mean values of all sampling dates were combined for tables and charts. Significant differences and correlation coefficients are significant at the $p<0.05$ level, unless otherwise noted.

Results

Mean values were calculated from the three sampling periods in order to analyze the effects of the canal inflows in LNWR, WCA-2A, WCA-3A, and ENP-TS. The results of mixed model repeated measures analysis on differences between enriched and reference sites are presented in Tables 4-1 and 4-2 for the benthic data and Tables 4-4 and 4-5 for the soil data. Due to the complexity of responses at the transitional sites, these results are presented separately.

Benthic Data

Nutrient data (Tables 4-1 & 4-2) for the benthic layers reflected significantly higher total phosphorus (TP) at the enriched sites in all four areas. The greatest P concentrations were present in the enriched LNWR and WCA-3A sites. TP concentrations were within the ranges reported in other studies (Koch and Reddy, 1992; Reddy et al., 1993; DeBusk et al., 1994; Wright et al., 2001b). Among the reference sites, the lowest average TP concentrations were within ENP-TS at 0.1 g kg^{-1}. These low values were also reported in an earlier study (Wright et al., 2001b).

Generally, there were not consistently significant differences between the enriched and reference TN and TOC values in the four areas (Tables 4-1 & 4-2). TN did vary significantly along the gradient in LNWR. TN and TOC concentrations were similar to

those reported in a previous study (Wright et al., 2001b). LNWR, WCA-2A, and WCA-3A exhibited a similar range of TN and TOC values. ENP-TS TN concentrations were as much as 64% lower at the ENR site and 70% lower at the REF site. Correspondingly, ENP-TS TOC concentrations were as much as 61% and 64% lower at the ENR and REF sites, respectively. TN was most strongly correlated with TOC and lignin and negatively correlated with calcium (Table 4-3). TOC was most strongly correlated with TP, TN, lignin, and negatively correlated with calcium (Table 4-3).

Calcium concentrations were the highest in ENP-TS, with values as great as 400 to 1800% higher in the enriched and reference sites, respectively. The only significant change along the gradient occurred in LNWR with a higher concentration at the enriched site. Increases in Ca have been shown to increase with P loading due to release from organic matter (Newman et al., 2001). Mg concentrations were between 150% and 338% greater in LNWR and WCA-2A than the other two areas sampled and did not vary significantly along the gradient.

Benthic C:P values were significantly higher at the reference sites in all four study areas. The largest difference in C:P values within an area occurred in ENP-TS with values of 236 and 1803 at the ENR and REF sites, respectively. Benthic C:N ratios ranged from 11.5 to 14.4 and were significantly lower at the reference sites (6 of 12 comparisons), with the exception of ENP-TS. The largest range of N:P values were 18.2 to 123.6 in ENP-TS, reflecting the minimum and maximum values among the areas. N:P values increased significantly with increasing distance from the canal in all areas.

Table 4-1. Benthic nutrient, enzyme, and ratio parameters in P enriched and reference sites in LNWR and WCA-2A. Values presented are means averaged over the three sampling periods with standard errors. Significant differences are given between the enriched and background sites in the respective areas. Each asterisk reflects differences present at the $p<0.05$ level per sampling period. Analysis was divided into the three sampling periods (1, 2, 3). Glucosidase, Leucine aminopeptidase, phosphatase units are µmoles AMC or MUF released g^{-1} AFDM h^{-1}. Phenol oxidase and peroxidase units are µmoles DICQ released g^{-1} AFDM h^{-1}. C:P= Total organic carbon to total phosphorus, C:N=Total organic carbon to total nitrogen, N:P=Total nitrogen to total phosphorus

		LNWR					WCA-2A				
Parameter	Units	Enriched (n=9)	Reference (n=9)	(P<0.05) 1	2	3	Enriched (n=9)	Reference (n=9)	(P<0.05) 1	2	3
Total Phosphorus	g kg^{-1}	1.65 ± 0.41	0.59 ± 0.02	*	*	*	1.36 ± 0.17	0.43 ± 0.04	*	*	*
Total Nitrogen	g kg^{-1}	34.99 ± 3.59	40.14 ± 1.40	*	*	*	30.99 ± 1.38	31.67 ± 0.61			
Total Organic Carbon	g kg^{-1}	440.44 ± 12.37	467.33 ± 20.34				447.00 ± 26.53	410.94 ± 22.86			
Calcium	g kg^{-1}	44.00 ± 10.50	13.78 ± 0.78	*	*	*	41.72 ± 10.72	42.39 ± 10.52			
Magnesium	g kg^{-1}	3.96 ± 0.28	2.37 ± 0.11				3.77 ± 0.49	3.68 ± 0.12			
C:P		312.6 ± 62.1	796.2 ± 9.3	*	*	*	347.7 ± 70.6	978.3 ± 140.2	*	*	*
C:N		12.8 ± 1.0	11.7 ± 0.2	*	*	*	14.4 ± 0.3	13.0 ± 1.0	*	*	
N:P		23.6 ± 3.2	68.2 ± 1.0	*	*	*	24.0 ± 4.4	74.3 ± 5.4	*	*	*
Lignin	%	40.5 ± 1.8	32.4 ± 1.3				42.5 ± 1.2	33.4 ± 7.5			
Cellulose	%	13.5 ± 0.9	12.8 ± 0.0				17.4 ± 1.6	18.1 ± 3.9			
Glucosidase		0.27 ± 0.01	0.27 ± 0.03				0.23 ± 0.01	0.11 ± 0.01		*	
Leucine aminopeptidase		3.49 ± 0.17	4.92 ± 0.40	*			3.00 ± 0.17	1.51 ± 0.08		*	
Phosphatase		2.77 ± 0.29	16.21 ± 0.39	*		*	3.18 ± 0.64	11.20 ± 2.56	*		
Phenol oxidase		59.45 ± 7.93	77.46 ± 5.02	*			25.05 ± 2.48	18.54 ± 1.21			*
Peroxidase		21.94 ± 4.45	15.41 ± 8.30				6.83 ± 1.10	4.43 ± 2.25			
Ecell/Ep		31.44 ± 14.11	0.97 ± 0.24	*	*	*	38.98 ± 20.59	0.39 ± 7.47	*	*	
Ecell/En		0.57 ± 0.14	0.37 ± 0.06				0.63 ± 0.15	0.20 ± 0.10		*	
Ecell/Eox		4.51 ± 2.47	4.30 ± 2.51				7.14 ± 2.28	17.58 ± 2.09		*	

Table 4-2. Benthic nutrient, enzyme, and ratio parameters in P enriched and reference sites in WCA-3A and ENP-TS. Values presented are means averaged over the three sampling periods with standard errors. Significant differences are given between the enriched and background sites in the respective areas. Each asterisk reflects differences present at the $p<0.05$ level per sampling period. Analysis was divided into the three sampling periods (1, 2, 3). Glucosidase, Leucine aminopeptidase and Phosphatase units are µmoles AMC-MUF released g^{-1} AFDM h^{-1}. Phenol oxidase and Peroxidase units are µmoles DICQ released g^{-1} AFDM h^{-1}.

		WCA-3A					ENP-TS				
Parameter		Impacted (n=9)	Reference (n=9)	(P<0.05) 1	2	3	Impacted (n=9)	Reference (n=9)	(P<0.05) 1	2	3
Total phosphorus	g kg^{-1}	1.66 ± 0.25	0.48 ± 0.05	*	*	*	0.74 ± 0.20	0.10 ± 0.01	*	*	*
Total nitrogen	g kg^{-1}	33.16 ± 1.12	37.53 ± 1.95				13.2 ± 4.77	11.98 ± 0.90			*
Total organic carbon	g kg^{-1}	438.11 ± 9.99	427.67 ± 16.88				173.63 ± 46.82	168.00 ± 8.19	*		*
Calcium	g kg^{-1}	64.72 ± 39.35	25.78 ± 6.34				166.44 ± 58.77	191.11 ± 28.04			
Magnesium	g kg^{-1}	1.17 ± 0.10	1.13 ± 0.16				1.43 ± 0.05	2.44 ± 0.16			
C:P		283.7 ± 48.8	840.8 ± 5.4	*	*	*	236.3 ± 21.6	1803.2 ± 137.9	*	*	*
C:N		13.2 ± 0.3	11.5 ± 0.3			*	13.1 ± 1.5	14.4 ± 0.1	*		
N:P		21.6 ± 4.3	75.2 ± 0.4	*	*	*	18.2 ± 1.1	123.6 ± 8.3	*	*	*
Lignin	%	21.6 ± 4.0	26.5 ± 2.7				20.1 ± 11.1	7.2 ± 4.5			
Cellulose	%	15.4 ± 4.7	11.5 ± 0.4				20.9 ± 4.8	8.4 ± 5.0			
Glucosidase		1.07 ± 0.29	0.26 ± 0.02		*		0.67 ± 0.01	0.15 ± 0.01		*	
Leucine aminopeptidase		4.39 ± 0.65	3.23 ± 0.10				8.13 ± 0.79	3.07 ± 0.05	*	*	
Phosphatase		2.32 ± 0.56	18.09 ± 3.23	*	*	*	11.19 ± 3.18	61.33 ± 0.48	*		*
Phenol oxidase		186.6 ± 46.0	105.2 ± 4.1				287.3 ± 89.1	94.9 ± 2.3		*	
Peroxidase		10.8 ± 4.3	17.3 ± 4.1				10.8 ± 2.2	10.4 ± 2.2			
Ecell/Ep		53.20 ± 22.73	1.51 ± 0.61	*	*	*	3.47 ± 0.80	0.11 ± 0.02	*	*	*
Ecell/En		1.33 ± 0.14	0.57 ± 0.09	*	*		1.59 ± 0.41	0.44 ± 0.09	*		*
Ecell/Eox		4.22 ± 1.01	1.44 ± 0.46		*		2.22 ± 0.43	0.96 ± 0.20	*	*	

Lignin and cellulose data is based upon data from the 2nd and 3rd sampling periods. Lignin values ranged from 7% to 43%. Decreases in lignin content occurred in LNWR, WCA-2A, and ENP-TS with increasing distance from the canal. The lowest lignin value was associated with the REF ENP-TS site as was the lowest cellulose content. Lignin was correlated with TP, TN, TOC, and negatively correlated with Ca. Cellulose content ranged from 8% to 21% with mixed relationships along the gradient. Various hydrolytic enzyme activities were associated with the P gradients in each area. Potential benthic BGL activities ranged from 0.11 to 1.07 (Table 4-1 & Table 4-2), and were higher in the ENR sites. However, these differences were only significant in 3 of 12 cases. There was no change in BGL along a gradient observed in an earlier study in WCA-2A (Wright and Reddy, 2001b). WCA-2A generally exhibited the lowest BGL activities. BGL was correlated with TP, LEU, and PHE. Similarly, LEU significantly decreased between the ENR and REF sites in only 1/3 of the cases, with the exception of LNWR, and ranged from 1.51 to 8.13 μmoles g^{-1} AFDM h^{-1}. The lowest LEU values were also generally found in WCA-2A and were only significantly correlated with PHE. PHO was significantly higher in the reference sites in 75% of the comparisons and ranged from 2.32 to 61.33 μmoles MUF released g^{-1} AFDM h^{-1}. The decrease in PHO with increased proximity to the canal has been observed in other studies in the Everglades (Wright and Reddy, 2001b) as well as with decreased phosphatase activities in periphyton associated P loading. Significant correlations were found with TP, TN, TOC, and lignin. The highest activities were associated with the ENP-TS sites, reflecting the lowest benthic TP values.

Table 4-3. Benthic layer nutrient and enzyme correlation coefficients. All values are significant at the $p<0.05$ level. TN=total nitrogen, TP=total phosphorus, TOC=total organic carbon, C:P=ratio of TOC to TP, Ca=calcium, Mg=magnesium, GLU=glucosidase, Ecell/En= apparent N control on carbon mineralization, Ecell/Ep=apparent P control on carbon mineralization LEU= leucine aminopeptidase, PHO=phosphatase, PHE=phenol oxidase, CRR=cotton rottenness rate.

	TP	TN	TOC	Lig	Cell	Ca	Mg	C:P	Ecell/ En	Ecell/ En	BGL	LEU	PHO
TN	.69												
TOC	.68	.98											
Lignin	.56	.66	.69										
Cellulose	.40			.41									
Ca	-.45	-.80	-.79	-.81									
Mg													
C:P	-.78				-.41								
Ecell/En							-.36	-.36					
Ecell/Ep	.76	.41	.44	.39				-.63	.46				
GLU	.39							-.54	.57	.42			
LEU											.60		
PHO	-.66	-.48	-.48	-.50		.10		.44		-.91			
PHE							-.23				.37	.51	
CRR	.70				.65			-.69		.62			-.57

Benthic phenol oxidase activities decreased between the ENR and REF sites in all areas but LNWR. However, this decrease was only significant in 25% of the comparisons. A lack of significant changes in PHE was previously documented within WCA-2A (Wright and Reddy, 2001b). The lowest PHE activities were present in WCA-2A with the largest range of activities in ENP-TS. Benthic PHE was correlated with BGL and PHO. Peroxidase activities were not significantly different between the ENR and REF sites and were not correlated with other enzymes or nutrients.

Average benthic Ecell/Ep values decreased with distance from the canal inflow (Tables 4-1 & 4-2) and were significantly lower in the reference sites in 11 of 12 cases. Values were as much as much as 20 times higher than those reported in an aquatic study

(Sinsabaugh et al., 1997). The lowest Ecell/Ep values were within ENP-TS, reflecting higher PHO activity in relation to BGL as well as the low TP values. Ecell/Ep was correlated with the nutrient parameters TP, TN, TOC, and lignin. The correlations with the enzymes inclusive in the ratio formulation, BGL and PHO, indicate the greater variability of phosphorus in this study.

Benthic Ecell/En significantly increased with P loading in all four areas in 5 of 12 comparisons. The largest range occurred within ENP-TS. There were no correlations to components that were not related to the ratio. The most significant changes in benthic Ecell/Eox values occurred within ENP-TS, with higher values at the enriched site. Conversely, Ecell/Eox was significantly higher in one time period at the reference site in WCA-2A. Ecell/Eox was not significantly correlated with any other parameters. Enzyme Index of Carbon Quality (EICQ) values generally did not predict increased C quality at the enriched sites, with the exception of WCA-2A and WCA-3A, though these differences were not significant.

Soil Data

Average 0 to –10 cm soil TP values ranged from 0.2 to 1.0 g kg^{-1} with significantly lower concentrations in 7 of 12 cases associated with the REF sites (Tables 4-4 & 4-5). The lowest TP values were associated with the ENP-TS sites. Soil TP was correlated with TN, TOC, and lignin, and was generally lower than the benthic layer (Table 4-6). The lowest TP and TN values were both associated with the ENP-TS sites. TN mean concentrations ranged from 8 to 37 g kg^{-1} and were significantly different in 5 of 12 cases with 2 of 3 instances reported in ENP-TS. TN was strongly correlated with TOC, lignin, and negatively correlated with Ca. There was no significant change in TN content with depth. TOC ranged from 86 to 473 g kg^{-1} with significantly lower values

once again associated with the ENP-TS sites in 1/3 of the comparisons. Soil TOC was correlated with lignin and negatively correlated with Ca. There was no consistent relationship between TOC content and depth.

Calcium concentrations ranged between 13 and 220 g kg^{-1}. The ENP-TS Ca concentrations were as much as 17 times greater than other areas. In relation to the gradient, Mg and Ca were not significantly lower at the reference sites. Mg values ranged from 0.9 to 3.6 g kg^{-1}. Ca was also negatively correlated with lignin. Neither Ca nor Mg demonstrated consistent relationships with depth.

Soil mass-mass C:P ratios were significantly higher (6 of 6 cases) at the WCA-3A and ENP-TS reference sites, reflecting the influence of the P gradient. C:P was weakly correlated with both TN and lignin, and was higher in the soil layer, with the exception of ENP-TS. Conversely, soil C:N values were significantly lower at 50% of the reference sites and ranged from 11 to 16. C:N ratios did not vary significantly within ENP-TS along the gradient. Like C:P, the soil C:N values were the lowest at the ENP-TS sites and exhibited higher values in the soil layer, with the exception of ENP-TS. N:P values were significantly higher at the reference sites in 11 of 12 comparisons and were the lowest in the ENP-TS sites. Additionally, soil N:P values were greater than the benthic layers, with the exception of the ENP-TS enriched site. These increases with soil depth are analogous to increases in N:P observed with increasing mass loss (Sinsabaugh et al., 1993). C:P and N:P values were comparable to soil data gathered in WCA-2A while C:N values were on the lower range (Reddy et al., 1993). Lignin data ranged from 11% to 52% in the soil layer and generally increased with depth. Cellulose content also

increased with depth, ranging from 13% to 27% and was generally higher at the reference sites, with the exception of ENP-TS.

Compared to the benthic layer, hydrolytic enzyme activities in the soil layer had fewer significant differences along the P gradient (Tables 4-4 & 4-5). Average soil layer BGL activity was consistently lower than the benthic activity. Soil BGL ranged from 0.05 to 0.39 μmoles g^{-1} AFDM h^{-1} and decreased from the ENR to REF site in all areas with significant differences in 2 of 3 sampling periods in WCA-3A and ENP-TS. The largest contrast between the ENR and REF sites occurred in ENP-TS. Soil BGL was negatively correlated with Mg.

Soil leucine aminopeptidase (LEU) activity was lower in 10 of 12 sites in comparison to the benthic layer and ranged from 1.92 to 3.58 μmoles g^{-1} AFDM h^{-1}. LEU was generally not significantly different between the enriched and reference sites. LEU was negatively correlated with TN, TOC, and lignin. There was not a consistent relationship between LEU activity and the P gradient. Soil PHO also did not significantly vary between the REF and ENR sites. Values were consistently lower in the soil layer, reflecting activities as great as 10 times lower those in the corresponding benthic layer. This depth relationship has been previously documented in other areas (Newman and Reddy, 1993; Wright and Reddy, 2001b). Soil PHO was negatively correlated with TP, TN, TOC, lignin, and positively correlated with Ca (Table 4-6). Soil PHE and PER activities increased with depth. PHE was weakly negatively correlated with TN, TOC, and lignin, while PER was not correlated with any parameters. The increase of oxidative activity with depth is in disagreement with earlier studies within the

Table 4-4. Soil layer nutrient, enzyme, and ratio parameters in P enriched and reference sites in LNWR and WCA-2A. Values presented are means averaged over the three sampling periods with standard errors. Significant differences are given between the enriched and background sites in the respective areas. Each asterisk reflects differences present at the $p<0.05$ level. Analysis was divided into the three sampling periods (1, 2, 3). Glucosidase, Leucine aminopeptidase, phosphatase units are µmoles AMC or MUF released g^{-1} AFDM h^{-1}. Phenol oxidase and peroxidase units are µmoles DICQ released g^{-1} AFDM h^{-1}. C:P= Total organic carbon to total phosphorus, C:N=Total organic carbon to total nitrogen, N:P=Total nitrogen to total phosphorus.

		LNWR					WCA-2A				
Parameter	Units	Impacted (n=9)	Reference (n=9)	(P<0.05) 1	2	3	Impacted (n=9)	Reference (n=9)	(P<0.05) 1	2	3
Total phosphorus	g kg^{-1}	0.91 ± 0.14	0.37 ± 0.09			*	0.72 ± 0.13	0.33 ± 0.04	*		*
Total nitrogen	g kg^{-1}	31.98 ± 1.74	35.58 ± 3.15		*		29.12 ± 2.77	33.61 ± 1.21	*		
Total organic carbon	g kg^{-1}	471.11 ± 3.74	462.11 ± 28.15	*			443.00 ± 17.14	464.67 ± 8.17			
Calcium	g kg^{-1}	30.33 ± 3.10	12.61 ± 0.20				34.83 ± 6.77	28.6 ± 2.69			
Magnesium	g kg^{-1}	3.41 ± 0.28	1.96 ± 0.08				3.64 ± 0.09	3.22 ± 0.21			
C:P		711.1 ± 122.3	1271.9 ± 58.7			*	743.9 ± 200.3	1478.4 ± 174.7			*
C:N		14.9 ± 0.9	13.2 ± 0.6	*	*		16.4 ± 1.3	13.9 ± 0.7	*		
N:P		47.1 ± 5.8	97.7 ± 8.1		*	*	46.0 ± 10.3	105.7 ± 7.6	*	*	*
Lignin	%	52.1 ± 3.1	45.7 ± 2.1				50.1 ± 2.8	46.5 ± 3.6			
Cellulose	%	17.3 ± 0.2	18.8 ± 0.1				18.2 ± 1.7	19.7 ± 0.7			
Glucosidase		0.24 ± 0.06	0.14 ± 0.06			*	0.08 ± 0.00	0.05 ± 0.01			
Leucine aminopeptidase		1.92 ± 0.13	2.69 ± 0.05		*		1.95 ± 0.03	2.16 ± 0.07			*
Phosphatase		1.89 ± 0.34	2.46 ± 0.07				1.35 ± 0.05	1.14 ± 0.06			*
Phenol oxidase		110.5 ± 8.33	223.0 ± 45.9				78.6 ± 4.0	142.8 ± 23.4			
Peroxidase		24.3 ± 0.8	36.1 ± 5.3				44.5 ± 9.1	41.1 ± 2.2			
Ecell/Ep		18.47 ± 8.40	8.52 ± 3.25				13.08 ± 5.47	0.84 ± 0.99	*		*
Ecell/En		0.60 ± 0.20	0.32 ± 0.15			*	0.27 ± 0.04	0.10 ± 0.03		*	
Ecell/Eox		0.88 ± 0.32	1.44 ± 1.11				0.24 ± 0.06	2.29 ± 0.06			

Table 4-5. Soil nutrient, enzyme, and ratio parameters in P enriched and reference sites in WCA-3A and ENP-TS. Values presented are means averaged over the three sampling periods with standard errors. Significant differences are given between the enriched and background sites in the respective areas. Each asterisk reflects differences present at the $p<0.05$ level per sampling period. Analysis was divided into the three sampling periods (1, 2, 3). Glucosidase, Leucine aminopeptidase and Phosphatase units are µmoles AMC-MUF released g^{-1} AFDM h^{-1}. Phenol oxidase and Peroxidase units are µmoles DICQ released g^{-1} AFDM h^{-1}.

		WCA-3A					ENP-TS				
Parameter		Impacted (n=9)	Reference (n=9)	(P<0.05) 1	2	3	Impacted (n=9)	Reference (n=9)	(P<0.05) 1	2	3
TP	g kg^{-1}	0.95 ± 0.42	0.31 ± 0.04	*	*	*	0.37 ± 0.11	0.23 ± 0.08			*
TN	g kg^{-1}	25.43 ± 9.13	37.21 ± 1.09	*			7.65 ± 1.63	12.74 ± 2.05	*	*	
TOC	g kg^{-1}	343.13 ± 129.19	473.39 ± 5.00	*			86.36 ± 25.78	153.48 ± 24.17	*	*	
Ca	g kg^{-1}	87.86 ± 72.95	19.06 ± 0.86				183.44 ± 78.51	219.89 ± 30.89			
Mg	g kg^{-1}	1.01 ± 0.20	0.88 ± 0.03				1.56 ± 0.33	2.92 ± 0.61			
C:P		489.3 ± 142.7	1601.9 ± 220.8	*	*	*	239.3 ± 6.4	775.9 ± 60.0	*	*	
C:N		13.1 ± 0.6	12.5 ± 0.3	*	*	*	10.9 ± 1.1	12.0 ± 0.1			
N:P		38.7 ± 13.2	127.4 ± 14.7	*	*	*	20.3 ± 0.4	62.5 ± 6.7	*	*	*
Lignin	%	20.5 ± 11.5	45.1 ± 1.8				19.0 ± 12.2	10.9 ± 1.8			
Cellulose	%	18.0 ± 1.6	18.7 ± 1.6				27.4 ± 8.4	13.3 ± 0.1			
BGL		0.39 ± 0.02	0.21 ± 0.02		*	*	0.28 ± 0.07	0.06 ± 0.01		*	*
LEU		2.87 ± 0.08	2.31 ± 0.02	*			3.48 ± 0.04	2.72 ± 0.17			*
PHO		0.80 ± 0.14	2.18 ± 0.23				3.87 ± 0.54	10.84 ± 0.20			
PHE		253.0 ± 32.0	222.1 ± 34.0				577.9 ± 76.0	252.4 ± 18.0		*	
PER		19.2 ± 3.6	32.6 ± 13.6				62.0 ± 14.0	24.9 ± 13.7			
Ecell/Ep		90.19 ± 39.15	9.37 ± 3.13		*	*	2.74 ± 0.71	0.32 ± 0.10	*	*	*
Ecell/En		1.28 ± 0.27	0.57 ± 0.11		*		0.63 ± 0.23	0.21 ± 0.07		*	*
Ecell/Eox		4.97 ± 3.19	1.29 ± 0.47				1.86 ± 1.20	0.34 ± 0.16			*

Everglades (McLatchey and Reddy, 1998). Soil PHE was highest in ENP-TS. Neither enzyme was observed to have a significant response across the gradient.

Soil Ecell/Ep values were lower in the reference sites in each area, however statistical significance was generally limited to WCA-3A and ENP-TS. The lowest Ecell/Ep values occurred in ENP-TS. Soil Ecell/Ep was higher than the benthic layer at the reference sites and lower at the enriched sites, with the exception of WCA-3A. Ecell/Ep was negatively correlated with PHE. As in the benthic layer, PHO was more linearly related to the Ecell/Ep ratio (r^2 =-0.65) than BGL (r^2 =0.32) (Table 4-6). Soil Ecell/En was also lower in the reference sites, although significant differences were confined to ENP-TS. The highest Ecell/En and Ecell/Eox values occurred within WCA-3A. Ecell/Eox was not significantly different along the gradient. Additionally, Ecell/Eox values were generally lower in the soil layer, possibly reflecting lower litter quality based on perceived lignin content. EICQ values predicted greater carbon quality at the enriched sites in WCA-2A, WCA-3A, and ENP-TS, although these differences were not significant.

CRR

Cotton Rottenness Rate (CRR) was averaged over the three sampling dates (Table 4-7). Benthic and soil CRR decreased with increasing distance from the canals in all areas. The greatest CRR in both the benthic and soil layers occurred at the WCA-2A ENR site. However, the most dynamic change in CRR was associated with the ENP-TS benthic layers between the enriched and reference sites. There were not consistent differences between the benthic and soil layers and values were within the ranges reported in the mesocosm P loading experiment in LNWR (Newman et al., 2001).

Table 4-6. Soil layer nutrient and enzyme correlation coefficients. Values in bold are significant at the p<0.0001 level, all other values are significant at the p<0.05 level. Lig=lignin, Cell=cellulose. TN=total nitrogen, TP=total phosphorus, TOC=total organic carbon, C:P=ratio of TOC to TP, Ca=calcium, Mg=magnesium, GLU=glucosidase, Ecell/En= apparent N control on carbon mineralization, Ecell/Ep=apparent P control on carbon mineralization LEU= leucine aminopeptidase, PHO=phosphatase, PHE=phenol oxidase, CRR=cotton rottenness rate.

	TP	TN	TOC	Lig	Ca	Mg	Ecell/ En	Ecell/ Ep	PHO
TP									
TN	.63								
TOC	.63	.99							
Lig	.48	.76	.79						
Cell									
Ca		-.59	-.62	-.78					
Mg									
C:P		.48	.51	.41					
EC/EN	.37					-.49			
EC/EP	.53	.61	.37				.62		
BGL						-.44	.93	.57	
LEU		-.69	-.54	-.39					
PHO	-.49	-.61	-.62	-.51	.57			-.81	
PHE		-.37	-.40	-.39				-.48	.57
CRR									

A model was created using enzyme data to test the validity of prediction against actual CRR values. Ecell/Ep and TP were found to be the most powerful predictors of CRR, accounting for 62% of the variability in the benthic layer. The resulting benthic model: Predicted CRR= $(0.04(Ln^{Ec:Ep})+1.4) + (0.35(Ln^{TP})+1)$, was strongly correlated in sampling period 1 (r^2 =0.92) and more weakly correlated in sampling period 3 (r^2=0.46). This benthic model predicted significantly greater cellulose decomposition at the enriched sites in all four areas at all time periods (p<0.0001). A separate significant model could not be created for the soil layer. However, the benthic model predicted significantly greater decomposition in the enriched sites in 75% of the contrasts.

Impact Index

An impact index was constructed to compare the varying parameters on the same scale and to allow the comparisons between the enriched and reference sites within each area (Table 4-8). In general, impact index values above 0.5 reflect severe changes in a specific parameter between the enriched and reference sites. Positive values reflect increased values at the enriched site while negative values reflect higher values at the reference site. The equation for each parameter is:

$$\text{Impact Index} = \log((\text{Enriched P site}) / (\text{Reference P site}))$$

As expected, the most responsive nutrient parameters to the P gradient were the P-related biogeochemical indicators TP, C:P, N:P, PHO, and Ecell/Ep in both the benthic and soil layers. TOC showed relatively little change in relation to the P gradient while TN demonstrated a small response in the soil layer. Differences were also observed with BGL in both layers. The majority of large impact indices in the benthic layer were within ENP-TS, even though there is a less pronounced P gradient in this area. WCA-2A, a heavily impacted area, did not exhibit the large differences that were expected. There were greater index values associated with the benthic layer, indicating that this layer is more responsive to changes in biogeochemical conditions.

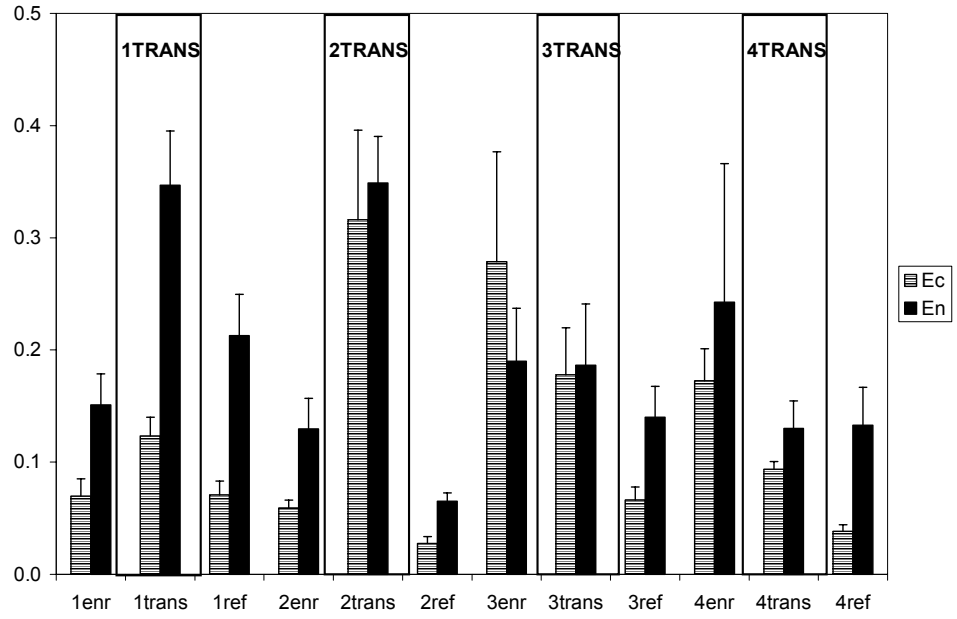
Transitional Sites

Large increases in several of the individual enzyme activities occurred at the transitional sites in the four hydrologic units (Figure 4-1a & 4-1b). The greatest increases in benthic activity occurred at the WCA-2A TRANS site with BGL and LEU with 10 of 12 comparisons significant. Spikes in LEU were also observed within the transitional sites in LNWR, WCA-2A, and ENP-TS. PHO also increased in the soil layer WCA-2A,

Table 4-7. Average benthic and soil layer CRR values over the three sampling periods with standard errors. Soil layer CRR values represent 2 of 3 sampling periods due to insufficient depth deployment of the cotton strip racks. ENR=Enriched, Trans=Transitional, REF=reference sites.

	Benthic layer	Soil layer
LNWR ENR	23.2 ± 0.5	24.7 ± 0.5
LNWR TRANS	22.9 ± 0.8	21.0 ± 0.7
LNWR REF	20.3 ± 0.8	19.4 ± 0.3
WCA-2A ENR	25.7 ± 0.1	25.4 ± 0.2
WCA-2A TRANS	20.7 ± 0.7	18.1 ± 1.0
WCA-2A REF	18.5 ± 1.3	18.7 ± 1.1
WCA-3A ENR	24.7 ± 0.1	25.1 ± 0.1
WCA-3A TRANS	23.5 ± 0.6	22.6 ± 0.4
WCA-3A REF	17.2 ± 1.1	20.7 ± 1.0
ENP-TS ENR	24.6 ± 0.5	22.2 ± 0.2
ENP-TS TRANS	17.8 ± 0.8	20.9 ± 0.6
ENP-TS REF	16.3 ± 1.3	19.6 ± 0.6

a.) Benthic layer



b.) Soil layer

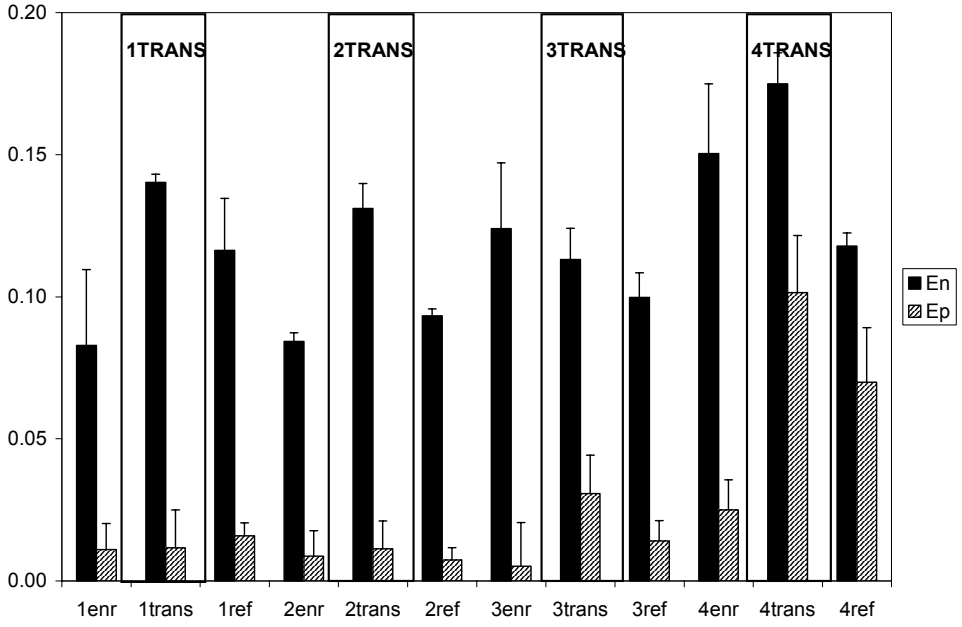


Figure 4-1. Graphs showing spikes in enzyme activities at the transitional sites. Mean enzyme activities have been normalized for visual comparison. Ec and En, normalized glucosidase and leucine aminopeptidase activities exhibited the largest spikes in the benthic layer while En and Ep (phosphatase) exhibited the largest in the soil layer. Bars represent standard errors. 1=LNWR, 2=WCA-2A, 3=WCA-3A, 4=ENP-TS. Enr=enriched, trans= transitional, ref=reference sites.

Table 4-8. Impact index (log(impacted/reference)) for nutrient and enzymic data in the soil and benthic layers for LNWR, WCA-2A, WCA-3A, and ENP-TS for averages calculated over the three sampling periods. Bolded items reflect parameters that are equal to or greater than 0.5, indicating large changes over the gradient. Negative values indicate larger parameters at the reference sites while positive values reflect larger values at the enriched sites.

	Benthic				Soil			
Parameter	LNWR	WCA-2A	WCA-3A	ENP-TS	LNWR	WCA-2A	WCA-3A	ENP-TS
TP	0.42	**0.49**	**0.53**	**0.83**	0.38	0.34	0.29	0.22
TN	-0.06	-0.01	-0.05	0.04	-0.04	-0.07	-0.38	-0.23
TOC	-0.03	0.04	0.01	-0.02	0.01	-0.02	-0.25	-0.28
Ca	**0.48**	-0.01	0.27	-0.14	0.38	0.07	0.22	-0.24
Mg	0.22	0.00	0.02	-0.23	0.24	0.06	0.05	-0.28
C:P	-0.42	**-0.46**	**-0.47**	**-0.88**	-0.27	-0.36	**-0.54**	**-0.49**
C:N	0.04	0.05	0.06	-0.05	0.05	0.05	0.02	0.00
N:P	**-0.5**	**-0.5**	**-0.53**	**-0.83**	-0.32	-0.40	**-0.56**	**-0.49**
Lignin	0.10	0.12	-0.09	**0.47**	0.06	0.03	**-0.47**	0.07
Cellulose	0.02	-0.01	0.10	**0.48**	-0.04	-0.04	-0.02	0.29
BGL	-0.04	**0.45**	**0.56**	**0.66**	0.15	0.27	0.26	**0.56**
LAP	-0.19	0.27	0.13	0.24	-0.16	-0.06	0.08	0.13
PHO	**-1.06**	**-0.49**	**-0.95**	**-0.81**	-0.03	-0.16	-0.44	-0.44
PHE	-0.29	-0.04	0.21	**0.51**	0.02	0.20	0.10	0.20
PER	0.21	0.03	0.13	-0.03	-0.13	-0.01	-0.11	0.39
Ecell:Ep	**1.03**	**1.04**	**1.54**	**1.42**	0.19	0.42	**0.85**	**0.96**
Ecell:En	0.13	0.24	0.41	0.44	0.28	0.32	0.29	**0.47**
Ecell:Eox	-0.09	0.28	**0.50**	0.40	0.28	0.19	0.38	**0.48**

WCA-3A, and ENP-TS sites. However, these spikes were not correlated with increased CRR.

The significant benthic BGL and LEU increase at the WCA-2A transitional site is accompanied by a generally much lower TN (31 g kg^{-1}), lignin (21%), cellulose (9%), the lowest TOC (352 g kg^{-1}) of any site not in ENP-TS, as well as elevated Ca (81 g kg^{-1}) and Mg (81 mg kg^{-1}). This site is also dominated by Chara spp., which is a characteristic algae in transitional P sites and is a level II indicator of P enrichment (U.S. EPA, 2002). The spike in the benthic LNWR transitional site is also accompanied by a lower TN (31.23 g kg^{-1}) and TOC (438.1 g kg^{-1}).

Increases also occurred in the soil LEU and PHO at the transitional sites in WCA-2A and ENP-TS. The lowest TP (0.2 g kg^{-1}) and TN (6 g kg^{-1}) of any site was recorded at the transitional site in ENP-TS which accounts for the greatest PHO and LEU activity of any soil layer. This relationship in the soil layer is not as clear at the WCA-2A transitional site.

Discussion

The following discussion has been grouped into several sections that first compare the general effects of the P gradient between the enriched and reference sites. The discussion generally pertains to the benthic layer results, as this layer is the most biologically active (White and Reddy, 2000) and is more responsive to changes in environmental conditions. Secondly, specific hydrologic units are compared in relation to their response to the P gradient. Lastly, a brief discussion of the observations made at the transitional sites is included.

Phosphorus Gradients

The nutrient and enzyme parameters of the benthic layer varied in differing degrees along the nutrient gradient. The correlations between TOC-TP and TOC–TN reflect increased vegetative input associated with increased TP values in both the benthic and soil layers. Aboveground production in cattail stands ranges from 3035 to 1077 g m^2 y^{-1} in the enriched and reference areas in WCA-2A, respectively, while sawgrass ranges from 1943 to 986 g m^2 y^{-1} (Davis, 1991). These changes in productivity and thus nutrient input from vegetative sources are reflected in soil nutrient contents. Decreases in P with increasing distance from the canals are reflected in the larger C:P values at the reference sites in both layers. Increases in C:P ratios in WCA-2A have been correlated with decreased C turnover rates (Koch and Reddy, 1992), while lower C:P ratios have been correlated with enhanced microbial respiration (Amador and Jones, 1993).

While individual enzyme activities can provide some insight into microbial nutrient perceptions, relative indices may provide a more productive look at community responses to environmental change. Ecell/En, Ecell/Ep, and Ecell/Eox relationships in the different areas are presented in Figure 4-2. The higher Ecell/Ep values at the enriched sites reflect a decrease in apparent P control on C mineralization. The greatest shift within WCA-3A suggests that this area has the largest P impact on the microbial community. The increase in Ecell/Ep that occurs in WCA-3A may be due to a the weighting effect of a higher C mineralization rate. This may be due to decreased inhibition from polyphenols, a generally lower water level during the sampling events, or the lower lignin content at the WCA-3A enriched site. The lowest shift in Ecell/Ep occurs in ENP-TS (Figure 4-2), suggesting that the microbial communities are less affected across the gradient by changes in P conditions. The impact index does not

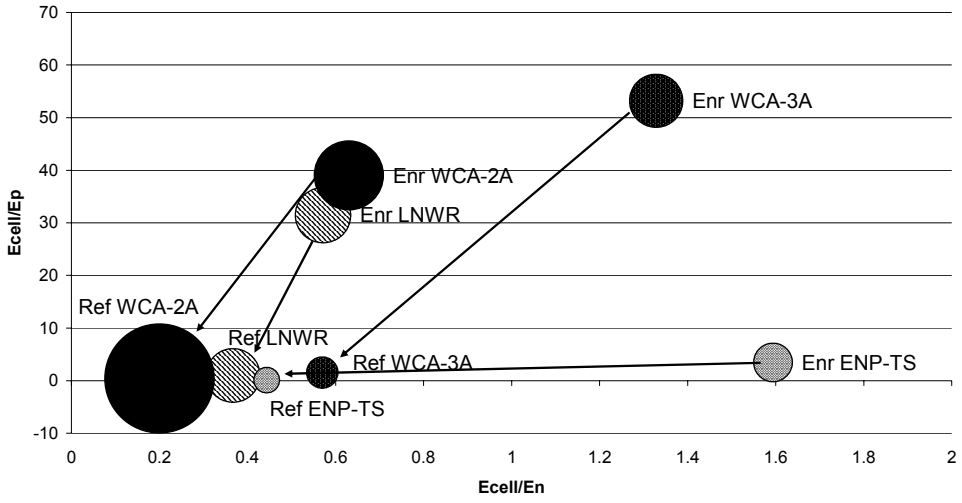


Figure 4-2. Benthic layer bubble plot of Ecell/Ep vs. Ecell/En with Ecell/Eox represented by the bubble size. Arrows denote shifts from the enriched to the corresponding reference sites.

support this by suggesting that the highest TP impact (0.83) occurs along the ENP-TS gradient. However, the TP values are lower at both the enriched and reference sites, so the increases may not be enough to induce resource allocation shifts along the gradient. In this case it does not appear that the impact index accurately reflects the severity of impacts in relation to significant changes that may be occurring within the communities. The greatest shift in P influence on C mineralization therefore appears to occur within WCA-3A with the smallest shift within ENP-TS.

Ecell/En values were higher at all the enriched sites, once again to differing degrees. The largest shift occurred within ENP-TS (Figure 4-2), which also had the highest Ecell/En impact index. The higher Ecell/En and Ecell/Ep values at all the enriched sites are concurrent with decreases in nutrient limitations that have been documented (McCormick et al., 1996), as well as lower P mineralization rates in enriched areas of the Everglades (Newman et al., 2003; White and Reddy, 2000).

Lower Ecell/En values at the reference sites may be attributed to shifts from primarily macrophyte to algal inputs at the reference sites (Figure 4-2). This would be manifested in higher LEU activity, in relation to BGL, since algae generally contain a higher protein content than macrophytes (Boschkler and Cappenberg, 1998). This is especially important since LEU may also function in C mineralization from protein sources. One driving force behind higher Ecell/En values at the enriched sites may be the generally elevated BGL activities, which has been correlated with microbial production in nutrient enriched mesocosms (Chróst and Rai, 1993). Overall, the increases in Ecell/En at the enriched sites mimic a southerly pattern: LNWR<WCA-2A<WCA-3A<ENP-TS, indicating that N pressures on the microbial community appear to become less important in the southern regions. Compared to the northern Everglades, significantly lower C/N ratios of the DOC/DON fraction of surface and shallow pore water were found within the southern Everglades (Qualls and Richardson, 2003), which mirrors the decreased apparent N control on C mineralization (Ecell/En). Several hypotheses concerning the southerly trend of decreasing DOC/DON ratios are presented by Qualls and Richardson (2003) and include increased rates of biodegradation, greater plant production of soluble organic matter, and greater production of soluble organic matter by-products where peat is being decomposed faster.

Plots of log Ecell/En vs. log Ecell/Ep indicate higher perceived P, and to a lesser extent, N in the enriched sites (Figure 4-3). Reference sites generally reside in the low N, low P quadrant. The general lack of sites residing in low P, high N conditions indicates that N loading may not be a substantial issue in the Everglades, which is consistent with the view that the system is P, not N limited.

The increase in Ecell/Eox values at the enriched sites, with the notable exception of WCA-2A, indicate decreased apparent lignin control on C mineralization. This would indicate a greater C mineralization potential. However, since enzyme activities in the view of a resource allocation model are interdependent, other factors may be influencing these trends. The higher Ecell/En values at the enriched sites in the four areas are a response to increased perceived N content. This is not apparent in the TN content of the soils which suggests that this parameter is not well suited for judging N availability, since there are varied forms of N within the system. A large portion of endogenous N may also be shielded by refractory compounds within the litter (Sinsabaugh and Moorhead, 1994) while exogenous N loading has been shown to repress lignin degradation (Eriksson et al., 1990; Blanchette, 1991; Sinsabaugh et al., 1993; Hammel, 1997; Carreiro et al., 2000) by the repression of phenol oxidase. Therefore a connection may be established between N and lignin influence on C mineralization whereas individual enzyme activities do not necessarily point to this conclusion. Therefore it appears that P loading has resulted in a generally lower level of lignin influence on C mineralization, although a higher level of microbially-perceived exogenous N may be repressing oxidative enzyme activity.

The combination of factors resulting from nutrient loading to the four areas of the Everglades appears to have resulted in a decrease in N, P, and lignin influence on the microbial community. This decrease of nutrient limitation on the community should result in greater mineralization rates of plant matter, which is supported by consistently higher CRR rates at the enriched sites as well as a greater microbial biomass in another study in WCA-2A (Wright and Reddy, 2001a). The CRR decomposition model, utilizing both TP and Ecell/Ep as parameters, indicates the relatively strong influences that the P

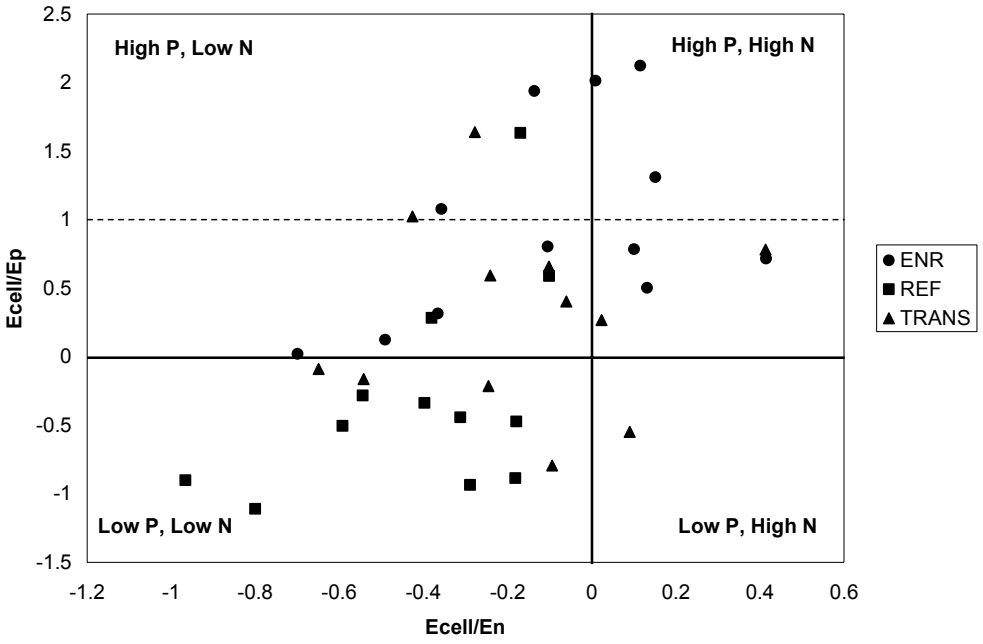


Figure 4-3. Benthic plot of Log Ecell/En vs. Log Ecell/Ep. Each point represents the mean from one sampling period. The X axis represents apparent N influence on C mineralization, the Y axis represent apparent P influence on C mineralization. Values greater than 1 on the Y axis indicates high perceived P availability.

gradient have on cellulose decomposition rates. The coupling of Ecell/Ep and TP in the CRR model indicates the relative strength of the enzyme parameters BGL and PHO in predicting cellulose decomposition. This is comparable to the correlation between BGL and PHO with bacterial production in nutrient enriched and un-enriched mesocosms, respectively (Chróst and Rai, 1993). However, as the interactions of other components within the system indicate, CRR may not be adequate to significantly predict actual litter decomposition rates, especially in relation to differing litter types.

The lack of consistent predictive power in EICQ values suggests that the dynamic changes in nutrient conditions relating to the canal inflows may influence the significance of the model. The more complex interactions that may be affecting the communities such

as microbial community shifts, vegetative changes, and differences in interactions between the heterotrophic and algal communities may outweigh the singular dynamics of the individual enzyme activity components of the EICQ model, especially in terms of lignin degradation. Therefore it does not appear that the EICQ model is a powerful predictor of microbial responses in this system among the different hydrologic units. Additionally, the use of only 5 enzymes in this study may be restrictive on the validity of the EICQ model.

ENP-TS

Special consideration is attributed to the ENP-TS sites due to the large shift in apparent N dynamics across the gradient, as compared to the relatively large shifts in P dynamics in the other areas. As previously discussed, ENP-TS had the greatest shift in apparent N influence and the smallest shift in apparent P influence on C mineralization. The highest Ecell/En and lowest Ecell/Ep values at the ENP-TS enriched sites suggests that P is the most limiting of the enriched sites, which is expected, and that N is the least limiting of any site, which is not expected. Although this site has a higher LEU activity, the relationship with the amount of C mineralization that appears to be occurring indicates that, energetically, this community is expending a smaller proportion of its resources on the acquisition of N from organic sources.

The lowest overall Ecell/Eox at the ENP-TS enriched site points to increased lignin control on C mineralization. This indicates that perceived lignin content is playing an important part in the C mineralization process, with increased combined oxidative enzyme activities, although % lignin content is lowest at this site. Interactions among environmental and microbial parameters may play an important part in the elevation of apparent lignin control. Elevated PHE activity may be partially due to the lack of

microbially available N, reflected in elevated LEU activity, which has been shown to possibly repress PHE activity.

Overall enzyme activities within ENP-TS were not drastically lower than the other enriched sites; in fact, the majority of activities were higher. One contributing factor to this elevated activity may be due to the UV photolysis of inhibitory polyphenols and other dissolved C compounds (Wetzel et al., 1995; Boavida and Wetzel, 1998; Wetzel, 2000). It has been reported that 90% of the solar radiation is absorbed at a depth of 2.5 cm in the northern Everglades while in the surface water of the southern Everglades the depth was approximately 10 cm (Qualls and Richardson, 2003). Greater UV penetration at the ENP-TS enriched site may serve to alleviate the inhibition by polyphenols, resulting in more efficient community energetics.

At the ENP-TS reference site, apparent P limitation on C mineralization is increased to the highest level of any site while TP, TN, TOC, lignin, and cellulose are all the lowest. It should be noted that phosphatase activities reflect the summation of algal and bacterial expressed enzymes (Jansson et al., 1988). The production of phosphatase is most probably the primary motive force, based on the resource allocation strategy, leading to the decreased production of the C and N acquiring enzymes at this site. However, BGL activity may also be tied to the resident periphyton community. Photosynthetically produced extracellular organic carbon (EOC) from the periphyton community may supply greater amounts of labile carbon that is sufficiently degraded for direct microbial uptake (Espeland et al., 2001). This may result in the markedly decreased production of BGL observed at these sites if the majority of DOC released is of sufficiently low molecular mass. When this readily utilizable carbon is available, there

may be no need for microorganisms to acquire it through enzymatic action (Chróst and Rai, 1993). The supplementation with EOC, resulting in lower BGL activities, may also be exhibited among the suite of cellulase enzymes, which would coincide with the lower CRR at the ENP-TS reference site.

In comparison with the other areas, ENP-TS appears to be a unique area of the Everglades that is relatively un-impacted from canal inflows. The relative lack of differences in apparent P limitation suggests very little P impacts to the resident communities. The large shifts in apparent N limitation cannot easily be explained through these techniques and suggests that some unique N anomaly is occurring near the inflow, in terms of the microbial resource allocation view. The fact that Ecell/En increases at the enriched sites with southerly canal flow suggests that this may be a consequence of agricultural loading, runoff from the northerly areas or processes occurring within the canals.

Transitional Sites

Transitional sites exhibit characteristics of both enriched and reference P regions, which may provide optimal conditions for microbial activity. The increases in enzyme activities among the transitional sites in the four areas suggest that different biogeochemical conditions support the microbial communities. Specifically, the lower nutrients present at the WCA-2A transitional site are even lower than the reference sites. The elevated Ca, as much as 2 times the concentration at the enriched and reference sites, may serve to relieve the inhibition by humic acids, which is analogous to the situation at the enriched site in ENP-TS. The lower TOC and cellulose suggests the possibility of relative C limitations, which are not necessarily supported by higher BGL activities, but once again must be viewed as a relationship to N and P mineralization. The unique algal

composition, with an abundant Chara spp. mat composing the majority of the benthic layer, has been shown to replace the Utricularia spp. and periphyton communities after one year of P loading (Craft and Richardson, 1995). Elevated Ca levels in the benthic and TP in the soil may be attributed to the $CaCO_3$ and $CaHPO_4$ bands of the cell walls of Chara spp. (Kiyosawa, 2001). The higher TP in the soil is supported by elevated Ecell/Ep, suggesting a lower P limitation on C mineralization than the enriched site in WCA-2A. Therefore, some of the enzyme activities reflected at this site may be attributed to the metabolic and structural attributes of the resident algal community.

Several reasons may account for the nutrient dynamics at the transitional sites. Upstream porewater dynamics may be exerting pressures on the transitional site. The increase in TP in the soil layer, which is greater than that at the enriched site, suggests that P is possibly being released from the soil at sites closer to the inflow. This may be due to the reduction of P loading from the canals in recent years to a concentration lower than that of the soil. The deposition of P downstream appears to result in the expansion of the P front and may continue until a relatively static equilibrium is reached. Secondly, and more specifically to the WCA-2A transitional site, the elevated TP may be due to P removal capabilities of the Chara spp. communities and eventual burial into the soil layer.

The most consistent increase at the transitional sites in both the soil and benthic layers was in relation to LEU. The decrease of average TN at the transitional sites in 6 of 8 cases supports these elevated activities. However, WCA-2A appears to be unique in this regard. This is the only area, in both layers, to show an increase in Ecell/En at the transitional site, indicating that N is less limiting to the microbial community as related to C mineralization.

Conclusions

Interpreting individual enzyme activities is often difficult due to the wide range of factors that control the induction and repression of activities. Additional factors include inhibitory and enhancing effects of organic and inorganic compounds as well as other environmental influences. The use of enzyme ratios that express activities in terms of microbially perceived C, N, and P relationships in conjunction with nutrient data appears to assist in beginning to understand some of the complex relationships.

The results of this study indicate that great care is required in any interpretations of raw enzyme activities to higher order processes such as decomposition processes. Local and regional influences must be accounted for before any conclusions are drawn. The comparison of enzyme activities to decomposition rates, either using litter bags, cotton strips, or other methods can assist in linking microbial activities to some of these higher order processes.

The relative impacts of P enrichment on microbial enzyme activities differs among the hydrologic units of the Everglades but generally results in decreased apparent N and P control (higher Ecell/Ep and Ecell/En) and greater cellulose decomposition rates (CRR). Site characteristics appear to contribute sharply to these differences. Plant matter induction, algal composition, UV photolysis of humic substances, and photosynthetically-produced extracellular organic carbon are some of the factors that are hyothesized to contribute to these differences. However, the increases in primary production, resulting from a greater C flow, may exceed any increases in decomposition, resulting in a net accumulation of organic matter (Davis, 1991; Reddy et al., 1993; Craft and Richardson, 1993).

It appears that the enzyme dynamics at the ENP-TS enriched site are mainly driven by the effects of a smaller P input relative to the other hydrologic units of the Everglades. The smaller P input has resulted in a smaller increase in NPP than the other enriched sites, leading to a relative C limitation in addition to maintaining a relative P limitation. This lower C input leads to a decreased input of detritus in the water and a more pristine water column which may increase UV inactivation of inhibitory polyphenols. Nitrogen needs are still being met as the microbial community does not appear to be limited by N in relation to C mineralization. Hypothetically, this condition would continue until increases in net primary production (NPP) equal or exceed microbial C mineralization.

The transitional sites frequently exhibit unique characteristics, manifested in elevated enzyme activities that appear to reflect changes that may be occurring in terms of structure and function of the algal, heterotrophic, and macrophytic communities. Variations in nutrient contents and enzyme activities at these sites from what would be expected from a quasi-linear reduction in nutrient contents from the enriched to reference sites suggests that there are more complex interactions occurring that may be early indicators of eutrophication.

The exponential model, using CRR as the defining decomposition criteria, utilized Ecell/Ep and TP as the major derivatives. However, the use of cotton strips restricts the model to account for only cellulose degradation. Litter bags or similar methods may be more suitable to develop a more robust enzyme model. Additionally, the use of a greater range of enzymes and more robust nutrient analyses may increase the resolution of the model as well as provide a more thorough understanding of the relationships within the system. Lastly, the fractionation of the algal, plant, and microbial decomposer enzyme

activities would allow for a much greater resolution into the sources and consequences of resource allocation dynamics.

CHAPTER 5
MICROBIAL ENZYME RESPONSES TO DECREASED WATER LEVELS IN EVERGLADES PEAT AND MARL SEDIMENTS

Introduction

Generally anaerobic conditions in wetlands are due to the decreased diffusion of O_2 in water as well as increased demand as a consequence of high carbon (C) availability. This results in an overall reduction in decomposition (Reddy and D'Angelo, 1994) which accounts for the role that inundated wetlands play in C sequestration. Organic matter accumulation in wetlands may be significantly affected by changes in water levels as a result of the characteristic hydroperiod which encompasses frequency, duration and depth. Organic mineralization rates are controlled by the microbial community that exert an appreciable influence on the energy flow of a system (Elliot et al., 1984) and have the potential to be affected by water level fluctuations.

The microbial degradation of organic matter been shown to be most influenced by the enzymes involved in lignocellulose degradation, P cycling, and N cycling (Sinsabaugh et al., 1991; Sinsabaugh and Moorhead, 1996), which are often considered the rate limiting steps in degradation (Chróst and Rai, 1993). Due to their functional role in the degradation of organic matter, enzyme activities may be especially significant in determining changes in environmental conditions through their subsequent effects on the resident microbial community (Frankenberger and Dick, 1983; Dick, 1997). Enzyme activities have the potential to affect all major wetland functions where decomposition is low: Peat accumulation is dependent upon a lower rate of enzymatic activity resulting in

C storage and inorganic nutrients remain sequestered within the poorly degraded peat matrix when decomposition is low. This impairment of nutrient cycling causes inorganic nutrients to accumulate and to be retained within the wetland (Freeman et al., 1996).

Phenol oxidase activity, which controls lignin degradation and is dependent on O_2 availability, has demonstrated varying results to water level decreases and has been referred to as an "enzymic latch" controlling C mineralization (Freeman et al., 2001). Phenol oxidase (PHE) has been observed to increase with depth (Lähdesmäki and Piispanen, 1988), decrease with decreased dissolved O_2 availability (Pind et al., 1994), exhibit no discernible variability with depth (Duxbury and Tate, 1981), increase in drought conditions (Freeman et al., 2001) and not respond predictably to drought conditions (Williams et al., 2000b). The mechanisms behind PHE variability may also include induction by the presence of certain phenolic materials among other inducible and repressible controls.

Other studies have investigated the effects of a water level decrease on flooded sediment microbial respiration. Linear increases in CO_2 flux were found with lowered water table depth down to 50 cm in Everglades sawgrass peat sediments (Volk, 1973). Increased microbial biomass and C flux in drought conditions has also been observed in peatland cores (Blodau et al., 2004). The highest total C flux as a function of combined CO_2 and CH_4 evolution was found under controlled drainage conditions 15 cm below an Everglades peat surface and was thought to be time dependent due to the water holding capacity of the peat matrix (DeBusk, 1996).

The mineralization of soil organic nitrogen (N) sources has been found to be greater in aerobic than anaerobic conditions (Reddy and Patrick, 1984; McLatchey and

Reddy, 1998) and to increase after the drying of wet soils (Cabrera 1993; Bridgham et al., 1998). N ammonification rates were specifically found to be 2-3 times greater in drained wetland soils (White and Reddy, 2001; Venterlink et al., 2002). Benthic ammonification rates have also been shown to be approximately 2 times greater in Everglades sediment cores than the corresponding deeper layers (White and Reddy, 2001).

The objectives of this study were to determine; (1) the response of enzyme activities to a controlled water level decrease in soil cores extracted from a marl dominated wetland (Taylor Slough in Everglades National Park (ENP-TS)) and a peat dominated wetland (Water Conservation Area 3A (WCA-3A)); (2) the differences in response to drained and flooded conditions between the two sites; and (3) any effects that an extended laboratory incubation has on enzyme activities.

Materials and Methods

Site Description

The Florida Everglades is an oligotrophic system with reference surface water total phosphorus (TP) levels averaging less than 10 $\mu g\ L^{-1}$ throughout the interior of the marsh (McCormick et al., 2003). Field study sites were located within the interior of WCA-3A and ENP-TS.

WCA-3A encompasses 2,012 km^2 and is predominantly a vast peat sawgrass marsh interspersed with sloughs, tree islands, and wet prairies. It is the only area not completely enclosed by levees. The highest annual mean surface water TP levels in the inflow and interior marsh were 67.3 and 20.3 $\mu g\ L^{-1}$, respectively for 1978-2000 (Newman et al., 2002). The vegetation at the sampling site is generally composed of water lilies, spikerush, and periphyton assemblages.

Everglades National Park is a 5,569 km^2 wetland consisting primarily of marl forming wet prairies, sawgrass stands, freshwater sloughs, and mangrove stands at the southern periphery. Marl forming prairies are characterized by the formation of calcitic mud, especially in the southern regions. The sampling site is characterized by dense periphyton assemblages, epiphyton and spikerush.

Sampling

Soil cores were obtained using a 12 cm thin-walled, serrated edge stainless steel corer with butyrate sleeves on March 3rd, 2003. Thirty cores were collected at each site. The coring procedure involved pushing the coring apparatus through the soil layer to a depth of approximately 50 cm. During insertion a serrated metal knife was used to cut around the perimeter of the corer to sever large roots and other plant matter. The core was removed intact with overlying water and transported to the laboratory for subsequent analysis.

Incubation Setup

Soil cores were prepared for an extended 12 week incubation to investigate the changes associated with decreased water levels. Aluminum foil was wrapped around the cores to the level of the soil to exclude light from penetrating into the soil profile. The cores were left unsealed at the top and holes were drilled in the cores at 20 cm below the soil surface for the dry treatments and 20 cm above the soil surface for the wet treatments, allowing any excess added water to be drained for water level maintenance. The dry treatment soil surface was generally within 5 cm of the top of the core to prevent any shading from the core walls. The cores were placed in a random arrangement in a Percival Scientific™ Growth Chamber Model e-36HID light chamber at a light intensity of 1500 micromole/m1/sec set on a diurnal light cycle. Water was transported from the

field study sites approximately once every two weeks to replenish water lost through evaporation.

Soil Preparation

Soil sample analysis for the initial time period was initiated within 24 hours of field collection. Each layer, corresponding to the -10 to -20 cm soil, 0 to –10 cm soil, and benthic layers, was prepared separately. 10 g sub-samples were placed in pre-weighed and pre-ashed aluminum pans for dry mass (DM) and ash free dry mass (AFDM) determination. Dry and ash weights were determined by incubating the pans in a drying oven for 36 hours at 105˚ C and ashing the samples at 500˚ C for 2 hours, respectively.

Fresh samples used for enzyme analysis were transferred to a 500 mL beaker and large objects, such as snail shells and rocks, were discarded. The samples were homogenized for 10 minutes with a Biospec Biohomogenizer™, resulting in a soil or benthic slurry. 10 g of the slurry was diluted to a concentration of 10^{-3} with deionized water and homogenized for an additional 5 minutes. The resulting suspension was transferred to a 100 mL centrifuge tube and refrigerated. Enzyme analysis began within 6 hours of sample preparation.

Enzyme Analysis

Hydrolytic enzyme activity was determined using methylumbelliferyl (MUF) and aminomethylcoumarin (AMC) substrates. Substrate concentrations were optimized at saturating conditions. The activities of β-glucosidase (BGL), phosphatase (PHO), leucine aminopeptidase (LEU), phenol oxidase (PHE) and peroxidase (PER) were assayed using MUF-β-D-glucoside (Sigma M3633), MUF-phosphate (Sigma M8168), L-Leucine amidomethylcoumarin (Sigma L2145), L-3,4-dihydroxyphenylalanine (DOPA), and DOPA + H_2O_2 as substrates, respectively.

MUF and AMC substrate enzymatic analysis was measured using a Cytofluor 600™ automated spectrofluorimeter (PerSeptive Biosystems, Inc., Framingham, MA) with Kineticalc™ software at 360 nm excitation and 460 nm emission at 20º C. Assays were performed using Corning ® 48-well culture plates in which 400 µL of sample, 360 µL of 10 mM Tris-HCl pH 8.5, and 40 µL of substrate were added. Stock substrate concentrations were 2000 µM for MUF-β-D glucoside, 1000 µM for MUF-phosphate, and 6000 µM for L-Leucine amidomethylcoumarin resulting in well concentrations of 100 µM, 50 µM, and 300 µM, respectively. Each sample run was performed in quadruplicate. Initial and final fluorescence measurements as well as measurements every five minutes were taken during the 1 hour incubation. Graphs produced from the readings taken every five minutes were analyzed to ensure that linear kinetics was being observed. Concentrations of MUF and AMC released were calculated by the application of standard curves to the initial and final fluoresences. The difference in concentrations yielded the substrate released during the incubation period.

The effects of quenching on MUF and AMC substrates were determined in order to account for fluorescence blocking or absorption effects caused by coloring, particle suspension, humic matter, self-quenching, or other inhibitions in the suspensions. MUF and AMC standards were placed in each sample suspension to determine the quench percentage of each matrix. The final and initial fluoresences were then converted using the appropriate quench percentage.

Phenol oxidase and peroxidase analyses were performed per Sinsabaugh (personal communication). 2.0 mL soil suspension was added to 2.0 mL 10 mM L-DOPA dissolved in 10 mM Tris-HCL pH 8.5 in 10 mL Eppendorf™ centrifuge tubes. The

solutions were vortexed for 30 seconds and placed on a shaker plate in a light-proof box for 45 minutes. The solutions were then centrifuged at 3000 rpm for 30 seconds, 500 µL supernatant was then extracted and placed in quadruplicate in a Corning™ 48-well culture plate. Controls consisting of 250 uL DI H_2O and 250 µL 10 mM L-DOPA solution were added to the remaining wells.

Sample nutrient analysis was performed by DB Labs, Rockledge, FL. Total phosphorus (TP) (EPA 365.2), total nitrogen (TN) (MVP), total organic carbon (TOC) (MVP), calcium (Ca) (SW7140), and lignin (AOAC 973.18) analyses were performed using standard methods on homogenized samples.

Potential enzyme activities are expressed in µmoles MUF released g^{-1} AFDM h^{-1} for BGL and PHO, µmoles AMC released g^{-1} AFDM h^{-1} for LEU, and µmoles DICQ released g^{-1} AFDM h^{-1} for PHE and PER.

Models

Extracellular enzymes were grouped into four categories: Ecell (BGL), En (LEU), Ep (PHO), and Eox (PHE and PER). This allowed the enzymes to be grouped to indicate C, N, and P mineralization as well as lignin degradation, respectively. Enzyme activities were normalized on a scale of 0-1 to eliminate the weighting effects of the more active enzymes. Enzyme ratios were formulated to examine resource allocation and were based on assumptions derived from the MARCIE (Microbial Allocation of Resources Among Community Indicator Enzymes) model (Sinsabaugh and Moorhead, 1994; Sinsabaugh and Findlay, 1995; Sinsabaugh et al., 1997; Sinsabaugh et al., 2002). The model is based on the premise that the enzyme mediated decomposition of complex molecules is the rate limiting step in C mineralization, indicating that the expression of enzymes are tied to environmental nutrient availabilities and that the distribution of enzyme activities can be

interpreted as a resource allocation strategy (Sinsabaugh et al., 2002). Ecell:Eox values were formulated by dividing the normalized BGL activity by the average of the normalized oxidative enzyme activities (PHE and PER). An Enzyme Index of Carbon Quality (EICQ) measure was calculated to assess the apparent microbial perceived carbon quality. EICQ is a relative index of the normalized activities of the hydrolytic enzymes to the oxidative or lignin degrading enzymes (Sinsabaugh and Linkins, 1990; Sinsabaugh et al., 1992b; Sinsabaugh, 1994; Sinsabaugh and Findlay, 1995). EICQ has been correlated with microbial biomass ($r=0.71$), productivity ($r=0.80$), and negatively correlated with particulate organic carbon (POC) turnover time ($r=-0.99$) (Sinsabaugh and Findlay, 1995).

Cumulative activities were calculated by integrating the area under a plot of time vs. enzyme activity (Sinsabaugh et al., 1993). The cumulative area corresponding to each time period was divided by the length of incubation. Regressions were performed on the cumulative activities for each treatment.

Statistics

Data was statistically analyzed with SAS™ v.8 (SAS, 1999) using repeated measures mixed models analysis to determine significant differences ($p<0.05$) between sampling periods, treatments, and sites. The different layers were analyzed independently and contrast statements were used to differentiate individual sites where interaction terms were significant. Data was log-transformed to improve normality and heteroscedescity. Regressions were performed using SYSTAT® 10.2 (SYSTAT, 2002). Mean values of the sampling periods were combined for tables and charts. Differences designated statistically significant were performed at the $p<0.05$ level.

Results

Enzyme Activities

By 12 weeks the moisture contents of the ENP-TS dry cores were 42 and 68%, compared to 89 and 76% for the wet cores in the benthic and 0 to -10 cm layers, respectively. The moisture contents at 12 weeks of the WCA-3A dry cores were 36 and 78%, compared to 93 and 90% for the wet cores in the benthic and 0 to -10 cm layers, respectively. BGL average values ranged from 0.005 to 0.252 μmoles MUF g^{-1} AFDM h^{-1} (Table 5-1). The highest enzyme activities were generally associated with the benthic layer in both WCA-3A and ENP-TS. Wet treatments generally exhibited higher activities across all time periods in ENP-TS and 4 of 9 comparisons in WCA-3A, although the difference was only significant in one case. Among the areas, significantly higher glucosidase activities were consistently associated with the WCA-3A cores in the benthic and 0 to -10 cm layers ($p<0.05$). Additionally, there were significant changes in enzyme activity over the incubation in both treatments within the benthic and 0 to -10 cm layers.

PHO exhibited a broad range of activities from 0 to 22.28 μmoles MUF g^{-1} AFDM h^{-1} (Table 5-1). Activities significantly decreased with depth in the 0, 2, and 4 week collections. However, by 12 weeks the greatest activity generally moved from the benthic to the 0-10 cm soil layer. Benthic wet treatments in both ENP-TS and WCA-3A were significantly higher at 2 and 4 weeks. However, at 12 weeks the trend was reversed with the dry treatments exhibiting significantly higher activities in both areas within the benthic layer. There was no clear difference between the two sites.

LEU activities ranged from 0 to 9.76 μmoles AMC g^{-1} AFDM h^{-1} and generally decreased with depth (Table 5-2). Benthic layer LEU did not vary significantly between treatments in ENP-TS. However, WCA-3A LEU activities were significantly higher in

Table 5-1. Mean enzyme activities expressed as μmoles substrate released g^{-1} AFDM h^{-1} with standard errors. Column heading numbers denote the sampling period in weeks (0, 2, 4, and 12) and enzyme measured. Note that time 0 values are replicated in the wet and dry rows as these serve as controls for both treatments. TS=ENP-TS. 3A=WCA-3A. BGL activities are expressed as 10^2. Zero enzyme activities reflect a lack of measurable activity.

			BGL				PHO			
			0	2	4	12	0	2	4	12
TS	WET	Benthic	7.8±2.5	5.0±1.9	5.2±0.2	9.9±6.1	22.28±3.76	17.09±12.26	13.73±0.90	0.01±0.01
		0 -10	2.9±0.1	2.5±0.9	3.2±0.5	0	1.35±0.94	1.18±0.03	1.76±0.21	1.08±1.08
		10-20	5.5±2.4	1.4±1.1	4.6±1.3	0.7±0.7	0.78±0.26	0.48±0.06	0.83±0.36	0.08±0.08
	DRY	Benthic	7.8±2.5	1.1±0.1	3.2±0.5	10.8±4.7	22.28±3.76	3.63±1.52	5.46±1.19	4.06±2.32
		0-10	2.9±0.1	1.5±0.9	1.1±0.2	3.7±2.4	1.35±0.94	0.61±0.07	0.87±0.20	5.02±1.84
		10-20	5.5±2.4	1.0±0.4	4.3±1.2	0.5±0.5	0.78±0.26	0.26±0.04	0.62±0.06	2.84±0.25
3A	WET	Benthic	9.9±1.2	8.9±0.8	23.8±2.4	20.7±0.5	8.56±1.43	15.40±1.96	26.52±4.35	0
		0-10	3.0±0.6	2.6±0.3	6.4±1.1	4.9±1.1	0.73±0.13	0.95±0.08	1.44±0.40	2.26±0.61
		10-20	1.0±0.1	0.5±0.1	3.1±0.1	0.5±0.5	0.43±0.04	0.36±0.06	0.77±0.19	1.58±0.07
	DRY	Benthic	9.9±1.2	12.6±3.1	15.0±2.2	25.2±4.8	8.56±1.43	7.92±2.36	4.93±1.30	5.66±0.73
		0-10	3.0±0.6	2.6±0.4	3.8±0.7	6.4±0.5	0.73±0.13	0.81±0.12	0.77±0.14	2.95±0.38
		10-20	1.0±0.1	0.5±0.1	1.3±0.1	0.9±0.1	0.43±0.04	0.33±0.03	0.43±0.02	1.62±0.15

Table 5-2. Mean enzyme activities expressed as μmoles substrate released g^{-1} AFDM h^{-1} with standard errors. Column heading numbers denote the sampling period in weeks (0, 2, 4, and 12) and enzyme measured. TS=ENP-TS. 3A=WCA-3A. Note that time 0 values are replicated in the wet and dry rows as these serve as controls for both treatments. Zero enzyme activities reflect a lack of measurable activity.

			LEU				PHE			
			0	2	4	12	0	2	4	12
TS	WET	Benthic	2.39±0.68	3.57±2.68	4.42±0.28	9.55±6.79	476.32±171.52	99.90±25.43	108.76±23.68	95.71±53.91
		0 -10	0.68±0.30	0.40±0.28	0.37±0.8	0.08±0.08	256.46±119.60	55.30±14.76	69.22±6.36	26.61±26.61
		10-20	0.80±0.57	0.56±0.26	0.93±0.42	0	256.31±119.75	50.05±16.50	86.17±16.68	58.25±58.24
	DRY	Benthic	2.39±0.68	1.49±0.53	1.55±0.20	8.06±6.07	476.32±171.52	62.07±4.58	81.52±10.43	71.58±6.30
		0-10	0.68±0.30	0.55±0.18	0.40±0.17	1.13±0.17	256.46±119.60	48.18±6.48	42.07±3.17	74.04±9.67
		10-20	0.80±0.57	0.43±0.11	1.13±0.54	0.55±0.55	256.31±119.75	62.84±8.36	91.77±13.44	31.45±31.45
3A	WET	Benthic	1.85±0.36	7.22±1.03	0	0	113.75±12.70	15.96±4.13	97.23±7.75	52.52±15.80
		0-10	0.21±0.07	0.35±0.08	0	0	21.49±9.12	9.78±0.61	22.69±22.36	26.44±2.76
		10-20	0.10±0.01	0.24±0.02	0	0	21.60±10.12	10.93±0.94	18.25±18.25	13.19±13.19
	DRY	Benthic	1.85±0.36	5.91±1.33	5.90±1.00	9.76±3.06	113.75±12.70	34.57±14.36	31.06±2.75	21.43±0.24
		0-10	0.21±0.07	1.20±0.03	2.59±0.48	7.46±2.46	21.49±9.12	6.95±4.03	23.92±5.32	15.67±1.64
		10-20	0.10±0.01	0.37±0.10	0.89±0.14	2.53±0.44	21.60±10.12	6.27±4.27	23.66±0.65	17.16±4.66

the dry treatments at 4 and 12 weeks. Dry treatment activities in the 0 to -10 cm layer were significantly higher in WCA-3A but were not significantly higher in ENP-TS until 12 weeks. There were significant time effects in the benthic but not the 0 to -10 cm layer. Comparing areas, there were generally no significant differences in the benthic layer. However, the WCA-3A dry treatments were significantly higher than the ENP-TS dry treatments in the 0 to -10 cm layer.

Phenol oxidase activities were widely variable and ranged from 6.95 to 476.32 μmoles DICQ g^{-1} AFDM h^{-1} (Table 5-2). The majority of benthic and 0 to -10 cm layer phenol oxidase activities were significantly higher in the ENP-TS cores. Significant differences between treatments were confined to the 4 and 12 week WCA-3A samples, with greater PHE activity in the wet cores. There was not a consistently clear relationship with depth.

Peroxidase activities ranged from 0 to 59.31 μmoles DICQ g^{-1} AFDM h^{-1} with the majority of enzyme activities unresolvable. There were no significant differences between treatments, time periods, or depth. This can be attributed to the very large replicate errors.

Ecell/Eox values reflect the apparent lignin influence on C mineralization. Significantly higher values were associated with the WCA-3A cores in all depths and treatments. Values generally decreased with depth, reflecting a greater lignin influence on C mineralization. WCA-3A dry treatments were significantly higher than the wet treatments at 4 and 12 weeks in the benthic layer and only at 2 weeks in the 0 to -10 cm layer. Conversely, the ENP-TS benthic and 0 to -10 cm layers generally exhibited higher values in the wet treatment with only 1 significant contrast.

EICQ values reflect the total apparent perceived carbon quality as a function of hydrolytic and oxidative enzyme activities (Table 5-3). WCA-3A benthic dry treatments were significantly greater than all the ENP-TS benthic dry treatments as were the wet treatments, although only in 50% of the cases. The effect of drying on the sediments was most apparent in the WCA-3A cores in both layers with significantly greater EICQ values in the dry treatments in 5 of 6 cases, compared to the wet. However, EICQ values were not generally significantly different between the treatments in the ENP-TS cores until 12 weeks.

Cumulative Activities

Cumulative enzyme activities were interpreted in two fashions. The slopes of the log regressions of each period's cumulative enzyme activity (CES) provide insight into the rate of total change occurring in each treatment over the total incubation period (Table 5-4). Cumulative enzyme activity was calculated according to the trapezoidal rule for integrating the cumulative activity under a curve. Secondly, net cumulative enzyme activity-day (CEA) was calculated by the cumulative integrated enzyme activity calculated using the trapezoidal rule for each time period divided by the number of days (Figure 5-1). While CES measures the net rate of changes of a particular treatment over the time period, CEA measures the average accumulation of enzyme activity per day.

BGL CES values exhibited the greatest changes over time in the WCA-3A cores in both the benthic and 0 to -10 cm layers (Table 5-4). There was not a consistent trend in rates of change among the treatments. BGL slopes were generally lower than that of the other enzymes, reflecting smaller net changes over time. CEA exhibited greater values associated with the WCA-3A cores and wet treatments within each site in the benthic

Table 5-3. Mean Enzyme Index of Carbon Quality (EICQ) values calculated as the ratio of normalized hydrolytic enzymes to normalized oxidative enzymes with standard errors. Ecell/Eox values calculated as the ratio of the mean normalized BGL to the mean normalized oxidative enzyme activities (PHE and PER) with standard errors. Values are unitless. B=benthic layer, 10=0 to -10 cm layer, 20=-10 to -20 cm layer. TS=ENP-TS, 3A=WCA-3A. Top row indicates the time period in weeks. X indicates a lack of standard error due to only one replicate included in the analysis.

			Ecell/Eox				EICQ			
			0	2	4	12	0	2	4	12
TS	WET	Benthic	2.06±0.21	0.64±0.17	0.62±0.11	0.88±0.47	2.50±0.78	0.89±0.51	1.26±0.25	0.69±0.43
		0 -10	0.96±0.34	0.51±0.22	0.52±0.11	0	0.60±0.23	0.27±0.04	0.32±0.04	0.14±0.14
		10-20	1.77±0.65	0.31±0.25	0.62±0.15	0.14±X	0.87±0.36	0.20±0.13	0.37±0.10	0.07±X
	DRY	Benthic	2.06±0.21	0.18±0.04	0.49±0.12	1.01±0.39	2.50±0.78	0.30±0.05	0.63±0.05	1.18±0.16
		0-10	0.96±0.34	0.20±0.11	0.30±0.06	0.63±0.36	0.60±0.23	0.14±0.04	0.29±0.05	0.88±0.40
		10-20	1.77±0.65	0.09±0.04	0.57±0.15	0.13±X	0.87±0.36	0.06±0.02	0.38±0.12	1.04±0.38
3A	WET	Benthic	10.51±1.84	5.78±4.36	2.90±0.06	4.37±0.88	6.57±0.64	6.42±4.70	1.88±0.10	1.46±0.29
		0-10	4.04±3.48	0.87±0.12	1.18±0.78	2.30±0.73	3.16±1.68	0.43±0.32	0.47±0.32	2.04±0.02
		10-20	2.86±1.43	0.13±0.04	0.99±X	0.48±0.09	1.39±0.69	0.10±0.03	0.38±X	3.97±2.93
	DRY	Benthic	10.51±1.84	6.47±1.21	5.73±0.73	11.01±1.35	6.57±0.64	5.09±1.17	4.93±0.52	8.77±1.08
		0-10	4.04±3.48	3.95±0.35	1.91±0.16	3.54±0.78	3.16±1.68	2.82±0.08	2.21±0.08	5.06±0.87
		10-20	2.86±1.43	1.33±0.89	0.68±0.08	0.47±X	1.39±0.69	0.92±0.40	0.79±0.11	1.85±0.44

Table 5-4. Cumulative enzyme activity slopes (CES) based on non-normalized enzyme activities. Values are unitless. Linear regressions were performed to determine the relationships over time.

			GLU	PHO	LEU	PHE	PER
TS	WET	Benthic	1.3 (r^2=0.93)	195.8 (r^2=0.99)	126.6 (r^2=0.98)	936.5 (r^2=0.45)	236.9 (r^2=0.97)
		0 -10	0.2 (r^2=0.98)	22.4 (r^2=0.98)	2.1 (r^2=0.78)	344.5 (r^2=0.32)	333.1 (r^2=0.98)
		10-20	0.4 (r^2=0.94)	6.0 (r^2=0.97)	6.0 (r^2=0.98)	843.5 (r^2=0.70)	2.1 (r^2=0.60)
	DRY	Benthic	1.25 (r^2=0.93)	45.8 (r^2=0.48)	89.6 (r^2=0.96)	528.7 (r^2=0.21)	158.8 (r^2=0.97)
		0-10	0.4 (r^2=0.92)	56.0 (r^2=0.96)	12.8 (r^2=0.94)	594.7 (r^2=0.49)	132.8 (r^2=0.94)
		10-20	0.3 (r^2=0.93)	33.0 (r^2=0.96)	13.8 (r^2=0.98)	587.7 (r^2=0.59)	79.8 (r^2=0.99)
3A	WET	Benthic	4.0 (r^2=0.99)	47.5 (r^2=0.73)	-21.8 (r^2=1)	1220.8 (r^2=0.95)	2.35 (r^2=0.96)
		0-10	1.0 (r^2=0.99)	33.1 (r^2=0.98)	-1.3 (r^2=0.97)	423.8 (r^2=0.97)	584.8 (r^2=0.99)
		10-20	0.3 (r^2=0.99)	21.9 (r^2=0.98)	-0.8 (r^2=0.99)	242.7 (r^2=0.95)	-5.6 (r^2=0.90)
	DRY	Benthic	3.5 (r^2=0.98)	69.7 (r^2=0.91)	137.7 (r^2=0.99)	229.4 (r^2=0.49)	17.2 (r^2=0.96)
		0-10	0.9 (r^2=0.97)	34.2 (r^2=0.97)	97.7 (r^2=0.98)	332.0 (r^2=0.97)	9.2 (r^2=0.15)
		10-20	0.2 (r^2=0.98)	19.2 (r^2=0.96)	33.3 (r^2=0.98)	346.5 (r^2=0.97)	10.5 (r^2=0.21)

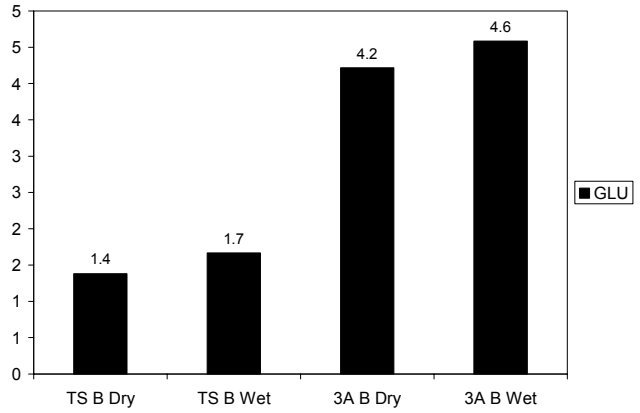
layer (Figure 5-1). This trend was also present in the 0 to -10 cm layer, with the exception of similar values among both the wet and dry ENP-TS treatments (Figure 5-2).

PHO CES values did not exhibit a clear trend between the TS and WCA-3A cores (Table 5-4) or between treatments, indicating that the rate of change was variable among cores among all layers. However, the benthic CEA showed that the wet treatments in both ENP-TS and WCA-3A resulted in a greater net accumulation of enzyme activity over time in the benthic layer (Figure 5-1). Dry treatments in both areas were similar while the WCA-3A cores were higher in the wet treatments. The relationship between ENP-TS CEA values treatments differed between the benthic and 0 to -10 cm layers (Figure 5-2). Benthic PHO CEA was higher in the wet treatment but the 0 to -10 cm PHO CEA was greater in the dry treatment. CES values decreased with depth in 3 of the four treatments.

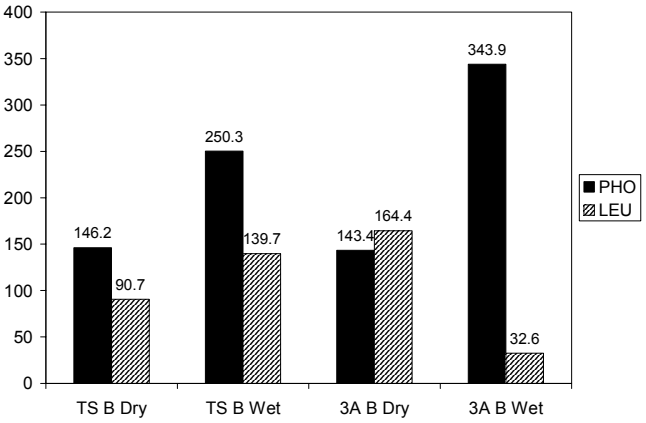
LEU CES values were variable in the benthic layer (Table 5-4) with a negative slope in the WCA-3A wet cores as a result of non-resolvable LEU activity at 4 and 12 weeks. The 0 to -10 cm and -10 to -20 cm layers exhibited larger CES values in the in the dry treatments in both sites. Benthic LEU CEA mirrored the CES values with the reduced accumulation in the WCA-3A wet treatment once again due to the non-resolvable activity at 4 and 12 weeks (Figure 5-1) as did the 0 to -10 cm layer (Figure 5-2). CEA and CES values generally decreased with depth, indicating reduced enzyme production and activity.

PHE CES values were lower in the dry treatments in the benthic layer (Table 5-4). However, in the 0 to -10 cm and -10 to -20 cm layers greater CES values were associated

A.)



B.)



C.)

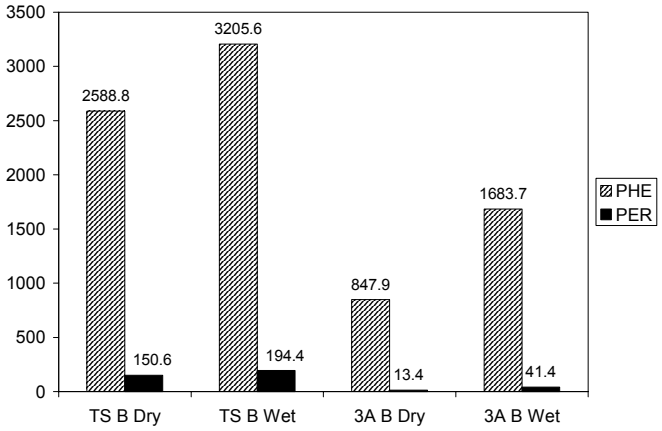
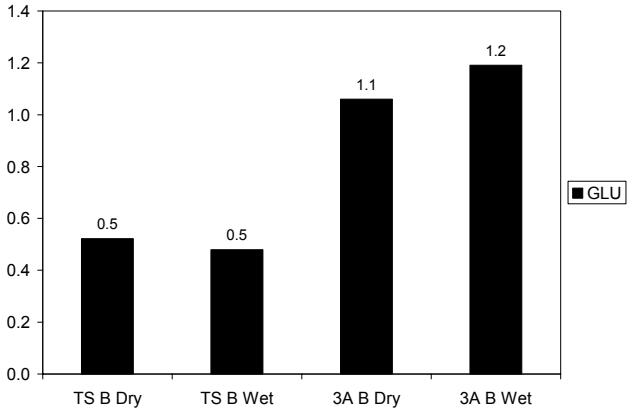
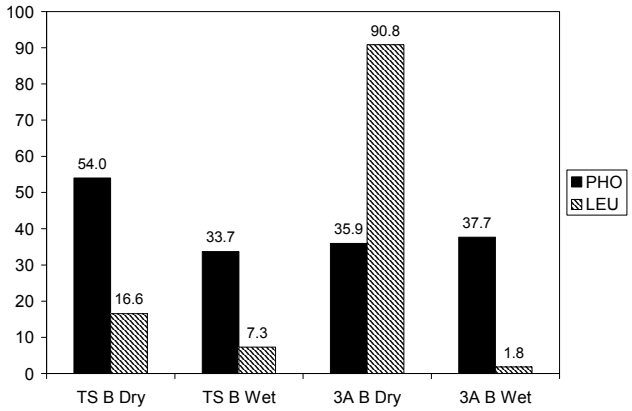


Figure 5-1. Benthic cumulative enzyme activity (CEA) expressed as the integrated area under the data points plotted against time based on mean values. A.) BGL, B.) PHO and LEU, and C.) PHE and PER. Units are cumulative enzyme activity expressed as total µmols MUF, AMC, or DICQ accumulated g^{-1} AFDM over 84 days.

A.)



B.)



C.)

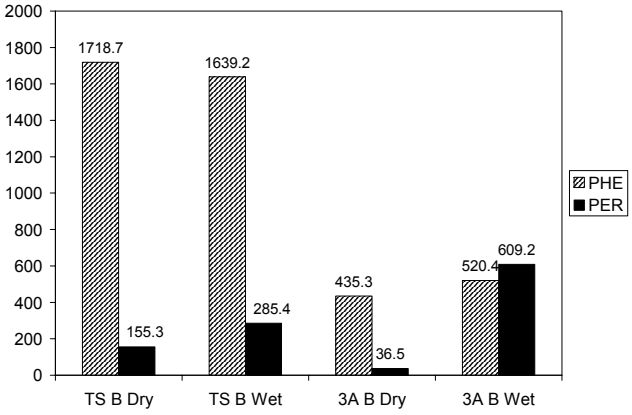


Figure 5-2. 0 to -10 cm cumulative enzyme activity (CEA) expressed as the integrated area under the data points plotted against time based on mean values. A.) BGL, B.) PHO and LEU, and C.) PHE and PER. Units are cumulative enzyme activity expressed as total µmols MUF, AMC, or DICQ accumulated g^{-1} AFDM over 84 days.

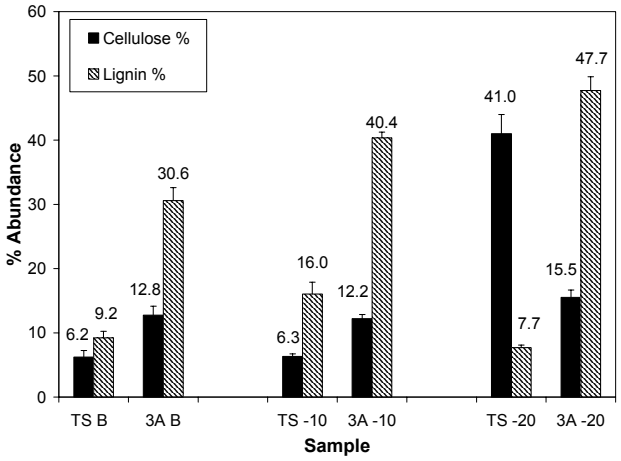
with the dry treatments. CEA values were greater in the ENP-TS cores in both the benthic and 0 to -10 cm layers (Figures 5-1 & 5-2) and mimicked the CES relationships between treatments with the exception of the WCA-3A cores in the 0 to -10 cm layer. There was not a consistent trend with depth in either the CES or CEA values.

The rate of increase of PER CES in the benthic layer was higher in the ENP-TS cores. ENP-TS wet treatments exhibited greater CES values in both the benthic and 0 to -10 cm layers (Table 5-4) which was also reflected in the CEA values (Figures 5-1 & 5-2). PER CEA values mirrored the relationships between treatments in a similar fashion to BGL, PHO, and PHE in the benthic layer. PER CES values decreased between the benthic and -10 to -20 cm layers, reflecting decreases in the rate of PER production with depth.

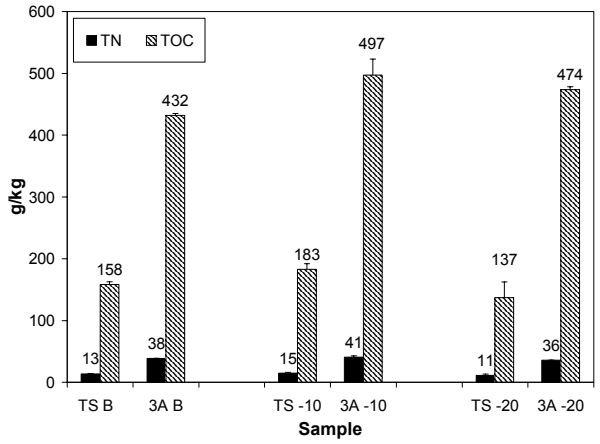
Nutrients

Site core variability generally exceeded any differences between wet/dry treatments as well as time effects. Therefore, the most consistent difference in nutrient concentrations as well as lignin and cellulose occurred in comparisons between WCA-3A and ENP-TS soil cores (Figure 5-3). Averages were calculated from a total of 15 cores per layer-site combination from 0, 2, and 12 week incubations. Average cellulose content ranged from 6.2 to 41% and was significantly greater in the WCA-3A cores, with an inverse relationship in the -10 to -20 cm layer. Cellulose content increased dramatically in the ENP-TS cores in the -10 to -20 cm layer, otherwise there was not a significant relationship with increasing depth. Average lignin content ranged from 7.7 to 47.7% with significantly higher concentrations found within the WCA-3A cores. Lignin content

A.)



B.)



C.)

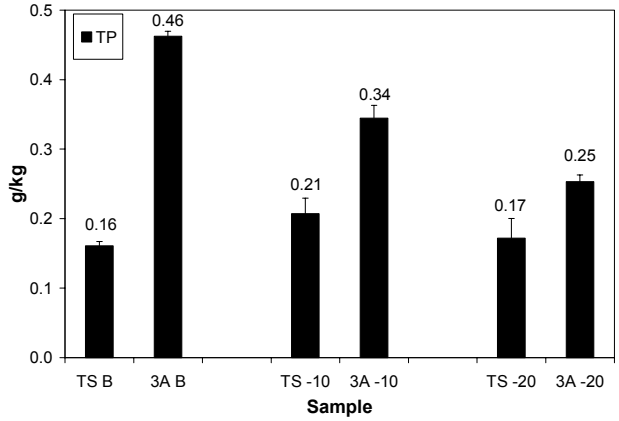


Figure 5-3. Mean lignin, cellulose, and nutrient contents of ENP-TS and WCA-3A cores benthic, 0 to -10 cm soil, and -10 to -20 soil layers. (A) cellulose and lignin content. (B) total nitrogen and total organic carbon (C) total phosphorus. Bars represent standard errors.

increased with depth in the WCA-3A cores but did not exhibit a consistent trend in the ENP-TS cores. Average total nitrogen (TN) and total organic carbon (TOC) concentrations exhibited the same type of relationship with ranges of 11 to 41 g kg^{-1} and 137 to 497 g kg^{-1}, respectively. WCA-3A cores had significantly greater TP with an overall range of 0.16 to 0.46 g kg^{-1}, and a significant decrease with depth.

Discussion

The surface layers are the most biologically active and important in terms of degradation in inundated systems. For this reason the majority of the discussion will pertain to findings related to the benthic and 0 to -10 cm layer since the water level decrease in the dry treatments involved a greater exposure of these layers to increased O_2 availability. The discussion is grouped according to the effects that lowered water levels and sediment types have on oxidative enzyme activity, N mineralization and microbially-perceived carbon quality (EICQ).

Oxidative Enzymes

The dependence of the oxidative enzymes on O_2 availability predicts that lowered water levels would result in increased activity (Pind et al., 1994). The results from this study demonstrate the apparent variability of PHE and PER activity in the field that has been previously documented as a response to depth and O_2 availability (Duxbury and Tate, 1981; Lähdesmaki and Piispanen, 1988; Pind et al., 1994; Freeman et al., 1991 & 2001; Williams et al., 2000b) with the dry cores exhibiting lower cumulative PHE and PER activities over the incubation period. This contradicts a recent study that showed a 7 fold increase in PHE activity in aerated conditions (Freeman et al., 2001) but agrees with another study (Williams et al., 2000b). The lack of a predictable increase within the

study time frame suggests that enzyme inhibition may be occurring or that an extended incubation time may be required. It has been suggested that persistent drought conditions may be required before PHE activity increases (Williams et al., 2000b), which appears to be the situation in this study.

While the oxidative enzymes do not appear to respond predictably to lowered water levels, the apparent lignin influence on C mineralization (Ecell/Eox), a function of the oxidative activities, does exhibit a treatment effect. After initial decreases at 2 weeks, the Ecell/Eox values increase at a more rapid rate in the dry treatments, especially in WCA-3A. This suggests that C mineralization in the dry treatments is less negatively influenced by lignin, which would result in more favorable potential decomposition. This is less significantly pronounced within ENP-TS, while the WCA-3A benthic layer exhibits much less lignin influence by 12 weeks.

The combination of greater C mineralization and lower oxidative enzyme activity in the WCA-3A cores results in a lower apparent lignin control on C mineralization in both the benthic and 0 to -10 cm layers. Thus, compared to the ENP-TS cores, the WCA-3A sediments appear to be more favorable for potential decomposition in terms of lignin influence and have a greater Ecell:Eox response with lower water levels. Much of this difference between areas is most probably due to differences in substrate composition (Williams et al., 2000a), phenolic concentrations (Freeman et al., 2001) and C availability.

N Mineralization

Based on other studies, N mineralization was expected to increase in simulated drought conditions (Reddy and Patrick, 1984; Cabrera 1993; Bridgham et al., 1998;

McLatchey and Reddy, 1998; White, 1999; Venterlink et al., 2002). Enhanced LEU activity was observed in the WCA-3A 0 to -10 cm layer dry treatments over the incubation period in the CES, CEA, and individual enzyme activities. However, this difference was only exhibited by the ENP-TS cores at 12 weeks. The relegation of this increase largely to the 0 to -10 cm layer is analogous to larger increases in N mineralization rates in this layer in aerobic versus methanogenic conditions (White, 1999). Therefore it appears that O_2 availability is limiting N mineralization in the WCA-3A cores with the ENP-TS cores responding slower to a decrease in water levels. The difference between the two sites may be due to the greater abundance of organic matter for C mineralization in the WCA-3A cores which would energetically lead to greater N mineralization.

EICQ

The Enzyme Index of Carbon Quality (EICQ) was expected to increase during a simulated drought as conditions became more favorable for microbial production. The largest predicted effect on the EICQ was exhibited by both layers in the WCA-3A cores where values were significantly higher in the dry treatments across the incubation period. However, the ENP-TS dry treatment in the 0 to -10 layer was only significantly higher at the 12 week collection. Thus drought duration appears to play a significant role in the cumulative effects on potential decomposition. Higher EICQ values in the dry treatments, which have been correlated to microbial biomass and productivity (Sinsabaugh and Findlay, 1995), are supported by greater C flux and soil respiration (DeBusk, 1996) as a result of drainage as well as increased microbial biomass and C flux (Blodau et al., 2004).

The time effect may be due to the slow dessication of the 0 to -10 cm layer due to the capillary draw of water from the saturated zone. Additionally, much of this layer may still contain a substantial portion of anaerobic microsites (Kettunen et al., 1998). Larger differences between the two treatments may therefore be expected over an extended incubation time. This is supported by a previous study in which increases of soil respiration rates due to soil drainage was seen to be time dependent (DeBusk, 1996). Lastly, the higher EICQ in the WCA-3A cores may be attributed to the soil composition differences between the two sites. WCA-3A is primarily an organic peat matrix while the ENP-TS profile is uniquely dominated by a robust periphyton mat in various stages of decay. Therefore, the nutritional basis for the resident heterotrophic microbial communities is distinct between the two sites.

It should be noted that the majority of enzyme activities showed a large reduction in activity between the initial collections following the field sampling event. This initial decrease in activity suggests that a core effect is occurring, possibly due to the lessening of nutrient and microbial waste exchange between the porewater and surface water due to the lack of a constantly replenished source. Additionally, the accumulation of DOC from mineralization processes may be occurring within the porewater (Blodau et al., 2004). This also suggests that a large effect of drought may occur within the initial time frame of hours or days following a treatment. The use of cumulative enzyme activities, either in the form of slopes or net activity reduces this effect in interpreting the data.

Conclusions

The ecological implications of these findings suggest that a lengthened hydroperiod with a significant drought period will result in decreased net organic matter accumulation

as a result of higher perceived C quality. These findings are based primarily on the use of the EICQ model in predicting potential decomposition rates as well as the apparent lignin influence on carbon mineralization. Increases in C quality with decreased water level appear to be especially apparent in the 0 to -10 cm layer in the organic peat dominated WCA-3A, where microbial activity is usually relatively limited due to anaerobic conditions. The increase of N mineralization in the dry treatments of the 0 to -10 cm layer in both areas points to decreased O_2 limitation. This points to overall increased microbial productivity, especially since the enzyme used in this study (LEU) to assay for N mineralization also has the capacity to function in the mineralization of C. Lastly, the higher organic matter WCA-3A soils respond greater to drought conditions with significantly higher N mineralization and EICQ in the dry treatments which was expected due to a higher organic matter content.

The results from enzyme studies are usually presented solely in terms of activity per period or treatment. The use of cumulative enzyme activities, expressed either as slopes representing the rate of change over time or as net cumulative activities conveys information regarding trends in terms of time series studies. These cumulative activities are usually expressed in relation to litter mass loss rates in decomposition studies. However, this study demonstrates the validity of using these comparisons, especially when significant differences between treatments are relegated to later time periods.

Due to the constraints of this study it is impossible to accurately model the effects of a prolonged dry-down. However, many of the differences between wet and dry treatments were becoming increasingly contrasting with time. The current trend suggests that a longer incubation period would result in larger EICQ and N mineralization

differences between the treatments as saturated pores empty. Conversely, the large differences between initial conditions and the 2 week cores suggest that significant changes occur within the cores as a result of containment. Therefore a more stringent series of assays on a time frame of hours or days are needed in order to further understand these microbial shifts as a function of laboratory containment. This has potential implications on the validity of numerous soil microcosm experiments where prolonged incubation times are involved.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Field and laboratory studies were conducted to examine the influences of nutrient loading, vegetative habitat, and simulated drought conditions on microbial enzyme activities in the Everglades. A summary of results as they relate to the study objectives is presented below.

1) Develop an appropriate experimental method for performing enzyme assays in wetland systems.

The investigation of the effects of varying substrate concentrations on measured enzyme activity was deemed important in developing assays that are accurate in estimating in situ activities. The inclusion of kinetic activity readings over time was found to be significant in determining the validity of measured enzyme activities and provided a method of discarding outlying values. The importance of incubation time was found to vary among substrate concentrations with a 45 minute incubation time deemed adequate in this study. Optimum substrate concentrations were determined to be 60 μM, 50 μM, 100 μM, and 300μM for cellobiohydrolase, phosphatase, glucosidase, and leucine aminopeptidase, respectively. These substrate concentrations exhibited a linear relationship over time in both enriched and reference P sediments and were utilized for further experimentation.

2) Determine the effects of vegetative habitats on microbial enzyme activities in Water Conservation Area 3A of the Everglades.

The apparent lignin influence on organic C mineralization (Ecell/Eox) appeared to be the driving force behind changes in microbial enzyme activities in the benthic layer among different vegetative habitats. The significantly higher Ecell/Eox values in the open water habitats, coupled with higher Enzyme Index of Carbon Quality (EICQ) measures indicate that this habitat is more suited to potential decomposition. Lower C:N ratios of the benthic matter associated with the open water habitats also predict greater potential decomposition. When coupled to the lower C input in the open water habitats, it is suggested that the increases in potential decomposition would result in a lower elevation over time that supports the current Everglades topography.

3) Determine the relationships between nutrient conditions and microbial enzyme activities among four hydrologic units of the Everglades.

Phosphorus was less limiting to C mineralization, expressed as Ecell/Ep values, at the enriched sites with the smallest apparent limitation within Water Conservation Area 3A. Conversely, the largest apparent P limitation occurred within Everglades National Park (ENP-TS). Shifts in apparent N limitation on C mineralization, expressed as Ecell/En, occurred between the enriched and reference sites in Loxahatchee National Wildlife Refuge (LNWR), Water Conservation Area 2A, Water Conservation Area 3A, and Taylor Slough within Everglades National Park. A trend of increasing Ecell/En values at the enriched sites in a southerly direction suggests that a decreasing N limitation

is occurring that is not reflected in soil nutrient conditions at these sites. A significantly larger Ecell/En difference between the enriched and reference sites within ENP-TS coupled with the lowest Ecell/Ep shift suggests that this area is unique in regards to microbial enzyme activities. The combination of decreased apparent N and P limitation on C mineralization results in greater potential decomposition at the enriched sites.

An enzyme decomposition model was constructed using tensile strength loss of cotton strips as a basis for cellulose decomposition. An exponential model utilizing Ecell/Ep and total P (TP) accounted for between 46% and 92% of the variability in cotton rottenness rates (CRR), reflecting the large influence of P availability on the microbial community within the Everglades ecosystem.

4) Determine the validity of different enzyme models in predicting potential decomposition among different litter qualities and in varying nutrient conditions.

The use of the EICQ, a relative measure of hydrolytic to oxidative enzyme activities, appears to most valid when comparing changes in vegetative types or changes in O_2 availability. This is due to the weight that the EICQ model places on the oxidative enzymes involved in lignin degradation. However, this model was found to be inconsistent when predicting potential decomposition along nutrient gradients in the Everglades. Rather, the model parameters Ecell/Ep and Ecell/En, which relate the apparent P and N influence on C mineralization respectively, predict cellulose decomposition rates, accounting for between 46% and 92% of the variability. The most appropriate model, which varies among the different comparisons in these studies,

suggests the need for the investigation of the underlying concepts and comparisons used for model development. Additionally, variations in the validity of the EICQ model were exhibited when comparing sites according to habitat or nutrient differences. These studies suggest that the use of this model appears to be restricted to comparisons between differing vegetative types where substrate structure and lignin content influence oxidative enzyme activities.

5.) Determine the effect of a simulated drought on enzyme activities in WCA-3A and ENP-TS cores.

The results from this study suggest that prolonged drought periods will result in increased C mineralization in the otherwise inundated surficial soil layers, especially in peat sediments. Elevated EICQ values, increases in individual enzyme activities, and decreased oxidative activities all suggest a greater microbial perceived carbon quality under dry conditions. Most of these changes between treatments occurred in the 0 to -10 cm layer. The primarily peat sediments of WCA-3A, compared to the marl sediments of ENP-TS, exhibited larger changes in the dry treatments. Relatively larger differences with time between the wet and dry treatments suggest that a longer incubation period may result in even greater changes in potential decomposition.

The microbial component of the ecosystem serves as the basis for the trophic food chain and is temporally the most responsive to alterations in environmental conditions. Changes in the microbial component are manifested in higher order trophic processes. As such, this study provides valuable data concerning the effects of vegetative habitats, nutrient loading and water table drawdown on microbial enzyme activities that are

responsible for nitrogen and phosphorus cycling, carbon mineralization and are the rate limiting steps in organic matter decomposition. The results from this study may be utilized to better understand the dynamics of the Everglades ecosystem, especially in terms of the Comprehensive Everglades Restoration Plan (CERP).

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BIOGRAPHICAL SKETCH

Christopher Ryan Penton was born in the deep fried southern town of Tuscaloosa, AL. After moving to Texas, Georgia and South Carolina he finally came to rest in the quaint, one redlight town of Lecanto, FL. His inquisitive mind, always taking toys apart, blowing up everything with his chemistry set, and asking his parents endless questions eventually propelled him into a B.S. degree in microbiology. He then went to work as a high school chemistry teacher where he continued with the fascination of watching things explode (much to the delight of his students) and then went on to work in the Everglades with the South Florida Water Management District. He has one brother Garrett, who attends the University of Florida on a track scholarship and two sisters, an Australian shepherd and a black lab. His wife Stephanie, who ardently supported him through the past several years, has also recently completed her education, culminating in an M.D. degree. He and his wife currently reside in Michigan, where the snow is as abundant as the Florida sun.