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Soil Carbon Dioxide and Methane Efflux From an Everglades Tree Island and Ridge Landscape

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FLORIDA INTERNATIONAL UNIVERSITY
Miami, Florida

SOIL CARBON DIOXIDE AND METHANE EFFLUX FROM AN EVERGLADES TREE ISLAND AND RIDGE LANDSCAPE

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

Robert Scott Schroeder

2012
To: Dean Kenneth G. Furton  
College of Arts and Sciences

This thesis, written by Robert Scott Schroeder, and entitled Soil Carbon Dioxide and Methane Efflux from Everglades Tree Island and Ridge Landscape, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Leonard J. Scinto, Major Professor

Date of Defense: November 2, 2012

The thesis of Robert Scott Schroeder is approved.

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Florida International University, 2012
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ABSTRACT OF THE THESIS

SOIL CARBON DIOXIDE AND METHANE EFFLUX FROM EVERGLADES TREE ISLAND AND RIDGE LANDSCAPE

by

Robert Scott Schroeder

Florida International University, 2012

Miami, Florida

Professor Leonard J. Scinto

The influence water levels have on CO₂ and CH₄ efflux were investigated at the Loxahatchee Impoundment Landscape Assessment (LILA) research facility, located in Boynton Beach, FL, USA. Measurements of CO₂ efflux were taken for 24 h periods four times for one year from study plots. Laboratory incubations of intact soil cores were sampled for CO₂, CH₄, and redox potential. Additionally, soil cores from wet and dry condition were incubated for determination of enzyme activity and macronutrient limitation on decomposition of organic matter from study soils. Water levels had a significant negative influence on CO₂ efflux and redox, but did not significantly influence CH₄ efflux. Study plots were significantly different in CH₄ efflux and redox potential. Labile carbon was more limiting to potential CO₂ and CH₄ production than phosphorus, with the effect significantly greater from dry conditions soils. Enzyme activity results were variable with greater macronutrient responses from dry condition soils.
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I. INTRODUCTION

A century of anthropogenic hydrologic alteration of the Everglades, to alleviate the economic and human losses due to flooding, has resulted in the construction of 2500 km of canals and levees (Light and Dineen, 1994; Sklar et al., 2001; Childers et al., 2003). Before anthropogenic alteration, the Everglades had developed a patterned landscape of ridges, sloughs, and tree islands where landscape features were oriented parallel to the direction of flow (Wu et al., 2006; Bernhardt and Willard, 2009). These patterns are characteristic of long-term environmental stability (Larsen et al., 2011) and formed under very wet conditions (Ogden, 2005). Alteration of water levels and timing of seasonal water delivery to the Everglades has caused the degradation of the distinct heterogeneous peat-based sawgrass ridge, slough and tree island landscape (Wu et al., 2006; Larsen et al., 2011). In 2000, nearly US$12 billion was designated for the Comprehensive Everglades Restoration Plan (CERP), which has a goal of “getting the water right” in quantity, quality, timing and distribution (Towery and Regalado, 2009).

Since hydrologic alteration of the Everglades, tree island total area has declined by ~67% and the ridge and slough landscape has degraded (Ogden, 2005; van der Valk et al., 2007; Wetzel et al., 2009; Larsen et al., 2011). Tree islands form a unique habitat within the Everglades and provide a refuge for flora and fauna that could not normally survive in the Everglades ridge and slough landscape. Because of the many uncertainties associated with tree island formation, and their abiotic and biotic processes, tree islands have not been included as a performance measure in Everglades’ restoration (Wetzel et al., 2009). Hydrologic alteration of the Everglades carbon-rich peat soils into urban and agricultural lands has caused large changes in mineralization of the soil carbon (C)
(DeBusk and Reddy, 2003). Soil aeration increases rates of microbial decomposition and subsequently changes the dynamics of nutrient cycling (Melling et al., 2005).

The Everglades Depth Estimation Network (EDEN) is a network of stage (water level) gauging equipment to monitor water levels in real time (Telis et al., 2006). The network can be used to adequately manage water for ecosystem stability, including that of tree islands. Water levels have been shown by many to greatly influence C efflux from wetland soils (e.g., DeBusk and Reddy, 2003; Hirano et al., 2009). Development of a relative water depth (RWD) driven soil C-efflux model would provide great insight into the water levels needed to maintain, or expand, tree island soils. Without the inclusion of tree island restoration in CERP, complete restoration of functionality in the Everglades may not occur (Wetzel et al., 2009).

Water leaving the Everglades Agricultural Area (EAA) before entering the Everglades contains elevated levels of nutrients from fertilizers (Childers et al., 2003; DeBusk and Reddy, 2003; Larsen et al., 2011). Historically, the Everglades was oligotrophic (nutrient poor) (Wright and Reddy, 2001; Childers et al., 2003; DeBusk and Reddy, 2003; Larsen et al., 2011), because phosphorus was limiting. The native plants and animals evolved strategies to overcome the environmental stress (Childers et al., 2003; Larsen et al., 2011), however, with the increased loading of phosphorus from agriculture, total phosphorus concentrations of the soil have risen, especially proximal to canal discharges (Reddy et al., 1993; Childers et al., 2003). These increases in phosphorus can facilitate changes in microbial community activity that control C cycling (DeBusk and Reddy, 1998).
The spatial coverage of the Everglades declined by 55% and that which remains has degraded as a result of flood mitigation and land-use change (Larsen et al., 2011). Efforts need to restore the Everglades under CERP are likely to be compounded by climate change (Schedlbauer et al., 2010). Peatlands and wetlands are also of critical concern for predicting global climate change because gaps in our knowledge about the rates of emission and sequestration of greenhouse gases (GHG) from these ecosystems. Carbon emissions from peatlands and wetlands are dominated by carbon dioxide (CO\(_2\)) and methane (CH\(_4\)) under aerobic and anaerobic conditions, respectively (Limpens et al., 2008). Peatlands cover about 3% of the Earth’s surface, but store between 270-370 Tg C (15-25% of terrestrial C; 1 Tg=10\(^{12}\) g C) (Turunen et al., 2002; Limpens et al., 2008), and accounting for 34-36% of the 796 Tg C stored in the atmosphere as CO\(_2\) (IPCC, 2007). Tropical and sub-tropical peatlands are estimated to store 52 Tg C of the total for peatlands (Hooijer et al., 2006), and account for 20% of the total peatland area (Limpens et al., 2002). Anthropogenic alterations of the water table and flooding and drainage patterns are some of the drivers of recent net C loss from wetlands and peatlands worldwide resulting from oxygen exposure increasing respiration rates (Furukawa et al., 2005).

Wetland and peatland inclusion into global climate models is hampered by the insufficient quantification of hydrologically-driven fluctuations in C release, which is a localized factor and would require a global universal correction factor (Limpens et al., 2002). Climate-carbon feedback models from peatlands and wetlands are inconsistent, as a result of the lack of reliable information linking carbon exchange of peatlands to climate, hydrology, ecosystem structure and function (Limpens et al., 2002), but are
consistent with respect to the positive feedback that peatlands and wetlands will have to climate (Sitch et al., 2008). Measurement of C efflux correlated to hydrology (e.g., DeBusk and Reddy, 2003; Hirano et al., 2009) can aid ecosystem management and restoration (Bridgham et al., 2006), and create a current environmental baseline for C efflux comparisons as climate change alters the processes and drivers of C efflux.

Soil CO$_2$ and CH$_4$ efflux is one large piece of the broader global carbon (C) cycle (Melling et al., 2005). Soil respiration consists of plant and microbial decomposition of carbon containing materials with CO$_2$ and CH$_4$ as gaseous end products (Raich and Schlesinger, 1992; Ryan and Law, 2005). Several biotic and abiotic factors influence rates of soil C (CO$_2$ + CH$_4$) efflux. Quality of organic matter inputs (Jauhiainen et al., 2005), types of microbes involved in decomposition (Bowling et al., 2002) and soil macroorganism presence (Bowling et al., 2002) are all examples of biotic controls of respiration, while water table level (Davidson et al., 2000; Furukawa, 2005), temperature (Davidson et al., 2000; Bahn et al., 2008), and nutrient availability (DeBusk and Reddy, 1998) are abiotic controls. Soil C efflux measurements quantify the rate of gas exchange between the soil surface and atmosphere per area per time. Further research is needed on the current carbon fluxes of US wetlands at the landscape level to determine soil C source/sink status, which can aid ecosystem restoration and management decisions (Bridgham et al., 2006). From the Everglades ecosystem, soil vial and intact core incubations for CO$_2$ and CH$_4$ efflux (e.g., Amador and Jones, 1993; Amador and Jones, 1995; DeBusk and Reddy, 2003), and ecosystem level CO$_2$ exchange (e.g., Schedlbauer et al., 2010; 2012) have been reported. Clark et al. (2009) have begun *in situ* CO$_2$ efflux measurements from Everglades ridges and sloughs. Quantification of *in situ* CO$_2$ and
CH₄ fluxes allow estimation of total annual C efflux rates (Jauhiainen et al., 2005). Similarly applied to the Everglades’ tree island and ridge landscape, soil C efflux can be incorporated into an integrated C budget estimation to help achieve CERP’s “getting the water right” goal.

II. BACKGROUND

2.1 Methods of Efflux Measurement

Considerable experimentation has been conducted around the world. This provides a rich database of information for comparison to results of this study. Numerous methods and techniques have been used to assess gaseous efflux from soil. Soil CO₂ efflux has been measured by trapping CO₂ efflux in an alkaline solution. The soil is commonly incubated in a closed chamber and the CO₂ is trapped in potassium hydroxide (KOH) or sodium hydroxide (NaOH) resulting in carbonate salts (e.g., Na₂CO₃). The solution is then titrated with hydrochloric acid (HCl) and the amount of CO₂ absorbed can be calculated from the difference between sealed and exposed beakers of alkaline solutions (King and Harrison, 2002).

A second method involves manual chambers where the chamber is moved to each location and the head-space gases are sampled over the course of an incubation interval. Gaseous efflux (e.g., CO₂ and/or CH₄) from these chambers is determined by measuring the head-space concentration change over time (multiple gas samples). Incubation times depend on chamber volume and soil porosity generating head-space gas concentration differences great enough to observe a measureable change in concentration. However, if incubation times are too great, the microclimate and efflux rates may be altered due to increased temperatures inside the chamber, high gas concentrations within the chamber.
limiting diffusion out of soil, and changes in pressure within the chamber affecting
diffusion rates.

To overcome limitations with manual chambers, automated systems have been
developed where chambers are fitted to an analyzer unites, typically an infrared gas
analyzer or IRGA (Savage and Davidson, 2003). Because of technological limitations of
CH₄ measurement, these systems are currently used only for CO₂ efflux measurement.
Associated with the analyzer unit are one to several vented chambers, each with a
different collar inserted into the soil, with a power supply capable of long-term
deployment (Savage and Davidson, 2003). Deployment of an automated system reduces
the human error associated with syringe sampling and can reduce incubation times
because of greater temporal resolution. Non-steady-state chamber systems pump air from
the chamber into the analyzer and back to the chamber (Fang and Moncrieff, 1996;
Savage and Davisdon, 2003). Automated non-steady-state systems are now incorporating
dynamic chambers that close for measurements and open afterwards so as to reduce
alteration of the microclimate within the chamber during long-term deployments (Savage
and Davidson, 2003).

Automated non-steady-state systems allow much greater temporal frequency of
measurements over manual systems, but they are spatially limited and expensive
(Goulden and Crill, 1997; King and Harrison, 2002; Savage and Davidson, 2003).
Manual chambers require the physical presence of a person for measuring and
transporting the analyzer to additional collars. Manual chambers may miss diurnal and
other short-term respiration responses to climatic variations, but they do provide much
greater spatial coverage over automated systems. Savage and Davidson (2003) found
that automated systems measured greater flux rates, ranging from 2-30% with a mean of 13% higher than manual chambers. Over a 58-day study period with weekly manual measurements, interpolated and summed efflux was 0.26 kg C m\(^{-2}\) for 58 days with the manual chamber while the automated system summed was 0.27 kg C m\(^{-2}\) for the same time (approximately 4% relative difference). Automated dynamic systems provide a more reliable estimate of flux, prevent microclimate modification, and overcome large portions of the variability associated with other systems of flux measurement (King and Harrison, 2002). Moreover, automated systems capture more of the temporal variability than manual measurements.

2.2 Soil Carbon Efflux

The major factors controlling carbon-release in peatlands and wetlands are organic-matter quality and hydrological conditions (Jauhiainen et al., 2005) that greatly influence nutrient availability (DeBusk and Reddy, 1998), oxidation-reduction potential (Thomas et al., 2009), and microbial community composition and activity (Bowling et al., 2002). *In situ* CO\(_2\) efflux measurements are often the combination of heterotrophic and autotrophic respiration, which varies with plant species and other phenological factors (Tang et al., 2003). Understanding the main drivers of CO\(_2\) and CH\(_4\) efflux from Everglades tree islands and ridges will aid ecosystem management. Tropical peat carbon flux measurements, until now, are rare (Jauhiainen et al., 2005), although there are currently active programs through Indonesia, South East Asia and South America to quantify the CO\(_2\) and CH\(_4\) fluxes. On the basis of the analysis of isotopic ratios of gaseous CO\(_2\) efflux, Bowling et al. (2002) found 75% of annual ecosystem respiration is
attributed to root and microbial respiration in soil, and about 20% occurs from foliage respiration in a temperate rain forest.

2.3 Environmental Controls

2.3.1 Organic Matter Quality

Organic matter (OM) contains a high percentage of C, and is used to provide energy to various microorganisms through respiration. Quality of litter falling onto the soil surface can play a major role in respiration rates (DeBusk and Reddy, 1998; Qualls and Richardson, 2000; Wright and Reddy, 2001). High detrital inputs can increase soil organic matter content or total carbon. Increased annual soil respiration has been found to coincide with higher soil carbon content (Bahn et al., 2008). Amador and Jones (1995) found that acetate, glucose and cellulose additions to Everglades’ soil enhanced carbon respiration (both CO₂ and CH₄) relative to sawgrass additions, which indicated that the low quality of organic matter constituting Everglades’ ridges might inhibit respiration. Nutrient availability of the litter can limit decomposition rates due to the limited microbial growth rates (DeBusk and Reddy, 2003).

2.3.2 Nutrient Availability

Macronutrients C, N, and P are required in a 106:16:1 molar ratio, respectfully, according to the Redfield ratio and can influence rates of CO₂ and CH₄ efflux (Mitsch and Gosselink, 2007). DeBusk and Reddy (1998) found that CO₂ production from litter was positively correlated with initial litter total phosphorus (TP) concentrations. Runoff from agricultural and urban areas contains P from excess fertilizer, which is increasing the availability of P in the Everglades (Davis, 1991; Qualls and Richardson, 2000; Childers et al., 2003). Nutrient enrichment is causing a shift in the Everglades plant
species communities (Davis, 1991; Childers et al., 2003; DeBusk and Reddy, 2003) and microbial biomass (DeBusk and Reddy, 1998). Increased phosphorous concentrations have been shown to increase microbial respiration in southern Everglades’ soil while nitrogen enrichment did not stimulate decomposition in low phosphorus soils (Amador and Jones, 1993). Phosphorus enrichment was also shown to significantly amplify the effects of water level. Methanogenic respiration in vial incubation experiments has been shown in low TP (C:P ratio of 2,052:1) and high TP (C:P ratio of 236:1) soils to lag four and two days respectively behind incubation initiation (Amador and Jones, 1995). The longer lag times of low TP soils indicated that conversion of organic C to CH₄ by microorganisms is limited by P. Additionally after substrate (various C compounds) and P amendments, P is considered a co-limiter of respiration with labile C (Amador and Jones, 1995).

However, P addition did not always increase CO₂ production in all of the incubation studies, presumably because of the variety of soil types in the Everglades, marl to organic peat (Amador and Jones, 1993; Amador and Jones, 1995; Drake et al., 1996). Tree island TP concentrations reach 1,500 to 3,000 µg g⁻¹ while pristine marsh concentrations range ≤200 to 500 µg g⁻¹ (Wetzel et al., 2009) where apatite P dominates tree island (Irick, 2012) and organic P dominates marsh P pools (Wetzel et al., 2009). Both of these forms are considered unavailable forms of P, and the P will only be released upon weathering and oxidation, indicating that P may still be a limiting nutrient on tree islands similar to marshes. Centers of tree islands are exposed to oxygen longer than any other ecotone in the Everglades allowing for litter and apatite P to become available as a result of the little or no flooding on a yearly basis.
2.3.3 Hydrology

Hydrology is a critical abiotic factor driving the carbon balance of both tropical and temperate peatlands (Blodau and Moore, 2003; Hirano et al., 2009). High water levels (peat flooding) bring anoxic conditions which reduce rates of respiration. Humid tropical rain forests and peatlands are among the most efficient at carbon sequestration (Sitch et al., 2003; Hirano et al., 2009) as a result of their year-round high soil water content. In tropical peatlands, soil moisture has a greater impact on soil respiration than soil temperature (Melling et al., 2005). Furukawa et al. (2005) showed on Sumatra Island, Indonesia that when the water table is lowered 10 cm below soil surface, CO$_2$ emission is 50% greater than when the water table was at soil surface. Kim and Verma (1992) found in Minnesota peatlands that 81% of soil CO$_2$ efflux was attributed to water table depth in hummocks and hollows. It has been proposed that Everglades tree islands and ridges are maintained by water levels influencing the relative rates of decomposition and production (Fig 1; Larsen et al., 2011).

The hydroperiod, or the periodicity and duration of drying and wetting, in peatlands and wetlands can have a major role in gas production and release (Ueda et al., 2000, Inubushi et al., 2003). Yearly, the Everglades experiences wet and dry periods based on seasonal precipitation inputs (Perry, 2004). Under prolonged flooding conditions, CO$_2$ production diminishes and CH$_4$ production increases as a result of changes in oxic status (Jauhiainen et al., 2005). Amazonian and Everglades soils have shown inhibited efflux rates under near saturated and saturated soil water content (Davidson et al., 2000; DeBusk and Reddy, 2003, respectively). However, Xu et al. (2004) found during and after a rain event, CO$_2$ concentrations near the soil surface
increased while those of deep soil decreased due to water filling pore space in a drier oak/grass savanna ecosystem. Dry soil conditions can also inhibit respiration in response to low microbial activity and reduced root respiration (Norman et al., 1992; Liu et al., 2002, Bahn et al., 2008). Low pore water levels limit microbial movement and cause community dormancy. Bahn et al. (2008) noticed a time lag after rain storms before CO₂ efflux increased and noted efflux rates dropped after soil moisture fell below 10%. Liu et al. (2002) showed in soil core incubations that increasing amounts of added water (simulating precipitation) had increasing effects on CO₂ efflux. Xu et al. (2004) found that the amount of carbon lost due to respiration after a rain event was proportional to the amount of rain that fell in an oak/grass savanna of California. The authors also found that sites with a greater soil carbon content and primary productivity lost more carbon after rainfall events, which was attributed to greater labile C pools.

Hirano et al. (2009) used a system of manual (syringe sampling) and automated chambers (IRGA sampling) to measure CO₂+CH₄ and CO₂ efflux, respectively. Samples were taken from several different land use/impact stages of an Indonesian tropical peat swamp forest in two elevational ecotones. In the forests studied, when water levels rose to -0.2 m below the soil surface, CO₂ efflux rates began to decrease. Overall, CO₂ flux was strongly influenced by groundwater level. Methane production from these soils was also found to be small (~1.5% of CO₂ equivalent emissions). Efflux (CO₂) ranged 3-8 µmol m⁻² s⁻¹ from high elevations and 2-6 µmol m⁻² s⁻¹ from low elevations when water levels were at or below soil surface. Annual CO₂ efflux from these soils ranged 640 to 764 g C m⁻² y⁻¹ from low elevation plots, 975 to to 1036 g C m⁻² y⁻¹ from combined high and low elevations, and had a mean of 1309 g C m⁻² y⁻¹ from high elevations, while total
CH$_4$ efflux ranged 1.06 to 1.30 g C m$^{-2}$ y$^{-1}$ from low elevations (not measured at high elevations).

Because roots can contribute substantially to \textit{in situ}-measured CO$_2$ efflux, few studies have successfully estimated the separate contributions of autotrophic and heterotrophic respiration \textit{in situ} (Tang et al., 2003). Root maintenance respiration has been hypothesized to decline with soil moisture stress (Burton et al., 1998). Tree island and ridge vegetation is determined by flood tolerance, and the root contribution to soil CO$_2$ efflux may vary similarly. Many factors influence \textit{in situ} soil CO$_2$ and CH$_4$ efflux measurements. Controlling environmental factors (e.g., removal of root respiration) in a laboratory setting may provide better insight into the role a single factor (e.g., RWD) has on efflux. Intact soil cores can provide a means to understanding the microbial respiration contribution of CO$_2$ and CH$_4$ efflux to total soil C efflux (Fang and Moncrieff, 2001). Equalization times are required before efflux measurements to minimize the influence of coring disturbance (Fang and Moncrieff, 2001; DeBusk and Reddy, 2003).

DeBusk and Reddy (2003) conducted an Everglades’ intact soil core incubation and found CO$_2$ respiration to vary significantly with water levels. Methane efflux from the same cores was also found to be lower at soil saturated conditions (0 cm water level) than for flooded (+ water levels) and drained conditions (- water levels). Methane flux had no consistent trend with water depth, and there was no significant difference between flooded and drained CH$_4$ flux rates. Overall, CO$_2$ flux from these soils accounted for 90 to 99% of total C efflux from Everglades’ soils due to CH$_4$ efflux being one-to-two orders of magnitude smaller than CO$_2$ efflux. Maximum rates of total C efflux were found at the lowest water levels (-15 cm). Frequent drying and wetting cycles have been
proposed to limit methanogenic conditions necessary for methane production in wetlands (Knorr and Blodau, 2009). Investigations into how hydrology influences oxidation-reduction with CO₂ and CH₄ can ultimately provide understanding in C storage (Thomas et al., 2009).

2.3.4 Oxidation-Reduction

Oxidation-reduction (redox) potential of wetland and peatland soils is important as an indicator of functions and processes occurring on multiple scales (Thomas et al., 2009). Saturation, or flooding, of soils limits the availability of oxygen (DeBusk and Reddy, 2003) because oxygen diffuses slower through water than through air, estimated at 10,000 times slower (Mitsch and Gosselink, 2007). Understanding redox potential aids understanding of C storage (Thomas et al., 2009). Soils with freely dissolved oxygen have redox potentials between +400 and +700 millivolts (mV) and are considered aerobic. After oxygen is consumed, redox ranges between +400 and -400 mV (Mitsch and Gosselink, 2007) and the soils are considered reduced or anaerobic. Methanogenesis, or the production of CH₄, only occurs at redox potentials below -200 mV (Mitsch and Gosselink, 2007). Redox potentials increase either linearly or exponentially with exposure to oxygen depending on marsh type and nutrient availability (Thomas et al., 2009).

Redox potential in Everglades’ soils from WCA2A have been shown to stabilize at about -200 mV at soil depths of 2 to 10 cm when flooded (Thomas et al., 2009). At unimpacted soil depths of 20 cm, redox potential was lower than -200 mV, indicating that Everglades’ soil reached the methanogenic redox range (Thomas et al., 2009). Moderately impacted soils from the Everglades have higher mean redox potentials (-134
mV) than highly impacted and reference soils (-185 mV) (Thomas et al., 2009). Within
the moderately impacted soils, root production was higher and decomposition slower,
which may explain the higher redox potentials (Thomas et al., 2009). Similarly, Qualls et
al. (2001) found no significant difference between P impacted (cattail dominant) and P
unimpacted (sawgrass dominant) redox potentials at 12.5 cm soil depth. Redox potential
is strongly influenced by microbial activity as determined by nutrient and substrate
quality (de Mars and Wassen, 1999).

2.3.5 Microbial Activity

Soil temperature, gross primary productivity, soil moisture and hydroperiod
(Penton and Newman, 2008; Vargas et al., 2010), litter quality and enzyme activities
(Penton and Newman, 2008), and soil management (Knight and Dick, 2004) influence
soil microbial activity. Microbial contribution to total ecosystem CO₂ efflux changes
with vegetation type and different substrates used for energy by the microbial community
(Law et al., 2001; Bowling et al., 2002). Enzyme diversity in soil strongly influences the
biological processes occurring in the soil, such as organic matter degradation and nutrient
cycling (Marx et al., 2001). Soil enzymes are good indicators for biological functional
diversity and quality in response to disturbance (Marx et al., 2001) and have been
proposed as a soil-quality indicator (Knight and Dick, 2004; Zhang et al., 2011). The use
of extracellular enzyme activity (EEA) assay analysis can provide insight into the
microbial nutrient requirements (Corstanje et al., 2006). Once enzymes are released by
microbes, EEA is governed by environmental controls and may persist in predictable
patterns at the community level (Sinsabaugh et al., 1997) such as ridge, slough, or tree
island communities. Feedback systems determine the metabolism and production of
extracellular enzymes (Sinsabaugh et al., 1997). Nutrient-limited environments, such as the Everglades, are dependent upon microbial decomposition of organic matter to release nutrients back into the system (Rejmánková and Sirová, 2007). The quality of decomposing organic matter determines release of nutrients by extracellular enzymes (Rejmánková and Sirová, 2007).

Abiotic enzymes are those enzymes of biological origin no longer associated with living cells (Skujins, 1976). A significant fraction of soil enzyme activity originates from abiotic enzymes sorbed to clays or humic colloids (Knight and Dick, 2004). Substrate utilization by bacteria is governed by extracellular enzymes (Sinsabaugh et al., 1997), and enzyme-catalyzed reactions in organic matter degradation are considered the rate-limiting step (Penton and Newman, 2008). Penton and Newman (2008) proposed that higher EEA and subsequent OM respiration may contribute to elevation differentiation of the Everglades ridge and slough landscape. They showed EEA from ridges (high C:N ratio) had lower activity than that in sloughs (low C:N ratio). Ridges have shorter hydroperiods than sloughs, and ridge vegetation litter has been shown to limit respiration (Amador and Jones, 1995). Similarly, tree islands are higher (drier) than ridges and may have lower enzyme activity associated with litter quality and nutrient availability.
III. OBJECTIVES AND HYPOTHESES

3.1 Objectives

Everglades tree island and ridge soil C efflux depends on the effects of water levels. Organic matter deposition and decomposition are dynamically interlinked with water levels on Everglades’ tree islands and ridges (Fig 1; Larsen et al., 2011). Soil CO$_2$ and CH$_4$ efflux research from tree islands and ridges can provide targets for “getting the water right” by indicating critical water levels for maintaining, or enhancing, the current extent of tree islands in the Everglades. This study evaluated whether water levels are a main driver of C efflux in the Everglades, and provide empirical evidence for rates of C efflux at varying water levels for use as a baseline in management decisions. The specific goals of this research were to estimate annual C efflux from Everglades’ tree islands and ridges, determine the significance of Everglades’ soils as sources of CO$_2$ and CH$_4$, determine nutrient or OM quality limitation of respiration seasonally, and to determine extracellular enzyme activity differences between tree islands and ridges seasonally.

Hypothesis I: Soil CO$_2$ respiration on tree islands and ridges varies with water level. Soil respiration is significantly influenced by hydrologic conditions, i.e., drying and wetting cycles (Blodau and Moore, 2003; Jauhiainen et al., 2005; Hirano et al., 2009). The anthropogenically altered subtropical peatland of the Florida Everglades has a temporal pattern of drying and wetting cycles (Stofella et al., 2010) which can greatly influence CO$_2$ efflux due to the availability of oxygen for respiration. Soil CO$_2$ efflux is expected to be negatively correlated to water levels (stage).
**Hypothesis II:** Soil OM decomposition by methanogenic pathways varies with water level.

As freely dissolved/available oxygen disappears, decomposition of OM requires the use of other electron acceptors, such as oxidized forms of nitrogen (e.g., NO$_3^-$, NO$_2^-$) and iron (Fe$^{3+}$), eventually reaching CO$_2$ as the terminal electron acceptor and producing CH$_4$ as the end product of the anaerobic OM decomposition pathway. Everglades soils have been shown to reach methanogenic redox potentials (Thomas et al., 2009) with CH$_4$ production 1/10$^{th}$ to 1/100$^{th}$ of CO$_2$ production (DeBusk and Reddy, 2003). Tree island and ridge soil used in this study is expected to produce 1-10% of total C efflux as CH$_4$, with higher rates of CH$_4$ efflux under flooded and low redox conditions.

**Hypothesis III:** Soil CO$_2$ and CH$_4$ production from Everglades tree islands and ridges varies between wet and dry conditions depending on changes in the quality of organic matter and quantity of labile P.

Flooded conditions slow OM decomposition, therefore allowing a build-up of labile C to occur. Microbial respiration of OM can additionally be regulated by nutrient availability (Debusk and Reddy 2003). High soil total P (DeBusk and Reddy, 2003), P amendments (Amador and Jones, 1995) and various labile C substrate amendments (Amador and Jones, 01993) all have been shown to increase CO$_2$ and CH$_4$ production from Everglades soil. Similarly, CO$_2$ and CH$_4$ production is expected to increase with labile C, P, and labile C + P enrichments to the peat soils used in this study, and influences are expected to be higher from ridges than tree islands. Furthermore, the response to labile C and
labile C + P enrichments is expected to be greater from dry condition soils because OM decomposition has been occurring in the presence of oxygen.

*Hypothesis IV: Soil extracellular enzyme activity from Everglades' tree islands and ridges varies with wet and dry conditions depending on changes in the quality of organic matter and labile P.*

Extracellular enzyme activity provides an indication, or the microbial demand, for nutrients (Marx et al., 2001). Autochthonous (i.e., in place) accretion of peat is theorized to be the dominant control of vertical accretion in the Everglades (Larsen et al., 2011). The litter falling on Everglades tree islands and ridges have poorer quality (i.e., high C:N ratios and lignin contents) than slough litter, which reduces microbial activity (Larsen et al., 2011) and may contribute to faster slough decomposition and the elevation differences (Penton and Newman, 2008). Relatively higher EEA (β-glucosidase, β-N-glucosaminidase, acid phosphatase, and sulfatase) is expected from ridge soil compared to tree island soil. Additionally, labile C, P, and labile C + P enrichment should increase EEA, other than acid phosphatase, by removing the P and labile C limitation. Conversely, any labile P enrichment will lower acid phosphatase activity from both tree island and ridge soils. Due to oxygen exposure, EEA of dry condition soils is expected to be higher.
4. METHODS

4.1 Site Description

This study was conducted at the Loxahatchee Impoundment Landscape Assessment (LILA) experimental landscape constructed at the Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR), Boynton Beach, Florida. Prior to the construction of LILA in 2002/2003, the site was actively managed until the early 1980’s by practicing conventional agriculture and left fallow over the next two decades. The LILA study site consists of four identical ‘macrocosms’, denoted as M1 (the northernmost), M2, M3 and M4 (the southernmost) (Fig. 2). Each macrocosm encompasses key features of the Everglades including ridges, sloughs, and tree islands. Macrocosms 1 and 2 were constructed from the same peat which is classified as 80% Okeelanta muck and 20% minor components (Sullivan et al., 2010). Prior to construction, the Okeelanta muck had an mean depth of 0.57 m and the mean TP level in the surface (0-10cm) soil was 575 mg kg\(^{-1}\). Except for TP in the upper 10 cm of soil, soil nutrients in the impoundment closely mimic the natural levels found in the Everglades. The hydrology within the macrocosms is managed by operating an electric pump (1.84 m\(^3\) s\(^{-1}\)) with a series of water control structures and recording stage gauges. The pump allows for manipulation and management of the stage, hyroperiod, and flow rate (Fig. 3; Stofella et al., 2010). One purpose of LILA is to study the responses of biological communities to the Everglades restoration strategies, including changes in hydrology and other critical processes associated with the CERP goal of “getting the water right” in the Everglades (Aich et al., 2011).
The landscape in each LILA macrocosm includes two 71 x 43 m islands, one limestone-based and the second peat-based. The limestone-based islands represent the ‘fixed’ tree islands formed around bedrock outcrops throughout the central and southern Everglades, and the peat-based islands resemble the ‘battery’ islands common in LNWR (van der Valk et al., 2007). Each island has a flat central plateau that is 0.9 m above the surrounding slough surface (4.2 m National Geodetic Vertical Datum [NGVD] 29) similar to the elevation difference in the Everglades (van der Valk et al., 2007; Aich et al., 2011). The central plateau of the limestone islands consists of the limestone core placed in a 14 x 49 x 0.6 m trench, with 0.3 m of peat fill placed on top of the limestone. While the relict soil found within the tree island footprint was not excavated, the peat that caps the islands was excavated from the sloughs in the immediate surroundings and the limestone was mined from the underlying bedrock near the site. All the islands have side slopes of 16:1 along the short (north-south) axis, and 12:1 in the east-west direction (van der Valk et al., 2007; Aich et al., 2011). Each tree island is divided into four quadrants for tree plantings with a spacing of 1, 1.66, 2.33 and 3 m between tree centers. Located in the high density plantings (1 m) are soil elevation tables (SETs) on the center portion (head high, HH) and edge portion (head low, HL), of each tree island. For this study, two western tree islands were studied with M1W being a peat based and M2W being a limestone based tree islands (Fig. 2). Installed around HH and HL SETs on M1W and M2W are four soil CO₂ efflux collars (A-D; Fig2 and 4). In addition to tree island plots, one ridge plot (MR) was sampled from each macrocosm (M1 and M2) with four collars in each plot.
4.2 Soil characteristics

Soils studied from tree islands are summarized in Table 1. Thirteen soil cores were collected in June 2010 around the tree island study plots on M1 and M2. Soils had a mean (n = 13) pH of 7.70, field bulk density of 0.64 g cm$^{-3}$, ash free (%) of 79.58, total phosphorus (TP) of 176.92 µg g$^{-1}$ dw, total nitrogen (TN) of 6.53 mg g$^{-1}$ dw, and total carbon (TC) of 111.96 mg g$^{-1}$ dw in 2010. The mean (n = 13) TC:TP ratio was 675:1 from 2010, indicating a high P limitation according to the required 106:1 Redfield ratio.

4.3 Stage

Water level (stage) at LILA is adjusted according to an operational hydrograph that mimics the seasonal flooding (high water levels) and dry down (low water levels) of water in Everglades (Stofella et al., 2010; Fig. 3). M1 had an mean stage of 4.68 m with a maximum of 4.94 m occurring 7-8 October, 2010 and a minimum of 4.22 m occurring 22 April, 2011 over the in situ study period (23 April, 2010 to 22 April, 2011).

Precipitation patterns in the Everglades drive the annual wet and dry cycle. Greater amounts of precipitation are received during the summer and fall months (June through September) and this period is considered the wet season (Duever et al., 1994). Peak water levels lag behind the precipitation and occur in October or November (Fig. 3; Stofella et al., 2010). According to the operational hydrograph for LILA, water levels remain highest from September through January, and lowest from April through June.

For the purposes of this study investigating the influence of water levels on CO$_2$ and CH$_4$ production, soils tested were from wet and dry conditions when water levels were high and low, respectively.
4.4 Soil Surface Elevations

A soil surface elevation map was generated as an ARC-GIS interpolation file from surveyed soil elevations made at the time of tree planting (Stofella et al., 2010). Soil elevations (Fig. 5) were the same for all collars from M1 and M2 -HH plots but varied within the HL and MR plots (Fig. 5). Relative water depth (RWD) was determined for each CO₂ efflux measurement from each collar by subtracting the collar soil elevation from the respective 15 minute macrocosm raw stage. Positive RWD values indicated water levels above soil surface and negative RWD values indicate water below soil surface.

4.5 In situ CO₂ Efflux

_In situ_ soil CO₂ efflux measurement from LILA tree island and ridge soils were conducted in May 2010 (low water level), August 2010 (rising water level), October 2010 (high water level) and March 2011 (falling water level). Soil CO₂ efflux was measured with an LI-8100 infrared gas analyzer (IRGA) and LI-8150 multiplexer with automated 104 long term chambers (LICOR, Lincoln, NB) sampling installed soil collars on M1W and M2W (Fig. 2). Collars (four collars per plot, three plots per macrocosm, and two macrocosms, 32 total collars) were measured once per sampling session for approximately 24 h. Over the 24 h period, samples were collected once every 3 h to conserve battery life (two 75 amp hour batteries in series). Each sample was taken over a 150 s period, with a dead band of 30 s. Before removal of bad values, individual collars had a minimum of 7-8 samples taken per season. Longer periods of deployment over weekends occurred for plots. Plots studied were M1 head high (M1HH), M1 head low (M1HL), M1 middle ridge (M1MR), M2 head high (M2HH), M2 head low (M2HL), and
M2 middle ridge (M2MR). When deployed on the tree islands, the LI-8100 allowed sampling on two HH and two HL collars per 24 h period. Therefore, samples from all four plot (i.e., HH) collars occurred over a 48 h period.

The hardware and software associated with the LI-8100 allowed for recording of additional parameters other than CO₂ efflux. These parameters include initial value, mean, and range of CO₂ concentration, along with relative humidity, voltage, date, time and flow rate of each sample. Should the machine stop recording, these parameters can help diagnose potential faults. Additionally, plotting initial CO₂ concentration and CO₂ efflux over time can show diurnal patterns associated with flora photosynthetic production and respiration.

On M2 East and West tree islands, trees were selected for a fertilization experiment with Control (C), Nitrogen (N), and Phosphorus (P) soil enrichments. An amount three times an individual tree incorporates annually was applied (72 g N or P) to the soil under the trees. The initial annual fertilization occurred June 2009-2010, and fertilization began again in June 2011. Before the first application in June 2011, soil CO₂ efflux collars were installed under three trees per soil enrichment per island, for a total of 18 collars, six per enrichment. Soil CO₂ efflux was measured by LI-8100 IRGA with 103 survey chamber (LICOR, Lincoln, NB), walked to the collars, and moved between each sample. Samples were collected for 150 seconds, with a 30 second dead band, with parameters recorded as discussed above. Measurements were made 3 d before, and 4, 11, 18, and 26 d after the fertilization.
4.6 Annual estimation of CO₂ efflux

All *in situ* CO₂ efflux and daily mean CO₂ efflux values were plotted against RWD for each plot and a linear regression calculated (Table 2 and 3, respectively). Daily mean stage values were used for the entire *in situ* study period (April 23, 2010 to April 22, 2011) to calculate daily mean RWD. Each plot’s linear regression equation was applied to the daily mean RWD to calculate CO₂ efflux in µmol m⁻² s⁻¹. Daily CO₂ efflux values were converted into µmol m⁻² d⁻¹, summed for the study period, and converted into g C m⁻² y⁻¹.

4.7 Soil core incubation

Intact soil core incubations were conducted in the laboratory with the LI-8100 and LI-8150 utilizing a multiplexed flask system. Triplicate intact cores of LILA soils were collected from study plots to a depth of 20 cm using 50 cm long clear acrylic tubing with a 5.7 cm inside diameter. Water levels in cores were raised to 15 cm above the soil surface in the cores by adding LILA surface water before transport to laboratory. Upon arrival at the laboratory, core tubes were fitted with rubber caps affixed with two quick connect fittings for connection to the LI-8150 and ambient air was flushed through a diffuser, into the water column and headspace, and exited out of the outflow. Intact cores were flushed for 24 d before any gas sampling occurred. Measurements of CO₂ efflux from soil cores occurred over 15 min duration with the LI-8100. Sampling for CH₄ measurements occurred simultaneously by collecting 10 mL column air samples with air-tight syringes, injecting sample into 20 mL vials with 10 mL N₂ gas headspace to maintain atmospheric equalization. Samples for CH₄ efflux were taken every 5 min.
during the 15 min CO₂ efflux measurement and analyzed by gas chromatography and methane (see below).

Oxidation-reduction (redox) probes were made by welding platinum (Pt; ~1.3 cm length) to an insulated copper wire and sealing with heat-shrink tubing (Thomas et al., 2009). Copper wire was cut to lengths of 55 cm to keep wire ends above the water in the core tubes. A total of 12 probes were made, and two were inserted to 10 cm soil depth in one of three replicate intact cores from each plot. Redox potential of intact soil cores was taken after each CH₄ incubation. Redox was measured by a multimeter with an Accumet 13-620-61 calomel reference electrode to complete the circuit. A +250 mV correction was applied to all readings (Thomas et al., 2009).

4.8 Field soil collection

Soil samples for physiochemical analysis were taken from around each of the soil collars in January (wet condition) and April 2012 (dry condition). Intact cores were taken by inserting a 2.3 cm i.d. cellulose-acetate-butyrate (CAB) tube to a depth of 10 cm below the soil surface. To minimize compaction, the core cutting edge was fitted with flexible razor blades to cut fine roots. Depth of the void and soil plug were verified by inserting a small ruler into the hole. Cores were extruded intact into a sample bag labeled with macrocosm, island, collar, date and collector and returned to the laboratory (at ambient temperature) for analysis within 72 hours.

4.9 Gas chromatography

Measurement of CO₂ and CH₄ production, and CH₄ efflux was performed with a Hewlett Packard 5890 Series II Gas Chromatograph (GC) fitted with an automated headspace sampler (HP-7694). Carbon-dioxide was converted to CH₄ via a methonizer
(Ni catalyst and H₂ gas stream, Shimadzu MTN-1) at 450°C (Amador and Jones 1992, Amador and Jones, 1995) and analyzed by flame ionization detection (FID) following retention on a HEYASEP-R column (Alltech, Inc.). Peak area was interpolated by ELAB software version 4.02R. Peak areas were converted into moles (vial enrichment) or ppm (CH₄ efflux) based on a standard curve of known gas concentrations.

4.10 Vial enrichment

Subsamples of soils collected during January (wet condition) and April (dry condition) 2012 were analyzed for CO₂ and CH₄ on GC. Nominal 4.5 g subsamples of 1:1 g freshweight soil g⁻¹ distilled deionized water (DDIH₂O) were incubated in 20 mL headspace vials fitted with rubber septum and aluminum cap. Replicate vials were amended with a water control (Con; 0.125 mL DDIH₂O), glucose (G; 0.125 mL 0.2M Glucose), phosphorus (P; 0.125 mL 0.2M K₂HPO₄), or glucose and phosphorus (GP; 0.125 mL of 0.2M Glucose and 0.2M K₂HPO₄) flushed with CO₂- free air for 1 min and evacuated five times. Samples were analyzed once a day for 5 d. After each analysis, samples were flushed and purged.

Tree island soils studied had a mean (n = 13) TP of 176.92 µg g⁻¹ dw and TC of 111.96 mg g⁻¹ dw (Table 1). The enrichments of vials by 0.125 mL of G and P in 0.2M concentrations equates to additions of +0.3 mg C g⁻¹ and +775 µg P g⁻¹, respectively. The mean TC:TP ratio before any enrichment was 676:1, with G enrichment was 678:1, with P enrichment was 117:1, and with GP enrichment was 117:1 (Table 1).

4.11 Extracellular Enzyme Activity

The measurement of EEA was performed using a Cytoflour 4000 96-well plate reader. A 1 mL sub-sample was taken from all vials (above) after the five days of
incubation and serially diluted to $10^{-3}$ DDIH$_2$O. Four methylumbelliferyl-based (MUF) substrates were added to the diluted and plated samples: MUF-phosphate (MUF-P), MUF-β-D-glucosidase (MUF-C), MUF-sulfate (MUF-S), and MUF-N-acetyl-β-D-glucosaminide (MUF-N). The plates amended with MUF-C, -S and –N were incubated for 24 hours and MUF-P for 2 hours in the dark at room temperature (Sinsabaugh et al., 1997). The MUF-C substrate tests for β-glucosidase enzyme activity, MUF-S tests for sulfatase enzyme activity, MUF-N tests for β-N-glucosaminidase enzyme activity, and MUF-P tests for phosphatase enzyme activity. Plates were read with excitation of 360 and emission of 460 nm, respectively. Values were converted to µmol MUF liberated per gram dry weight of soil per hour ($\mu$mol gdw$^{-1}$ h$^{-1}$).

4.12 Statistical Analysis

All statistical analyses were conducted with SPSS (18.0, Chicago, Illinois, USA). Results were considered statistically significant with $p < 0.05$. The effect RWD had on soil CO$_2$ efflux was compared by linear regression ($r^2$). Collinearity of other independent variables with RWD was tested.

Differences in CO$_2$ production, CH$_4$ production, and all EEA were tested with ANOVA and considered significant if $p < 0.05$. Multiple comparisons for enrichment (Con, G, P, and GP) were evaluated with Tukey HSD for CO$_2$ production, CH$_4$ production, and all EEA. Before ANOVA’s were run, all data were tested for normality with Shapiro-Wilk’s tests. Data that were not normal were log10 or square root transformed to approximate normality and outliers were removed before ANOVA analysis. For CH$_4$ production, the Kruskal-Wallis multiple nonparametric comparison tests of means was done because this data was not normally distributed after
transformation. After the Kruskal-Wallis test, ANOVA was conducted and had the same results, and Tukey HSD was used to determine enrichment comparisons.

The differences between wet and dry conditions in CO₂ production, CH₄ production, and all EEA were tested by t-test after normalization.
V. RESULTS

5.1 In situ CO$_2$ efflux

In situ soil CO$_2$ efflux from M1- and M2- HH and HL plots did not show the diurnal pattern common in other studies (e.g., Hirano et al., 2009). From M1HL (Fig. 6), CO$_2$ efflux was variable over the 48 h shown (Fig. 6 A), but remained more or less constant. However, the concentration of CO$_2$ in the chamber at the beginning of samplings did show the diurnal pattern (Fig. 6 B).

Within each plot replicate collars (A-D) vary in measured CO$_2$ efflux rates, with M1HL collar A frequently having CO$_2$ efflux rates higher than collars B, C, and D (Fig. 6 A). Overall, in situ soil CO$_2$ efflux ranged from 0.5 to 23.3 µmol m$^{-2}$ s$^{-1}$ from HH plots, 0.1 to 21.8 µmol m$^{-2}$ s$^{-1}$ from HL plots, and from 0.4 to 32.9 µmol m$^{-2}$ s$^{-1}$ from MR plots (e.g., Fig. 8 and 9). Values provided by LI-8100 can be both positive (CO$_2$ leaving soil surface) or negative (CO$_2$ entering soil) and were all rounded to the nearest 0.1 µmol m$^{-2}$ s$^{-1}$. Occasionally negative values were generated. This indicates CO$_2$ uptake by the soil or water surface (when flooded). However, post-processing in the LICOR system showed these did not meet a signal-to-noise criteria (as determined by linearity of efflux with time in the LICOR system) and were, therefore, eliminated.

5.2 Influence of water levels on CO$_2$ efflux

The HH plots from M1- and M2- experienced no flooding (RWD > 0) but did experience near-saturated conditions (RWD ≥ -0.2), while all other plots experienced at least 140 days of flooding (Fig. 5). In situ CO$_2$ efflux was significantly (p < 0.001)
negatively influenced by water levels, as represented by RWD, from all study plots (Table 2, Fig. 7 and 8). Combining tree island (HH and HL) CO₂ efflux into one linear regression shows that RWD significantly (p < 0.001) influences CO₂ efflux (Table 2; Fig. 7). Similarly, combining M1- and M2- MR plots yields an equally significant influence of RWD on CO₂ efflux (Table 2, Fig. 9). Tree island substrate had a significant (p < 0.001) influence on \textit{in situ} CO₂ efflux between the M1- and M2-HH plots during the April 2010 sampling when relative water depth (RWD) was < -0.4 m. The RWD effect on CO₂ efflux is significant (p < 0.001) when using mean daily CO₂ efflux rates for each plot (Table 3, Figure 11).

5.3 Estimating annual C efflux

All \textit{in situ} soil CO₂ efflux and mean daily CO₂ efflux linear regressions used for interpolation of annual C loss estimates (as g C m⁻² y⁻¹) are significantly influenced by RWD (p < 0.001, Table 2 and 3). Estimates were greater from the daily mean CO₂ efflux interpolation by 64 – 599 g C m⁻² y⁻¹. The estimates were greatest from the two HH plots, but differed by more than 1,000 g C m⁻² y⁻¹ with M1 greater than M2 (Tables 1 and 2). The HL plots had smaller estimates of loss than HH plots due to their flooding. Additionally, HL plots had similar estimates even though their slopes (rates) were different (Tables 1 and 2). Combining tree island plots into one linear regression produces highly significant and similar estimates of C loss. The MR plots had different estimates due to \textit{in situ} efflux variability from M2. The mean daily CO₂ efflux linear regression removes a substantial portion of this variability and yields an estimate from
M2 closer to that of M1 (Table 3) than was found with the use of all in situ CO₂ efflux values (Table 2).

5.4 Intact soil core

Intact soil core CO₂ efflux was significantly (p ≤ 0.008) influenced by RWD, and plot soil (elevation) but not significantly (p = 0.102) influenced by their interaction (Table 4). Mean of CO₂ efflux rates from the replicate intact cores and in situ studies by plot are presented in Table 6. Intact core CO₂ efflux rates represent between 16 and 54% of mean in situ mean CO₂ efflux rates (Table 6). Intact soil core CH₄ efflux was not significantly influenced by RWD (p = 0.177) or the combination of RWD and elevation (p = 0.264). However, CH₄ efflux was significantly (p = 0.038) influenced by the elevation that soil was collected (Table 4). The ratio of CH₄ to CO₂, as a percentage, ranged from below detection (BD) to over 3000% (Table 7).

Redox potential was significantly (p < 0.001) influenced by RWD, elevation soil was collected, and their combination is shown in Table 3. Redox potential decreased into anaerobic ranges (Eh = -200 to +400 mV) in all intact soil cores (Table 5). Methanogenic redox range (Eh < -200) was reached by all soil cores. However, redox potential did not have a significant influence on rates of CH₄ efflux (data not presented).

5.5 Vial enrichment incubation

Soils collected from HH and HL during both wet (January) and dry (April) conditions showed a significant (p < 0.05) increase in potential CO₂ production with the G and GP enrichment (Fig. 12). Soils from MR were the most variable and showed no
significant effect from enrichment in the wet condition. However, MR soils showed significant (p < 0.05) increases in potential CO₂ production under the dry condition with G and GP enrichments (Fig. 12). Between wet and dry condition soils, HH soils under Con and P enrichment had significantly (p < 0.05) more CO₂ production under wet than dry condition soils (Fig. 12). Conversely, HH soils under G enrichment had significantly (p < 0.05) greater CO₂ production under dry than wet condition soils (Fig. 12). From HL soils, dry condition CO₂ production was significantly (p < 0.05) greater than wet condition soils greater under G and GP enrichments (Fig. 12).

Dry condition soils showed a significant (p < 0.05) increase in potential CH₄ production in the G and GP enrichments from all plot soils (Fig. 14). No significant increases in CH₄ potential production from wet condition soils were found from any plot (Fig. 17 A). Only the GP wet condition enrichment and G dry condition enrichment showed significant increases in potential CH₄ production from HH to HL to MR (Fig. 17 A and B). Wet condition soils had greater CH₄ potential production than dry season condition soils from C and P enrichments, while only the G enrichment from HH dry condition was greater than wet condition soil. Wet condition soils showed a CH₄/CO₂ ratio percentage [(CH₄/CO₂)*100] mean of 0.95% across all enrichments, while dry condition soils had a mean of 21.61%.

5.6 Extracellular Enzyme Activity

Glucosidase EEA was most variable from MR soils from both the wet and dry conditions (Fig. 15). During the wet condition, enrichment had no significant effect on glucosidase EEA from any plots (Fig. 15). During the dry condition, HL soils showed
significant (p < 0.05) enrichment effects from G and GP enrichments (Fig. 15). The GP enrichment from HL dry condition soils encouraged significantly (p < 0.05) greater glucosidase EEA than wet condition soils (Fig. 15).

Glucosaminidase EEA from wet and dry condition soils show no significant increases with Con, G, P, or GP enrichments (Fig. 16). The P enrichment from HL and MR soils showed significantly (p < 0.05) higher activity from wet condition soil (Fig. 16).

Wet condition soils from HH and MR showed no significant phosphatase EEA enrichment effect (Fig. 17). However, from HL wet condition soils, the G, P, and GP enrichments had significantly (p < 0.05) lower activity than Con. Soils from dry condition under the G enrichment appear to have significantly enhanced phosphatase EEA from all plot soils, but none were significant due to variability (Fig. 17). Conversely, all plot soils from dry conditions had significant (p < 0.05) reductions of phosphatase EEA under the P and GP enrichments (Fig. 17). The P and GP enrichments had significantly greater activity from all plot wet condition soils than dry condition soils (Fig. 17). Additionally, HH and HL dry condition soils had a significantly (p < 0.05) greater activity with G enrichment over wet condition soils (Fig. 17).

Sulfatase EEA was not significantly increased with G, P, or GP enrichment over Con from any plot wet condition soils (Fig. 18). Dry condition GP enrichment from HH and HL soils had significantly (p < 0.05) increased activity over Con (Fig. 18). Wet condition soils all had significantly greater sulfatase EEA than dry condition soils (Fig. 18).
VI. DISCUSSION

6.1 Factors influencing in situ CO₂ efflux

Hydrology is one of the main drivers of soil CO₂ and CH₄ efflux from peatland and wetland soils worldwide (Davidson et al., 2000; Blodau and Moore, 2003; Furukawa et al., 2005; Jauhiainen et al., 2005; Hirano et al., 2009). High soil moisture and flooded soil limit oxygen’s ability to diffuse into the soil (Mitsch and Gosselink, 2007). Both in situ and laboratory studies have shown that high RWD, or water above the soil surface, reduces rates of CO₂ efflux from peatland and wetland soils (DeBusk and Reddy, 2003; Furukawa et al., 2005; Melling et al., 2005; Hirano et al., 2009). The in situ CO₂ efflux data presented here were significantly influenced by RWD from all LILA tree island and ridge plots studied (Table 2; Fig. 8 and 9). Combining CO₂-C efflux and C tree production data into a C budget can be used to find where equilibrium with water levels slow or reverse tree island and ridge loss in the Everglades (Fig. 1; Larsen et al., 2011). These results indicate that water levels are a significant driver of CO₂ efflux from the Everglades (Hypothesis I). However, only 21-30% of M1W, 68-73% of M2W, and 40-65% of MR CO₂ efflux variability are explained by RWD.

In situ CO₂ efflux values from LILA tree island and ridge soils have high variability (Fig. 9), with peak rates of CO₂ efflux two to four times higher than many other literature rates (e.g., Jauhiainen et al., 2005; Hirano et al., 2009). Over the study period, rates of CO₂ efflux ranged 0.5 to 23.3 µmol CO₂ m⁻² s⁻¹ from HH, 0.1 to 21.8 µmol CO₂ m⁻² s⁻¹ from HL, and 0.4 to 32.9 µmol CO₂ m⁻² s⁻¹ from the MR soils studied. Rates of CO₂ efflux presented here range more than literature values of 2.32 to 12.34
µmol m\(^{-2}\) s\(^{-1}\) found in a mixed peat swamp forest in Malaysia (Melling et al., 2005). Additionally, annual mean CO\(_2\) efflux values (Table 6) are higher than the mean rates found in wet tropical peatland forests of Indonesia which ranged 0.06 to 3.16 µmol m\(^{-2}\) s\(^{-1}\) (Furukawa et al., 2005), 3.06 to 3.85 µmol m\(^{-2}\) s\(^{-1}\) (Jauhiainen et al., 2005), and 2.98 to 4.02 µmol m\(^{-2}\) s\(^{-1}\) (Hirano et al., 2009). Furthermore, from peat on a volcanic island, CO\(_2\) efflux ranged 1.07 to 2.15 µmol m\(^{-2}\) s\(^{-1}\) (Chimner, 2004). However, efflux rates ranged 0 to 20 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) from papayrus wetlands in Africa (Jones and Humphries, 2002) and hitchcock wetlands in the Amazon (Morison et al., 2000). These two ecosystems are characterized by high productivity and seasonal water fluctuations similar to the Everglades. As has been mentioned previously, high rates of primary productivity and a build-up of OM with flooded conditions can produce high rates of CO\(_2\) efflux.

From LILA soils, CO\(_2\) efflux did not show a diurnal signal (Fig. 6 A). Many studies have shown a diurnal signal to CO\(_2\) efflux, where higher rates are associated with low- to no- light conditions due to tree respiration (e.g., Hirano et al., 2009). Trees were planted at LILA <5 years before this study takes place. Young stands have been shown to have higher rates of soil CO\(_2\) efflux than older stands (Saiz et al., 2006). In contrast to no CO\(_2\) efflux diurnal signal, the atmospheric concentration of CO\(_2\) at measurement initiation shows the diurnal signal expected (Fig. 6 B). Concentration of CO\(_2\) begins to rise around sunset (~1800 hrs), when trees can no longer photosynthesize and respire CO\(_2\), peaking in the early morning hours (0100-0500 hrs). As light returns (~0600 hrs), concentrations of CO\(_2\) fall with initiation of photosynthesis and remain low until sunset.
Mean daily CO₂ efflux from LILA tree islands also has a significantly (p < 0.001) negative influence from RWD (Table 3, Fig. 10). With the substantial reduction in n from Table 2 to Table 3, there was no less significance in confirmation that RWD does significantly influence CO₂ efflux (Hypothesis I), indicating that inclusion of all *in situ* efflux values may not influence results substantially. Additionally, the amount of variability accounted for by RWD was only slightly different, with M1 having consistently the lowest accountability.

Dinsmore et al. (2009) found that CH₄ and N₂O built up in the water and soil matrix of flooded mesocosms are released upon drawdown of water levels. The peat core tree island (M1) consists of about 90 cm of peat piled up in the center (HH) that is potentially contributing to the trapping of CO₂ from soil respiration during flooded conditions, while the limestone core tree island (M2) only has 30 cm of peat on top of 60 cm of limestone core that could contribute to this phenomenon. During the dry season when RWD declines below -0.4 m, M2 had significantly (p < 0.001) lower peak efflux rates than M1 (Fig. 8 and 9). With M1HH having more soil, not rock, the potential to slowly release trapped CO₂ from soil depth is greater than that of M2HH. When water levels are lowered below the soil depth of M2HH (30 cm, or in RWD terms, -0.30 m), the water table lies in the limestone portion of the tree island. Sullivan et al. (2010) showed that groundwater levels in the center of M2 during the dry season are drawn down more dramatically than M1 (mean of 6.33 cm and 1.21 cm, respectively). When soils are not flooded, drops in soil moisture limit both root and microbial respiration (Liu et al., 2002, Bahn et al., 2008). *In situ* CO₂ efflux rates below -0.4 m RWD range between 4 and 8
μmol CO₂ m⁻² s⁻¹ from M2HH, while M1HH CO₂ efflux rates continue to rise from the 4 to 12 μmol CO₂ m⁻² s⁻¹ range to between 9 and 18 μmol CO₂ m⁻² s⁻¹ (Fig. 9). With a 6.33 cm water table draw down mean in the center of M2 (the HH plot), the -0.4 m RWD calculation is no longer accurate and lower soil moisture may be limiting soil respiration from this plot.

Root and microbial respiration can contribute substantially to overall soil CO₂ efflux (an estimated 75% in a temperate rain forest; Bowling et al., 2002). Krauss et al. (2012) found 79% of the variation in CO₂ efflux from mesocosms and tidal freshwater cypress swamp in situ measurements was due to root biomass and root length, respectively, which aligns with several other studies reporting increased soil respiration with higher biomass (e.g., Chimner and Ewel, 2004). Tree biomass work conducted at LILA shows M1 has greater aboveground biomass than M2 (Ross et al., unpublished), which coincides with M1 being planted one year earlier than M2 (Stofella et al., 2010). Biomass estimates above each LICOR tree island collar from an inverse distance weighted (IDW) ArcGIS calculation indicate biomass is significantly (p = 0.005) correlated with annual mean CO₂ efflux from each collar (Fig. 19). The IDW used may not properly calculate biomass, but for the purposes of correlating biomass to mean CO₂ efflux, the results are significant and show CO₂ efflux is greater from higher biomass areas, as has been shown previously (Chimner and Ewel, 2004).

Furthermore, higher amounts of C inputs, and thus more labile C, have been shown to increase soil respiration rates (Allen et al., 2000; Trumbore, 2000; Bahn et al., 2008). Scinto et al. (unpublished) have been collecting litter fall mass in litter traps from
LILA tree islands within one meter of respiration collars sampled during this study. The sum total of litter fall (g C m\(^{-2}\) y\(^{-1}\)) from traps adjacent to respiration collars is also significantly (p = 0.005) correlated with annual mean CO\(_2\) efflux (Fig. 20). Biomass differences between M1 and M2 –HH can also be seen in Fig. 20 where M1HH has mean litter fall (359 g C m\(^{-2}\) y\(^{-1}\)) more than double M2HH (159 g C m\(^{-2}\) y\(^{-1}\)). Higher biomass and litter fall help identify, through literature, potential causes for M1HH to have higher \textit{in situ} CO\(_2\) efflux than M2HH. Biomass and litter fall from M1HL, M2HH and M2HL are clustered closely to each other (Fig. 19 and 20), and these three plots have similar annual mean CO\(_2\) efflux rates of 4.4-4.6 \(\mu\)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\) (Table 6). Tropical and subtropical systems receive a more constant supply of litter than temperate and boreal systems which allows tropical and subtropical systems to maintain more constant soil respiration rates (Lovelock, 2008). Due to the annual wet and dry cycles the Everglades experiences, litter can build during flooded conditions on HL and MR plots, eventually to be released during dry conditions. The plot M1HH received the most litter fall and experienced no flooding contributing to this plots highest annual mean CO\(_2\) efflux rate (Table 6).

Carbonate dissolution from the calcareous Everglades soil was not something investigated in the present study. Tamir et al. (2009) found that dissolution of Mediterranean soils following wetting can influence CO\(_2\) efflux rates. Furthermore, acidic conditions of both the soil and surface and ground water from OM decomposition can cause dissolution of carbonate (Tamir et al., 2009). Underlying the Everglades basin is limestone bedrock contributing to high carbonate concentrations in solution,
particularly in areas exposed to the underlying bedrock (Noe et al., 2001).
Photosynthesis by periphyton in flooded plots drives changes in water column pH, thus
contributing changes in CO₂ partial pressure (Noe et al., 2001) and, potentially, efflux
into the atmosphere.

Due to the limited amount of variability of CO₂ efflux values explained by RWD
from M1 plots, further investigation is warranted into the role the peat substrate and tree
biomass of these plots plays in the variability of CO₂ efflux. Additionally, long-term
monitoring of CO₂ efflux from LILA islands and ridges with develop will further define
the role tree stand age and root respiration play in tree island CO₂ efflux.

6.2 Annual CO₂ efflux estimates

The interpolated estimates of annual and daily mean C efflux (Table 2 and 3) vary
by plot. Linear regressions used to interpolate the annual estimates in Table 2 include all
in situ measurements. Hirano et al. (2009) used mean daily efflux to eliminate the
diurnal variation for annual estimation. Presented in this study are both complete annual
and daily mean CO₂ efflux estimates to provide comparison. The greatest C loss was
from M1HH which had some of the highest rates of efflux and was never flooded (Fig.
9), which is further indication that RWD limits CO₂ efflux. Because M2HH had lower
efflux rates than M1HH when RWD < -0.4 m (discussed above; Fig. 8 and 9), the annual
estimate of efflux was also lower from this plot and was about half of the M1HH estimate
(Table 2 and 3). Annual estimates of C loss from M1- and M2-HL were similar (Table 2
and 3). These estimates are similar even though M2HL mean elevation (4.70 m) is
higher than M1HL (4.59 m) with 59 fewer days of flooding. Additionally, M1HL has a
more negative slope than M2HL (Table 2), influencing the interpolations due to the greater variability of efflux values (discussed above).

For the MR plots, the annual estimate of C efflux based on the relationship between individual sample models differs strongly between M1 and M2 in Table 2, but does not differ as much based on the daily mean model (Table 3). Soil elevation from M1MR was lower than M2MR (4.37 m versus 4.51 m; Fig. 5). Variability of CO₂ efflux was much higher from M2MR (Fig. 9 and 11) during dry conditions which strongly influenced the slope and C loss estimate of the linear regression (Table 2). The linear regression of M2MR produced negative interpolated efflux values when RWD > 0.2 m, which occurred for 208 of the 292 days of flooding. By combining M1- and M2- MR CO₂ efflux rates into a single model produces an interpolated annual C loss estimate of 991 g C m⁻² y⁻¹ (Table 2) which minimizes the influence variability. This removed some of the interpolated negative efflux rates.

Annual estimates of C efflux from LILA soils range 149 to 2250 g C m⁻² y⁻¹ (Table 2) and 748 to 2403 g C m⁻² y⁻¹ (Table 3) for all annual and daily mean interpolations, respectively. Estimates of increasing loss are associated with shorter hydroperiod length for both annual and daily mean CO₂ efflux (Tables 2 and 3, respectively). This provides further indication that long periods of flooding annually reduce CO₂ emissions, and thus C loss, confirming predictions in Hypothesis I. These C loss values are within the range of other reported values: Melling et al. (2005) reported 2100 g C m⁻² y⁻¹ from a Malaysian peat swamp forest, and Jauhiainen et al. (2005) reported 898 to 1061 g C m⁻² y⁻¹ and Hirano et al. (2009) reported 640 to 1309 g C m⁻² y⁻¹
from an Indonesian peat forest. LILA trees during the study were 4-5 and 3-4 years old for M1 and M2, respectively, while the literature annual estimate values presented were from more mature forests (> 10 years). Saiz et al. (2006) found greater stand ages tend to reduce annual estimates of C efflux in Sitka spruce first generation plantations. The annual estimate of C loss from M1HH is higher than the highest reported value. As LILA tree island stands age, the estimate of C loss may decrease and fall within the range of reported values. Continued CO2 efflux measurements over many years from these plots are needed to track CO2 effluxes response to stand age.

An attempt was made to provide an additional method to estimate annual efflux from tree island plots for comparison. Seasonally, mean efflux per plot was linearly regressed between each successive season, which yielded four equations to use for estimation based on day of the year (data not shown). Estimates, in g C m^-2 y^-1, were 3,586 from M1HH, 1,359 from M2HH, 1,634 from M2HL and 714 from M2HL (data not presented otherwise). Estimates of C efflux from M1HH were much greater based on this method than the previously discussed method. LILA was creased to mimic historical flows and to monitor the influence of water level and flow on biological processes in an Everglades ecosystem. However, baseline water levels differ year to year, as a result the LILA control system cannot reproduce the exact same water regime each year. Dry season 2011 (April – June 2011, Fig. 3) was the driest period ever recorded at LILA, and one of the driest in the Everglades’ recorded history. During the final measurements of the study period, stage was falling rapidly (Fig. 3 and 5) and eventually fell to below 3.9 m. This seasonal interpolation method of estimation does not have the ability to
compensate for fluctuations in water levels. Consequently, use of this approach was discontinued.

6.3 Intact soil cores

Intact soil cores were used to isolate the microbial respiration portion of CO2 efflux. Water levels (RWD) significantly influenced core CO2 efflux (Table 4) similar to in situ CO2 efflux, and is further confirmation of Hypothesis I. Cores collected from all plots contained live roots, which could not be removed without destruction of the intact core. The cores were equalized for 24 days before efflux sampling. Destruction of the core to remove the roots would have homogenized the soil profile and exposed all soil to oxygen which would have influenced CO2 and CH4 efflux measurements. Rates of CO2 efflux found in this study are comparable to rates presented by DeBusk and Reddy (2003). Based on mean intact core CO2 efflux rates and mean annual in situ CO2 efflux (Table 6), the theoretical microbial contribution to in situ efflux ranges from 15-24% of HH, 23-35% of HL, and 30-54% of MR. Therefore, between 46 and 85% of in situ efflux can be classified as “other” and could include root (Bowling et al., 2002; Krauss et al., 2012), macroorganism (Bowling et al., 2002) and dissolution of carbonate from limestone and calcareous soil (Tamir et al., 2011). As the trees age, their contribution to total soil CO2 efflux may become smaller (discussed above). Therefore, the microbial portion of total soil CO2 efflux may increase. This warrants continued long-term investigation.

Elevation from which the soil was collected also had a significant effect on intact core CO2 efflux (Table 4), with the highest mean rates from MR cores. Soil for both the
tree islands and ridges originated from the same location, experienced the same management, and were piled up to create the tree island (see Site Description). However, after construction, not only were the soils disturbed during construction, but the soils have been colonized by various flora species, depending on location and hydroperiod, which can have major influences on soil development, microorganisms, etc. These differences may be a major driver of the significant influence soil location has on CO₂ efflux from the cores.

Water levels (RWD) did not significantly influence the rates of CH₄ efflux, (Table 4), similar to DeBusk and Reddy (2003), and rejecting Hypothesis II. The intact core study presented here relied on an oxidized water and air column above the soil surface creating the potential for CH₄ to be oxidized before sample collection. The range of CH₄ efflux rates were variable, ranging from 0.1 to 29.2 µmol CH₄ m⁻² s⁻¹ from intact Everglades’ soil cores (DeBusk and Reddy, 2003) and 0.07 to 0.11 µmol CH₄ kg⁻¹ s⁻¹ from intact Floridian tidal freshwater peat cores (Chambers et al., 2011). These are within range of the rates found in this study. Rates of CH₄ efflux were highest with 0 RWD in this study, opposite the results presented by DeBusk and Reddy (2003). This may be due to sampling procedure error, no oxygenated water column or methanotrophic bacteria to oxidize CH₄, or a release of CH₄ from the soil matrix (discussed 6.1 above). Elevation was a significant treatment for CH₄ efflux (Table 4). As mentioned above, the soil from tree islands and ridges originated from the same location and management. The MR cores produced the highest CH₄ efflux rates and have the longest hydroperiod.
studied, which likely contributes to (larger) populations of methanogenic organisms responsible for the greater CH$_4$ efflux.

The annual cycle of flooding and draining tree islands and ridges of the Everglades can dictate the ratio of CO$_2$ and CH$_4$ gas emitted to the atmosphere based on oxidation-reduction (redox) potential (Thomas et al., 2009). All soil cores reached redox potentials (Eh) necessary for methanogensis (Table 5). However, intact core CH$_4$ efflux was not influenced by redox potential (data not presented). All soil cores stabilized around -200 mV at 10 cm soil depth. Thomas et al. (2009) showed similar results from P impacted and unimpacted soils of WCA2 at depths of 2-10 cm, and reported Eh below -200 mV at depths of 20 cm. With redox potentials in the methanogenic range and no significant interaction with CH$_4$ efflux, there is another force driving CH$_4$ efflux from these soils requiring further investigation. Unlike CH$_4$, redox was significantly influenced by RWD, elevation and the combination (Table 4). The significance of RWD’s effect on redox is expected due to oxygen availability. Dry and wet cycles in the Everglades annually expose soil to oxygen (Eh > +400 mV), re-flooding and force soil into anaerobic conditions (Eh < +400 mV). Redox potential can provide insight into C cycling dynamics (Thomas et al., 2009), but no such insight was found with this current study.

6.4 Vial incubation

LILA soils are known to have lower P concentrations than typically found in the Everglades soil (Stofella et al., 2010). Before the soil was used to create LILA, the soil was used for agricultural purposes (Stofella et al., 2010) which may have altered the soil
from its historical properties. *In situ* CO₂ efflux measurements of soil amended with P in a fertilization study indicate that P enrichment had no significant influence on CO₂ efflux over Con enrichment (Fig. 13). Incubation experiments conducted on both wet and dry condition soils indicate that labile P generally is not limiting CO₂ and CH₄ potential production from any study plot (Fig. 12 and 14), thus rejecting the labile P portion of Hypothesis III. The only soils to show a P limitation to potential CO₂ and CH₄ production were found from HL wet condition (Fig. 12 and 14). Other work has shown that P addition to Everglades’ soil does not always increase respiration (Amador and Jones, 1993; Amador and Jones, 1995; Drake et al., 1996). Soils analyzed from 2010 show that LILA has a P limitation with an mean TC:TP ratio of 676:1. The Redfield ratio states that the ideal TC:TP ratio is 106:1. After the addition of P to vials, the TC:TP ratio fell to a mean (n = 13) of 117:1, close to the ideal Redfield ratio. Only the HL soils showed a response to this reduction in the TC:TP ratio, which may indicate that another macronutrient is limiting respiration.

Everglades soil has also been shown to be labile carbon (C) limited (e.g., Amador and Jones, 1995; DeBusk and Reddy, 1998). The G and GP enrichments did significantly enhance CO₂ production potential from wet (only HH and HL plots) and dry (all plots) condition soils (Fig. 12), accepting the labile C portion of Hypothesis III. Additionally, CH₄ production from dry condition soils had significantly greater by G and GP enrichments than wet condition soils (Fig. 14) indicating that OM quality is limiting during dry conditions. Quality of OM (C) may be limiting microbial respiration from LILA soils, as evidenced here, even with the small addition of C (0.3 mg C g⁻¹) equating
to 0.3% of TC. The response of dry condition G and GP enrichment was significantly greater than that of wet condition soils from HH and HL plots for CO₂ production and all plots for CH₄ production (Fig. 12 and 14, respectively). Greater response from dry condition soils indicates that during these conditions, LILA tree islands and ridges have a C limitation to respiration. In the wet condition soils, the GP enrichment soils also significantly had more CO₂ potential production than just the G enrichment indicating that when C is not limiting, P is the next limit to respiration, as has been found by Amador and Jones (1995). The potential CH₄ and CO₂ production rates increase with hydroperiod (Fig. 12 and 14), indicating that longer hydroperiod plots have the potential to release C to the atmosphere once exposed to oxygen.

6.5 Extracellular enzyme activity

Glucosidase EEA showed one significant matrix enrichment (GP) effect from dry condition HL soils (Fig. 15). All other enrichments were not significantly different than Con due to variability. The β-glucosidase enzyme is responsible for hydrolyzing glucose from chains for uptake by the microbial community; its activity is considered to be partly responsible for limiting respiration (Penton and Newman, 2008). Glucosidase EEA is in range with Troxler et al. (2012) for Panamanian peat soils with TP concentrations similar to those found at LILA. Rates from this study are also within range that Corstanje et al. (2006) found from decomposing sawgrass and cattail litter in mesocosms. From LILA soil incubations, both wet and dry condition Con enrichment glucosidase EEA was highly (r²=0.60 and 0.75 respectively) and significantly (both: p < 0.001) positively correlated with Con enrichment potential CO₂ production rates (Fig. 21). Similarly, Rejmánková
and Sirová (2007) found glucosidase EEA to linearly correlate to litter decay rates across a salinity and nutrient enrichment gradient. These trends indicate that higher glucosidase EEA will foster greater amounts of CO₂ production. With only HL dry condition soils showing a G and GP enrichment response, further investigation is recommended into glucosidases’ role in respiration of OM, and whether inputs of labile C from greater litter inputs as stands age will increase activity and CO₂ and CH₄ efflux.

The phosphatase enzyme is responsible for hydrolyzing phosphate groups from organic molecules, and its activity is inversely related to P availability in the soil. Wet condition phosphatase EEA is significantly inhibited by G, P and GP enrichments from HL soils (Fig. 17). No significant enrichment effects were found for HH and MR soils from wet conditions (Fig. 17). Conversely, dry condition phosphatase EEA appears to be enhanced under the G enrichment from all soils, but was not significantly different (Fig. 17). While this result is not significant, G enrichment should enhance phosphatase EEA because P is the next limiting nutrient for respiration (discussed above). Additionally, from all dry condition soils, phosphatase EEA is inhibited under the P and GP enrichments (Fig. 17). The reduction in phosphatase EEA with any addition of labile P occurs because orthophosphate (PO₄) is readily taken up by the microbial community without a need for phosphatase enzymes to free organically bound phosphate groups (Fig. 17). Between wet and dry condition soils from all plots, dry condition G enrichment has significantly greater phosphatase EEA than that of wet condition, with no significant difference between Con enrichment (Fig. 17). Conversely, wet condition P and GP enrichments have significantly greater phosphatase EEA than dry condition soils (Fig.
Should conditions remain unchanged at LILA, phosphatase EEA will remain the same between wet and dry conditions (Con enrichment). However, if a form of labile C becomes present, the potential to pull more P from soil is greater.

Glucosaminidase EEA had no significant enrichment effects from any soil in both the wet and dry conditions (Fig 16). This was unexpected because labile C additions should raise the C:N ratio, driving demand for N. From the wet condition HL and MR soils, glucosaminidase EEA was higher than dry condition soils. Wet condition phosphatase EEA was only significantly lower from HL soils with P additions (Fig. 17), and the glucosaminidase EEA results here may be indicating that N limitation may be the cause.

Sulfatase EEA had no significant response to enrichment from all wet condition soils (Fig. 18). Sulfur in the Everglades is not as limiting as P, which may be why results show little enrichment effect on sulfatase EEA. Dry condition soils, however, show an enrichment response from HH and HL soils with GP enrichment (Fig. 18). After labile C and P microbial demands are met, sulfur may be the next limiting nutrient under dry conditions. Wet condition soils all had significantly greater sulfatase EEA than dry conditions soils.

6.6 Implications

The ability to determine georectified relative elevation, knowing stage within 0.3 cm of every collar, and macrocosms sampled for CO₂ efflux benefits estimation of annual C emissions from LILA tree islands. Models generated from LILA tree islands and
ridges can be field evaluated on Everglades’ tree islands and ridges with measures of RWD from any sampling location in the Everglades. These CO₂ efflux models estimating C loss based on accurate RWD at LILA can provide evidence for adaptive management of Everglades’ water levels to slow, or stop, degradation of the tree island and ridge landscapes. Combining C loss estimates with change in biomass estimates (i.e., production) in relation to RWD can indicate what water levels are needed to maintain or build tree island and ridge peat material (Fig. 1; Larsen et al., 2011).

Furthermore, because the interpolations of annual C loss use stage, as RWD, to generate the estimate, it provides an opportunity to estimate annual C loss under various water level scenarios. Use of this model with a rise in daily mean stage of 0.03 m (0.1 ft), the annual loss of C estimates from tree island HH and HL plots are 74 to 160 g C m⁻² yr⁻¹ lower. Conversely, a drop of the same amount in daily mean stage would increase HH and HL C loss by 74 to 160 g C m⁻² yr⁻¹. This indicates that water levels can be managed to reduce or increase C loss depending on management needs. With further investigation into the various other components of the C budget of LILA tree islands and ridges, these estimates can be used to indicate whether tree islands and ridges are building or disappearing. The knowledge gained in respect to the C budget based on water levels can subsequently be applied to Everglades management to slow or reverse tree island loss.
VII. CONCLUSIONS

Soil CO$_2$ efflux, as measured by both *in situ* and laboratory intact soil core incubations, was significantly influenced by water levels, or RWD. Of the two tree islands studied *in situ*, M1 had 21-30% and M2 had 68-73% of variability in soil CO$_2$ efflux explained by RWD. The MR soils also had a significant response to RWD, with 40-65% of variability explained by RWD. Rates of CO$_2$ efflux were lowest when RWD $\geq$ 0 and highest when RWD $<$ 0. Beyond RWD, variability of CO$_2$ efflux presented in this study can potentially be explained by aboveground biomass, litterfall, evapotranspiration-driven water table draw down, and soils building up gas concentrations due to flooding.

*In situ* CO$_2$ efflux measures both root and microbial respiration. The intact soil cores were used to isolate microbial respiration rates from total CO$_2$ efflux. The resulting mean CO$_2$ core efflux ranged from 15-54% of *in situ* efflux which indicates that 46-85% of *in situ* efflux comes from non-microbial forms of respiration. Methane efflux from intact cores was not significantly influenced by RWD, however soils were different by plot collected. Redox potentials were sufficiently low to support methanogenesis, but CH$_4$ efflux was not significantly related to redox.

Annual estimates of C efflux range from 960 to 2,403 and 149 to 921 g C m$^{-2}$ y$^{-1}$ from LILA tree islands and ridges, respectively. These estimates fall within other reported values but as a result of the young age of the tree stands (three-to-five years old) CO$_2$ efflux rates fall are expected to fall as the stand ages.
Quality of organic matter (OM) may also be limiting soil respiration from LILA tree islands. Vial enrichments of tree island soils show significant increases in CO₂ and CH₄ production with labile C, and labile C and P enrichments even though very small amounts of labile C were added. Phosphorus-only enrichments did not significantly influence CO₂ or CH₄ production from wet and dry condition soils with a substantial reduction in the C:P ratio. Potential rates of CO₂ production increased positively with hydroperiod length, indicating that with exposure to oxygen, long hydroperiod sections of LILA could release C.

Due to the variability of EEA in this study, further investigation is recommended for LILA soils. The positive correlation of glucosidase EEA with CO₂ production was significant for both wet and dry condition soils. Dry condition glucosidase EEA was significantly increased by labile C, and labile C and P enrichments, indicating the microbial community may be devoting a greater allocation of energy to less favorable C compound degradation. Wet condition phosphatase EEA was inhibited from Con enrichment HL soils by all other enrichments, while HH and MR soils were not significantly inhibited. Dry condition phosphatase EEA was significantly inhibited by any labile P enrichment, showing that once P limitation is removed, phosphatase EEA is no longer required. Glucosaminidase EEA had no significant enrichment effects. However, glucosaminidase EEA was significantly higher from HL and MR wet condition P enrichment than from the dry condition, which may indicate an N limitation for OM degradation. Sulfatase EEA was significantly greater in all wet condition soils than those of the dry condition. No significant enrichment effects were found from wet condition
soils; however, dry condition HH and HL soils show increased sulfatase EEA with a labile C and P enrichment indicating that dry condition soils have a sulfur limitation.

Results from these experiments combine one-year of in situ measurements and laboratory incubations. LILA tree islands and ridges are still in their developmental stages. Further investigation into soil CO$_2$ efflux, limitations to respiration, enzyme activity, and root and microbial contributions to CO$_2$ and CH$_4$ efflux should be considered. Incorporation of these results, specifically the annual C efflux estimates, into a C budget can indicate whether tree islands and ridges are sequestering or emitting C and help identify water levels that maintain the equilibrium between C production and emission.
LIST OF REFERENCES


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Table 2. Mean annual soil CO₂ efflux estimates from plots on two LIL A tree islands and ridges (mean). P (peat), L (limestone) and R (ridge) indicate substrate of plot. Equation terms are ±SD.

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Table 3. Daily mean soil CO$_2$ efflux annual soil CO$_2$ efflux estimates from study plots on two LILA tree islands and ridges (mean). P (peat), L (limestone) and R (ridge) indicate substrate of plot. Equation terms are $\pm$SD.

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Table 4. Two-way ANOVA table for intact core CO₂, CH₄ and redox response to relative water depth and elevation effects. Response variable: \( \text{CO}_2 = \mu\text{mol CO}_2 \, \text{m}^2 \, \text{s}^{-1} \), \( \text{CH}_4 = \mu\text{mol CH}_4 \, \text{m}^2 \, \text{s}^{-1} \), and redox = Eh; Treatment effect: RWD = relative water depth (m), Elevation = HH, HL or MR.

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Table 5. Redox potential (Eh) averaged (±SD) by intact core after each CH₄ measurement. n = 6

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Table 6. Intact Core and In situ average CO$_2$ efflux rates (µmol m$^{-2}$ s$^{-1}$), and intact cores percentage of in situ efflux.

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Table 7. Methane (CH$_4$) to Carbon Dioxide (CO$_2$) percentage

\[ ([\text{CH}_4/\text{CO}_2] \times 100] \text{ from intact soil cores.} \]

<table>
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<th>RWD (m)</th>
<th>M1HH</th>
<th>M1HL</th>
<th>M2HH</th>
<th>M2HL</th>
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<th>M2R</th>
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<td>BD</td>
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</table>
Figure 1. Diagram showing organic matter production and decomposition relative to water level for tree islands (A) and ridges (B). Figure modified from Larsen et al. (2011).
Figure 2. The Loxahatchee Impoundment Landscape Assessment study area located at the Arthur R Marshall Loxahatchee National Wildlife Area. Study tree islands are indicated (M1W and M2W) and have similar Head High (HH) and Head Low (HL) collar layout. The macrocosm 1 west (M1W) study tree island is a peat core and macrocosm 2 west (M2W) is a limestone core. The middle ridge (MR) plots sampled are located due south of each study tree island.
Figure 3. The recorded stage (m) and rainfall (cm) from Loxahatchee Impoundment Landscape Assessment study area from macrocosm 1 (M1; black) and macrocosm 2 (M2; grey) over the study period April 2010 to May 2012. Grey vertical bars represent periods of in situ measurements with the LICOR LI-8100 or soil collection. The horizontal dashed lines show the mean elevation of study plots Head High (HH), Head Low (HL), and Middle Ridge (MR) in relation to stage.
Figure 4. LICOR 20 cm PVC collar inserted into ground with a 104 long term chamber in open position.
Figure 5. Detailed elevation of stage and plots from the in study period. Vertical grey bars represent seasonal *in situ* field samplings of CO$_2$ efflux with the LICOR LI-8100 infra-red gas analyzer. Shaded regions around Head Low (HL) and Middle Ridge (MR) mean elevations represent the standard deviation of the elevation.
Figure 6. Diurnal pattern of macrocosum 1 (M1) - Head Low (HL) replicate collar CO$_2$ efflux (A) and concentration of CO$_2$ at measurement initiation (B) from measurements taken June 2010. While CO$_2$ efflux (A) is variable throughout the 48 hours measured, it does not show a diurnal pattern like CO$_2$ concentration at measurement initiation (B).
Figure 7. All seasonal measurements of *in situ* CO$_2$ efflux from LILA tree island soils based on relative water depth (RWD). The solid regression line includes M2HH limestone core efflux values, “all tree island” regression presented in Table 2, while the dashed line only contains efflux values from peat sections of tree islands (y = -9.24x + 3.54, $r^2 = 0.34$, n = 624, p < 0.001).
Figure 8. All in situ efflux measurements from the study plots M1HH, M2HH, M1HL, M2HL, M1MR, and M2MR for the study period. The line indicates the interpolated line used in annual CO₂ efflux estimation (Table 2).
Figure 9. Combined macrocosm 1 (M1) and 2 (M2) middle ridge (MR) CO₂ efflux from entire study period. The line indicates the interpolated line used in “all ridge” annual CO₂ efflux estimation (Table 2), n = 270.
Figure 10. Daily mean CO$_2$ efflux from the M1HH, M2HH, M1HL, M2HL, M1MR, and M2MR study plots. The line indicates the interpolated line used in annual CO$_2$ efflux estimation (Table 3).
Figure 11. Daily mean measurements of *in situ* CO$_2$ efflux from LILA tree island soils based on daily mean relative water depth (RWD). The solid regression line includes M2HH limestone core efflux values, “all tree island” regression presented in Table 3, while the dashed line only contains efflux values from peat sections of tree islands ($y = -10.03x + 3.54$, $r^2 = 0.42$, $n = 127$, $p < 0.001$).
Figure 12. Potential CO₂ production (μmol g dw⁻¹ h⁻¹) from Head High (HH), Head Low (HL), and Middle Ridge (MR) soils collected from wet and dry conditions. n = 15; lower case = enrichment significantly different per seasonal condition; + = enrichment significantly higher between conditions; and * = enrichment significantly lower between conditions.
Figure 13. *In situ* CO$_2$ efflux taken from soils treated with Control (C), Nitrogen (N) and Phosphorus (P). Lower case letter = significant difference between treatment (p < 0.05).
Figure 14. Potential CH₄ production (µmol gdw⁻¹ h⁻¹) from Head High (HH), Head Low (HL), and Middle Ridge (MR) soils collected from wet and dry conditions. n = 15; lower case = enrichment significantly different per seasonal condition; + = enrichment significantly higher between conditions; and * = enrichment significantly lower between conditions.
Figure 15. $\beta$-glucosidase extracellular enzyme activity (EEA; $\mu$mol gdw$^{-1}$ h$^{-1}$) from Head High (HH), Head Low (HL), and Middle Ridge (MR) soils collected from wet and dry conditions. HH and HL n = 6, MR n = 4; lower case = enrichment significantly different per seasonal condition; + = enrichment significantly higher between conditions; and * = enrichment significantly lower between conditions.
Figure 16. Glucosaminidase extracellular enzyme activity (EEA; µmol gdw⁻¹ h⁻¹) from Head High (HH), Head Low (HL), and Middle Ridge (MR) soils collected from wet and dry conditions. HH and HL n = 6, MR n = 4; lower case = enrichment significantly different per seasonal condition; + = enrichment significantly higher between conditions and; * = enrichment significantly lower between conditions.
Figure 17. Phosphatase extracellular enzyme activity (EEA; µmol gdw⁻¹ h⁻¹) from Head High (HH), Head Low (HL), and Middle Ridge (MR) soils collected from wet and dry conditions. HH and HL n = 6, MR n = 4; lower case = enrichment significantly different per seasonal condition; + = enrichment significantly higher between conditions; and * = enrichment significantly lower between conditions.
Figure 18. Sulfatase extracellular enzyme activity (EEA; µmol gdw⁻¹ h⁻¹) from Head High (HH), Head Low (HL), and Middle Ridge (MR) soils collected from wet and dry conditions. HH and HL n = 6, MR n = 4; lower case = enrichment significantly different per seasonal condition; + = enrichment significantly higher between conditions; and * = enrichment significantly lower between conditions.
Figure 19. Inverse distant weighted (IDW) of tree aboveground biomass at each LICOR collar from M1and M2 –Head High (HH) and –Head Low (HL) tree island plots. Linear regression: $y = 0.001x + 3.334$, $r^2 = 0.448$, $n = 16$, $p = 0.005$. 
Figure 20. Yearly mean of in situ efflux from tree island LICOR collars vs annual sum of Litter Traps located next to LICOR collars on M1 and M2 –Head High (HH) and –Head Low (HL) plots. Linear regression: $y = 0.014x + 2.925$, $r^2 = 0.56$, $n = 12$, $p = 0.005$. 
Figure 21. β-glucosidase extracellular enzyme activity (EEA; μmol liberated gdw⁻¹ h⁻¹) is highly correlated to potential CO₂ production (μmol CO₂ produced gdw⁻¹ h⁻¹) in wet and dry season. n = 16 for wet and dry conditions. Linear regressions: Dry condition (Blue) y=0.57x + 0.03, R² = 0.75, p < 0.001; Wet condition (Green) y=0.57x + 0.00, R² = 0.60, p = 0.001.