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The Role of Spatiotemporal Heterogeneity in Water and Nutrient Pools in Everglades Plant Community Diversity and Function

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THE ROLE OF SPATIOTEMPORAL HETEROGENEITY IN WATER AND
NUTRIENT POOLS IN EVERGLADES PLANT COMMUNITY DIVERSITY AND
FUNCTION

By

Amartya K. Saha

A DISSERTATION

Submitted to the Faculty
of the University of Miami
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THE ROLE OF SPATIOTEMPORAL HETEROGENEITY IN WATER AND
NUTRIENT POOLS IN EVERGLADES PLANT COMMUNITY DIVERSITY AND
FUNCTION

Amartya K. Saha

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The Role of Spatiotemporal Heterogeneity in Water and   (May 2009)
Nutrient Pools in Everglades Plant Community Diversity
and Function

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Spatial and temporal heterogeneity of water and nutrient pools is closely associated

with the existence of different plant communities in hydrologically-controlled ecosystems

such as the Everglades. These various communities differ in their flooding and fire

tolerances as well as nutrient requirements. Upland plant communities are of particular

ecological significance since they have high biodiversity and provide habitat to terrestrial

fauna, yet comprise less than 10 % of the total area. Restoration and maintenance of such

communities requires an understanding of their water and nutrient requirements. Chapter

2 compares water source utilization in hammocks and pine rocklands on the Miami Rock

Ridge using stable isotopes of water. Hammocks do not flood, while adjacent pinelands

may flood between 2-3 months. In the wet season, hammocks were found to use

phosphorus (P) rich soilwater, a local pool of water and nutrients while pineland plants

primarily relied upon groundwater, the regional pool. Access to a rich pool of P in the

oligotrophic Everglades was associated with higher community-level foliar P

concentration in hammocks. However in the dry season, hammocks utilized groundwater,

which suggests sensitivity to extended droughts. Chapter 3 compares the hammock

(upland or head) and swamp forests (lowland or tail) on tree islands in the Shark River

Slough. Uplands were associated with P-rich soilwater uptake in the wet season, with

regional water uptake in the dry season. Accordingly, tree island heads are rich in foliar P and thereby P-hotspots in the Everglades. Foliar nutrient concentrations can thus indicate limiting nutrient availability in the Everglades. Chapter 4 looks at how leaf phenology patterns are tied to water and nutrient pools. Leaf fall in ridge hammocks is associated with high foliar carbon isotope values over the dry season, which is not the case for tree island hammocks. However, in some species, high levels of foliar nitrogen are also associated with high foliar C^{13} values indicating stomatal limitation of photosynthesis. Growing season for most hammock species is the wet season coinciding with high availability of P, as reflected in high foliar P in this season. Linking water sources to foliar nutrients elucidates roles of water and nutrient pools in leading to different plant communities within an ecosystem.

It was many years ago that I came across "A River of Grass" by Marjorie Stoneman Douglas on a Bombay sidewalk; that was my introduction to the "mysterious" Everglades. After having worked there for the last five years, the mystery has deepened.

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CHAPTER 1

Introduction

Prologue

The 1900s has seen an accelerated worldwide destruction of wetlands largely by
drainage for land development. It is only since the 1970s that the tremendous
ecological importance of wetlands began to be widely realized thereby leading to
efforts towards preservation and restoration. Wetlands have been described as
kidneys of the landscape on account of their role in sequestering waterborne
sediment, nutrients, organics and heavy metals in watersheds. Ecosystem functions of
wetlands include storm/flood water retention, shoreline protection, water-quality
improvement, and wildlife habitat (www.usgs.gov). The Ramsar Convention
(www.ramsar.org) has initiated the compilation of a global list of natural wetlands
with high biodiversity and ecosystem service values. Wetlands and estuaries
typically have a mosaic of diverse plant communities, many of which can tolerate
inundation to various extents, while a few (upland communities) cannot tolerate any
flooding. This diversity results from the interaction of topography with water levels
that determines soil moisture, hydroperiod, water depth and nutrient availability. Fire
and biotic effects, such as alligator holes and bird rookeries can also influence the
heterogeneity of nutrient distribution. Thus, plant community distribution, especially
in hydrologically-controlled ecosystems, is primarily governed by the temporal and
spatial distribution of water and nutrient pools. Identifying the pools used by each
plant community is central to the restoration and conservation of these plant

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communities. This understanding can also help predict the fate of these communities with altered hydrological and nutrient flow processes as a consequence of direct management and/or climate change.

Water and nutrients exist in different pools on the landscape

In any ecosystem, plant community composition is influenced by a variety of biotic and abiotic factors. The former include presence of pollination and seed dispersal agents, herbivory, allelopathy and competition for light and nutrients. Abiotic factors comprise of light availability, climate, soil types, water, nutrient availability and fire. Of these, water and nutrients are often the environmental factors that most strongly constrain terrestrial productivity (Lambert *et al.*1998).

Variability in soil and topography together with the hydrological and nutrient cycles can create different pools of water and nutrients over space and time (Poulsen *et al.* 2006, Grimm *et al.*2003). For instance the flat topography of the Caribbean basin leads to intermixing of saline seawater and rain-derived freshwater thereby giving rise to a mosaic of different vegetation types exhibiting different salinity tolerances (Sternberg *et al.* 1991, Sternberg & Swart 1987). Pools of water may be spatially distinct, such as shallow rain-derived soilwater and deep groundwater in Venezuelan savannas, where shallow-rooted grasses and shrubs depend upon the former while deep-rooted trees can also access groundwater (eg. Sarmiento 1984). Gallery forests along watercourses in the Cerrado savannas in Brazil act as sinks for nutrients leached from higher plateaus, trapping them within their biomass (Haridasan 2002), thus functioning as a capacitor in nutrient flux in the ecosystem. Biotic

processes can also contribute to the formation of nutrient pools; leaf cutter ants concentrating nutrients in their nests (Moutinho *et al.* 2003), termite mounds in savannas (Haridasan 2002) and fecal droppings in bird roosting sites (Gann et al.2005) are some examples of nutrient patches on the landscape arising from the activities of fauna. Hydrologically controlled ecosystems such as wetlands, estuaries and savannas often have patterns of forested islands on a grassland or marshy landscape, which represent local concentrations of nutrients on a relatively oligotrophic matrix. Such a juxtaposition of local and regional pools are illustrated in Figure 1.1. In addition to spatial variation, there can be seasonal shrinking or expansion of these pools. The existence of multiple pools of water allows species within a community to differ in their sources and modes of water uptake which in turn determines differential access to nutrients since water and nutrient uptake are coupled processes.

Water and nutrient pools are coupled together

Discerning the actual water and nutrient sources utilized by plants in wildland ecosystems can be complex given the numerous possible sources, competition with other plants and soil microorganisms and the seasonal/diurnal transience of these sources. The presence of roots does not necessarily imply water and nutrient uptake from that zone at a point in time (eg. Romero-Saltos *et al.*2005). Stable isotope analysis (described below) has been widely used to infer water sources since different water pools in an ecosystem often have different isotopic identities. While ^{15}N stable isotopes can be used as a tracer to track nutrient uptake from a particular depth of the soil, it will be unfeasible to infer all the nutrient sources for a single plant. Scaling up

to ecosystem level nutrient cycling represents a further challenge owing to interspecific differences in nutrient uptake and translocation (Haridasan 2002). One possible approach can be to couple potential water sources and the nutrients made available through these sources in order to infer nutrient sources of a plant, since most nutrient uptake happens in the dissolved form.

Absorption of nutrients from the soil via roots is the most common and significant pathway for nutrient uptake. Other pathways include foliar absorption of nutrients, association with rhizobia and mycorrhizae and entrapment of insects by carnivorous plants (Lambert et al 1998). What links the water pools to the nutrient pools from a plant uptake perspective is that in almost all situations, presence of water in the rhizosphere appears to be a prerequisite for nutrient uptake. Practically all nutrient uptake occurs from the soil solution (Baldwin 1975, Comerford 2005) where they occur as ions. Furthermore, interception of nutrients by growing roots is on a lower scale as compared to the volume of nutrients that arrive at the root surface via massflow and diffusion, both processes that require presence of soil water (Lambert *et al.* 1998, Comerford 2005). Mycorrhizal associations are also able to absorb nutrients in a wider zone outside the rhizosphere and are especially significant for relatively immobile P (Lambert *et al* 1998, Allen 1991). They too require soil moisture to stay alive. Thus soil moisture is inextricably connected with nutrient uptake. As an instance, the lack of surface water in the dry season in seasonal ecosystems such as savannas implies little or no nutrient uptake in this period. (eg. Haridasan 2002, Scholz 2002).

Areas of research in this dissertation

For this dissertation I focus on the upland and adjacent flooded plant communities in the Everglades National Park at both the community and the species level (Fig 1.2). The terrestrial portion of the Everglades National Park (ENP) ranges from sea level to just over 3 meters above sea level. Within this elevation span exists a mosaic of plant communities – mangroves, cypress domes, long and short hydroperiod marshes, sawgrass savannas, bayheads, pine rocklands (or pinelands) and tropical hardwood hammocks. This mosaic is primarily the result of the interplay of topography with water levels that leads to a range of hydroperiods and depths as well as spatial heterogeneity in nutrient availability and protection from fire. Most of the ecosystem is oligotrophic with small patches of high nutrient availability that are primarily associated with the upland or unflooded communities, which are hammock forests on the highest elevations in the Park, ie the tree islands in the sloughs and the Miami Rock Ridge that is the eastern boundary of the Park. These upland communities comprise less than 10 % of the park area, yet are high in floral diversity as well as provide critical habitat to fauna since these are the only areas above water in the wet season. The significance of hammocks in the Everglades ecosystem has led to their restoration being one of the major objectives of the Comprehensive Everglades Restoration Program (CERP).

The Everglades is an ideal system to identify the various pools of water and nutrients associated with the diverse plant communities that are subject to the same climatic forcing function. In the second chapter, I investigate the water sources and

foliar nutrient status of hardwood hammocks and pine rocklands that are adjacent yet

vastly different plant communities on the Miami Rock Ridge. Hammocks never

flood, while pinelands vary in their hydroperiod depending on the site from 0-3

months. While fire is the main delineating factor, hammocks have a much higher

productivity and biomass suggesting different water source usage and attendant

nutrient stocks as compared to pinelands. In the third chapter, I look at the two

distinct plant communities on tree islands in the Shark River Slough -- upland

hammock and flooded swamp forests, in how they differ in their water sources and

foliar nutrients, to understand better the precarious positioning of flood-intolerant

hammocks in the slough that is flooded all year round. In the fourth chapter, I relate

the variety of leaf phenological patterns (leaf fall and leaf growth) present in the

seasonal moist hammock and swamp forest communities with the periods of water

and nutrient availability. I conclude with how the technique of identifying water and

nutrient pools can be applied towards understanding the structure and function of

plant communities in any ecosystem along with further areas of work.

The use of stable isotopes in ecology

As mentioned earlier, pools of water can differ in their isotopic composition, and

thereby the relative contribution of each source to plant stemwater may be calculated.

Here I briefly introduce the use of stable isotopes in the areas of ecology relevant to

this research.

A chemical element is said to have isotopes if atoms differ in the number of

neutrons while having the same number of protons and electrons. Thus isotopes differ

in mass but not in electrical charge. The similarity in charge between isotopes of an element results in their chemical properties being the same. Stable isotopes are those that do not decay over time. For instance, carbon has three isotopes, ^{12}C, ^{13}C and ^{14}C. ^{14}C is unstable being radioactive, while ^{12}C and ^{13}C are the stable isotopes, with 6 and 7 neutrons respectively. For an element, the heavier isotope is usually much less abundant than the lighter isotope. So in the biosphere, the heavier ^{13}C constitutes 1.11% of total carbon while the lighter ^{12}C adds up to 98.9% (Fritz and Fontes, 1980). Both the component elements of a water molecule, hydrogen and oxygen have isotopes. Hydrogen has two isotopes, ^{1}H (with one neutron) and deuterium, D or ^{2}H (with two neutrons), while oxygen has three – ^{16}O, ^{17}O and ^{18}O with 8, 9 and 10 neutrons respectively. ^{17}O is extremely rare. Water molecules having ^{18}O or D would be heavier than ones without the heavy isotopes. This has significance in evaporation and precipitation processes where lighter molecules evaporate at a faster rate while heavier molecules condense earlier (Clark & Fritz, 1997). Lighter molecules require lesser energy to leave the liquid phase and thus evaporate at a faster rate than the heavier molecules. Thus a partially evaporated pool of water gets enriched in heavier isotopes. This incomplete transfer of mass between phases is termed fractionation and is the reason why different bodies of water can have different isotopic signatures at an instant of time.

The isotopic signature or composition, δ is expressed as a ratio (R) of the heavier to the lighter isotope, with reference to an internationally defined standard as follows:

δ (parts per thousand or ‰) $=$ $\{(R_{sample}/R_{standard}) - 1 \} * 1000$

For water, the international standard is Vienna-Standard Mean Ocean Water (V-SMOW).

In most ecosystems, plants do not discriminate against either the heavier or lighter isotopes; hence the isotopic composition of plant stemwater (xylem) is a reflection of the sources taken up by the plant (Wershaw et al 1970). Exceptions to this have been discovered in mangroves (Lin & Sternberg 1993) and some desert halophytes (Ellsworth &Williams 2007) that discriminate against the heavier deuterium. To date no plant water uptake fractionation has been noted against ^{18}O which is why ^{18}O can be employed for determining the sources taken up by a plant.

Leaf water can get evaporatively enriched due to transpiration from stomata. Similarly, water in green or unsuberized stems can get evaporatively enriched. Hence it is imperative to sample well- suberized stems for plant stemwater, as far away from leaf petioles as possible.

Another isotope widely used is the stable isotope of carbon, ^{13}C that can be employed in discerning photosynthetic processes and in recording photosynthetic limitations caused either by water stress in plants (Farquhar *et al* 1982) or by high levels of foliar nitrogen (eg. Cordell et al 1999, Bai et al 2008). The universal standard for carbon is the PeeDee belemnite formation of South Carolina. Sucrose and other plant compounds have a lower ^{13}C content relative to atmospheric CO_2 as a result of a two-step discrimination against the heavier ^{13}C isotope: an initial

fractionation of around 4‰ in stomatal diffusion of CO_2 and then further discrimination depending on the photosynthetic pathway. The carboxylation enzyme ribulose-1, 5-biphosphate carboxylase (Rubisco) is associated with a discrimination factor ~ 30‰ in C3 plants which have an average value of -28‰; while in C4 plants the primary carboxylation enzyme, phosphoenolpyruvate carboxylase (PEP) has a much smaller discrimination (Deines, 1980), and thus C4 plants have an isotopic composition of ~ 12‰. Furthermore, there exists considerable variation in carbon isotope signatures within C3 plants that results from variation in intercellular concentrations of carbon dioxide inside the leaf stemming from environmental conditions. For instance, low atmospheric humidity in the dry season creates a high vapor pressure deficit that can induce stomatal closure in leaves so as to decrease transpirational losses of water. Stomatal closure stops further entry of CO_2 into the leaf from the atmosphere, thereby limiting available intercellular CO_2 for fixation, which in turn then leads to lower discrimination by Rubisco against the heavier ^{13}C isotope (Farquhar et al 1982).

Carbon isotope fractionation in C3 plants is described by the following equation

$$\delta^{13}C_{plant} \ = \ \delta^{13}C_{atmosphere} - a - (b-a) \ c_i/c_a$$

where c_i/c_a refers to the ratio of the partial pressure of carbon dioxide outside and inside the leaf, a symbolizes the difference in diffusivities through stomatal pores of ^{13}C and b represents the isotopic fractionation by Rubisco (Farquhar et al, 1982). High values of $\delta^{13}C$ can also result from high foliar nitrogen concentrations that are associated with high levels of photosynthesis (Field &Mooney, 1986). Stomata then pose a limit to the amount of carbon dioxide entering the leaf, even when stomata are

fully open. The limited availability of carbon dioxide inside the leaf then results in reduced discrimination as described earlier.

The stable isotope of nitrogen, $\delta^{15}N$ is used as a tracer for nutrient uptake. Atmospheric N_2 is considered as the global standard for nitrogen. Plants fractionate against ^{15}N both during uptake as well as in biochemical reactions leading to the incorporation of N in plant compounds. Foliar $\delta^{15}N$ has also been used as an indicator of phosphorus availability (eg. Crews 1993, McKee *et al* 2002, Inglett *et al* 2006) with an increase in $\delta^{15}N$ (more enriched, less negative values) being associated with increasing P availability. An increase in P uptake requires a concomitant increase in N uptake, since plant tissue has a certain stoichiometric range of P and N that depends upon the constituent compounds present in the tissue. The resulting increased demand for N reduces discrimination against ^{15}N if the amount of available N is limited.

Foliar nutrient concentrations as indicators of nutrient availability

Foliar nutrient concentrations are widely used as a measure of nutrient status and productive potential (eg. Vitousek et al, 1995) provided sampling takes into account temporal variations in concentration due to phenology and leaf age. The response of plant communities to increased nutrient availability can be complex: (i) higher foliar concentrations (ii) greater growth and thus greater foliar area and/or (iii) altered competition within the communities possibly leading to species replacements (eg. Aerts and Berendse, 1988). Numerous studies concerning plants in the wild (involving either a comparison across an existing soil nutrient gradient or experimental fertilization) have shown that higher nitrogen (N) and phosphorus (P)

availability can lead to higher foliar N and P concentrations (Aerts and Chapin, 2000, Campo and Dirzo, 2003, Chapin, 1980, Fisher *et al.*, 2006, Harrington *et al.*, 2001, Lower *et al.*, 2003, Vitousek et al 1995). Periods of high nutrient availability in oligotophic ecosystems can also lead to luxury consumption by plants with attendant increase in foliar concentrations because of storage in vacuoles (Bertiller *et al.*, 2006, Tripler *et al,* 2002, Chapin, 1980). Studies have also shown that often high soil nutrient concentration areas are populated by communities of species having inherently higher foliar nutrient concentrations (Boerner, 1984, Fensham and Bowman, 1995, Santiago *et al.*, 2005). At the same time there also exist species with very low plasticity in their foliar nutrient concentrations, such as sawgrass that does not respond as much to an increase in P as cattails.
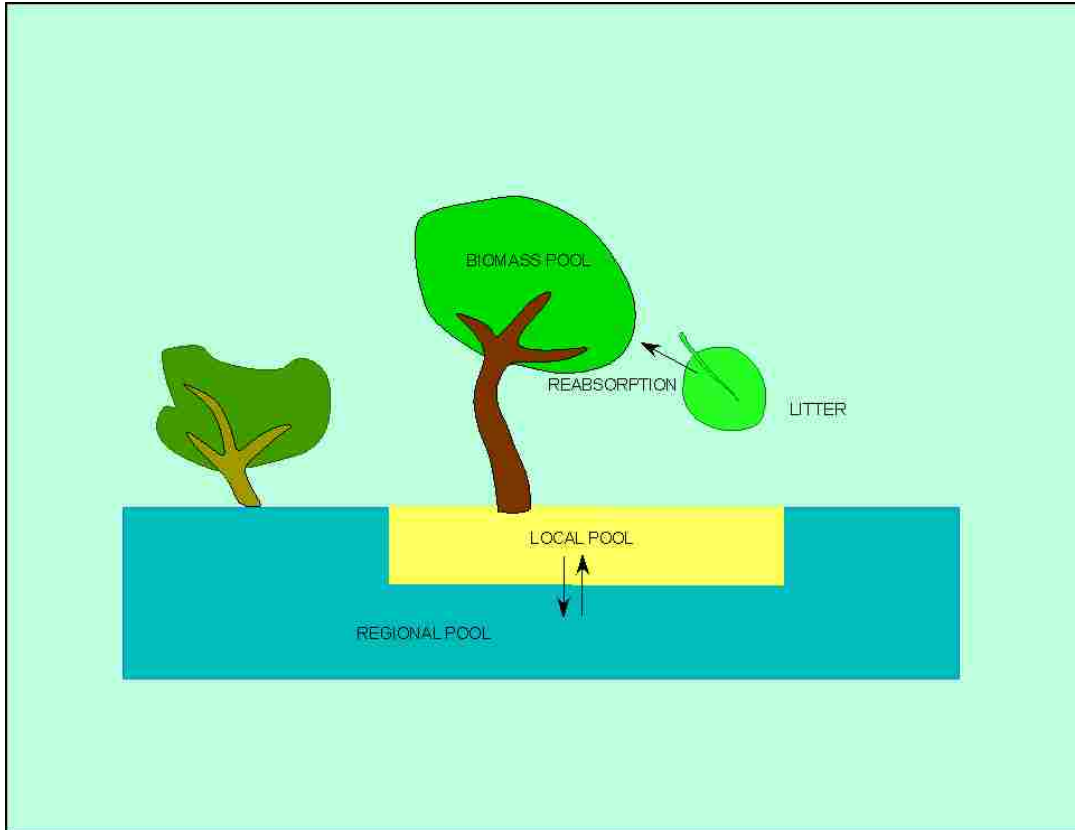
Figure 1.1: Conceptual illustration of local and regional pools of water and nutrients on the landscape. Local pools of water and/or nutrients are caused by abiotic factors, such as topographical highs or lows, or by biotic factors such as termite mounds, and represent concentrations or hotspots, while the landscape has a typically lower background nutrient concentration, that is the regional pool.
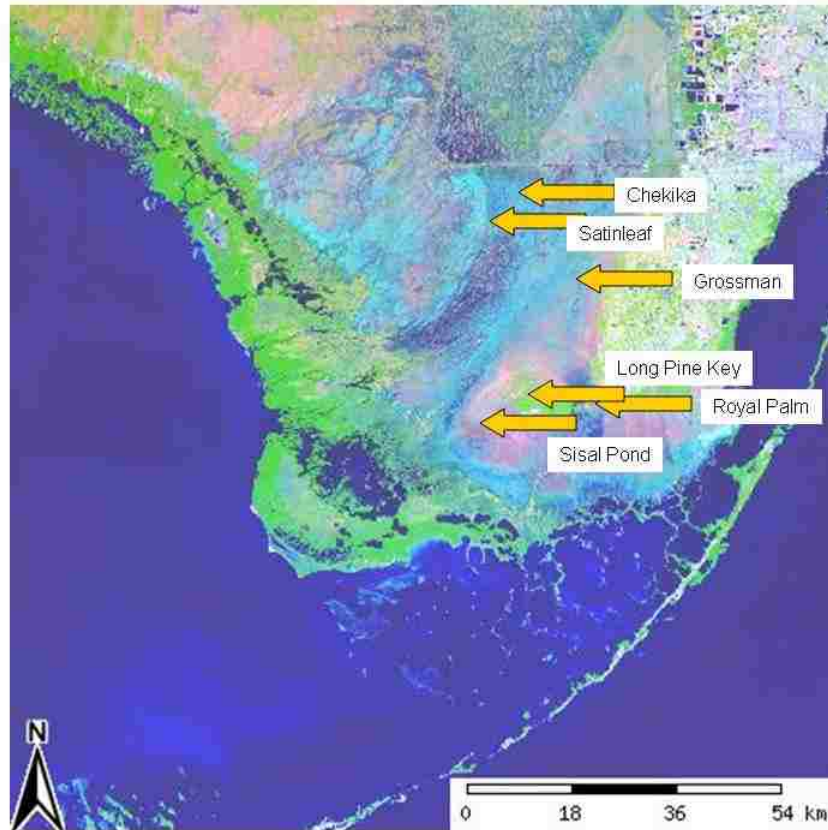
Figure 1.2: Study sites at Everglades National Park, Florida, USA. The hammock and pineland sites were at Royal Palm, Long Pine Key and Sisal Pond, while the Shark River Slough tree islands studied were Chekika, Satinleaf and Grossman Hammock. Locations shown on this map are approximate.

CHAPTER 2

Linking water sources with foliar nutrient status in hardwood hammock and pine rockland plant communities in the Everglades National Park, USA[1]

Summary

Plant communities within an ecosystem can vary in water source usage and nutrient availabilities, thus permitting community coexistence and diversity. I investigated the differences in water source utilization in two ecologically important upland plant communities: tropical hardwood hammocks and pine rocklands in the predominantly flooded Everglades ecosystem. I then linked these differences with their foliar nutrient levels and photosynthetic performance as measured by $\delta^{13}C$ abundance. Based on a comparison of $\delta^{18}O$ of plant stem waters with those of potential water sources (nutrient-poor groundwater and nutrient-rich water in organic litter referred to as soilwater), I observed that during the wet season hammock plants relied on soilwater while in the dry season they relied on groundwater. A similar seasonal shift was observed in pineland plants, however groundwater constituted the major part of water uptake throughout the year except for late wet season. Consistent with the nutrient concentration of different water sources used in the two communities, hammocks had a greater annual mean foliar nitrogen and phosphorus concentration at the community level over pinelands as well as a higher leaf area index. High foliar N concentration in hammock plants was associated with eventual stomatal limitation of photosynthesis. Hammock species being intolerant of flooded soils are restricted to

water uptake in the shallow unsaturated soil layer in the wet season, yet access the lowered groundwater table in the dry season. This dependence on a relatively narrow annual range of water table levels should be considered in South Florida water management and Everglades restoration.

Background

Coexisting plant communities in an ecosystem can differ widely in their water sources and nutrient availabilities thereby leading to distinct ecohydrological functioning patterns within the same ecosystem. This is especially evident in hydrologically controlled ecosystems such as the Everglades in South Florida. The Everglades ecosystem is the largest subtropical wetland savanna in North America with a high degree of floral endemism and direct links with the marine ecosystem of Florida Bay. The terrestrial portion of the Everglades National Park (ENP) ranges from sea level to just over 3 meters above sea level. Within this elevation span exists a mosaic of plant communities – mangroves, cypress domes, long and short hydroperiod marshes, sawgrass savannas, bayheads, pine rocklands (or pinelands) and tropical hardwood hammocks in ascending order of elevation. Such a diversity of communities is largely a consequence of the wide range of interactions between water flow, hydroperiod, nutrients, fire and faunal effects that change with minor differences in topography, typically less than 1 meter (Wetzel *et al.*, 2005; White, 1994). Each community is associated with a certain hydroperiod range, and the small elevational differences between communities can result in a high degree of sensitivity to water level fluctuation. This is especially true for upland (emergent) communities which usually do not flood, or do so for very short periods of time. An example of

the sensitivity of upland communities is the loss of tree islands in the Everglades over 1950-1970 (Lodge, 2005). This has been attributed to both prolonged flooding by water releases from canals that led to mortality of flood-intolerant hardwood species as well as prolonged dry conditions that led to peat fires lowering tree island elevation rendering them more vulnerable to subsequent flooding (Sklar *et al.*, 2004, Wetzel *et al.*, 2005). Because of this, knowledge of the sensitivity of upland communities to changing hydroperiods is critical in the management of water resources, which has to address both South Florida urban needs and ecosystem preservation (Lockwood *et al.*, 2003, Wilcox *et al.*, 2004).

Because of its low elevation and relatively flat topography most of the Everglades are inundated in the wet season and part of the dry season with the exception of the highest parts of limestone outcrops and the Miami Rock Ridge to the east. The Miami Rock Ridge is part of the Atlantic coastal ridge, a mid-Pleistocene marine limestone ridge < 3 m.a.s.l. running along the SE coast of Florida and ending in the southeastern Everglades (Hoffmeister, 1974) as a series of transverse ridges interspersed with glades. Hammocks occur on the highest parts of this ridge that rarely flood, while pinelands often occur adjacent to the hammocks, and depending on the site, are prone to flooding for 0-3 months (Gunderson, 1994). These two upland plant communities differ in many aspects, key ones including the presence of flood-intolerant species in hardwood hammocks (Carr, 1973) and the paucity of soil with mainly exposed limestone in fire adapted pinelands. With the exception of a previous study in which comparative measurements were taken once during the dry season and once during the wet season (Ewe *et al.*, 1999), not much is known about

pineland and hammock ecohydrological functioning throughout the year.

Specifically, little is known about their water and nutrient sources and how these may

change with water levels over the course of the seasons.

Hardwood hammocks are composed of neotropical evergreen broadleaf trees with

a profusion of epiphytes. The closed canopy maintains a humid microclimate inside

the hammock that, along with the moist organic soil horizon, is largely responsible

for deterring most fires (both lightening-induced and anthropogenic) ranging in from

pinelands (Snyder, 1990). Only large episodic fires can burn down hammocks (Carr,

1973, Craighead, 1974, Robertson and Platt, 2001). Thus fires maintain a distinct

boundary between hammocks and pinelands (Lodge 2005, Slocum et al 2003). The

organic soil layer that has built up by litter deposition and decomposition directly

over the limestone bedrock in hammocks is in general about 5000 years old

(Loveless, 1959) and on average 10-30 cm thick. In contrast, high fire frequency in

open canopy pine rocklands prevents any substantial organic soil formation (Fig. 2.1),

except in sinkholes and temporary surface accumulations between successive fires.

This relative paucity of soil in pinelands implies that pineland plants depend largely

on groundwater (water table at 0-1 meter depth) as the main water source (Ewe et al,

1999). Hardwood hammock plants, in addition to groundwater may also utilize rain

water trapped in the litter layer (henceforth referred to as soilwater), a potentially

significant amount owing to the high water holding capacity of the spatially

continuous litter horizon.

From a nutrient availability perspective, the Everglades is an oligotrophic

ecosystem (Davis, 1994, Wetzel *et al.*, 2005) with extremely low levels of

phosphorus. Because plant nutrient uptake mainly occurs in solution (Baldwin, 1975), pineland plants are thought to have access to limited amounts of nutrients owing to their primary reliance on groundwater that has low nutrient concentrations. Association with ectomycorrhizae can increase nutrient availability to pineland plants than just dependance on roots alone (Janos-personal communication); even so, the pool of nutrients available is extremely dilute. Hammock plants, on the other hand, may have access to a more concentrated pool of nutrients that is present in soilwater (trapped rainwater) stemming from litter decomposition and faunal sources in the litter layer.

In this study, I characterize soil depth and plant-available nitrogen (N) and phosphorus (P) in water sources of hammock and pineland plants. I then investigate the potential water sources (groundwater and soilwater) used by the hammock and pineland plants over a year using stable isotope techniques that have been widely used for plant source water determination in coastal ecosystems (Ewe *et al.*, 1999, Ewe and Sternberg, 2002, Greaver and Sternberg, 2006, Lin and Sternberg, 1992, Sternberg *et al.*, 1991). I also test whether the higher levels of exchangeable nutrients in hammock soils compared to groundwater would be reflected in higher foliar nutrient concentrations in hammock communities relative to pineland communities. Foliar nutrient concentrations are widely used as a measure of nutrient status and productive potential (eg. Vitousek et al, 1995) by ensuring proper sampling that takes into account temporal variations in concentration due to phenology and leaf age. The response of plant communities to increased nutrient availability can be complex: (i) higher foliar concentrations (ii) greater growth and thus greater foliar area and/or (iii)

altered competition within the communities possibly leading to species replacements (eg. Aerts and Berendse, 1988). Numerous studies concerning plants in the wild (involving either a comparison across an existing soil nutrient gradient or experimental fertilization) have shown that higher nitrogen (N) and phosphorus (P) availability can lead to higher foliar N and P concentrations (Aerts and Chapin, 2000, Campo and Dirzo, 2003, Chapin, 1980, Fisher *et al.*, 2006, Harrington *et al.*, 2001, Lower *et al.*, 2003, Vitousek et al 1995). Periods of high nutrient availability in oligotophic ecosystems can also lead to luxury consumption by plants with attendant increase in foliar concentrations because of storage in vacuoles (Bertiller *et al.*, 2006, Tripler et al 2002, Chapin, 1980). Studies have also shown that often high soil nutrient concentration areas are populated by communities of species having inherently higher foliar nutrient concentrations (Boerner, 1984, Fensham and Bowman, 1995, Santiago *et al.*, 2005). I refer to this effect as a community-level effect. Since higher soil nutrient availability can also lead to greater growth and leaf biomass, I indirectly compare foliar biomass in hammocks and pinelands using the Leaf Area Index (LAI) (Jordan, 1969) as a proxy in each community.

I specifically test the following hypotheses:

Hypothesis 1: Hammocks and pinelands differ in their water utilization throughout the year. In the wet season, hammock plants depend more on soilwater while pineland plants depend more on groundwater. In the dry season, plants in both communities increasingly utilize groundwater

Hypothesis 2: The foliar nutrient (N and P) content at the community level will reflect the nutrient status of water sources utilized in the respective communities, with hammocks showing higher foliar nutrient levels than pinelands

Hypothesis 3: The differences in nutrient status will affect photosynthetic properties as measured by the carbon isotope ratios of leaf tissues.

Material and methods

Study area: The study took place in the Everglades National Park (ENP) from March 2005 to March 2006. The climate is subtropical and humid, with an average annual rainfall of 1450 mm (http://sofia.usgs.gov), more than half of which occurs in the wet season that lasts from June to October. The hammocks on the Miami rock ridge and tree islands in the Shark River Slough constitute the northern boundary of the range of the neotropical tree species that have established owing to winters being free of frost in most years (Lodge, 2005). Periodic hurricanes pass through south Florida every 3-5 years and can cause considerable structural damage in hammocks and pinelands by toppling over shallow-rooted trees (mostly in hammocks) and by breakage of the main trunks known as snap-offs (mostly in pinelands).

Three previously studied sites (Ewe *et al.*, 1999) differing in elevation and hydroperiod were selected on the Miami Rock Ridge within ENP along an east-west increasing rainfall gradient at Royal Palm (25°23'39"N, 80°37'17"W), Long Pine Key (25°24'10"N, 80°39'05"W) and Sisal pond (25°23'29"N, 80°37'45"W). Long Pine Key had the highest elevation (Ewe *et al.*, 1999) and neither the hammock nor the pineland was flooded during the study period, although the groundwater level in

pineland sinkholes was within 10 cm of the mean surface in July 2005. Parts of

Royal Palm pineland were briefly flooded, while the pineland at Sisal Pond remained

continually flooded (water table above ground surface) from mid June to the end of

September. Each site was located at the interface between a hammock and a

pineland. Pinelands in the ENP are subject to a burn cycle of 3-7 years, either

lightening-induced or set by the National Park Service. The pineland site on Long

Pine Key experienced fire in June 2006 that completely burned the understory to

expose bedrock.

Characterization of soil depth to bedrock: A line transect was laid out perpendicular

to the border between the hammock and pineland at each site, extending 100 m into

each community. Every 2 meters a 1 cm diameter metal rod was pushed down until

bedrock or an impenetrable layer was reached, and the depth noted to the nearest

centimeter.

Plant-available nutrient concentration in soil and groundwater: In May 2005 at the

onset of the wet season, 5 samples of soil (0-15 cm depth) per site from Royal Palm

and Long Pine Key were analyzed at the Agricultural Analytical Services Laboratory,

University of Pennsylvania, USA for exchangeable P (Mehlich 3 soil test), nitrate

nitrogen (NO_3-N) and ammonium nitrogen (NH_4-N) (ion electrode method) with the

precision of analysis reported as $\pm$ 0.1 ppm ($\pm 1\sigma$). for P, 0.6 ppm and 1 ppm for

nitrate and ammonium nitrogen respectively.   Soil solution concentrations of the

above nutrients were calculated from the above lab measurements (dry soil values)

based on a wet season water content of 230% (dry wt. of soil), a value chosen within

the range 28% -250% measured by Ewe et al (1999) and the assumption that all the

plant-available nutrients would be in solution. Groundwater was sampled from wells in the same sites at the same time as soils and analyzed at the University of Georgia Soil, Plant and Water laboratory, Athens, GA for NH_4-N, NO_3-N and phosphate (PO_4) with precision of analysis reported to be within ± 0.001ppm (1σ).

Collection of sourcewaters and plant stemwater: Groundwater was sampled from wells at Royal Palm and Long Pine Key every two weeks over the study period after pumping out the standing water (that could be evaporatively enriched in ^{18}O and D) and allowing recharge. The Sisal Pond site did not have a well to sample groundwater. Rainwater collectors (glass bottles with a 1cm layer of mineral oil to prevent evaporation) were placed in both the hammock and the pineland at each of the three sites and collected at the same time as groundwater. Both rain and groundwater samples were placed in scintillation vials, sealed with Parafilm (Pechiney, Chicago, IL, USA) to prevent evaporation and refrigerated in the lab. Five replicates of soils in each community were collected at each site every two months over a range of 0-20 cm depth. These were placed in stoppered glass tubes, sealed with Parafilm and stored in a freezer to avoid fungal/microbial respiration.

Every two months over the year, 25 plants (5 replicates per species for 5 species) were sampled in the hammock as well as in the pineland for each site, to make up a total of 50 plants per site. Individuals were marked with flagging tape for future sampling. Plants were sampled to 50 meters inside each community. I sampled species that were the most abundant at each site, keeping in mind that species diversity is higher in the interface of the two communities than inside the hammock (Alexander, 1967). Species sampled in the pineland were: (i) Royal Palm site:

Guettarda scabra, Lysiloma latisiliqua, Myrica cerifera, Quercus pumila, Pinus elliottii var densa (ii) Long Pine Key: *G. scabra, L. latisiliqua, Tetrazyga bicolor, M. cerifera, Myrsine guianensis (iii)* Sisal Pond site: *Ilex cassine, Crossopetalum ilicifolium, M. guianensis, M. cerifera, Persea borbonia, P. elliottii var densa.* For hammocks, I sampled the following *(i)* Royal Palm: *Bursera simarouba, L. latisiliqua, G. scabra, Quercus virginiana, M. cerifera (ii)* Long Pine Key: *B. simarouba, Chrysobalanus icaco, G. scabra, L. latisiliqua, M. cerifera (iii)* Sisal Pond: *L. latisiliqua, M. cerifera, Q. virginiana, P. borbonia, Annona glabra.* Despite being flood-tolerant and thus uncharacteristic of hammocks where the majority of species cannot survive on flooded soils, *Annona glabra* was included simply because numerous individuals existed at the Sisal Pond hammock site. For each plant, a well suberized stem was cut and after removal of the bark and phloem, placed in a sealed glass tube, parafilmed and frozen on return to the lab. Branches high up in the canopy were accessed using a 5 m extensible pole pruner (Corona, CA, USA).

Water extraction and isotopic analysis: Stem and soilwaters were extracted either by the method of West et al. (2006) or Vendramini & Sternberg (2007). Extracted water was analyzed at the Laboratory of Stable Isotope Ecology in Tropical Ecosystems (LSIETE) at the University of Miami for oxygen and hydrogen isotope ratios by equilibration on an Isoprime© Isotope Ratio Mass Spectrometer connected to a Multiflow© system (Elementar, Germany) as described by Vendramini & Sternberg (2007). Oxygen and hydrogen isotope ratios are reported here as $\delta^{18}O$ and δD values respectively and calculated as:

$$\delta^{18}O \text{ or } \delta D \;=\; \{(R_{sample}/R_{SMOW}) - 1\} * 1000 \qquad\qquad \text{---(1)}$$

where R_{sample} and R_{SMOW} represents the heavy to light isotope ratio of the sample and the standard respectively. The standard for water isotope ratios used here is Vienna standard mean ocean water (vSMOW) and the precision of analysis of ±0.1‰ and ±2.0‰ (1σ) for oxygen and hydrogen isotopes respectively.

Foliar nutrient analysis: 10-15 mature leaves for each individual sampled for stemwater were collected from the same branch as the stem for water, placed in paper bags and subsequently dried in an oven at 50° C for 72 hours. Dried leaves were then ground and mixed well to homogenize the samples, in preparation for foliar N, P and $\delta^{13}C$ analysis at LSIETE. Preparation of samples for P analysis consisted of ashing 700 mg of the dried ground leaves in porcelain cups in a furnace maintained at $600^{O}C$ for 6 hours followed by digestion in 4% sulfuric acid and filtration through glass fiber filter paper (Whatman, UK). Analysis was carried out on the filtrate. Foliar P concentration was obtained by the USEPA method 365.1 (USEPA, 1984) based upon the molybdate blue colorimetric approach (Fiske and SubbaRao, 1925) using an Alpkem 3000 Phosphorus analyzer (Alpkem, OI Analytical, Texas, USA) and expressed on a weight percentage basis. The precision of analysis was ± 0.1 ppm (±1σ). Foliar N concentration in 5 mg ground leaf samples was obtained using an NC 2100 analyzer (Thermoquest CE Instruments, Milan, Italy) and expressed as the percent weight ratio of total N relative to total leaf dry mass, with precision of analysis 1 ppm (±1σ).

Foliar $\delta^{13}C$ analysis: Ground leaf samples (5 mg) were loaded in individual tin cups (Elemental Micro-analysis, Milan, Italy), rolled into tight balls which were placed in

an automated elemental analyzer (Euro-EA-Elemental Analyzer, Eurovector, Milan, Italy) connected to a continuous flow isotope ratio mass spectrometer (Isoprime, GV, Manchester, England).

Carbon-13 abundances are expressed as $\delta^{13}C$ values:

$$\delta^{13}C(‰) = [(R_{sample}/R_{PDB})-1]\times1000 \qquad\qquad\text{---(2)}$$

in which R_{sample} and R_{PDB} represent the $^{13}C/^{12}C$ ratios of the sample and the universal standard from the PeeDee belemnite formation of South Carolina. The precision of analysis was $\pm 0.1‰$ $(\pm1\sigma)$.

Leaf area index (LAI) analysis: Hemispherical (fisheye) images of the canopy in hammocks and pinelands were obtained at dawn using a Nikon Coolpix 4500 with an 8 mm Nikon LC-ER1 fisheye lens. The camera was positioned at a height of 1 m from the ground surface. LAI estimates were obtained from these images by the software Gap Light Analyzer (Frazer et al 1999). The emphasis was more on comparing LAI between hammocks and pinelands rather than obtaining the absolute values.

Data analysis: To examine how similar the plant stemwater of pinelands and hammocks are to groundwater, a potential source, I regressed the average $\delta^{18}O$ values of stemwater from each community and site throughout the year against those of groundwater (SPSS, Chicago, USA) using Model II regression, despite having just one groundwater $\delta^{18}O$ value per site due to one well per site. This was carried out only for Royal Palm and Long Pine Key sites where groundwater was collected.

 For quantifying the relative contribution of soilwater and groundwater to stemwater, and to see how this contribution may change with season, I computed the proportion of the groundwater fraction in stemwater, x, using an end member mass balance equation of the form

$$\delta_{plant} = x\ \delta_{groundwater} + (1\text{-}x)\ \delta_{soilwater} \qquad\qquad \text{---- (3)}$$

Here δ signifies $\delta^{18}O$ values of the plant stemwater and the respective sourcewater pools. The fraction x has a value between 0 and 1 that corresponds to 0-100% groundwater fraction in stemwater. When calculations yielded values of $x < 0$ or > 1, I considered them as 0 % and 100% groundwater usage respectively.

 δD values have not been considered in the above regression and mass balance equation since they exhibit considerably more variation than $\delta^{18}O$. In addition, there is the possibility that certain species of plants discriminate against deuterium during water uptake (Ellsworth and Williams, 2007, Lin and Sternberg, 1993)

 To compare foliar nutrient levels between hammock and pineland communities, leaf P averaged over all individuals per species that were sampled in each community and site was plotted against the corresponding leaf N average value. Data obtained over the entire study period was considered to avoid over or underestimation of nutrient concentrations caused by temporal variation due to species phenology or fire. A Euclidean distance analysis was then carried out on another plot of foliar P versus foliar N, where each point on the plot represents the leaf N and P values averaged over 5 individuals of a species per sampling. The distance d, of each point i from the

plot origin (zero values of N and P) was computed using the Cartesian distance

formula

$$d_i = (N_i{}^2 + P_i{}^2)^{0.5}$$
-----(4)

where N_i and P_i represent the foliar N and P values of the point i as mentioned. A one

way ANOVA was performed to infer if the Euclidean distances of the pineland points

were less than those for hammock datapoints, thus indicating whether the pineland

plants had lower combined (N and P) foliar nutrient values relative to hammock

plants. Model II regression and correlation of leaf $\delta^{13}C$ vs. leaf N was carried out to

examine for differences between hammock and pineland plants in stomatal limitation

as a function of foliar nitrogen concentration.

Results

Soil depth and nutrient characterization: Hammocks had a significantly greater depth

of soil over bedrock compared to pinelands as confirmed by a 2-way ANOVA with

replication (F = 65, P < 0.01), with a significant site effect (F = 11, P <0.01, Table

2.1) This soil, mainly leaf litter in various stages of decomposition mixed with some

crushed marl, forms a continuous layer in hammocks. In pinelands, soil is present in

scattered sinkholes and sometimes as a 1-5 cm deep layer in areas that have not been

recently burned. The concentration of plant available phosphorus, ammonium and

nitrate nitrogen in hammock soil at the date of sampling was found to be greater than

that in groundwater by 1-2 orders (Table 2.2).

Sourcewater isotopic composition

The isotopic composition of rainfall varied according to the season with wet season precipitation $\delta^{18}O$ and δD values being lower than those of the dry season (Fig. 2.2 showing only $\delta^{18}O$ values). From the end of May 2005, when the wet season commences, rainfall becomes isotopically lighter until July. Isotope ratios of precipitation become higher around mid-wet season (July-August, Fig 2.2) coincident with a lull in precipitation as shown in Fig.2.5A. Precipitation isotope ratios decrease again towards the end of the wet season (September-October) coincident with resumption of high rainfall. The bimodal peak in rainfall amount over the wet season is typical of South Florida precipitation (Duever *et al.*, 1994) and can be observed in rainfall records (1949-2005) at Royal Palm Ranger Station in the ENP (SERCC, 2008). Groundwater displays the same temporal and seasonal isotopic pattern as rain in Royal Palm and Long Pine Key, but the amplitude of isotopic variation between successive samplings is dampened, particularly for the Royal Palm site (Fig. 2.2). Soilwater $\delta^{18}O$ values in both hammocks and pinelands follow rainwater $\delta^{18}O$ trends with lighter isotopic composition during the wet season.

Regression of δD against $\delta^{18}O$ values for rain samples collected at all three sites over the entire year forms the Everglades Local Meteoric Water Line (ELMWL- Fig.2.3) having a slope of 8.5 and deuterium excess of 17 ($r^2 = 0.93$). The ELMWL falls within the 95% confidence bounds of the Global Meteoric Water Line (GMWL, slope = 8) and thus is not significantly different; this has been also seen in Wilcox et al (2004). The larger deuterium excess of the ELMWL, 17 as compared to GMWL's 10 could indicate re-evaporation of shallow Everglades surfacewater. During the dry

season, groundwater values lie to the right of this line, indicative of evaporative

enrichment (Friedman et al, 1962). Soilwater values lie further to the right, indicating

a greater evaporative enrichment.

Plant stemwater and sourcewater correlation

When considering the dataset of hammock and pineland plant stemwater isotopic

values over the year, pineland plant $\delta^{18}O$ values were better correlated with

groundwater ($r^2 = 0.82$), with a Model II regression slope (1.11) and intercept (0.3)

close to 1 and 0 respectively, indicating a 1:1 relationship (Fig. 2.4). Hammock

plants had a poorer correlation with groundwater ($r^2 = 0.57$), with a slope (0.82) and

intercept (-0.13). Both pinelands and hammocks exhibit a seasonal variation with

increasing groundwater use in the dry season (Fig. 2.5B) although pineland plants

were utilizing a greater fraction of groundwater on every sampling date except during

late dry season (May 2005). The difference in groundwater utilization between

pineland and hammocks was greatest during the wet season.

Foliar nitrogen and phosphorus

Foliar N is related to foliar P by a Model II regression N = 55P -0.43, r = 0.78 (Fig 2.

6). The community average foliar N and P is highest for the group of species present

only in hammocks (1.70 and 0.056 % respectively) followed by the species group that

is present in both hammocks and pinelands (1.59 and 0.032 % in hammocks, 1.5 and

0.032% in pinelands) (Table 2.3). The species group restricted to pinelands has the

lowest average foliar N and P values (1.22 and 0.037%). There is also considerable

interspecific variation in foliar N and P within each community. Foliar N and P

values at the community level for pinelands (including all species sampled in

pinelands, not just those restricted to pinelands) averaged over the entire year were

significantly lower than the same for hammocks (Fig. 2.6), based on the ANOVA of

Euclidean distances showing pineland plants being significantly closer to the plot

origin (P and N = 0) as compared to hammock plants (F = 10.51, P = 0.0014).

Average annual N:P ratios of 45 ± 1.15 and 42 ± 1.16 (± SEM) were obtained for

hammock and pineland communities respectively, with no significant difference in

N:P ratio between the two communities (single factor ANOVA, F = 3.56, P = 0.059).

Foliar $\delta^{13}C$

The correlation of $\delta^{13}C$ against foliar N was highly significant for hammock plants (r^2

= 0.41, P < 0.01) with a Model II regression having a slope of 1.73 (Fig. 2.7). There

was no correlation between foliage $\delta^{13}C$ values of pineland plants and their respective

nitrogen concentrations (r^2 = 0.15). At low levels of N (>2%), pineland leaves have

higher $\delta^{13}C$ values than hammock plants.

Leaf area index (LAI)

Hammocks had a higher LAI (2.49±0.27, n=10) than pinelands (0.35±0.04, n = 5).

Values calculated were LAI 4 ring, that is the effective leaf area index of the canopy

intergrated over the zenith angles 0˚to 60˚ (Frazer et al, 1999).

Discussion

Sourcewaters are isotopically distinct

The greater depth of soil throughout the hammocks compared to pinelands (Table 2.1) implies a much larger volume of soil in hammocks per unit area as compared to pinelands. In the wet season, rainwater entrapped in this soil ends up bearing nutrients in dissolved form that have been measured and estimated to be 1-2 orders of magnitude higher than groundwater (Table 2.2). Thus hammocks are expected to contain a larger volume of nutrient-rich soilwater than pinelands.

Soilwater was isotopically distinct from groundwater at every instance of sampling, thereby allowing determination of the contribution of each source to plant stemwater by the mass balance approach. Soilwater is essentially rainwater trapped in litter which is subject to evaporation between successive rain events and so becomes isotopically enriched relative to rain. At the same time, soilwater was lighter than groundwater in the wet season with correspondingly lower $\delta^{18}O$ values (Fig 2.2 and 2.3). While groundwater also receives rain inputs, the extensive volume of the perennial groundwater pool dampens the lighter rain isotopic signal, thus remaining isotopically higher than rain over the wet season (Fig 2.2). Over the dry season, soilwater (a far smaller pool in comparison with groundwater) undergoes greater evaporative enrichment resulting in higher $\delta^{18}O$ values than groundwater (Fig. 2.2 and 2.3). Soilwater $\delta^{18}O$ values appear less enriched in March 2006 as compared to March 2005 probably due to differences in precipitation amounts, intervals and evaporative conditions in the months preceding the samplings. For instance there

were larger than normal rain events in the 2005-2006 dry season (December 2005 and February of 2006).

Water usage by plant communities

The higher correlation and near 1:1 relationship between pineland plant stemwater and groundwater compared to the lower correlation of hammock plant stemwater and groundwater is consistent with groundwater being the major water source for pineland plants (Fig. 2.4) in comparison to hammock plants except for late in the dry season. This conclusion is further corroborated by the relative utilization of groundwater and soilwater by plants in these two communities at different times of the year according to the mass balance equation (Fig. 2.5B). I demonstrate the seasonal variation in *both* communities in the proportions of soilwater and groundwater used, noting that pineland plants *also* use soilwater (about 50% on average) in mid to late wet season (Fig. 2.5B).

The 2005 wet season commenced with a large pulse in rainfall over June and July (Fig. 2.5A), with a slightly delayed increase in the groundwater table level (Fig. 2.5C). A concomitant decrease in groundwater fraction of stemwater was observed for hammock plants in July (Fig. 2.5B), which can be construed as an increase in soilwater uptake. This could be due to wetting of the soil litter by rain, and/or because the rising water table may restrict hammock plants to water uptake only to the unsaturated surface soil layer, since hammock hardwoods are flood intolerant (Jones *et al.*, 2006, Snyder, 1990). During the early wet season, pineland plants continued to rely upon groundwater, and started to absorb soil water in significant

amounts only after July, until almost half of their water uptake comprises of soilwater during October (late wet season). This suggests the possibility that pineland plants can tolerate some period of inundation when they almost entirely take up groundwater, after which they gradually increase soilwater uptake. Indeed the pineland at Sisal Pond was flooded continuously from June to September. Soilwater in pinelands occurs in litter-filled holes in the limestone bedrock and is probably richer in nutrients than groundwater.

With the advent of the dry season around mid-October, plants in both communities begin to increase groundwater uptake. This is probably a response to decreasing plant-available water in the litter layer because of gradual drying. Another possibility is that the falling water table increases the vadose zone thereby permitting re-growth of roots downwards. The flood-intolerant nature of hardwood hammock species precludes the presence of root strategies to tolerate inundation. Water levels rise again after December, possibly in response to rain showers that occurred in December 2005 and February 2006 (Fig. 2.5A and C) which could once again flood roots and force plants to shift towards newly available soilwater as seen in hammocks (Fig. 2.5B).

If there is substantial root mortality in hammock plants because of their intolerance of flooding, then the rate of root regrowth must keep pace with the falling water table over the dry season. If the water levels descend rapidly to levels lower than what is normal in the late dry season, groundwater may become inaccessible to hammock plants, causing water stress. Given that the thin organic soil layer overlies bedrock and that roots can grow downwards only through existing cracks (at least in

the short term), it is possible that roots in many cases may not be able to encounter suitable pathways down to the water table at a rate adequate to meet water demand by the plant. Ewe *et al* (1999) observed a greater decrease in predawn water potential in hammock trees over pineland trees in the transition from wet to dry season. This indicates a larger seasonal decrease in water availability for hammock trees. Thus the existence of hammock plant communities appears to be precariously positioned between two critical water levels: too high a water level will lead to flooding stress while too low a level would engender drought stress. Deviation outside this range over time can have irreversible effects culminating in mortality of trees. Lower than normal water levels caused by droughts or increased water withholdings/diversions from canals can also affect pine rocklands (Oberbauer *et al.*, 1997, USFWS, 1999), possibly due to water table access problems similar to hammocks.

Foliar N and P at the community level

As hypothesized, the pooled average foliar nutrient concentration for all plants sampled in the hammock is significantly higher than that in pinelands (Fig. 2.6). This could be a consequence of greater availability and greater uptake of nutrient-rich soilwater in hammocks (Fig. 2.5B). The far smaller volume of soil in pinelands, together with the observation that nutrient-poor groundwater is the main water source in pinelands most of the year positions pinelands below hammocks in terms of available nutrient pools (N and P). Other factors can also influence foliar nutrient concentrations – for instance, fire can increase availability of certain nutrients to plants with attendant increase in foliar nutrient concentrations in resprouts. This would be expected mainly in pinelands that experience fires every 3-7 years and is

probably applicable to the Long Pine Key site that underwent a fire during the study period.

A preliminary consideration of the foliar nutrient data at the species group level suggests a nutrient gradient increasing from pinelands to hammocks (Table 2.3 averages): (i) Pinelands: The dominant species (canopy and biomass) in the pinelands, *Pinus elliottii,* has relatively low values of foliar N and P in comparison to other pineland and hammock species (ii) Species present in the hammock-pineland interface have mostly intermediate foliar nutrient values and (iii) Hammocks: At the higher foliar nutrient concentration end is *Bursera simarouba,* one of the few species occurring throughout the range of rockland hammocks (Snyder, 1990) with high abundance (Alexander, 1967). Indeed, the pineland community average would have been even lower than our calculated value had the foliar nutrient concentration of each pineland species been weighted by the relative abundance of foliar biomass of that species.

Despite differing soil nutrient availabilities between hammocks and pinelands, foliar nutrient concentrations as well as N:P ratios were seen to be similar in individuals of a species common to both communities. Plasticity in foliar N and P concentrations is constrained by species-specific stoichiometric relationships between foliar N and P that are in turn driven by physiological function and the anatomy of leaves. A plant faced with lower nutrient availability (in comparison to a site with higher nutrient availability) could be restrained by lower growth and have a lower number of leaves, rather than manifest a significant change in either concentrations or N:P ratios. Greater nutrient availability (as proposed in hammocks) may also result in

greater growth and biomass instead of higher foliar nutrient concentrations. Individuals of the co-occuring species are much larger in the hammocks; however fire is a confounding factor since periodic fires keep burning off these species in pinelands, while hammocks for the most part are spared from these fires. In their review (2000), Aerts and Chapin mention that oligotrophic sites are dominated by slow growing species with low foliar nutrient concentrations, notably evergreens. Sites with higher levels of nutrient availability have species with higher leaf nutrient concentrations (forbs, graminoids and deciduous species). Thus increasing nutrient availability can lead to higher foliar nutrient concentrations at the community level due to both phenotypic responses and species replacements. Species found exclusively in the hammocks at the three study sites in general had higher foliar nutrient concentrations as compared to those found exclusively in the pinelands (Table 2.3). In addition to possibly higher foliar nutrient concentrations, higher soil nutrient availability can also result in greater number of leaves per unit area that is quantified by the leaf area index (LAI) (Jordan, 1969). The higher LAI values in hammocks as compared to pinelands lends further support to hammocks having greater availability of nutrients to plants.

Foliar N:P ratios can be indicative of the relative availability of N and P to the plant, with N:P ratios being inversely correlated with soil P availability (Gusewell, 2004, Han *et al.*, 2005). The literature on N:P ratios in ecosystems in other parts of the world range from 3 to 89 with a mean N:P ratio in the range of 13-16 (Reich and Oleksyn, 2004, Wright *et al.*, 2005). Plants in European herbaceous freshwater wetlands were found to have N:P ratios between 7 and 30, with ratios greater than 16

signifying community P limitation in those ecosystems (Koerselman & Meuleman, 1996). Both hammocks and pinelands in the Everglades have high mean N:P values (45 and 42 respectively in this study) indicating a very low level of foliar P, which could result from low soil P availability. Other plant communities in the Everglades have even higher N:P ratios, possibly indicating even lower P availability than upland areas. Wet graminoid prairies are flooded most of the year, cover much of the Everglades and are oligotrophic (Brown *et al.*, 2006, Newman *et al.*, 1996). They are dominated by sawgrass (*Cladium jamaicense*) which has extremely low levels of P in its tissue, with foliar N:P ratios of 70-84 in areas relatively unaffected by agricultural nutrient enrichment (Richardson *et al.*, 1999).

Foliar nutrients and photosynthesis

The correlation between foliar $\delta^{13}C$ and foliar nitrogen concentration in hammock plants (Fig. 2.7) can be explained as follows: higher foliar N concentration indicates higher chlorophyll and carboxylation enzyme content in leaves (Duursma & Marshall, 2006) which in turn leads to higher photosynthesis; a positive relationship between photosynthesis and leaf nitrogen content has been widely reported (eg. Field & Mooney, 1986). Higher levels of photosynthesis can increase the proportion of ^{13}C incorporated in leaf biomass, a consequence of the ensuing stomatal limitation on atmospheric carbon dioxide CO_2 inflow, thus forcing decreased discrimination by Ribulose Bis-Phosphate Carboxylase against ^{13}C (Farquhar *et al.*, 1982). Stomatal limitation could be caused either by a greater photosynthetic demand of CO_2 over supply, even with stomata fully open, or by some degree of stomatal closure triggered by the need to reduce transpirational water loss in periods when water is limiting.

While positive correlations between foliar δ^{13}C and foliar nitrogen have been observed in other ecosystems (eg. Li *et al.*, 2007), there also have been negative or absence of correlations reported (eg. Ma *et al.*, 2007). The foliar δ^{13}C – N correlation varies with species, with leaf age and canopy position and thus the precise mechanisms underlying the correlation are not well understood (Li *et al.*, 2007). The absence of the above correlation in pineland plants indicates that some other factor could be affecting photosynthesis in pineland plants, such as higher light intensity in pinelands as compared to hammocks. Nevertheless, leaves of pineland plants have higher δ^{13}C values at low levels of foliar N (<2%) than hammock plants, indicating greater stomatal limitation of photosynthesis at low N.

Conclusions

The reliance of hammock trees upon nutrient-rich soilwater in the wet season together with their higher levels of foliar N and P supports the idea of the litter layer in hammocks being a nutrient hotspot in the oligotrophic Everglades. The organic soil layer in hammocks thus facilitates establishment and propagation of flood-intolerant neotropical hardwood species by (i) serving as an unsaturated substrate allowing roots to survive in the wet season and (ii) furnishing a significant water and nutrient source. In addition, the moist nature of this organic layer is partly responsible for preventing ingress of fires that range through pinelands every few years. Thus the organic soil layer in hammocks is an instance of how vegetation can modify the local environment to foster conditions enabling establishment and growth of species that otherwise would not be able to survive as has been observed elsewhere (Scheffer *et al.*, 2005).

Being the only abovewater communities along with the emergent areas of tree

islands, hammocks and pinelands are critically important to the Everglades ecosystem

from both a floral and faunal perspective, but in 1984 they constituted only about 4%

of the total ENP area based on data in Olmsted & Loope (1984). In addition, almost

all the pine rocklands outside ENP have been eliminated by logging and urban

development (Herndon, 1998, Slocum *et al.*, 2003, Snyder, 1990) with barely 2%

remaining as of 2007 (WWF, 2007). Maintenance of these critical upland

components of the unique Everglades ecosystem requires that the seasonal water level

regime be maintained. The dangers of flooding to hardwood hammocks are well

known. This study provides isotopic evidence of the almost exclusive dependence of

hardwood hammocks and pinelands on groundwater in the late dry season, thus

supporting the contention of Alexander (1967) that droughts due to lowering of the

South Florida water table is the most serious deterrent to the continued development

and survival of hardwood hammocks. It is thus necessary to manage dry season

groundwater levels so that they remain accessible to upland community plants.

Table 2.1: Average soil depth to bedrock (cm) in hammocks and pinelands, ±
standard error of the mean (SEM). A 2-way ANOVA with replication confirmed that
(i) hammocks have greater soil depth than pinelands (F = 65, P < 0.01) and (ii) a site
effect exists (F = 11, P <0.01).

Site	Hammock	Pineland
Royal Palm	7.7 ± 0.7 (n = 70)	5.7 ± 0.5 (n = 104)
Long Pine Key	32.1 ± 3.0 (n = 32)	2.8 ± 0.8 (n = 24)
Sisal Pond	15.9 ± 1.5 (n = 51)	5.5 ± 2.1 (n = 51)

Table 2.2: Plant-available phosphorus, ammonium and nitrate concentration ranges (ppm) in hammock soil and groundwater in the Everglades National Park (wet season). Soil pore water concentrations are calculated based upon the following assumptions: 1 – the entire pool of plant available nutrients are in solution and 2 - hammock soils contain 230% water by dry soil weight in the wet season (based on Ewe et al, 1999).

Nutrient	Hammock		Groundwater
	Soil	Porewater	
Available P	2.1 - 44.5	0.9 – 19	0.03 - 0.05
Nitrate	5.5 – 126	2.4 – 54	0.01 –0.15
Ammonium	6.52 – 150	2.8 – 64	0.09 - 0.15

Table 2.3: Species level foliar N and P (averaged for all individuals of a species for all sites sampled 6 times over a year) for plants in hammocks and pinelands (n ranges from 30 to 90, depending on the presence of the species in one to three sites). The SEM was calculated for the entire pool of samples collected over the year.

Species	Foliar N (%) ± SEM		Foliar P (%) ± SEM	
	Hammock	Pineland	Hammock	Pineland
Species found both in hammocks & pinelands				
Lysiloma latisiliqua	2.59 ± 0.16	2.62 ± 0.15	0.047 ± 0.000	0.046 ± 0.002
Guettarda scabra	1.32 ± 0.08	1.07 ± 0.10	0.030 ± 0.004	0.032 ± 0.005
Myrica cerifera	1.45 ± 0.09	1.42 ± 0.11	0.0191 ± 0.0031	0.0182 ± 0.0026
Tetrazyga bicolor	1.34 ± 0.1	1.10 ± 0.16	0.0321 ± 0.0059	0.0284 ± 0.0028
Persea borbonia	1.26 ± 0.15	1.28 ± 0.08	0.0332 ± 0.0038	0.0370 ± 0.0079
Average over species common to hammocks and pinelands	**1.59 ± 0.12**	**1.50 ± 0.12**	**0.0324 ± 0.0046**	**0.0324 ± 0.0051**
Species found only in hammocks				
Annona glabra	2.25 ± 0.12	-	0.0832 ± 0.0177	
Chrysobalanus icaco	1.33 ± 0.12		0.0370 ± 0.0045	
Quercus virginiana	1.44 ± 0.12		0.0378 ± 0.0073	
Bursera simarouba	1.80 ± 0.13		0.0661 ± 0.0137	
Average over species found only in hammocks	**1.70 ± 0.12**		**0.0560 ± 0.0108**	
Species found only in pinelands				
Pinus elliottii var densa		0.79 ± 0.08		0.0289 ± 0.0039
Quercus pumila		1.57 ± 0.12		0.0588 ± 0.0079
Myrsene guianensis		1.06 ± 0.09	-	0.0297 ± 0.0045
Ilex cassine		1.18 ± 0.08		0.0330 ± 0.0039
Crossopetalum ilicifolium		1.51 ± 0.09	-	0.0367 ± 0.0059
Average over species found only in pinelands		**1.22 ± 0.09**		**0.0374 ± 0.0052**
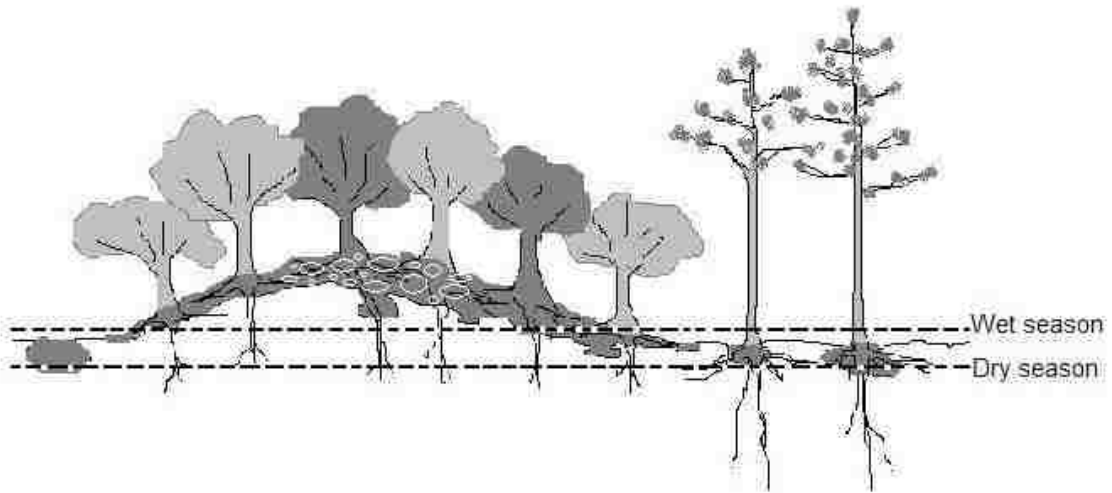
Figure 2.1: Schematic illustration of a tropical hardwood hammock (left) and a pine rockland (right). Dashed lines indicate relative wet season and dry season water levels. The thin mantle of soil overlying limestone bedrock in the hammock and in sinkholes in the pineland is shaded grey. In the hammock, the wet season soilwater pool is denoted by small ellipses. Understory plants are not shown.
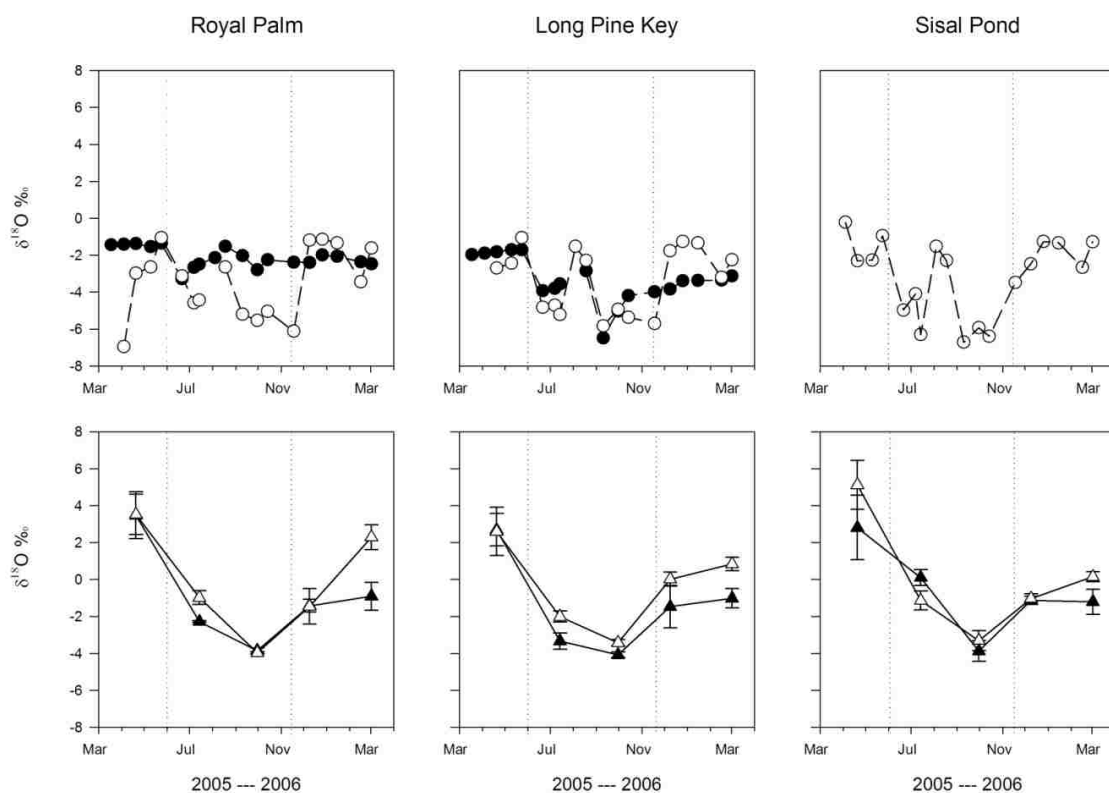
Figure 2.2: Upper panel: plots of $\delta^{18}O$ values of rain ($\circ$) and groundwater ($\bullet$) for the study sites in the Everglades National Park for the period March 18, 2005 to March 3, 2006. Sisal pond did not have a groundwater well. The lower panel depicts soilwater $\delta^{18}O$ values -- hammock ($\blacktriangle$) and pineland ($\triangle$) from April 22, 2005 to March 18, 2006. Error bars indicate SEM. Dotted vertical lines denote the wet season (June-November).
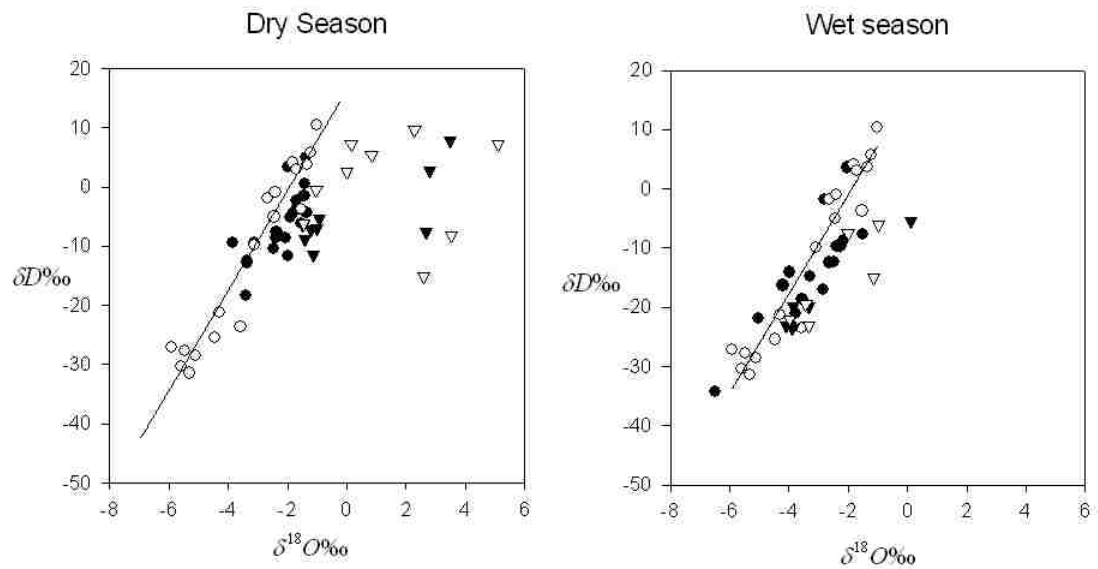
Figure 2.3: Plot of δD vs. δ ^{18}O for rain (○), groundwater (●) and soilwater (hammock (▼) and pineland (▽) for all 3 sites in the Everglades National Park with the Everglades local meteoric water line shown (δD = 8.5 δ ^{18}O + 17 ‰, r^2 = 0.93, P < 0.01).
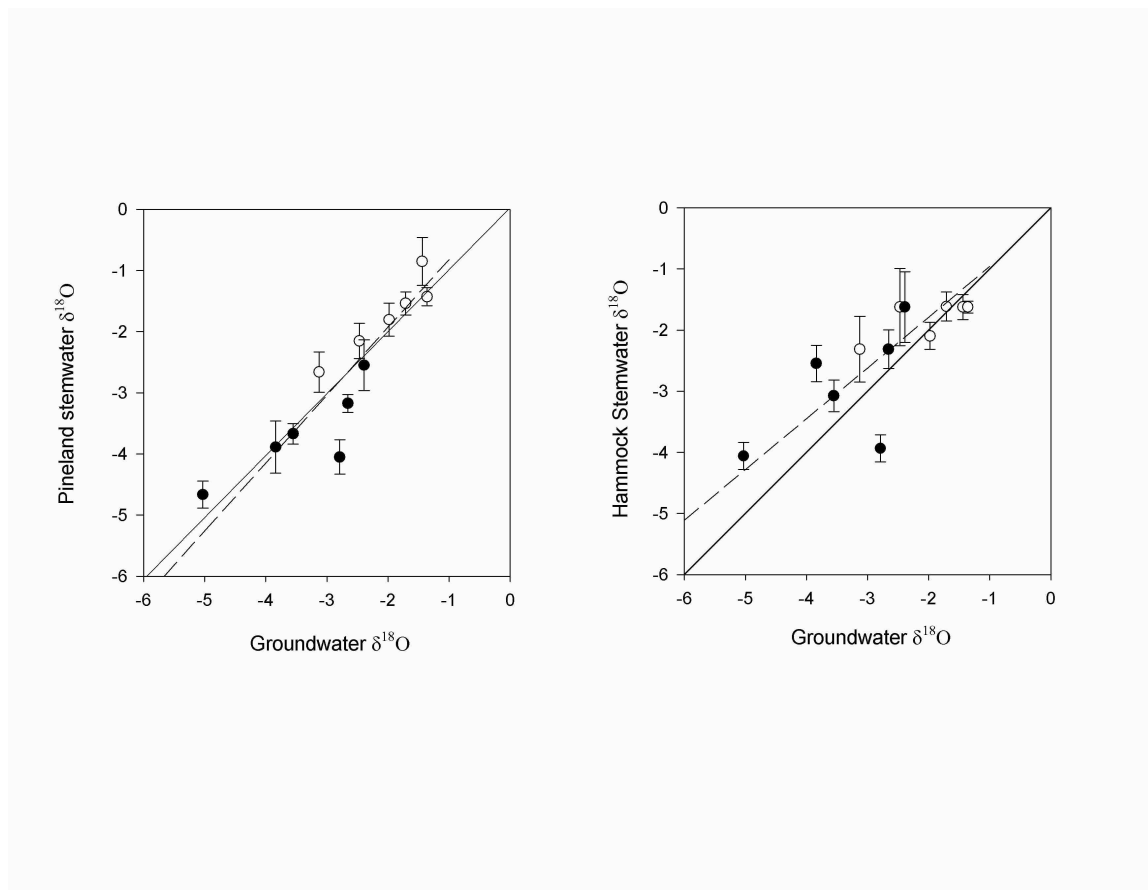
Figure 2.4: Relationship of average pineland plant $\delta^{18}O$ values (left) and hammock plant $\delta^{18}O$ values (right) with groundwater $\delta^{18}O$ values bimonthly over a year. Filled circles (●) denote wet season data while empty circles (○) dry season values. Each datapoint is the average of all plants in a community (hammock or pineland) at a site. Error bars indicate SEM. There are no error bars for groundwater $\delta^{18}O$ on the x-axis since we had just one sample (one well) per site. The pineland plant stemwater Model II regression equation is $\delta_{plant} = 1.10 \; \delta_{groundwater} + 0.03$, $r^2 = 0.82$, $P < 0.01$ and this regression line (dashed line) is similar to the 1:1 relationship solid line. The hammock plant stemwater Model II regression equation is $\delta_{plant} = 0.61 \; \delta_{groundwater} + 0.9$, $r^2 = 0.57$, $P < 0.01$.
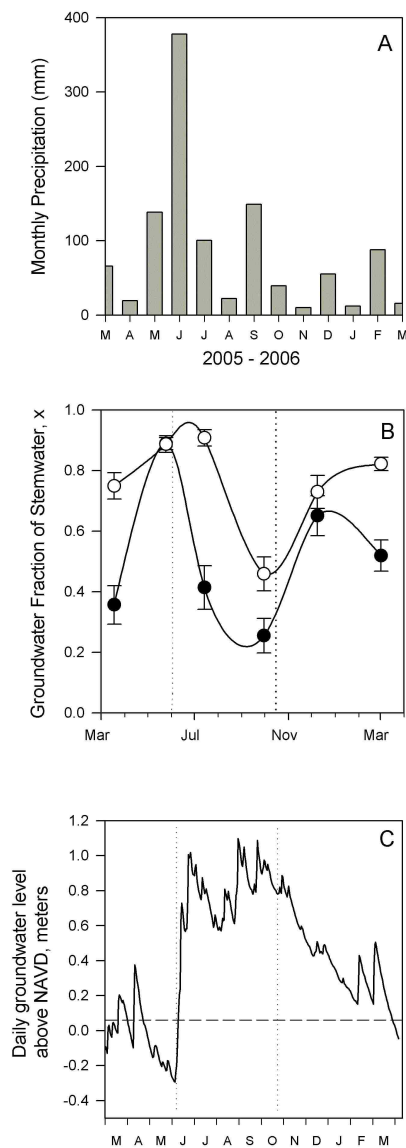
Figure 2.5: A: monthly rainfall over the study period (www.usgs.gov). B: Plot of average groundwater fraction utilized by hammock (●) and pineland (○) communities through the year. A value of 1 implies 100% of stemwater is groundwater. Sampling months indicated on the horizontal axis. Vertical dotted lines indicate the wet season, and error bars depict ± SEM. C: Daily water table values in meters above North American Vertical Datum (NAVD 1988) at well NP72 in Long Pine Key, ENP (latitude 25°23'46" N, longitude 80°42'11" W)
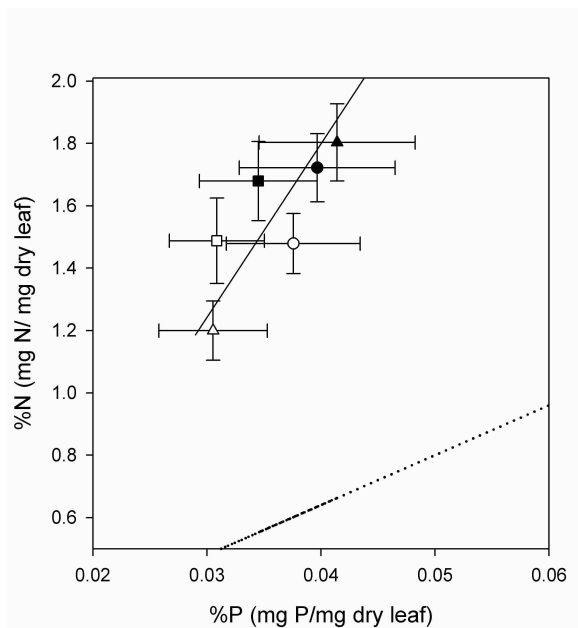
Figure 2.6: Relationship between average foliar N and P in hammocks (●,■,▲) and pinelands (○,□,Δ) from March 2005 to March 2006 at Royal Palm, Long Pine Key and Sisal Pond respectively in ENP as shown by a Model II regression line (N = 55 P - 0.43, r^2 = 0.61, P < 0.01). Each datapoint in the plot represents the average of all plants in a community and site sampled over the year (n = 150). Error bars indicate ± SEM. The dashed line in the lower part of the plot represents an N:P ratio of 16, beyond which signifies P limitation for European freshwater wetlands (Koerselman and Meuleman, 1996).
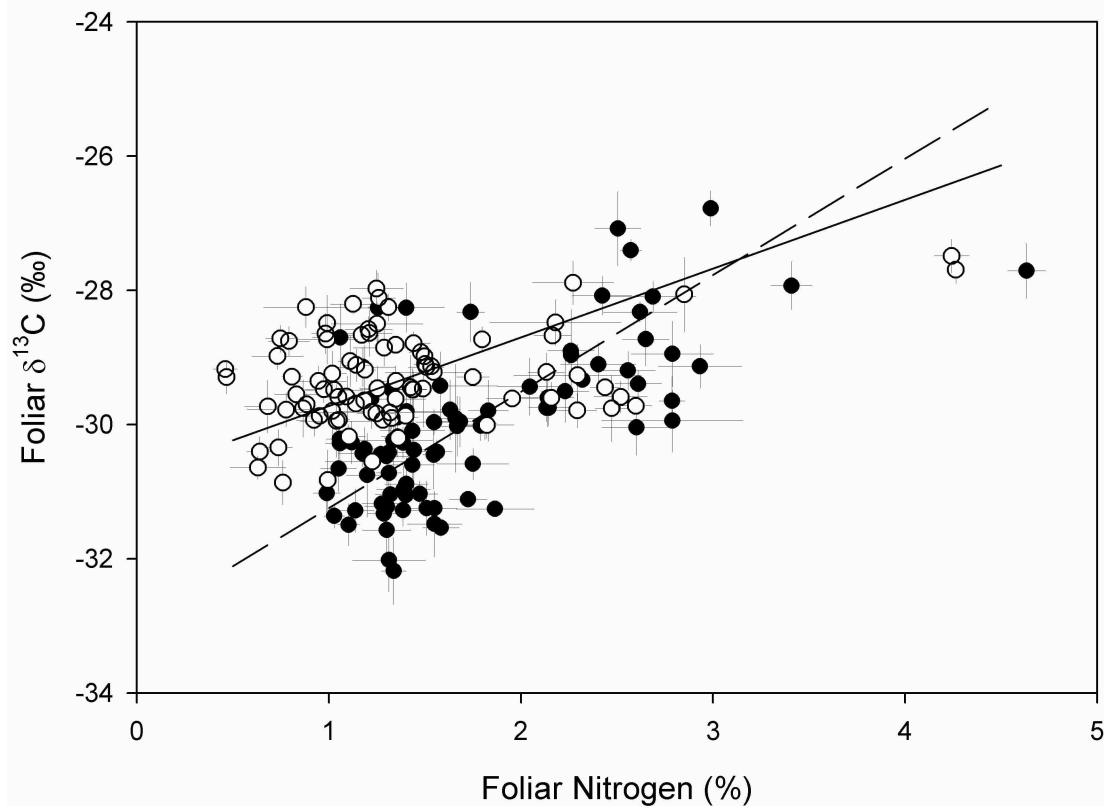
Figure 2.7: Model II regression of foliar $\delta^{13}C$ (‰) against foliar nitrogen for hammock (●) and pineland plants (○). Each datapoint is an average of 5 individuals of a species per sampling event. All six bimonthly sampling events over March 2005 – March 2006 are included. Correlation was significant in hammocks ($r^2 = 0.41$, $P < 0.01$) but not in pinelands ($r^2 = 0.15$). Error bars indicate ±SEM. The Model II regression equations are $\delta^{13}C = 1.73\ N - 33$ (dashed line) and $\delta^{13}C = 1.02N - 31$ (solid line) for hammock and pineland communities respectively.

CHAPTER 3

Water source utilization and foliar nutrient status differs between upland and flooded
plant communities in wetland tree islands

Summary

Tree islands in the southern Everglades are centers of biodiversity and targets of

Everglades restoration, yet little is known about plant water sources and nutrient

requirements. These tree islands have two distinct, hydrologically defined woody plant

communities: (i) an upland community, locally known as hammock, consisting of flood

intolerant neotropical species, and (ii) a lowland or swamp forest community flooded for

1-9 months per year, with flood-tolerant species. Two potential water sources exist: (i)

entrapped rainwater in the vadose zone of the organic soil (referred to as litter water), that

becomes enriched in phosphorus, and (ii) phosphorus-poor regional

groundwater/surfacewater (referred to as regional water). Using natural stable isotope

abundance as a tracer, I tested the hypothesis that upland communities access litter water

while lowland communities have access to only regional water. I linked access to the

different water sources with foliar nutrient levels. Upland plants used litter water in the

wet season and shifted to greater regional water uptake in the dry season, while lowland

plants used regional water throughout the year. Consistent with the phosphorus

concentrations of the two water sources, upland plants had a greater annual mean foliar

phosphorus concentration over lowland plants, thereby supporting the idea of tree islands

being nutrient hotspots in the oligotrophic, phosphorus-limited Everglades. Contrary to

the pattern exhibited by foliar phosphorus, lowland plants had higher foliar nitrogen

levels than upland plants. The high concentration was associated with stomatal limitation

of photosynthesis only for lowland plants. Linking water sources with foliar nutrient concentrations can indicate nutrient sources and periods of nutrient uptake, thereby linking hydrology with the nutrient regimes of different plant communities in wetland ecosystems. Our results support the hypotheses that (i) upland tree island communities incrementally increase their nutrient concentration by harvesting marsh nutrients through transpiration seasonally, and (ii) small differences in microtopography in a wetland ecosystem can lead to large differences in water and nutrient cycles.

Background:

Plant species vary widely in their water requirements and tolerance to flooding/drought. Hence the spatial and temporal distribution of water and soil moisture is a major influence on the diversity of plant community types and species in ecosystems throughout the world (eg. Conner *et al.*, 2002; Furley & Ratter, 1988; Rodriguez-Iturbe *et al.*, 2001; Saha *et al.*, 2008). The conjunction of multiple water sources, e.g., precipitation and groundwater, with variable topography, soils, and vegetation, may result in separate pools of water in close proximity. These pools in turn allow different forms of vegetation to coexist in the same ecosystem. For instance, hardwood hammocks on the Florida Keys depend upon a rain-derived freshwater pool while neighboring mangroves utilize seawater (Sternberg & Swart, 1987). Pools of water can also differ in their dissolved nutrient concentrations. Since plant nutrient uptake happens in solution (Baldwin, 1975), the existence of multiple pools of water can create heterogeneity in nutrient availability to plants, which in turn may increase species diversity (Tilman, 1999). Identification of these pools of water and their chemical composition is thus a fundamental step in understanding the structure and function of plant communities, how plants adapt to their

environment, as well as how these communities may cope with climate change. In this study I look at water source utilization and foliar nutrient status of flooded (lowland) and upland plant communities in wetland tree islands in the Everglades National Park, USA.

Tree islands in wetlands and savanna ecosystems are particularly appropriate for study of the association of different pools of water and nutrients with vegetation pattern, because they exhibit sharp contrasts in plant community types along a relatively mild topographic gradient. Tree islands are patches of woody vegetation in a landscape matrix dominated by non-woody species (Tomlinson, 1980) and occur globally, from the tropics to the tundra (Wetzel, 2002). Distinct boundaries of vegetation communities on tree islands are aligned along abrupt changes in environmental conditions and biogeochemical processes. In hydrologically-controlled ecosystems, such as the Everglades, tree islands are often the only mesic communities in a flooded landscape, thus resulting in the presence of a mosaic of communities with different tolerances to flooding. Emergent tree islands support terrestrial, flood-intolerant flora, provide critical habitat for fauna (Heisler *et al.*, 2002) and increase ecological complexity by incorporating aerobic biogeochemical processes in a predominantly anaerobic system (McClain *et al.*, 2003; Sklar *et al.*, 2004). The slight difference in elevation between tree island and surrounding wetland, typically ≤ 1 m in the Everglades (Loveless, 1959; Willard *et al.*, 2006), makes tree islands extremely sensitive to water level changes (Conner *et al.*, 2002). Hydrologic alterations in the Everglades resulted in a large loss of tree islands from 1950 to 1995 (Alexander & Crook, 1974; Craighead, 1971; Lodge, 2005; Sklar *et al.*, 2004). The prevention of further losses and the restoration of tree islands is an important part of the Comprehensive Everglades Restoration Plan (CERP), requiring the development of ecological performance measures for the adaptive assessment of restoration (Sklar *et al.*,

2001). Conservation and restoration of such water-controlled ecosystems necessitates identification of the water pools utilized by vegetation, an understanding of their seasonal variability, and knowledge of their effects on nutrient mineralization and nutrient availability to plants.

Tree islands are considered nutrient hotspots in the oligotrophic, phosphorus-limited Everglades landscape (Davis, 1994; Gann *et al.*, 2005; Wetzel *et al.*, 2005) because soil phosphorus levels (bulk soil as well as soil porewater) in the highest parts of the island (typically not flooded) are several orders of magnitude higher than the surrounding marsh (Jayachandran *et al.*, 2004; Ross *et al.*, 2006). Greater nutrient availability to plants in one area over another can be manifested in three ways – (i) increased foliar nutrient concentrations (ii) increase in growth and leaf area and /or (iii) species replacements. Many studies on wildland plants, involving existing soil nutrient gradients or experimental fertilizations, have shown that higher nitrogen (N) and phosphorus (P) availability in soil can cause species to take on higher foliar N and P concentrations (Aerts & Chapin, 2000; Campo & Dirzo, 2003; Chapin, 1980; Fisher *et al.*, 2006; Harrington *et al.*, 2001; Lower *et al.*, 2003). Several studies have shown that sites with high soil nutrient concentrations are populated by species of inherently high foliar nutrient concentrations (eg. Boerner, 1984; Fensham & Bowman, 1995; Santiago *et al.*, 2005). I refer to this effect as a community-level effect. Because there is very little species overlap between upland and lowland communities in these Everglades tree islands, I propose to investigate whether the upland community is composed of species with a higher aggregate foliar P than the lowland community. Thus from a plant community perspective, if phosphorus-rich soil porewater from heads constitutes an

important water source for upland plants in the wet season, then upland plants can potentially access a larger pool of phosphorus than can lowland plants.

Linking the water sources and nutrient status of the different plant communities on tree islands will therefore enable understanding and prediction of community shifts on tree islands in consequence of hydrological change. The objectives of this study are to test the following hypotheses: (a) upland trees have access to rainwater entrapped in soil which is P-rich, in contrast to lowland plants that only have access to P-poor regional water (b) foliar P concentrations are related to the water sources utilized by each community within a tree island, and (c) different nutrient availabilities can, in turn, affect photosynthetic processes. For (a), I used the natural abundance of stable isotopes of water (^{2}H, ^{18}O) , which have been widely used in coastal ecosystems to identify the proportion of each sourcewater in plant stemwater (Ewe *et al.*, 1999; Greaver & Sternberg, 2006; Lin & Sternberg, 1993; Saha *et al.*, 2009; Sternberg *et al.*, 1991; Sternberg & Swart, 1987). For (b), foliar nutrient concentrations were measured. In addition, the stable isotope of nitrogen (^{15}N) was employed as a proxy for phosphorus, whereby increased P uptake stoichiometrically drives greater N uptake that in turn results in decreased discrimination against ^{15}N in the assimilation of N in cells (Inglett & Reddy, 2006; Inglett *et al.*, 2007; McKee *et al.*, 2002). For (c), the stable isotope of carbon (^{13}C) was used as an indicator of stomatal limitation to photosynthesis.

Material and methods

Study area: The study took place from November 2006 to November 2007 in the Shark River Slough, the main hydrological channel in the Everglades National Park (ENP). The climate is subtropical and humid, with an average annual rainfall of 1450 mm

(http://sofia.usgs.gov), most of which occurs in the wet season that lasts from June to

October. Three tree islands were selected along a west-east gradient from slough to

prairie; Satinleaf (80°45'22.21"W, 25°39'35.21"N) and Chekika (80°39'25.58"W,

25°44'40.52"N, Fig. 3.1) are within the slough while Grossman Hammock

(80°35'0.54"W, 25°36'56.95"N) lies in the prairies that border the slough on the east.

Emergent tree islands present in the Shark River Slough of the southern Everglades are

fixed islands, ie. they are thought to have originated on limestone outcrops in the marsh

whose tops or heads remain above the wet season high water level (Loveless, 1959)

(Fig.3.1, 3.2). These islands have two clearly demarcated plant communities with

practically no species overlap. On the highest part of the island or head are found flood-

intolerant hardwoods (also referred to as upland plants) that are mostly neotropical in

origin. The tropical species in the tree islands and hardwood hammocks of the

Everglades National Park (ENP) are near the northern limit of their global range, because

the southernmost parts of Florida are practically frostfree (Craighead, 1971; Gunderson,

1994; Lodge, 2005). Soils on island heads are 10-40 cm deep on average overlying

bedrock or carbonate layers and are composed of a mix of decomposing leaf litter and

weathered bedrock limestone that has formed over a span of about 500-6000 years BP

(Ross et al 2004, Willard *et al.*, 2006). The lowland community consisting of swamp

forest surrounds the upland portion of the tree island, and experiences a hydroperiod

varying from approximately 1 month at its border with the hardwood hammock, to

around 9 months where it grades into the sawgrass dominated marsh. Soils in lowlands

are peat accumulations from 0.5 – 2 m thick (Craighead, 1971).

Water pools in tree islands: There are two water inputs to tree islands (Fig. 3.2) – direct

precipitation and the regional surfacewater/groundwater pool, the bulk of which flows

from Lake Okeechobee. Hardwood hammocks can potentially access two distinct water sources - rainwater trapped in the upland soil layer (henceforth termed litter water) and groundwater that is part of the regional pool. Swamp forests on the other hand are probably restricted to groundwater that is also in contact with standing surface water from the surrounding marsh, both being part of the regional pool. Upland soilwater being in contact with decomposing leaves and animal feces is thereby enriched in phosphorus with concentrations of soluble reactive phosphorus several orders of magnitude higher than regional ground/surface water (Ross *et al.*, 2008). Additionally, the head of a tree island typically has greater biomass per unit area than the lowland and surrounding marsh.

Collection of soilwaters and plant stemwater: Soilwaters were sampled every two months as follows: In the uplands, *5* soil cores (0-15 cm depth) per island were randomly obtained and placed in stoppered glass tubes, sealed with Parafilm and stored in a freezer to avoid fungal/microbial respiration until distillation. In the lowlands, 5 soil samples were taken along a transect from the upland-lowland ecotone to the outer edge of the island, the lowland-sawgrass marsh ecotone. In addition, 5 surface water samples were collected from the marsh just outside the tree island, at a depth of 15 cm. Water samples were placed in scintillation vials, sealed with Parafilm (Pechiney, Chicago, IL, USA) to prevent evaporation and refrigerated in the lab. A rainwater collector (glass bottle with a 1cm layer of mineral oil to prevent evaporation) was placed in Grossman Hammock, and the isotopic composition of these samples was combined with that of rainfall collected every 2 weeks for a year (March 2005 – March 2006) during an earlier study (Saha et al, 2009) to construct the Everglades Meteoric Water Line.

Every two months over the year, 20 plants were sampled in the upland and 30
plants in the lowland of each tree island using a 5m. extensible pole pruner (Corona, CA,
USA). Because the topographic gradient in the upland appears to influence species
composition, plants were sampled at random locations from the highest point of the
island to the lowland/upland ecotone. Lowland plants were selected along a transect
from the lowland/upland ecotone to the marsh, thus incorporating a range of
hydroperiods. Species sampled in the uplands were: (i) Chekika: *Eugenia axillaris*
(Sw.)Willd., Myrsine guianensis (Aubl.) Kuntze, Celtis laevigata Willd., Sideroxylon
foetidissimum (Jacq.)Cronquist (ii) Satinleaf: *E. axillaris, Bursera simaruba (L.) Sarg.,*
Coccoloba diversifolia Jacq., C. laevigata, Chrysophyllum oliviforme L. (iii) Grossman
Hammock: *Ardisia elliptica, C. diversifolia, B. simaruba, Nectandra coreacea (Sw.)*
Griseb., Simarouba glauca DC. For lowlands, I sampled the following (i) Chekika:
Annona glabra L., Salix caroliniensis Michx., Persea borbonia (L.)Spreng., Morella
cerifera L., Schinus terebinthifolius Raddi. (ii) Satinleaf: *A. glabra, S. carolinensis,*
Chrysobalanus icacoL., P. borbonia, M. cerifera (iii) Grossman Hammock: *A. glabra, S.*
carolinensis, M. guinensis, M. cerifera, S. terebinthifolius. For each plant, a well
suberized stem was cut and after removal of the bark and phloem, placed in a sealed glass
tube, parafilmed and frozen on return to the lab.

Water extraction and isotopic analysis: Stem and soilwaters were extracted by the
method of Vendramini & Sternberg (2007). Extracted water was analyzed at the
Laboratory of Stable Isotope Ecology in Tropical Ecosystems (LSIETE) at the University
of Miami for oxygen and hydrogen isotope ratios by equilibration on an Isoprime©
Isotope Ratio Mass Spectrometer connected to a Multiflow© system (Elementar,

Germany) as described by Vendramini & Sternberg (2007). Oxygen and hydrogen isotope ratios are reported here as $\delta^{18}O$ and δD values respectively and calculated as:

$$\delta^{18}O \text{ or } \delta D \; = \; \{(R_{sample}/R_{SMOW}) - 1 \} * 1000$$

where R_{sample} and R_{SMOW} represents the ratio of heavy to light isotopes in the sample and the standard respectively. The standard for water isotope ratios used here is Vienna standard mean ocean water (vSMOW) and the precision of analysis ±0.1‰ and ±2.0‰ for oxygen and hydrogen isotopes, respectively (±1standard deviation).

The use of oxygen isotopes yields a more precise value of water use than deuterium isotopes because δD exhibits considerably more variation than $\delta^{18}O$. In addition, there is the possibility that certain species of plants discriminate against deuterium during water uptake (Ellsworth & Williams, 2007; Lin & Sternberg, 1993).

Foliar nutrient and isotopic analysis: Mature leaves for each individual sampled for stemwater were collected from the same branch, placed in paper bags and subsequently dried in an oven at $50^\circ C$ for 72 hours. Dried leaves were then ground and homogenized, in preparation for foliar N, P, $\delta^{15}N$ and $\delta^{13}C$ analysis at LSIETE. Preparation of samples for P analysis consisted of ashing 700 mg of the dried ground leaves in porcelain cups in a furnace kept at $600^O C$ for 6 hours, followed by digestion in 4% sulfuric acid and filtration through glass fiber filter paper (Whatman, UK). Analysis was carried out on the filtrate. Foliar P concentration was obtained by the USEPA method 365.1 (USEPA, 1984) based upon the molybdate blue colorimetric approach (Fiske & SubbaRao, 1925) using an Alpkem 3000 Phosphorus analyzer (Alpkem, OI Analytical, TX, USA), and expressed as the percent ratio of the mass of P in the sample to the mass of the sample (dry leaf). The precision of analysis was ± 0.1 ppm (1standard deviation).

Foliar N, $\delta^{13}C$ and $\delta^{15}N$ analysis: Ground leaf samples (5 mg) were loaded in individual tin cups (Elemental Micro-analysis, Milan, Italy), rolled into tight balls and placed in an automated elemental analyzer (Euro-EA-Elemental Analyzer, Eurovector, Milan, Italy) connected to a continuous flow isotope ratio mass spectrometer (Elementar, Germany). Foliar N concentration was expressed on a per weight percent basis, similar to foliar P.

Carbon-13 and Nitrogen-15 abundances are expressed as $\delta^{13}C$ and $\delta^{15}N$ values respectively, as represented by x in the generic formula:

$x(‰) = [(R_{sample}/R_{standard})-1] \times 1000$

in which R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ ratios of the sample and the universal standard (the PeeDee belemnite formation of South Carolina for $^{13}C/^{12}C$ and atmospheric nitrogen isotopic composition for $^{15}N/^{14}N$). The precision of analysis was $\pm\ 0.1‰$.

Data Analysis: To examine whether there were any differences in isotopic ($\delta^{18}O$) composition in stemwater between upland and lowland communities, a 3-way analysis of variance (SPSS 12.0, Chicago, USA) was performed using $\delta^{18}O$ as the dependant variable and community (upland/lowland), tree island (Chekika/Satinleaf/Grossman) and time (6 sampling events over 1 year) as three independent variables. A linear correlation was carried out to observe whether there was any relation between surfacewater and stemwater from upland and lowland communities separately. Four 3-way ANOVAs were employed to examine differences in Foliar P, N, $\delta^{15}N$ and $\delta^{13}C$ as the dependant variables respectively, with community, tree island and time being the independent variables.

Tukey post-hoc tests and Bonferroni corrections were applied to analyze the significant

interactions.

Results

Sourcewater isotopic composition: $\delta^{18}O$ values for upland soil water, rain, lowland soil

water and marshwater (regional surfacewater) averaged for all three islands over a year

represented an increasing gradient with rain and upland soil having lower values than

lowland soil water and marshwater. Values were -3.22 ± 0.2 (± SEM) ‰ for rain, -4.18 ±

0.65 ‰ for upland soil waters, -1.03 ± 1.03 ‰ for lowland soil waters and 0.35 ± 0.45 ‰

for marshwater.

Plant stemwater isotopic composition in upland and lowland plant communities: The

isotopic composition of upland plant stemwater (both $\delta^{18}O$ and δD) were found to be

significantly lower than those of lowland plant stemwaters throughout the year (Fig. 3.3);

this was corroborated by the results of a 3-way ANOVA carried out on plant stemwater

$\delta^{18}O$ values with community, tree island and time as independent factors (Table 3.1).

There was a significant interaction between community and time with a greater difference

between $\delta^{18}O$ values of stemwater from upland and lowland communities during the end

of the wet season (November, Fig. 3.3). As the dry season proceeds, upland plant

stemwater values tend toward the lowland plant values (January and March, Fig. 3.3) and

almost overlap at the end of the dry season (May, Fig. 3.3). Then as the wet season

follows, upland plant stemwater $\delta^{18}O$ values start to diverge from lowland values (July

and Sept, Fig. 3.3). There was also a tree island effect, a time effect as well as

interactions between community type x tree island and tree island x time on stemwater

$\delta^{18}O$ values (Table 3.1). Tree islands differed significantly in their mean stemwater $\delta^{18}O$

values (Table 3.1), with both Tukey and Bonferroni post-hoc tests identifying Satinleaf island as having the most enriched values followed by Grossman and Chekika (P<0.01). The difference in stemwater $\delta^{18}O$ between upland and lowland communities was similar for Chekika and Satinleaf (2 ‰ and 1.93‰ respectively) while Grossman had lower difference on average (1.32‰). Mean $\delta^{18}O$ values of all islands varied significantly with season with dry season mean $\delta^{18}O$ values more enriched than wet season values. Lowland plant stemwater $\delta^{18}O$ values were correlated wth those of surface water/regional water pool while upland plant stemwaters were not (Fig. 3.4).

Foliar nutrients: The average foliar P concentration in upland communities was found to be significantly higher than in lowland communities (Table 3.2, Fig. 3.5). There was also a significant tree island effect upon foliar P concentration (Table 3.2), with Satinleaf having the highest values followed by Grossman and then Chekika. The difference in foliar P between upland and lowland communities also varied with tree island.   Season (time) had no significant effect on foliar P at a community level (averaging over species). Foliar N concentrations, unlike foliar P, were significantly less in uplands at the community level as compared to lowlands (Table 3.2, Fig. 3.5). There was no effect of either tree islands or seasons. The N:P ratio was lower in uplands than in lowlands for each island (Fig. 3.6; one-way ANOVA: F = 39, P<0.001) and were 12-20 for upland plants while being greater than 30 for lowland plants.

Foliar $\delta^{15}N$: Upland communities had higher foliar $\delta^{15}N$ levels than lowland plant communities in both seasons (Table 3.12). A large difference in foliar $\delta^{15}N$ was observed between tree islands, with the islands within the slough, Satinleaf and Chekika having significantly higher $\delta^{15}N$ than Grossman Hammock, that is on the edge of the slough.

The difference in foliar $\delta^{15}N$ between upland and lowland communities also depended upon the tree island owing to an interaction between community and tree island. A small but significant difference existed between seasons with $\delta^{15}N$ values higher in the wet season (Table 3.2).

Foliar $\delta^{13}C$: Upland plant communities had significantly higher foliar $\delta^{13}C$ values than lowland communities (Table 3.2) in both wet and dry seasons. For each community, dry season values were higher than wet season values as indicated by a significant time effect. There was also a tree island effect whereby foliar $\delta^{13}C$ values increase along the gradient from slough to prairie (Satinleaf < Chekika < Grossman Hammock – Tukey posthoc test). Lowland plant communities had significant correlations (P < 0.01) between $\delta^{13}C$ values and foliar N at the end of the dry season (Fig. 3.7). Upland plants had no significant correlation in Satinleaf and marginally significant in Chekika and Grossman Hammock at the P=0.05 level.

Discussion

Water source usage by upland and lowland plant communities:

The water sources, litter water and regional water, have distinct isotopic compositions that reflect different exposures to hydrological processes. This consistent difference in sourcewater composition permits determination of the water sources utilized by plants. Marsh or surface water has more enriched values (a greater proportion of heavier isotopes ^{18}O and D) than litter water, an outcome of greater exposure leading to much higher evaporation rates than litter water which is shaded by the tree canopy. Surface water values also vary less with season than litter water, because the large volume of this regional pool buffers the isotopic signature of the much smaller rain pool (Saha *et al.*,

2009). Lowland soilwater is a mix of rain and surrounding marshwater that is drawn in from around the island by a transpiration-driven potential gradient, and it has an attendant decrease in exposure to further evaporation. Thus lowland soilwater has isotopic values between marshwater and litter water.

The difference between upland and lowland plant stemwater isotopic composition through most of the year (Fig. 3.3) suggests that different proportions of the two sourcewater pools are utilized by these two plant communities. During the wet season, upland plants are able to access the rainwater trapped in the organic soil on the tree island head – a conclusion arrived at from three observations: (i) upland plant stemwater $\delta^{18}O$ composition is closer to the Everglades Meteoric Water Line than lowland plant stemwaters (Fig. 3.3), implying that upland plant stemwater composition is close to rainwater; (ii) upland plant stemwater is isotopically similar to upland soilwater composition and (iii) $\delta^{18}O$ values of upland stemwater are not correlated with those of marshwater (Fig. 3.4). This is expected because roots preferentially take up water close to them (ie. in the shallow soil). As the dry season progresses, I propose that the water in the tree island head soil begins to dry up, and upland plants start taking up more of the regional water (groundwater in the rhizosphere). This is suggested by upland stemwater $\delta^{18}O$ composition approaching that of lowland plants (Fig. 3.3) starting from January with the closest values in this study being in May near the end of the dry season. Lowland plants use regional water throughout the year as suggested by their stemwater $\delta^{18}O$ values being consistently more enriched than those of upland plant stemwater (Fig. 3.3) as well as being correlated with marshwater (Fig. 3.4). This conclusion is further supported by the relatively static nature of lowland plant stemwater $\delta^{18}O$ and δD composition throughout the year versus that of upland plants (Fig. 3.3), which reflects

marshwater composition varying less with season than soilwater. Being subject to a hydroperiod of 3-9 months, lowland species have various adaptations to flooding, such as the formation of adventitious roots in *Salix carolinensis*.

Extended drought can negatively affect upland trees on tree islands: Our results indicate that upland trees use significant amounts of groundwater in the dry season. Given that in the dry season the water table exists below the organic soil horizon (that is just 30 cm thick on average, Ross *et al.*, 2008), roots must be penetrating the limestone bedrock or through hard precipitated carbonate layers (Graf *et al.*, 2008) to access groundwater. Root distribution in tree island hammocks is unknown; the flood-intolerant nature of these species precludes root strategies to tolerate prolonged flooding. However, there could be a selective pressure on these species for maintaining dormant main roots in the limestone that would be submerged in the wet season. While fine roots branching off these main roots are likely to die off under anoxic conditions, they could regrow in the dry season when the water table recedes, and take up water from the capillary-recharged vadose zone . Now, a lower than normal water table could result in water stress or even permanent wilting if roots are unable to find suitable pathways or grow at a rate fast enough to access the lowered water table. Peat-filled sinkholes also are present on tree island heads; lowering of the water table can result in peat fires that can destroy plant roots as has been described for the Long Pine Key hammock fires in 1959 (Craighead, 1974).

Plant communities and foliar nutrients

Higher foliar phosphorus concentration at the community level in upland plants compared to lowland plants is consistent with P-rich litter water being an important water

source for upland plants, especially during the wet season which is the growing season for many of the upland plants. Besides, greater availability of P on tree island heads also explains the higher foliar $\delta^{15}N$ levels in upland plants than in lowland plants (Table 3.2), because higher P uptake is accompanied by a correspondingly higher N uptake in order to satisfy stoichiometric nutrient relationships for leaf function. The higher N uptake in turn can decrease the discrimination against ^{15}N if available N becomes limiting, thus leading to higher foliar $\delta^{15}N$ values in upland plants.

The link between soil nutrient availability and community level foliar nutrient concentration is also seen for foliar N. The lower community-level foliar N concentrations in upland plants compared to lowland plant species seen in this study agrees with the trend in soil, whereupon soil porewater nitrogen was found to be lower in tree island heads as compared to lowlands (Ross et al 2006). The unsaturated soil zones of uplands can have higher rates of nitrification and subsequent loss of nitrogen to the atmosphere than in flooded soils, where the reducing environment can store nitrogen as ammonia.

The significantly higher N:P ratio of lowland plants than upland plants (Fig. 3.6) (community averages 30-45 for lowlands and 12-20 for uplands) suggests that lowland zones are more phosphorus-limited than upland zones as also found by Ross et al (2008). Flooding for 3-9 months can result in P transport out of lowland soils, whereas uplands retain much of their P. Comparing the community N:P ratios of upland plant communities in tree islands (12-20) with those of the only other upland communities in the Everglades -- hardwood hammocks located further south amidst pine rocklands on the Miami Rock Ridge (N:P around 45- Saha et al 2009) suggests that hammock

communities in the slough have higher phosphorus availability than hammock

communities on the Miami Rock Ridge. The difference in N:P ratios is also because of

differences in species composition, although both types of upland communities have

neotropical hardwoods. Other plant communities in the Everglades have ratios higher

than that for tree island hammocks such as approximately 42 for pine rocklands (Saha *et*

al., 2009) and 84 for sawgrass (Richardson *et al.*, 1999) in the northern Everglades, thus

supporting the idea of tree islands as localized nutrient hotspots in the oligotrophic

Everglades landscape.

Nutrient utilization and photosynthetic performance: The positive correlation of $\delta^{13}C$

with foliar N in lowland plant communities at the end of the dry season (Fig. 3.7)

suggests that there is stomatal limitation of photosynthesis . High foliar N concentration

implies high chlorophyll and carboxylation enzyme content (Duursma & Marshall, 2006)

which in turn leads to high photosynthesis that increases the proportion of ^{13}C

incorporated in leaf cellulose. The high ^{13}C values are a consequence of the ensuing

stomatal limitation on atmospheric carbon dioxide CO_2 inflow to leaves, thereby

lessening the discrimination against ^{13}C by Ribulose Bis-Phosphate Carboxylase

(Farquhar *et al.*, 1982). Stomatal limitation can be caused either by elevated

photosynthetic demand of CO_2 over incoming supply, even with the stomata fully open

(here the stomata constitute a resistance to free inflow), or by some degree of stomatal

closure triggered by the need to reduce transpirational water loss when water is limiting.

For lowland plants, the former possibility would seem to apply. The lower correlation

values for upland plant communities suggests that photosynthesis is not limited by N but

by some other factor, possibly access to water in the dry season or extreme events such as

droughts or hurricanes (Wetzel *et al.*, 2008).

Nutrient input into tree islands: Three pathways are thought to exist for nutrient input to tree islands: (i) the transpiration-driven pathway whereby groundwater and marshwater (regional water) is drawn to tree islands ; dissolved nutrients in this regional water get assimilated into foliar biomass which then decomposes to contribute to organic soil over time; ii) atmospheric deposition and iii) animal feces (Wetzel *et al.*, 2005). These inputs can co-occur but how the relative magnitude of each process differs from island to island is not yet known. Our results show that upland plants utilize P-rich litter water during the wet season and P-poor regional water during the dry season; this is consistent with the transpiration-driven pathway of P buildup in Everglades tree islands. Our results add a temporal dimension to this hypothesis consisting of a nutrient utilization phase during the wet season and a nutrient harvesting phase during the dry season.

Conclusions

Based on our findings, certain predictions can be made regarding the impact of hydrological changes on tree islands: if water levels in the wet and/or dry season are much higher than normal, mortality of flood-intolerant upland trees and nutrient loss via transport can occur. At the other extreme, if water levels are much lower than normal, drought-induced mortality of upland trees and nutrient loss because of peat fires can occur. Our findings also support the contention that the organic soil layer on tree island heads is an instance of a vegetation feedback upon the barely emergent surface. This feedback allows the establishment and survival of neotropical flood intolerant hardwood species (Jones *et al.*, 2006) by specifically providing an unsaturated soil environment (Armentano *et al.*, 2002), and by acting as a nutrient sink over time.

Coupling water sources and foliar nutrient status can be an effective tool to identify nutrient sources and periods of nutrient uptake by plants. This information can enable scientists and restoration ecologists to discern how coexisting plant communities in an ecosystem differ in the partitioning of nutrient pools and how seasonal changes in the sizes of water pools can result in plants altering their water sources. This understanding of water and nutrient pools can also be used to make predictions on the fate of plant communities when subject to modifications of water and nutrient regimes.

Table 3.1: Results of a 3-way univariate analysis of variance carried out on plant stemwater δ^{18}O value as the dependant variable in each case with community (upland/lowland), tree island (Chekika/Satinleaf/Grossman) and time (6 sampling dates bimonthly over one year) as independent factors. The entire dataset over 1 year was included in this analysis.

Dependant variable	*Source*	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Stemwater δ^{18}O	Community (upland/lowland)	715.988	1	715.988	349.725	**.000**
	Tree Island	155.465	2	77.733	37.969	**.000**
	Time	167.754	6	27.959	13.657	**.000**
	Community * Tree Island	29.042	2	14.521	7.093	**.001**
	Community * Time(season)	63.619	6	10.603	5.179	**.000**
	Tree Island * Time	154.654	8	19.332	9.443	**.000**
	Community * Tree Island * Time	7.542	8	.943	.460	.884

Table 3.2: Results of four 3-way univariate analyses of variance carried out on foliar P, foliar N, foliar $\delta^{15}N$ and foliar $\delta^{13}C$ values as the dependant variable respectively, with community (upland/lowland), tree island (Chekika/Satinleaf/Grossman) and time (wet and dry season) as independent factors. The entire dataset over 1 year was included in these analyses.

Source	*df*	*Foliar P*		*Foliar N*		*Foliar $\delta^{15}N$*		*Foliar $\delta^{13}C$*	
		F	*Sig.*	*F*	*Sig.*	*F*	*Sig.*	*F*	*Sig.*
Community	1	87.131	.000	21.749	.000	57.438	.000	45.164	**.000**
Tree island	2	4.285	.015	.903	.407	65.529	.000	5.222	**.006**
Time	1	.184	.669	.627	.429	5.092	.025	4.582	**.033**
Community * Tree island	2	5.917	.003	4.313	.014	38.982	.000	.607	.546
Community * Time	1	.053	.818	3.250	.073	2.562	.111	.541	.463
Tree island * Time	2	1.165	.314	4.101	.018	1.003	.368	1.513	.222
Community * Tree island * Time	2	.399	.671	1.676	.189	.039	.962	.218	.804

Figure 3.1: Aerial view of Chekika tree island showing the head (upland/hammock species) and tail (swamp species).The block arrow indicates direction of water flow (southwesterly direction) along which is aligned the longitudinal axis of the tree island.
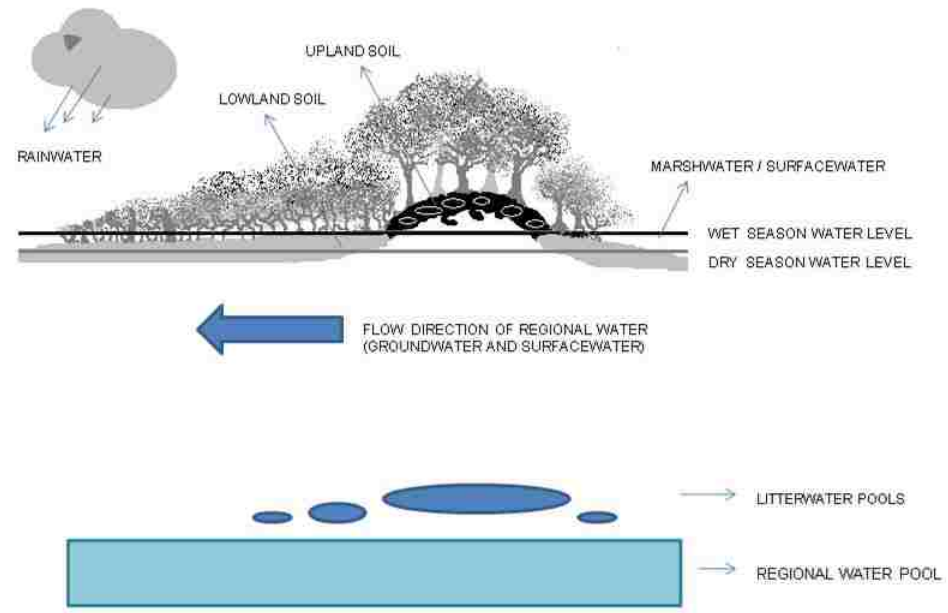
Figure 3.2: Exaggerated profile of a fixed tree island in Shark River Slough, ENP showing the two distinct plant communities: the flood intolerant hammock vegetation on the head (above wet season water level) and the flood tolerant swamp forest in the rest of the island submerged in the wet season. The organic soil horizon on the head (upland soil) is shown in black while flooded peat sediments (lowland soil) in gray. The two water sources are rainwater trapped in the organic soil layer on the tree island head (shown in ellipses within the organic soil layer) and the regional surface/groundwater pool. These two sources are schematically illustrated at the bottom of the Figure. Water in the upland soil (litter water) is mainly entrapped rainwater (wet season) while lowland soilwater is primarily regional water with some rainwater.
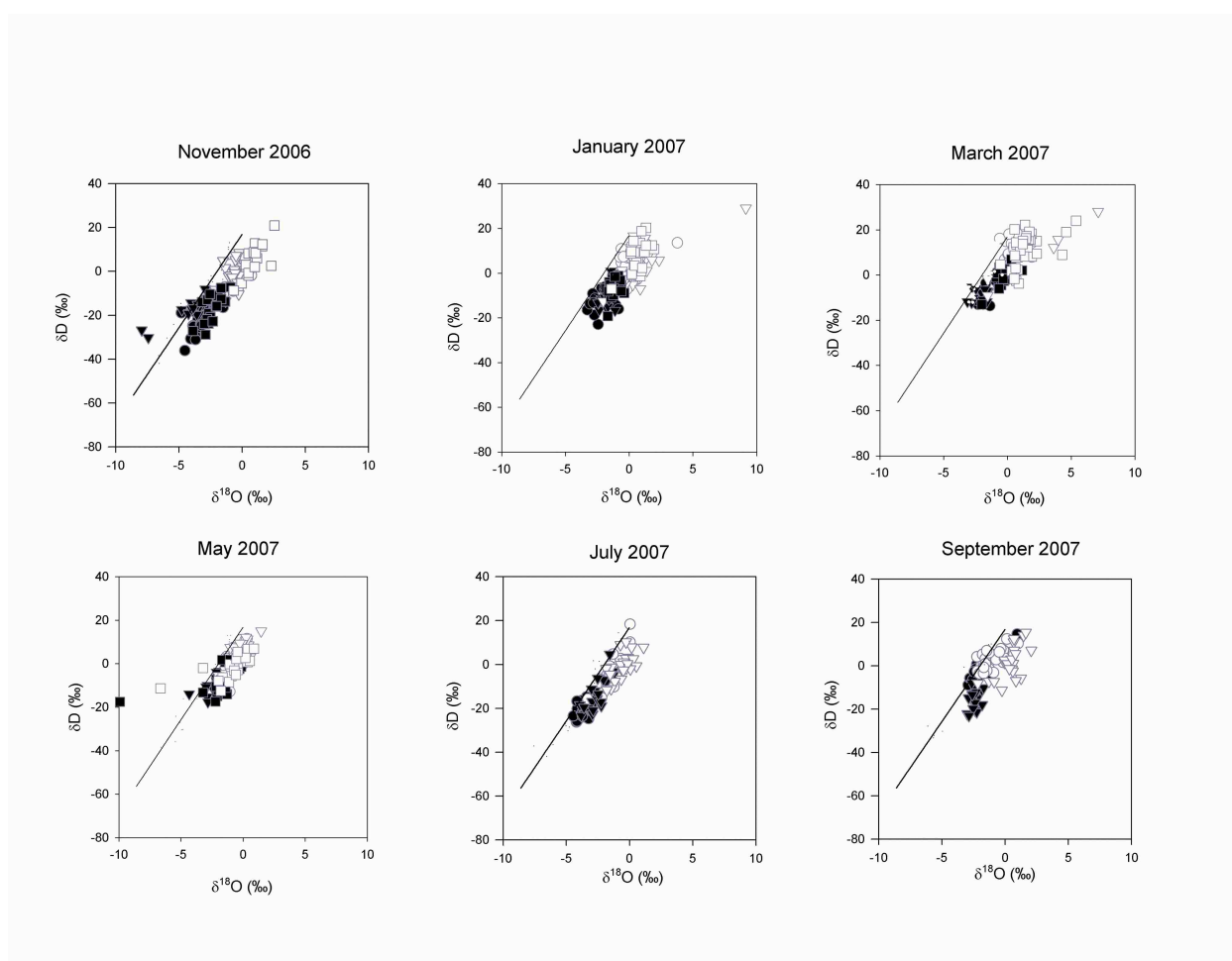
Figure 3.3: Plots of δD vs. δ ^{18}O of plant stemwater in upland (shaded symbols) and lowland (open symbols) communities for Chekika (circles), Satinleaf (squares) and Grossman Hammock (triangles) sampled every two months from November 2006 to September 2007 in Shark River slough, ENP (n = 20 for upland plants and 30 for lowland plants per sampling). Also shown is the Everglades local meteoric water line (δD = 8.5 δ ^{18}O + 17 ‰, r^2 = 0.93, P < 0.01) for rainfall collected 2005 -2006 (n = 72).

Figure 3.4: Relationship between lowland stemwater δ^{18}O (o) and upland stemwater δ^{18}O (•) values with surface water δ^{18}O. Each point in the plot represents stemwater and surface water values averaged over all three tree islands for each sampling month (n= 60 for upland plants, = 90 for lowland plants and 6 for surface water respectively); seven sampling events are shown here (November 2006 to November 2007 inclusive). Error bars represent standard error of the means for stemwaters. The correlation between lowland stemwaters and surface water (r = 0.74, barely significant at P=0.05) was stronger than that between upland stemwaters and surface water (r = 0.11, not significant).

Figure 3.5: Average foliar P (left) and average foliar N (right) in lowland (solid) and upland communities (hatched) in all three tree islands at the end of the dry season (May). Values expressed as concentration (eg. milligrams P/milligrams dry leaf tissue). Upland plant communities have a significantly higher foliar P concentration and significantly lower foliar N concentration than lowland plant communities. Error bars depict the standard error of the mean. n = 20 for uplands and 30 for lowlands respectively.

Figure 3.6: Foliar N:P ratios in lowland (solid) and upland (hatched) communities in all three tree islands at the end of the wet and dry seasons (November and May). Lowlands plants have significantly higher N:P ratios than upland plants. Error bars depict the standard error of the mean. N = 20 for uplands and 30 for lowlands respectively.

Figure 3.7: Foliar $\delta^{13}C$ vs. foliar N in upland (•) and lowland (o) plant communities in three tree islands at the end of the dry season (May 2007). Correlation was found to be significant at the 0.01 level for lowlands in all three tree islands (Satinleaf n = 30, r =0.75, P<0.01; Grossman n = 20, r=0.66, P<0.01; Chekika n=34, r=0.48, P<0.01). Uplands had marginally significant correlation in Grossman (n=20, r = 0.47, P = 0.05) and Chekika (n=18, r = 0.54, P = 0.05) while Satinleaf island was found to be not significant.

CHAPTER 4

Relating water sources and foliar nutrients explains leaf phenological patterns in hammock and swamp forests in the Everglades.

Summary

Temporal and spatial variation in water and nutrient availability is a major determinant of vegetative phenology of plants in tropical forests. Studies so far have attempted to correlate soil moisture with leaf phenology stages. Here, I identified the water sources and nutrient pools used by plants using stable isotopes of water, and related that to the period of leaf growth. The stable isotope of carbon was used as an indicator of water stress during the period of leaf fall. This study was carried out in hammock forests and lowland swamp forests in the Everglades National Park (ENP). Leaf fall in hammock forests on the Miami Rock Ridge within ENP occurs in the dry season, and is associated water stress as indicated by elevated foliar carbon isotope values. Hammock trees use phosphorus (P)-rich soil water from the organic horizon in the wet season, and upon cessation of that, are driven to P-poor groundwater/regional water use. There is an increase in foliar P in the wet season that indicates the period of leaf growth and expansion. Thus most species assimilate P in leaves in conjunction with increasing soilwater uptake. Hammock forests on tree islands in Shark River Slough exhibited much lower seasonal differences (both foliar $\delta^{13}C$ and foliar P), possibly arising from the more mesic nature of slough tree islands than hammock forests on the Miami Rock Ridge. Swamp forests species exhibited a trend opposite to hammock forests, whereby some species lose their leaves in the wet season and get new leaves at the beginning of the dry season. Foliar carbon isotopes, however, did not indicate flooding stress at the

end of the wet season. The increase in foliar P over the dry season identifies this season as the period of leaf growth in swamp forests. Linking water sources and nutrient pools can indicate periods of availability of these resources critical to leaf phenology at both species and community levels. This technique has the potential to further the understanding of the function in tropical forests, especially seasonal or moist tropical forests where there is a high diversity of phenological patterns in coexisting species.

Background:

Temporal and spatial variation in water and nutrient availability is a major factor determining the vegetative phenology of plants in tropical forests. The sequence of leaf fall, budbreak, flowering and fruiting is an adaptation of a plant to its local environment (Van Schaik 1993). Water has been identified as the main limiting resource, with leaf abscission being the most significant drought response of trees in dry tropical forests (Lieberman 1982, Holbrook 1995, Williams et al 1997) as well as in seasonal subtropical forests (eg. Frankie 1974, Shukla & Ramachandran 1982, Williams-Linera 1997). Leaf fall occurs in order to limit water loss to the plant via transpiration. Similarly, leaf flushing and growth requires water (Lieberman 1982, Borchert 1994), prompting most species in dry tropical forests and seasonal subtropical forests to flush only on arrival of the wet season (eg. Lieberman 1982, Shukla & Ramakrishnan 1982, Funch et al 2002). At the other extreme of the water availability gradient, flooding stress also causes leaf fall as has been reported in Amazonian flooded forests (eg Parolin 2000, De Simone et al 2003, Schongart et al 2002, Haugaasen 2005) and elsewhere (eg Blom et al 1990, Kozlowski 1997).

Studies linking plant and soil water status/rainfall to patterns in leaf phenology have been largely observational, correlating the measures of soil and plant water status to phenological stages (eg. Borchert 1994, Chapotin et al 2003, Dunham 1991, Alvim & Alvim 1978, Duff et al 1997). Such correlations are easily observed in temperate and seasonal dry tropical forests where most species share similar patterns of leaf fall, leaflessness and leaf renewal. However in moist tropical and subtropical forests, as the seasonality decreases, the diversity of both inter- and intra-specific phenological patterns increases, (eg Tomlinson 1980, Reich & Borchert 1982, Huxley & Van Eck 1974, Wright 1996) owing to a wide variety in controlling factors (Corlett & LaFrankie 1998). Here we present a novel approach of linking leaf phenology events with the identity of the water sources used by the tree, along with the seasonal variation in these sources. Stable isotopes of water have been widely used to determine water sources of plants (refs). One can then relate the onset of leaf fall and subsequent leaf flush and growth to water source usage and availability. Tracking water sources provides a more definitive indication of water availability and uptake by a plant than soil moisture values alone because the isotopic composition of plant stemwater can reflect the composition and thus the identities of the specific sources of water taken up by the plant at an instant of time. This can be especially useful when water uptake occurs at specific depths in the soil profile. For instance, the coexistence of deciduous and evergreen trees has been thought to reflect the different rooting depths of each group, with evergreen trees accessing deeper, less variable water sources (eg. Medina 1982, Sarmiento 1984). Indeed, a detailed understanding of dry forest ecosystems is hampered by the lack of knowledge of water sources of trees (Sayer & Newberry 2003). The stable isotope of carbon has been widely used to detect water stress in plants that is accompanied by an increase in the $\delta^{13}C$ values

of sucrose and other plant compounds produced in the leaf. This is because stomatal contraction and/or closure limits the supply of CO_2 for photosynthesis which, in turn, lessens the carboxylation enzyme (Rubisco) discrimination against ^{13}C (Farquhar et al 1982).

The production of new leaves, flowers and fruits requires nutrients, in addition to adequate amounts of water (Chapotin et al 2003). Nutrients and water, however, are closely linked because nutrient uptake happens in the dissolved form (Baldwin 1975) and different pools of water differ in their nutrient availabilities (eg. Saha et al 2009a). Thus the knowledge of the water sources or combination of sources that are used at different times over the year can indicate periods of relatively high nutrient availability. Relating the periods of water and nutrient availability with the timing of leaf growth, flowering and fruiting can indicate whether the plant relies upon direct uptake of water and nutrients during these phenological events, or whether the plant uses stored resources for these events. Foliar nutrient concentrations have been used as an indicator of nutrient availability to plants (eg Aerts & Chapin 2000, Campo & Dirzo 2003, Bertiller et al 2006, Saha et al 2009a), and thus, in addition to water source uptake, can indicate periods of high nutrient assimilation, and by inference, nutrient availability. From a phenological perspective, an increase in foliar nutrient concentration indicates the leaf growth stage; diminished foliar nutrient concentrations indicate leaf maturity followed by leaf senescence.

Hydrologically controlled ecosystems such as the Everglades have a mosaic of upland (hammock) and flooded (swamp forest) plant communities exhibiting a variety of phenology patterns, which may result from the tremendous spatial and temporal

heterogeneity in water and nutrient pools. Hardwood hammocks on elevated rocklands and tree islands potentially can access two distinct water sources – (i) rainwater trapped in the upland soil layer positioned always above the water table (henceforth termed soilwater) and (ii) groundwater that is part of the regional pool (Saha et al 2009a, 2009b). Swamp forests on the other hand most probably are restricted to the regional pool. Soilwater being in contact with decomposing leaves and animal feces is enriched in phosphorus, with concentrations of soluble phosphorus several orders of magnitude higher than in the regional water pool (Ross *et al.*, 2008). Swamp forest species often flush new leaves at a time when hammock species are losing theirs (Table 4.1). Furthermore, gradients in elevation lead to large differences in soil moisture, that have been associated with deciduousness in several studies (eg. Reich & Borchert 1984**,** Kapos 1986**,** Swaine 1992**).** Hammocks vary in their mix of deciduous and evergreen species (Gilchrist 2006) with Ross et al (2001) suggesting the replacement of deciduous by evergreen species may reflect soil conditions that provide buffering from dry season water stress.

Given that changes in the hydrological regime in the Everglades have affected spatial distribution and extent of plant communities through species displacements, the understanding of species-specific patterns of water sources, leaf nutrient concentrations and phenological behavior will elucidate current patterns of species distribution and might predict the fate of species under modified water and climate regimes. In this study we link water sources with foliar nutrients for species in both terrestrial (upland) and adjacent flooded forest communities in the Everglades. We relate this information with phenological data (both published literature and field observations), in particular focusing on leaf fall and leaf growth periods. We specifically test the following hypotheses: (1)

Leaf fall in the Everglades hammocks and swamp forests is associated with water stress, as determined by foliar carbon isotopes, with hammocks being stressed in mid-late dry season and the swamp forests being flood-stressed in the late wet season. (2) Leaf growth coincides with the period when water with a relatively high nutrient concentration is available in the wet season for hammock forests and in the dry season for swamp forests. Foliar P and stable isotopes of water will be used to identify the period of high nutrient availability along with phenological data from the literature and field observations.

Materials and methods

Study area: This study took place in hammock and swamp forests in the Everglades National Park (ENP). The climate is subtropical and humid, with an average annual rainfall of 1450 mm (http://sofia.usgs.gov), most of which occurs in the wet season that lasts from June to October. Hammock forests occur on the highest elevations in the Everglades, and are found on the Miami Rock Ridge (henceforth referred to as rockland hammocks, Saha et al 2009a) as well as on the unflooded parts or the heads of tree islands in the Shark River Slough (henceforth called tree island hammocks, Saha et al 2009b). However the difference in elevation between the hammock soil surface and the water table in the wet season is less than 1 m.

Rockland Hammocks: Hardwood hammocks on the Miami Rock Ridge in the eastern part of ENP were studied from March 2005 to March 2006 at three previously studied sites (Ewe *et al.* 1999, Saha et al 2009a) differing in elevation and hydroperiod along an east-west increasing rainfall gradient at Royal Palm (25˚23'39"N, 80˚37'17"W), Long Pine Key (25˚24'10"N, 80˚37'45"W) and Sisal pond (25˚23'29"N, 80˚39'05"W). These

hammock forests do not flood and are surrounded by pine rocklands. Soils are about 30

cm in depth on average (Saha et al 2009a) and overlie limestone bedrock.

Tree islands: Tree islands in the Shark River Slough, the main hydrological channel in

the central ENP were studied from November 2006 to November 2007. (Saha et al

2009b). Three tree islands were selected along a east-west gradient from prairie to

slough; Satinleaf (80°45'22.21"W, 25°39'35.21"N) and Chekika (80°39'25.58"W,

25°44'40.52"N) are within the slough while Grossman Hammock (80°35'0.54"W,

25°36'56.95"N) lies on in the eastern edge of the slough bordering prairies. These islands

have two clearly demarcated plant communities: hammock forests and swamp forests

with practically no species overlap. Soils on island heads are 10-40 cm deep on average,

overlying bedrock or carbonate layers and are composed of a mix of decomposing leaf

litter and weathered bedrock limestone that has formed over a span of about 500-6000

years BP (Ross et al 2004, Willard *et al.*, 2006). Swamp forest surrounds the upland

portion of the tree island and has a hydroperiod ranging from approximately 1 month at

the hammock-swamp ecotone to around 9 months at the edge of the swamp-sawgrass

marsh edge. Soils in lowlands are peat accumulations from 0.5 – 2 m thick (Craighead,

1971).

Species: Most of the species on both rockland and tree island hammocks are of

neotropical origin, and their occurrence on the frost-free southern tip of Florida

constitutes the northern boundary of their biogeographical range. These species are

intolerant of flooding but are thought to have dispersed to South Florida over the sea

from the Caribbean via zoochoric pathways (Gilchrist, 2006). Lowland swamp forests,

on the other hand, are a mix of temperate and tropical species that differ in their

tolerances to flooding. Species in both hammocks and swamp forests differ in their leaf

phenology patterns that can be classified (Shukla & Ramakrishnan 1982) as deciduous

trees that have a distinct leaf less period, as semi-deciduous or evergreen periodic trees

that have a leaf fall and flush period, and as evergreen-evergrowing trees that continually

exchange leaves and do not have a distinct leaf fall or flush season. Table 4.1 lists the

species in this study; species selected were the most abundant species at each site and

differed from site to site because rockland hammocks have a high beta diversity (Snyder

1990, Koptur 2002). Every two months from March 2005 to March 2006, 5 individuals

of 5 species at each rockland hammock site were sampled (Saha et al 2009a) while in

each tree island, 20 plants (5-10 per species) were sampled in the hammock forest and 30

plants in the swamp forest (Saha et al 2009b) over November 2006-November 2007.

Foliar nutrient and isotopic analysis: 10-15 youngest fully expanded leaves for each

individual sampled for stemwater were collected from the same branch, placed in paper

bags and subsequently dried in an oven at 50° C for 72 hours. If the individual was

emerging out of a leafless period, young immature leaves were taken since they were all

that was present. Dried leaves were then ground and homogenized, in preparation for

foliar N, P and δ^{13}C analysis at The Laboratory of Stable Isotope Ecology in Tropical

Ecosystems (LSIETE) at the University of Miami. Preparation of samples for P analysis

consisted of ashing 700 mg of the dried ground leaves in porcelain cups in a furnace kept

at 600°C for 6 hours, followed by digestion in 4% sulfuric acid and filtration through

glass fiber filter paper (Whatman, UK). Analysis was carried out on the filtrate. Foliar P

concentration was obtained by the USEPA method 365.1 (USEPA, 1984) based upon the

molybdate blue colorimetric approach (Fiske & SubbaRao, 1925) using an Alpkem 3000

Phosphorus analyzer (Alpkem, OI Analytical, TX, USA), and expressed as the percent

ratio of the mass of P in the sample to the mass of the sample (dry leaf). The precision of analysis was ± 0.1 ppm (1standard deviation).

Foliar N and $\delta^{13}C$ analysis: Ground leaf samples (5 mg) were loaded in individual tin cups (Elemental Micro-analysis, Milan, Italy), rolled into tight balls and placed in an automated elemental analyzer (Euro-EA-Elemental Analyzer, Eurovector, Milan, Italy) connected to a continuous flow isotope ratio mass spectrometer (Elementar, Germany). Foliar N concentration was expressed on a per weight percent basis, similar to foliar P.

Carbon-13 abundances are expressed as $\delta^{13}C$ values:

$$\delta^{13}C(‰) = [(R_{sample}/R_{PDB})-1] \times 1000$$

in which R_{sample} and R_{PDB} represent the $^{13}C/^{12}C$ ratios of the sample and the universal standard from the PeeDee belemnite formation of South Carolina. The precision of analysis was ± 0.1‰ (±1σ).

High foliar $\delta^{13}C$ is an indicator of water stress (Farquhar 1982) but is also associated with high foliar N (Cordell et al, Bai et al 2008) due to stomatal limitation of photosynthesis; hence foliar N is analyzed in this study to be able to infer whether high $\delta^{13}C$ values in the dry season are attributable to water stress or to high foliar N in the dry season.

Sourcewaters and plant stemwater: For both hammocks and swamp forests, 5 soil samples were collected per site at 0-20 cm depth. These were placed in stoppered glass tubes, sealed with Parafilm and stored in a freezer to avoid fungal/microbial respiration. Groundwater was collected every 2 weeks at Royal Pam and Long Pine Key sites in rockland hammocks (Saha et al 2009a) where there was access to a well at each site.

Groundwater samples were placed in scintillation vials, sealed with Parafilm to prevent

evaporation and refrigerated in the lab. For each plant, a well suberized stem was cut and

after removal of the bark and phloem, was placed in a sealed glass tube, sealed with

Parafilm (Pechiney, Chicago, Il, USA) and kept frozen in the lab until extraction of the

stemwater via distillation.

Water extraction and isotopic analysis: Stem and soilwaters were extracted by the

method of Vendramini & Sternberg (2007). Extracted water was analyzed at LSIETE for

oxygen and hydrogen isotope ratios by equilibration on an Isoprime© Isotope Ratio Mass

Spectrometer connected to a Multiflow© system (Elementar, Germany) as described by

Vendramini & Sternberg (2007). Oxygen and hydrogen isotope ratios are reported here

as $\delta^{18}O$ and δD values respectively and calculated as:

$$\delta^{18}O \text{ or } \delta D = \{(R_{sample}/R_{SMOW}) - 1\} * 1000$$

where R_{sample} and R_{SMOW} represents the heavy to light isotope ratio of the sample and the

standard respectively. The standard for water isotope ratios used here is Vienna standard

mean ocean water (vSMOW) and the precision of analysis of ±0.1‰ and ±2.0‰ (1σ) for

oxygen and hydrogen isotopes respectively.

Phenological field observations – The stage of leaf development (fall, flushing) and

visual estimates of leaf cover extent were censused every 2 weeks in rockland hammocks

and every 2 months in tree islands, because the latter were logistically difficult to access.

In addition, we rely upon the published literature for additional phenology information

that serves as an approximate guideline for the species. Some publications are based

upon the species being grown in urban thoroughfares and gardens where irrigation can

change water availability and thus the extent of leaf fall.

Data Analysis:

Foliar $\delta^{13}C$ and foliar P: Two separate 2-way ANOVAs were performed to examine seasonal and site differences in foliar $\delta^{13}C$ and foliar P respectively, for each of the three communities: rockland hammock forests, tree island hammock forests and tree island swamp forests. In addition, differences in foliar $\delta^{13}C$ or foliar P between end-dry season (May) and end-wet season (November) at the species-level was examined using one-way ANOVAs and Bonferroni-corrected. In order to examine for any significant difference in foliar P between rockland hammocks and tree island hammocks, four one-way ANOVAs were performed, one at the community level and the other three analyses for the three co-occurring species in both hammock communities– *Bursera, Chrysobalanus* and *Morella.*

Foliar N: Seasonal difference in foliar N concentrations were examined with one-way ANOVAs that were carried out for each of the rockland hammock species having significant seasonal differences in foliar $\delta^{13}C$.

Soilwater fraction of plant stemwater in rockland hammocks: The proportion of the soilwater fraction in stemwater, *x*, was calculated using an end member mass balance equation (Saha et al 2009a). δD values have not been considered in the analysis because they exhibit considerably more variation than $\delta^{18}O$. In addition, there is the possibility that certain species of plants discriminate against deuterium during water uptake (Ellsworth and Williams, 2007, Lin and Sternberg, 1993). A 2-way ANOVA was then performed on a pooled sample set (all samples in rockland hammocks) to examine date and species differences in the soilwater fraction of plant stemwater. One-way ANOVAs were carried out for each species individually to examine if soilwater uptake changed significantly with time/season.

Results

Foliar $\delta^{13}C$: Hammock forests on the rockland showed significantly higher foliar $\delta^{13}C$ values at the community level in the dry season than in the wet season (F = 21, P < 0.001, Table 4.2). *Lysiloma, Morella, Annona* and *Quercus* showed statistically significant higher values in the dry than in the wet season (Fig. 4.1). Other species did have the above seasonal trend, but the seasonal difference in foliar $\delta^{13}C$ was not significant. In comparison to rockland hammocks, the seasonal difference in foliar $\delta^{13}C$ in tree island hammock forests was much smaller (Fig. 4.1) although still significant (F = 4.37, P < 0.05, Table 4.2) with no significant site differences. *Bursera* at Grossman Hammock was the only species with a large seasonal difference ($F_{1,7}$=4.95, P = 0.06, Fig 4.1). There was no significant seasonal difference in foliar $\delta^{13}C$ for swamp forests in tree islands while there was a site effect on foliar $\delta^{13}C$ (F=3.97, P < 0.05, Table 4.2).

Foliar Nitrogen: The only species (*Annona*, *Morella* and *Quercus*) that showed significant seasonal differences were all in the hammock at Sisal Pond (Table 4.3; Fig. 4.2).

Foliar Phosphorus: Hammock forests on the rockland showed a strong seasonal trend at the community level ($F_{5,336}$ = 14.96, P < 0.05) with most of the species (7 out of 9) showing higher foliar P concentration in the wet season than in the dry season (Fig. 4.3). Unlike rockland hammocks, there was no significant seasonal difference at the community level in foliar P for hammock forests in tree islands, although, like the rockland hammocks, there was the trend of higher foliar P in the wet season (mean foliar P g/g/dry leaf mass = 0.17 ± 0.010)　than dry season (0.14% ± 0.009). Swamp forests on tree islands did not have a significant seasonal difference in foliar P at the community

level, although individual species exhibited differences. *Annona* had significantly higher

P in the dry season than in the wet season at all three islands (F=6.69, P=0.02 at Chekika,

F=16, P=0.002 at Satinleaf and F=8.1, P=0.01 at Grossman) while *Morella* showed

significant difference on Chekika (F=7.69, P=0.03) but not on Grossman. *Salix* and

Chrysobalanus did have higher foliar P in the dry season but this was not significant at P

= 0.05 (Fig 4.3).

Comparison of foliar P between tree island and rockland hammock forests

Tree island hammock forests had significantly higher foliar P concentrations averaged

over seasons as compared to those in rockland hammock forests ($F_{2, 578}$=242, P<0.001).

Bursera had significantly higher foliar P on Grossman Hammock than in Royal Palm

hammock ($F_{7,29}$ = 6.82, P<0.001). The differences were dramatic with *Bursera* foliar P in

Grossman Hammock (0.24 ± 0.02) up to three times higher than in Royal Palm (0.07 ±

0.013). *Chrysobalanus* had significantly higher foliar P on Satinleaf island than in Long

Pine Key hammock ($F_{7,30}$ = 11.24, P<0.001) while *Morella* had significantly higher foliar

P in the tree islands (0.03 ± 0.003) than hammocks (0.02 ± 0.002; t = 8.76, df = 1, 94, P <

0.01).

Soil water fraction of stem water in hammock forests

Rockland Hammocks: All species utilized maximum upland soil water in early and

middle of the wet season, with little soil water uptake during the late dry season (Fig.

4.4). The seasonal difference was significant ($F_{5,242}$=20, P<0.001).

Tree island hammocks: Similar to rockland hammocks, the majority of the tree island

hammocks species sampled showed greater soilwater uptake over the wet season (Fig.

4.5).

Discussion

Hypothesis 1: Leaf fall is caused by water stress in hammock and swamp forests

Higher foliar $\delta^{13}C$ over the dry season than over the wet season can be an indication of

water stress (Farquhar et al 1982). This seasonal difference in foliar $\delta^{13}C$ was found to be

highly significant for rockland hammocks, marginally significant for tree island

hammocks and not significant for swamp forests (Fig. 4.1, Table 4.2). Therefore, no

generalization is possible regarding whether water stress is responsible for leaf fall in all

these three plant communities. While drought stress in the dry season can cause leaf fall

in most species in rockland hammock forests, the marginally significant seasonal

differences in foliar $\delta^{13}C$ for tree island hammock forests indicate that drought stress may

not necessarily be the main causative factor for leaf fall in tree island hammock forests.

Similarly, plants in swamp forests may be receiving other environmental cues resulting in

leaf drop.

Drought stress in rockland hammocks during the dry season has been documented

earlier (Alexander 1967, Ewe *et al* 1999); the basis for which is the lowering of the

groundwater table and the dessication of the litter layer present in these hammocks.

Hammock trees are shallow rooted (Lodge 2002, personal observations on trees uprooted

during hurricanes) because the main rooting zone, the organic soil layer, is just 30 cm

deep on average (Saha et al 2009 a) and directly overlies limestone bedrock. While it is

likely that some sinker roots penetrate into the limestone via cracks, their fate in the wet

season, when the water table is high (< 1m from surface – Saha et al 2009a) is unknown.

These species occupy well-drained areas elsewhere in their range in the Neotropics and

are known to be flood-intolerant, which suggests that it is unlikely that these species

possess strategies for roots to tolerate inundation, such as dormancy or suberization of

the outer root surface. However in Everglades hammocks, there exists a selective

pressure for main roots to develop tolerance to some degree of flooding. Their presence

would then allow fine roots to grow back once the water table recedes downwards. Fine

roots, responsible for the bulk of water and nutrient uptake would certainly perish in wet

season flooding, which can then affect the root-shoot ratio, with consequent lowering of

drought-resistance in the plant (Gillespie 2006). Prior studies have shown that trees in

rockland hammocks shift to groundwater uptake during the dry season (Ewe et al 1999,

Saha et al 2009a) on drying of the shallow soilwater. It is likely that the rate of root

regrowth (either just fine roots, or main roots as well) may limit the amount of

groundwater uptake. Ewe et al (1999) have observed low leaf water potentials at the

same sites in an earlier study. Another feature of the limestone substrate is its very rapid

drainage and lack of water holding capacity (Beard 1944) which prompted Alexander

(1967) to observe that lowered water tables in the Everglades could endanger hammocks.

Hence it is very plausible that hammock trees on rocklands become water stressed during

the dry season and thus experience their highest extent of leaf loss, as is the case in other

seasonal tropical forests (eg. Daubenmare 1972, Frankie et al 1974, Lieberman 1982,

Bullock & Solis-Magallanes 1990). The drought stress signal in rockland hammocks

may be species-specific. While all species exhibited the seasonal trend of high $\delta^{13}C$

values in the dry season, they differed in significance (Fig 4.1). *Lysiloma* had the most

enriched $\delta^{13}C$ values with an annual average of -28.72 ‰ while most other species had

annual values more negative than -30 ‰. This agrees with *Lysiloma* being a brevi-

deciduous species (Table 4.1) that loses most its leaf cover over the dry season, as also

noted in other neotropical forests where *Lysiloma* occurs (eg. Borchert 1988, Olivares &

Medina 1992, Holbrook 1995, Greenberg et al 1995, McLaren & McDonald 2005).

Other species showing a significant seasonal difference in foliar $\delta^{13}C$, such as *Morella, Quercus* and *Annona* could also lose their leaves because of drought stress. However, there can be other environmental factors causing or contributing to leaf fall, such as declining solar photoperiod that is seen to reduce vegetative growth to a very slow rate in the tropics (eg. Downs and Piringer 1958) as well as lead to leaf senescence and fall (eg. Stubblebine *et al* 1978). The roles of drought stress and photoperiod in causing leaf fall would depend upon water availability, as has been observed in connection with intraspecific variation within the range of the species (eg. Stubblebine 1978, Reich and Borchert 1982); the relative roles of each factor also could depend upon the species that can differ in their strategies concerning water relations. Hence, it may be declining photoperiod that may cause leaf fall in rockland hammock species that do not show a significant seasonal difference in foliar $\delta^{13}C$.

Apart from drought stress, high foliar nitrogen concentration has also been associated with high foliar $\delta^{13}C$ indicating stomatal limitation of photosynthesis in situations where water is not a limiting factor (Cordell et al 1999, Bai et al 2008). Three species at Sisal Pond (Table 4.3, Fig. 4.2) had significantly higher foliar N in the dry season than in the wet season; thus it is possible that it is high foliar N rather than drought stress that causes high foliar $\delta^{13}C$ values in these particular individuals at Sisal Pond. In such cases, additional physiological measurements can indicate the existence of water stress, such as leaf water potential. Other individuals did not have any significant seasonal differences in foliar N, thereby suggesting drought stress as an interpretation of high $\delta^{13}C$ values in the dry season.

In contrast to rockland hammocks, tree island hammock forests are not as water stressed; the location of tree islands in the slough with year-round water availability can

buffer the hammock forests against drought stress. The absence of *Lysiloma* from

hammock forests on tree islands may reflect the mesic nature of tree islands. *Bursera*

was the only species on tree island hammocks that had marginally significant seasonal

difference in foliar $\delta^{13}C$ (F=4.95, P = 0.06, df = 7), that complements the observation that

individuals (n=5) on Grossman Hammock were completely leafless in March. Thus other

factors such as declining photoperiod could play a greater role in causing leaf fall in tree

island hammock forests than in rockland hammock forests.

Woody species in wetland forests have a variety of strategies to withstand

anerobic conditions, exposure to phytotoxins and energetically unfavorable growth

conditions that result from continuous inundation (De Simone et al 2003). In numerous

species, flooding stress has been widely shown to result in reduced water conductance,

leaf chlorosis, abscission and eventual leaf fall (eg Worbes 1997, Kozlowski 1997,

Pezeshki 2001, Parolin et al 2004, Haugaasen & Peres 2005) as a precursor to dormancy.

Two woody species abundant in Everglades tree island swamp forests, *Annona* and *Salix,*

completely shed their leaves in late wet season (November and January respectively),

after 6-7 months of inundation, while flooding still persisted. However, the lack of

significant seasonal differences in foliar $\delta^{13}C$ at either the community or the species level

(Fig. 4. 1, Table 4.2) in swamp forests suggests that foliar $\delta^{13}C$ did not record any water

stress in these plants. There are two possibilities: that flooding stress did not exist, or that

flooding stress did exist but was not recorded by foliar $\delta^{13}C$. The latter possibility can be

explained as follows: flooding stress can reduce photosynthesis by decreasing the activity

of Rubisco in the leaves (eg. Vu & Yelenowski 1992, Kozlowski 1997, Pezeshki 2001),

with attendant decrease in photosynthetic demand for CO_2. Despite stomatal closure that

occurs with flooding stress, the amount of intercellular CO_2 inside the leaf is not limiting

to the extent it would be under high photosynthesis rates, thereby the level of isotopic discrimination against C^{13} by Rubisco is not greatly lessened. Hence other techniques could be used to investigate potential flooding stress. For instance, annual growth rings have been observed in Amazonian varzea and igapo flooded forests (Parolin 2000) that have been associated with periods of dormancy induced by flooding stress.

Hypothesis 2: Maximum leaf production coincides with water and nutrient availability

On an annual timescale, the period of high foliar nutrient concentration is associated with the leaf growth and expansion phase in plants. Nutrient concentration (per leaf mass) is higher in young growing leaves than in fully mature leaves, because in the early growing stages a large amount of nutrients are allocated to enable the basic biochemical growth processes of photosynthesis, protein synthesis, DNA duplication and transcription (eg. Gusewell 2004). Thereafter, carbon fixation and structural development of the leaf increases the carbon content thereby incrementally lowering the nutrient-to-carbon ratio in the leaf (eg. Chapin 1980) until the leaf reaches maturity. Leaf senescence further lowers foliar P concentration because nucleic acid P is eventually translocated to younger growing leaves. Thus foliar P averaged over the samples has been used in this study as an indicator of the period of leaf expansion at the population or community level.

The period of leaf expansion in rockland hammocks clearly coincided with the period of P-rich soilwater uptake, which was the wet season (Table 4.4, Fig. 4.3, and 4.4). Tree island hammock forests have a less marked but longer period of leaf expansion that can be related to their use of P-rich soilwater (Fig. 4.5, Saha et al 2009b). Leaf expansion happens in the dry season in most of the swamp forest woody species (Fig. 4.3), when water and nutrient availability to plants is thought to increase on account of cessation of

flooding stress in these plants. However, further research is required to ascertain whether flooding stress really does occur in swamp forest plants; this would then indicate whether the leaf growth happens due to an increase in resource availability in the soil, or are the nutrients obtained from stored reserves.

The indication of the growth phase by high foliar P levels in rockland hammocks agrees with field observations of highest foliage cover in the wet season relative to the dry season (Koptur et al 2002, Saha – pers obs). The availability of water and nutrients is also higher in the wet than in the dry season, as seen by P-rich soilwater that constitutes a greater portion of the water uptake of rockland hammock plants over this period (Fig. 4.4). This also has been observed at the community level (Saha et al 2009). The arrival of rains creates a soilwater pool in the hammock soil that is rich in P (Ross et al 2008, Saha et al 2009a), owing to dissolution and accelerated decomposition of organic matter. It is highly likely that the P required for the formation of new leaves and continued leaf expansion comes directly from this soilwater uptake for most species.

The lack of significant seasonal difference in foliar P at the species level in tree island hammocks indicates more or less uniform leaf growth and exchange conditions through the year. The location of tree islands in the slough imparts a more mesic environment to the hammocks than those in the rocklands, thereby buffering hammocks to some degree against dry season water stress. Water and nutrients are thus available in tree island hammocks over a longer period than in rockland hammocks, thereby increasing the evergreen nature of tree island hammocks as mentioned by Gillespie (2006). Foliar P is slightly higher in the wet season than in the dry season for some species - *Ardisia, Bursera, Eugenia* and *Nectandra* (Fig. 4.3), although this seasonal difference was significant only for Bursera in Grossman Hammock, whose location on

the edge of the Slough makes it the least mesic of the three islands studied. *Coccoloba* and *Chrysophyllum* showed the reverse trend, with higher foliar P in the dry season, but again these differences were not significant. Thus the tree island hammocks display no clear seasonal period of growth, owing to the aseasonality of water and nutrient availability. Other factors can influence growth once water limitations are removed; for instance temporal and spatial differences in photosynthetically active radiation (PAR) was associated with changes in leaf production for different species in a Panamanian lowland moist forest under well watered conditions (Barone 1998). The longer period of water availability in tree island hammocks can lead to nutrient uptake over a longer duration as compared to rockland hammocks. Comparison of hammock species co-occurring in rocklands and tree islands, ie *Bursera, Chrysobalanus* and *Morella* shows significantly higher foliar P concentrations in tree island populations than in rockland hammocks.

Similar to hammocks, the species in swamp forests present a spectrum from evergreen to deciduous habits. The deciduous species, *Annona* and *Salix* showed significantly higher foliar P in the dry season (with the exception of *Salix* at Grossman), that complements field observations of leaf growth and expansion over the same period. Leaf renewal in these two species started at the beginning of the dry season after 1-2 months of leaflessness, when water levels were lower, although the soil was still flooded in many instances. A similar pattern has been observed in Amazonian floodplain forests (Parolin et al 2004, Haugaasen & Peres 2005). It is likely that cessation of flooding stress permits the resumption of water and nutrient uptake, thereby leading to leaf renewal and growth. The evergreen species *Myrica* and *Chrysobalanus* showed higher but statistically insignificant foliar P in the dry season in the slough tree islands, Chekika and Satinleaf.

This indicates that while the dry season does see more leaf growth, some leaf growth also happens in the wet season, as is expected for an evergreen habit. *Rapanea* did not show any difference between dry and wet seasons, which might indicate similar rates of leaf growth and exchange in both seasons. Swamp forest plants primarily use the P-poor regional water pool all year (Saha et al 2009 b). Hence, further research can examine whether nutrient availability in lowland water sources increases during dry season, when water levels recede. This could either happen because the cessation of surface flow can lead to nutrients not being transported away from the tree island (Troxler & Childers, 2009), or the newly emergent soil surface fosters conditions for enhanced mineralization. Otherwise the nutrients for leaf flush and expansion must have been stored in plants.

Conclusions

This study has shown that water stress leads to leaf fall in rockland hammocks, while a combination of water stress and other environmental factors such as declining photoperiod may be responsible for leaf abscission in the more mesic tree island hammocks. Furthermore, the period of high water and nutrient availability coincides with the period of leaf growth for the majority of the species in rockland hammocks. Increasingly mesic conditions can decrease water and nutrient limitation in much of the dry season, thereby permitting other environmental factors to influence growth. This results in species differing in their patterns of leaf phenology. The diversity of such patterns increases with decrease in seasonality, reaching a bewildering extent in moist tropical forests. The approach of identifying water and nutrient sources and linking that to leaf phenology could be focused at the individual level, ideally over several years to account for variations in phenology with both changes in weather as well as nutrient

stocks in the trees. This could then help determine whether leaf phenology for a species in such forests is driven directly by water and nutrient availability or by some other factors such as photoperiod.

Table 4.1**:** Leaf phenology data for hammock and swamp forest species in the Everglades National Park, from field observations over 2005-2007 and from the literature. Most species listed as evergreen in the literature had the highest leaf growth and leaf cover in the wet season (field observation). Annona, Morella and Chrysobalanus sometimes occurred in hammocks.   Literature sources are Tomlinson 1980, Nelson 1994, Haehle & Brookwell 1999, Osorio 2001, Long & Lakela 1991, MacCubbin &Tusker 2002, Scurlock 1987, Sayer et al, Borchert 1994, Holbrook 1995, Broschat & Meerow 1991, Huegel 1995 and Koptur et al 2002.

	Species (FAMILY)	Phenology Habit, leaf fall	New leaf growth
	Hammock forest species		
CL	*Celtis laevigata(ULMACEAE)*	Deciduous, March	May-September
GS	*Guttardia Scabra (RUBIACEAE)*	Brevi-deciduous	May – September
LL	*Lysiloma latisiliquum (LEGUMINOSEAE)*	Brevi-deciduous; Late Jan – March	May – September
AE	*Ardisia escallionoides (MYRSINACEAE)*	Evergreen	All year
QV	*Quercus virginiana (FAGACEAE)*	Evergreen	July - September
CO	*Chrysophyllum oliviforme (SAPOTACEAE)*	Evergreen	All year
CD	*Coccoloba diversifolia (POLYGONACEAE)*	Evergreen	All year
EA	*Eugenia axillaries (MYRTACEAE)*	Evergreen	All year
NC	*Nectandra coreopsis (LAURACEAE)*	Evergreen	All year
TB	*Tetrazyga bicolor(MELASTOMATACEAE)*	Evergreen	May- September
	Swamp forest species		
SC	*Salix carolinensis(SALICACEAE)*	Deciduous, December-January	March-May
PB	*Persea borbonea (LAURACEAE)*	Evergreen	All year
AG	*Annona glabra (ANNONACEAE)*	Deciduous, January	February - May
RG	*Rapanea guianensis(MYRSINACEAE)*	Evergreen	All year
CI	*Chrysobalanus icaco (CHRYSOBALANACEAE)*	Evergreen	All year
MC	*Morella cerifera=Myrica cerifera (MYRICACEAE)*	Evergreen	All year

Table 4.2: Results of univariate 2-way ANOVAs examining the seasonal and site difference in foliar $\delta^{13}C$ at the community level in rockland hammock forests, tree island hammock forests and swamp forests. Significance at $P < 0.05$ shown in bold.

Community	Df	MS	F	P
Rockland hammock forests				
Season	1, 138	31.06	21	**0.000**
Site	2, 138	13.03	8	**0.000**
Season x Site	2	1.368	0.926	0.399
Tree island hammock forests				
Season	1,110	6.897	4.37	**0.039**
Site	2,110	3.529	2.23	0.112
Season x Site	2	1.159	0.735	0.482
Tree island swamp forests				
Season	1,156	2.108	0.967	0.327
Site	2,156	8.663	3.974	**0.021**
Season x Site	2	2.108	0.967	0.383

Table 4.3: Results of one-way ANOVAs examining seasonal differences in foliar N in rockland hammock species that had significant seasonal differences in foliar $\delta^{13}C$. Significance at $P < 0.05$ shown in bold

Site	Species	df	MS	F	P
Royal Palm	*Lysiloma latisiliquum*	1, 8	0.008	0.045	0.83
Royal Palm	*Morella cerifera*	1, 8	0.078	0.316	0.29
Royal Palm	*Quercus virginiana*	1, 8	0.084	0.738	0.41
Long Pine Key	*Lysiloma latisiliquum*	1, 8	0.195	0.551	0.49
Sisal Pond	*Annona glabra*	1, 8	3.495	81.27	**0.00**
Sisal Pond	*Lysiloma latisiliquum*	1, 8	0.178	2.572	0.15
Sisal Pond	*Morella cerifera*	1, 8	0.491	7.763	**0.02**
Sisal Pond	*Quercus virginiana*	1, 8	0.194	7.948	**0.02**

Table 4.4: Results of univariate 2-way ANOVAs examining the seasonal and site difference in foliar P at the community level in rockland hammock forests, tree island hammock forests and swamp forests. Significance at P < 0.05 shown in bold.

Community	Df	MS	F	P
Rockland hammock forests				
Season	1, 135	0.015	9.01	**0.003**
Site	2,135	0.003	1.955	0.146
Season x Site	2	0.004	2.15	0.12
Tree island hammock forests				
Season	1. 102	0.001	0.27	0.604
Site	2, 102	0.029	5.569	**0.005**
Season x Site	2	0.004	0.692	0.503
Tree island swamp forests				
Season	1, 147	0.003	2.816	0.095
Site	2, 147	0.002	1.917	0.151
Season x Site	2	6.14E-05	0.064	0.938

Figure 4.1: Foliar δ^{13}C concentration in dry (black) and wet seasons (gray) for rockland hammock species (top), tree island hammock species (middle) and tree island swamp forest species (bottom). Error bars indicate standard error of the mean (SEM). n = 5-10 individuals per species. Table 1 lists species names for the abbreviations shown here. * indicates significant difference at P < 0.05

Figure 4.2: Foliar N concentration in dry (May) and wet (Dec) seasons for rockland hammock species that had significant seasonal differences in foliar $\delta^{13}C$ (Figure 1). Error bars indicate standard error of the mean (SEM). The first two letters of the code refers to the species (Table 1), while the suffixes S1, S2 and S3 refer to Royal Palm, Long Pine Key and Sisal Pond respectively. Seasonal differences in foliar N were found to be significant only at Sisal Pond for AG, MC and QV respectively (Table 3).

Figure 4.3: Foliar P concentration concentration in dry (black) and wet (gray) seasons for rockland hammock species (top), tree island hammock species (middle) and tree island swamp forest species (bottom). Error bars indicate standard error of the mean (SEM). n = 5-10 individuals per species. Table 1 lists species names for the abbreviations shown here. * indicates significant difference at P < 0.05

Figure 4.4: Soilwater fraction of plant stemwater for hammock species on the rocklands. Wet season is shown by line under X axis (between June and November). Error bars on top panel indicate SEM.
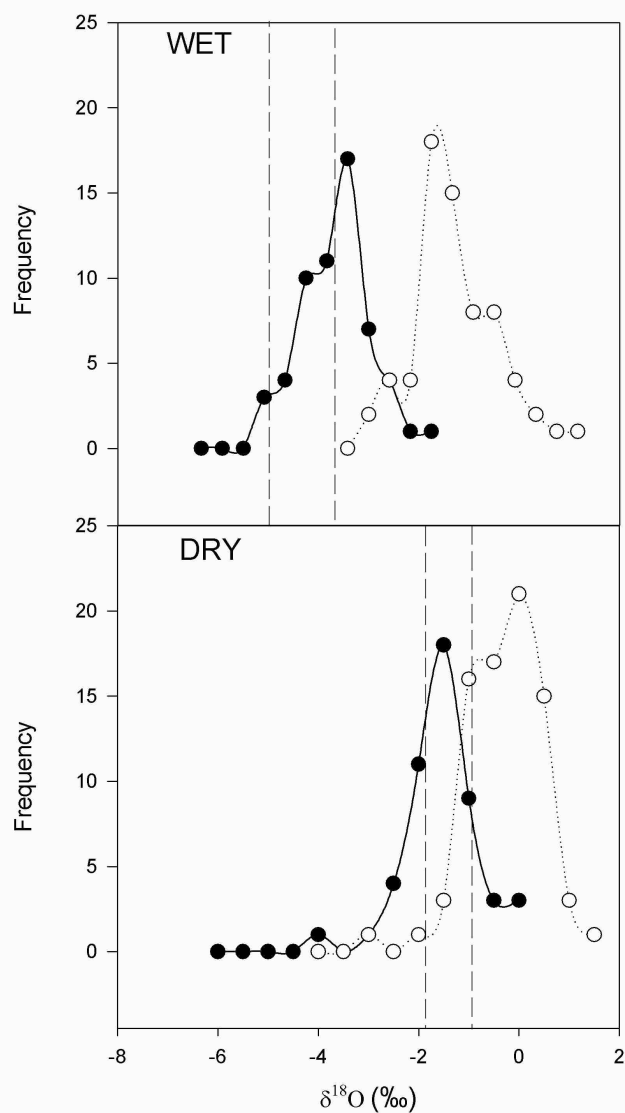
Figure 4.5: Frequency distribution of $\delta^{18}O$ composition of hammock forest plant stemwater (●) and swamp forest plant stemwater (○) in tree islands of the Shark River Slough, ENP in wet (November) and dry (May) seasons; n = 60 for hammocks and 90 for swamp forests in each season. Vertical dashed lines indicate the range of $\delta^{18}O$ composition of shallow soilwater (litter horizon) in hammocks.

OVERALL CONCLUSIONS

The studies in this dissertation have examined how heterogeneity in water and nutrient pools is closely associated with terrestrial and adjoining flooded plant communities in the Everglades ecosystem. Water sources for upland and lowland woody plant communities were identified, their seasonal variation noted and linked to foliar nutrient concentrations at the community and species levels. Foliar nutrient concentrations were found to indicate nutrient availability to plants, at least for the limiting nutrients. This knowledge of resource availability was then used to understand water and nutrient uptake and phenology of these communities that often have very different species composition. Here I summarize the results of the studies and then discuss how this technique of coupling water sources with foliar nutrient concentration values enables understanding the role of water and nutrients in the coexistence of diverse communities in an ecosystem.

Heterogeneity in water and nutrient pools supports community diversity in the Everglades ecosystem

We saw that the distinct pools of water in the Everglades, ie the upland soilwater and the regional groundwater-surfacewater pools vastly differ in their nutrient concentrations (Ch 2). The upland soil layer, present in the very few areas that do not flood, furnishes not only a relatively rich source of nutrients to plants, especially phosphorus, the limiting nutrient in the Everglades, but also an unsaturated rhizosphere during the wet season for the neotropical hammock species, most of which are flood-intolerant.

 On the Miami Rock Ridge, hammocks differ vastly in species composition and biomass from adjacent pine rocklands, which are also mostly unflooded but oligotrophic environments on account of natural fires that periodically burn off accumulated litter, except in sinkholes. The small quantity of soil thus present in sinkholes constitutes the

only source of nutrient-rich soilwater for pineland plants, which we have seen is used in late wet season. For the rest of the year, pineland plants are constrained to make do with groundwater (the regional pool) that is very low in nutrients (Ch 2).

Hammocks on tree islands are even more distinct in species compostion from adjacent swamp forests, and it is the wet season water level that maintains the distinct boundary between these two communities. The access to the P-rich soilwater results in hammock plants having higher foliar P concentrations than swamp forest plants at the community level (Ch 3). Interestingly, soil nitrogen in upland hammocks is less than in the swamp forest, a fact that is also reflected in hammock plants having lower foliar N than swamp forest plants.

The dependence of hammock plants, both on the Rock Ridge as well as on the tree islands on soil water indicates that this soilwater is their major nutrient source, with groundwater taken up in the dry season being the secondary nutrient source when considering over the lifespan of an individual plant. However, over the long term formation of hammocks, groundwater uptake provides the major pathway for nutrient input to hammocks whereupon nutrients present in groundwater get concentrated in plant biomass, which on decomposition forms soil biomass, and thereafter sets forth a tight cycling between plant and soil. Phosphorus being relatively immobile as compared to nitrogen gets retained in hammock soils.

Susceptibility to droughts for hammock communities

The susceptibility to flooding for hammock communities is well known. This study shows that they are susceptible to droughts that could be either natural or caused by lowered water tables. As early as 1960, Taylor Alexander had mentioned the dangers

posed by lowering water tables to hammocks. The study here has supported that

contention by showing that almost 50% of water uptake over the dry season in hammocks

is groundwater (Ch 2). These trees being flood-intolerant, it is almost certain that their

roots would not survive prolonged immersion below the water table in the wet season.

Given that the trees have to re-grow their roots once the wet season water table recedes,

and do so through the rocky limestone substrate indicates that it may not be easy to

encounter or chase the falling water table. Ewe et al (1999) found hammock plants get

water stressed over the dry season, while in Ch 4 I note the prevalence of high foliar δ^{13}C

values over the dry season in hammock plants indicating water stress. Careful

excavations could reveal how deep functioning roots of hammock plants go to, but these

are difficult to carry out given the rock and carbonate layer horizons present. Besides

excavation being a destructive technique, a statistically significant sample size may not

be achievable given the vastly reduced area of hammocks in the present day.

Foliar nutrient concentrations as indicators

We have seen that foliar nutrient concentrations can serve as indicators of relative

nutrient availability in soil for plants at both the community level (hammocks vs swamp

forest, hammocks vs pinelands) in the Everglades. They can also serve as similar

indicators at the species level, but this depends upon the species. Species differ in their

response to increased nutrient availability, with some showing elevated foliar

concentrations accompanying an increase in availability, while others do not show any

significant difference. For instance, *Bursera simaruba* showed a three-fold difference in

foliar P between rockland hammocks and tree island hammocks (Ch 4) while *Morella*

cerifera had consistently one of the lowest foliar P concentrations of any species sampled

in hammocks, pinelands and tree island swamp forests (Ch 2, 3, 4). This plasticity in

foliar nutrient concentrations reflects the range of nutrient availability conditions the

species has evolved over, and not much is known about that.

Phenology

Water and nutrients being major drivers of leaf fall, leaf flush, flowering and fruiting, the

knowledge of water source usage across different seasons aids understanding of the

timing of phonological events at the individual, species and community level. We have

seen that many species time their leaf growth phase to the period of high water and

nutrient availability. For hammocks this is the wet season, while for swamp forests this

appears to be the dry season, on the cessation of flooding stress. However, species within

a community vary in their strategies, for instance, Bursera flushes new leaves while still

in the dry season, by virtue of stored water in the stem. Why it does so could be an

interesting study investigating the advantage of early leaf flush.

Broader applicability of this technique

Identifying the water and nutrient sources, and how they vary over the seasons, can thus

indicate how species with differing water and nutrient requirements coexist within a

community, either through spatial partitioning or temporal partitioning of these resources.

Community diversity is controlled by a whole suite of abiotic and biotic factors operating

simultaneously. Efforts to understand which factors drive community structure and

function, and in what manner can therefore be complex. Ascertaining water and nutrient

use by various species in the community can provide part of the answer, thereby paving

the way to identify other factors that may be also involved. A similar exercise can be

performed at the ecosystem level, with communities as units. I have done this in a

hydrologically-controlled ecosystem; however this technique can be employed in any ecosystem, as long as the water sources differ in their isotopic composition. Spatial mapping of the isotopic composition of water sources in an ecosystem can be done at various times of the year to yield a database, which can then be compared to plant stemwaters for rapid studies. However caution should be study the possible variation within a water pool, such as soilwater that may be subject to different stages of evaporation, especially if the rainfall is patchy over a scale smaller than the soil area.

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