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Water and Nutrient Uptake of Deciduous and Evergreen Trees in a Dry Seasonal Forest: Interrelationship Between Root Structure and Leaf Phenology

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UNIVERSITY OF MIAMI

WATER AND NUTRIENT UPTAKE OF DECIDUOUS AND EVERGREEN TREES IN A DRY SEASONAL FOREST: INTERRELATIONSHIP BETWEEN ROOT STRUCTURE AND LEAF PHENOLOGY

By

Patrick Z. Ellsworth

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Water and Nutrient Uptake of Deciduous and Evergreen Trees in a Dry Seasonal Forest: Interrelationship Between Root Structure and Leaf Phenology

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Dry seasonal ecosystems are defined by a pronounced and consistent dry season, which reduces water availability in the dry season. Plants must adapt to water limitation in the dry season and high water availability during the wet season. In addition, the soil is nutrient-poor with the majority of nutrients concentrated in shallow soil. Considering the temporal drying of these layers, nutrient availability is dependent on the climatic conditions that govern water availability in the shallow soil. In this dissertation I address differences in fine root density, water source use, and nutrient uptake between deciduous and evergreen species in a dry seasonal ecosystem. My five study species are the deciduous *Carya floridana and Quercus laevis* and the evergreen *Lyonia ferruginea*, *Q. geminata*, and *Q. myrtifolia*. Chapter 2 compares the change in fine root density from dry to wet season (fine root turnover) and the distribution of fine root density with soil depth within dry and wet seasons. I tested the hypotheses that 1) root density would increase from dry to wet season and 2) that the increase in root density from dry to wet season would be smaller in deciduous species than in evergreen species. Fine root density decreased in all species and all root orders, except in the fourth order of *C. floridana*. High water availability appears to be the cue for high fine root production. Fine root turnover from dry to wet season was lowest in the deciduous *C. floridana,* possibly

because aboveground dormancy reduced transpiration, which increased the amount of water available for shallow roots during the dry season. Chapter Three compares water uptake during the year between deciduous and evergreen species using stable isotopes. I examined the hypothesis that evergreen and deciduous woody species take up shallow soil water in the wet season, but when shallow soil water is unavailable evergreen species switch to deep soil water, while deciduous species remain dormant. Evergreen and deciduous species used the same water sources through the year. During the dry season, four of the five species took up water based on the distribution of available water, while during the onset of the wet season and wet season water source use was based on fine root distribution. During the dry season *Lyonia ferruginea* took up more shallow soil water than expected if uptake was based on the distribution of available water. Deep water (50-150cm) was the most important water source during the dry season and an essential water source throughout the year. Chapter Four compares PO_4^{3-}, NH_4^+, and NO_3^- availability in the top 150cm of the soil profile using ion-exchange resins. I hypothesized that phosphorus and nitrogen availability would be highest in the shallow soil. This was true for PO_4^{3-}, but NH_4^+ and NO_3^- did not differ with depth. Nitrogen uptake by evergreen and deciduous species was measured by using ^{15}N as a tracer of nitrogen uptake. I hypothesized that nitrogen uptake rate would be highest among deciduous species because of the nitrogen demands of new leaf growth. During the late dry season ^{15}N uptake rate was highest among evergreen species. Low N uptake during leaf-out may mean that deciduous species cannot take up sufficient N to meet the demands of leaf growth. This temporal uncoupling of nutrient demand and uptake may be why deciduous species are more common in fertile sites. From this research, it appears

that the most important driver controlling rooting depth and distribution was water availability, while root density was controlled by nutrient availability.

To my mother who did not live to see me finish my doctorate but has been and always will be my inspiration

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Introduction

Leaf phenology in a seasonal environment

A seasonal environment consists of a growing season and an inhospitable season to growth because of freezing temperatures in the northern latitudes or because of drought in tropical, subtropical, and Mediterranean environments (Specht and Rundel 1990, Murphy and Lugo 1986). The dry season in dry seasonal ecosystems is characterized by little to no precipitation for several months leading to low water availability in the shallow soil and sometimes extending to the deep soil (Weekley et al. 2008). Soils are oligotrophic, and organic matter on the soil surface is the principal source of nutrients (Jobbagy and Jackson 2004). For nutrients to be available for plant uptake, water has to be available in the same soil layer. Because of the concentration of nutrients in the shallow soil layers and the temporal drying of the soil surface, nutrient availability is dependent on the climatic conditions that govern water availability in the shallow soil. One of the ways that plants adapt to these conditions is by modifying leaf phenology (Chabot and Hicks 1982). Several hypotheses have been proposed to explain the effects nutrients and water limitation and acquisition have on leaf phenology.

Evergreen species maintain transpiring, photosynthetically-active canopies during the dry season, which means the leaves tolerate low nutrient availability in the soil and drought conditions during the dry season. This leaf durability and longer leaf lifespan is accomplished by higher allocation of carbon and secondary compounds in the leaf structure (Sobrado 1991). One example of this is sclerophylly, a leaf adaptation to nutrient-poor soils and drought (Barchuk and Valiente-Banuet 2006, Read et al. 2006,

Serrano et al. 2005, Groom and Lamont 1997, Salleo et al. 1997). Scleromorphous leaves have high mass per leaf area and increased hardness (Wright and Cannon 2001). Longer leaf lifespan compensates for low photosynthetic rate - increasing net carbon gain over the life of the leaf (Orians and Solbrig 1977). Long leaf lifespan in evergreen species also delays nutrient loss and increases carbon gain per unit of nutrient (Chabot and Hicks 1982). Evergreen species also have higher resorption efficiency than deciduous species, which decreases need for additional nutrient uptake (Gray 1983).

Deciduous leaf phenology is hypothesized to be a drought avoidance strategy in water-limited environments (Chabot and Hicks 1982). Deciduous species lose their leaves during the dry season and by so doing, avoid low water availability (Bullock and Solis-Magallanes 1990, Givnish 2002, Haugaasen and Peres 2005, Lei and Koike 1998, Opler et al. 1980). Drought avoidance plausibly explains why deciduous woody plants lose their leaves in the dry season, but drought avoidance cannot be the entire explanation, because many deciduous species leaf out in the late dry season (Borchert 1994, Rojas-Jimenez et al. 2007, Borchert et al. 2002, Lieberman 1982). They experience from one to three months of the dry season when soil water availability is at its lowest point. These species, in some cases, experience very low midday water potentials of -7 MPa after leafing out (Borchert et al. 2002).

Growing and operating leaves only during the wet season, the season optimal for growth, reduces the structural requirements leaves would require to withstand biological, environmental, and climatological stresses associated with a long leaf lifespan, especially the harsh dry season conditions (Choat et al. 2006, Gray 1983, Schlesinger and Chabot 1977). In many ways, the deciduous strategy is the opposite of the evergreen strategy in

that they contrast in many specific leaf characteristics. For example, in contrast with evergreen leaves, deciduous leaves with a shorter lifespan have lower mass per leaf area, a lower number of sclerenchymatic cells per area (Van Arendonk and Poorter 1994), and higher water content than evergreen leaves. The relative decline of leaf water potential per unit loss of water from leaves is higher in deciduous than in evergreen leaves. The water requirement for maintenance of normal leaf function is higher in deciduous than in evergreen species. Avoidance of drought conditions may incur less carbon cost in leaf construction and maintenance, but deciduous species remain dormant throughout most of the dry season and photosynthesize for fewer days per year than evergreen species.

Deciduous species have adapted to compensate for the loss in photosynthesis from having leaves with a shorter lifespan. Deciduous species have higher CO_2 assimilation capacity and nutrient use efficiency because they have higher productivity per unit of nutrient (Sobrado 1991). In some seasonal dry ecosystems, deciduous species allocate more nutrients per leaf mass than evergreen species to maximize photosynthetic output per leaf mass per unit time (Chabot and Hicks 1982). However, evergreen species sometimes have higher nitrogen content than deciduous species. In the dry seasonal forests in Venezuela evergreen leaves have slightly higher nitrogen content than deciduous species, but deciduous species still had higher CO_2 assimilation capacity and nutrient use efficiency because deciduous leaf construction cost was lower than the construction costs in evergreen leaves (Sobrado 1991).

Deciduous and evergreen species differ in timing of nutrient demand. At the time of leaf out, deciduous species must meet the nutrient requirements of constructing new leaves. The source of the nutrients required for leaf growth can come from storage or

from uptake. If the nutrients used for leaf growth come from storage, the origin of the nutrients is either from nutrients resorbed from senesced leaves or nutrients taken up and stored during the last wet season (Aerts 1996). If nutrients for leaf growth come from current uptake, woody plants must be able to take up adequate nutrients in the late dry season when deciduous woody plants leaf out. Many evergreen species do not leaf out at once like deciduous species, so nutrients required for evergreen leaf construction can be taken up over a longer period than in deciduous species. Therefore, evergreen species can more easily meet the nutrient demands from uptake (Dyckmans and Flessa 2002).

Root structure and function in a seasonal environment

Fine roots respond to fluctuations in water and nutrient availability by modifying rooting density and depth to maximize nutrient and water uptake (Kavanagh and Kellman 1992, Yanagisawa and Fujita 1999). Fine root density is a function of root production and mortality, which have a seasonal effect and vary with edaphic conditions present (Borchert 1994). Low dry season water availability can result in high fine root mortality depending on whether plants shed roots in dry soil or maintain them (Bauerle et al. 2008, Eissenstat and Yanai 1997). Shedding roots in dry soil is common in drought tolerant plants, which produce new roots once the soil is re-wetted (Huang and Nobel 1994). Whether plants shed or maintain their roots in the dry season depends on the potential benefit for incurring high maintenance costs (Eissenstat and Yanai 1997). Citrus maintain their roots in dry soil for long periods of time with no reduction in ability to take up water and nutrients (Kosola and Eissenstat 1994, Eissenstat et al. 1999). Phosphorous uptake

was actually high, while roots were in dry soil (Eissenstat et al. 1999). Maintaining roots during the dry season can be advantageous in taking up the nutrient pulse associated with the first rains of the wet season (Sarmiento et al. 2008).

Another way that plants can manipulate root density is by proliferating in nutrient patches (Hodge 2004). Increasing root density is an important way to take up immobile nutrients such as PO_4^{3-} from the soil. A low diffusion coefficient of PO_4^{3-} in the soil means that increasing fine root density increases the soil volume that is being mined (Fitter 1994). Mobile nutrients such as NO_3^- and NH_4^+ can be taken up under low root density, but in the presence of competition with other roots, uptake is proportional to the total root length in the nitrogen patch (Robinson et al. 1999). High root density in the shallow soil layers where most nutrients are found can increase greatly nutrient uptake, especially if the soil is poor in phosphorus. The timing of root proliferation is likely in response to nutrient availability, which leads to high wet season fine root production in the shallow mineral soil layers and organic horizon (Soethe et al. 2006, Kummerow et al. 1990a, Kummerow et al. 1990b, Valverde-Barrantes et al. 2007).

Root structure and function in dry seasonal ecosystems cannot be explained by a need to maximize nutrient uptake alone. If water availability is low in the shallow soil, evergreen species and deciduous species, when they have leaves, require access to a permanent source of water. The hypothesis that evergreen species have deeper roots than deciduous species to access deep soil water sources and to meet the dry season transpirational demands was not corroborated. In one study evergreen species took up water from relatively deeper layers in the soil profile than deciduous trees, although both relied on the top 1 m of the soil profile for water (Jackson et al. 1995, Meinzer et al.

1999). Other studies have shown that deciduous species take up water deeper in the soil profile than sympatric evergreen tree species (Stratton et al. 2000, Jackson et al. 1999c). Two studies in the Florida scrub found that evergreen trees took up water from 1-2m (Li et al. 2002, Saha et al. 2008). In another study in the Brazilian Cerrado, both phenological groups relied largely on the top 1.5m of the soil profile, but can differ in where in the top 1.5m that water uptake occurs (Romero-Saltos et al. 2005). If nutrient are principally taken up from the shallow soil and the shallow soil cannot meet the transpiration demands for water, water and nutrient uptake are uncoupled.

Interrelationship of leaf and root traits

Rooting structure and function and leaf phenology are both ways in which plants adapt to environmental conditions that affect nutrient and water availability. Likely the linkage between these processes explains how the whole plant functions with water and nutrient limitation. Studying above- and belowground processes separately would be short-sighted, because above and belowground responses to the same environmental conditions are interconnected in determining the plant's fitness. Evergreen species may have tighter coupling between nutrient demand and internal nutrient cycling at the early dry season than deciduous species. Any nutrients required for leaf production that are more than what is stored can be replenished by nutrient uptake in the wet season (Lamaze et al. 2003). Deciduous species have high nutrient demands at the early dry season to grow nutrient-rich leaves (Gray 1983). Therefore nutrient uptake and active surface fine roots are more important early in the growing season for deciduous species than for evergreen species in nutrient-poor ecosystems where nutrients are principally in the surface soil layers.

Leaf traits may have an additional effect on fine surface root structure by limiting carbon allocation to fine roots from recent photosynthates. If this is the case, deciduous trees lose fine surface roots to a greater extent than evergreen trees because fine roots of deciduous trees would lack translocated photosynthates. However, deciduous and evergreen tree species in seasonal forests have a large storage of non-structural carbohydrates (Chapin et al. 1990, Langley et al. 2002, Newell et al. 2002, Würth et al. 2005). In a labeling experiment in the Florida scrub, stored carbon was available for a period of two years in the evergreen *Quercus myrtifolia* and *Q. geminata* (Langley et al. 2002). Species existing in seasonal or unpredictable environments such as fire-prone ecosystems like the Florida scrub and sandhill require greater carbon stores, so lacking carbon stores to maintain dry season fine root respiration is unlikely (Iwasa and Kubo 1997, Kozlowski et al. 1991, Verdaguer and Ojeda 1989).

In a study in the Brazilian Cerrado, the aboveground stem and shallow lateral roots were in competition for deep water from the tap root (Moreira et al. 2003). This leads to question of whether deciduous and evergreen species differ in competition for water acquired by deeper tap roots during the dry season . Deciduous species lose their leaves during the dry season, decreasing the transpirational demand on the plant and may increase the allocation of moisture to other tissues such as surface lateral fine roots. This allocation of moisture to the shallow lateral roots during the dry season in deciduous plants may promote a greater survival of shallow roots compared with those of evergreen plants. Live shallow fine roots at the early dry season would allow greater uptake of nutrients from relatively nutrient-rich shallow soil layers.

Research hypotheses of this dissertation

My dissertation research focused in comparing several structural and functional characteristics of roots between deciduous and evergreen species in a seasonally dry scrub. In the second chapter of this dissertation, I tested the hypothesis that deciduous species have lower root turnover rates between dry and wet season than those of evergreen species. To test this hypothesis, I quantified fine root density in the dry and wet seasons of five species: the evergreen *Quercus myrtifolia* Willd., *Q. geminata* Small, and *Lyonia ferruginea* (Walter) Nutt. and the deciduous *Carya floridana* Sarg. and *Q. laevis* Walter. I used restriction fragment length polymorphism (RFLP) to identify the root segments to species. Fine root standing crop in dry and wet seasons was quantified by counting the root branches in each of the first four terminal orders. In the third chapter of this dissertation, I tested the hypothesis that evergreen species would use water proportionally deeper in the soil profile than deciduous species during the dry season and that wet season water source use would be predominately shallow water for both deciduous and evergreen species. I measured water source use of these same five species during the entire year. I used stable isotopes of oxygen and hydrogen in water to measure water source use and the model IsoSource to calculate proportional contributions of each soil depth to water use for each species. In the fourth chapter, I tested the hypothesis that deciduous species would have higher nitrogen uptake in the late dry season because it is during this time that they leaf out and require nutrients for leaf growth. I measured PO_4^{3-}, NH_4^+, and NO_3^- availability at six depths in the top 150 cm of the soil profile throughout the year. During late dry season and early wet season, I labeled the shallow soil nitrogen with ^{15}N and measured ^{15}N uptake rate for each of the five species.

Research area and study species of this dissertation

The Florida scrub and sandhill plant communities at Archbold Biological Station (ABS) in south-central Florida provide an ideal place for studying the concept of linkage between rooting function and structure and leaf phenology. First, the soils are deep, extremely well-drained, acid, quartzipsamments sands (Kalisz and Stone 1984), have very low nutrient and organic matter content (Huck 1987), and experience periodic drought (Saha et al. 2008, Menges and Gallo 1991). The sandhill plant community, which contain my research sites, are located along the southern ridge of the Lake Wales Ridge, which represents the ancient beaches from the late Pliocene (Weekley et al. 2008). The shallow sandy soil layers are dry during the dry season, but deep soil layers are an accessible water source year-round. The groundwater table is approximately 2 to 24m in depth. Second, Florida scrub has distinct wet and dry seasons. The climate of the site is characterized by hot, wet summers and mild, dry winters that extend from December through April. Deciduous species lose their leaves between December and February. Mean annual rainfall at Archbold is 1345mm, with approximately 61% falling between June and September (ABS weather records, 1932-2011). Third, the sandhill plant communities are dominated by a small group of deciduous (*Carya floridana* Sarg.*, Q. laevis* Walter, and *Asimina obovata* (Willd.) Nash, and *Vitis rotundifolia* Michx.) and evergreen woody species (*Quercus myrtifolia* Willd., *Q. inopina* Ashe, *Q. geminata* Small*, Q. chapmanii* Sarg., *Serenoa repens* (Bartram) Small, *Lyonia ferruginea* (Walter) Nutt.*, Pinus elliottii* Engelm. var. *densa* Little & Dorman*, and P. clausa* (Chapm. ex

Engelm.) Vasey ex Sarg.). A simple system, such as this, facilitated studying my hypotheses. In addition, this ecosystem appears to have similar climatic and edaphic characteristics with other dry seasonal ecosystems.

The station encompasses 3,653 ha of vegetation in a relatively undisturbed state. The varied topography of Lake Wales Ridge found at Archbold Biological Station creates a diverse matrix of plant communities, ranging from wetlands and flatwoods to oak-hickory scrub, Florida rosemary scrub, and sandhill (Menges 1999). Fires are a common and an important factor in plant community structure and species adaptation (Abrahamson 1984). Most species withstand frequent fires by resprouting, being fire tolerant (e.g. *P. elliottii* var. *densa*) or regenerating from seed banks.

The species studied in this dissertation are two deciduous (*Quercus laevis* and *Carya floridana*) and three evergreen species (*Q. myrtifolia, Q. geminata,* and *Lyonia ferruginea*). These species were used because of their abundance in the southern ridge sandhill plant community. Also *C. floridana* and the *Quercus* species are ectomycorrhizal species and *L. ferruginea* is an ericoid mycorrhizal species. Colonization by ectomycorrhiza and ericoid mycorrhiza facilitates the uptake of nutrients from organic matter, especially nitrogen (Chapin 1995, Read 1993). The deciduous species are generally shrubs or small trees, growing to maximum height of 10-12m. The evergreen species are large, multi-stemmed shrubs and can reach a height of 7m. However, these deciduous and evergreen woody species do not often attain maximum height in the Florida scrub because of frequent fires. *Lyonia ferruginea, Q. myrtifolia, Q. geminata, Q. laevis,* and *C. floridana* regenerate mostly by sprouting either from roots or rhizomes (Menges and Kohfeldt 1995).

Background on techniques used in this research

In this dissertation research I used three techniques: 1) stable isotopes of oxygen and hydrogen in water as a tracer of water use, 2) stable isotopes of nitrogen as a tracer of nutrient uptake, and 3) restriction fragment length polymorphism as a means of identifying roots to species. Because these techniques are complex, I give a brief background of each below to facilitate the interpretation of the results in the chapters 2, 3, 4.

Stable isotopes of oxygen and hydrogen in water as a tracer of water use

Stable isotopes are a powerful tool in ecology because they act as integrators, recorders, and tracers of physical and biological processes (Clark and Fritz 1997). Stable isotope analysis is possible because each element studied (in this dissertation H, O, N) has at least two naturally-occurring stable isotopes. Isotopes of an element differ in the number of neutrons their nucleus contains, affecting atomic mass. In all cases, the light isotope of each element is naturally most abundant. For example, hydrogen has two stable isotopes: the light isotope has no neutrons, ^{1}H, which comprises 99.98% of all the hydrogen, and the heavy isotope has one neutron, ^{2}H which only comprises 0.015% of all hydrogen. Oxygen has three stable isotopes: ^{16}O (99.762%), ^{17}O (0.0373%), and ^{18}O (0.2%). Stable isotopes are chemically identical but differ physically because of the difference in mass between isotopes. The heavy isotope has a lower vibrational energy, which makes bonds formed with the heavy isotope slightly more stable than those formed with the light isotope, so they break at a slower rate than bonds formed with the light isotope. Also $^{2}H^{1}H^{16}O$ and $^{1}H_{2}{}^{18}O$ (heavy water) preferentially remain in forms that are more stable (e.g. liquid water is more stable than water vapor).

Evaporation and condensation are the physical processes that change the isotopic composition of water in the environment, or in other words, fractionate the isotopes of oxygen and hydrogen in water. In evaporation a light water molecule ($^{1}H^{1}H^{16}O$) breaks the hydrogen bonds that it shares with other water molecules in the liquid phase and enters the vapor phase at a faster rate than heavy water ($^{1}H_{2}{}^{18}O$ and $^{2}H^{1}H^{16}O$) . The water that evaporates is isotopically lighter than the water that remains in the liquid phase. Condensation is a physical process that occurs in the opposite direction (i.e. water vapor condenses to form liquid water). Condensation favors heavy water, meaning that condensed water is heavier than the pool of vapor from which it condensed. The processes of evaporation and condensation control and create pools of water in the environment that are isotopically distinct.

Isotopic composition or signature is expressed in δ or per mil notation (‰):

$$\delta^{2}H \text{ or } \delta^{18}O = \left[\frac{R_{sample}}{R_{standard}} - 1\right] X 1000 \quad ,$$

where R is the ratio of quantity of the heavy isotope to the light isotope. The international standard of hydrogen and oxygen in water is Vienna-standard mean ocean water (VSMOW), which is defined as $\delta^{2}H$ and $\delta^{18}O$ of 0‰.

Stable isotopes can be used to measure the proportional water source use of plants because water sources available to the plant can vary isotopically. In a given region, precipitation varies isotopically, depending on the cloud origin and the season (Dansgaard 1964). Once precipitation enters the soil, it undergoes evaporation. The isotopic signature of the soil water depends on the signature of rain in a rain-fed system

such as the Florida Sandhill and the fractionating process of evaporation. Evaporation occurs more at the soil surface and decreases with depth, so fractionation of hydrogen and oxygen isotopes at the soil surface is greatest and decreases with depth (Zimmermann et al. 1966, Barnes and Allison 1983, Allison 1982). Groundwater also may have an isotopic signature different than the vadose zone soil water or it may be identical to the deep soil water.

Xylem water is a mixture of its water sources, and its isotopic signature reflects proportionally its mixture of water sources. This important principle that xylem water accurately records the isotopic signature of its water sources has some exceptions in hydrogen. Mangrove species and some halophytes and xerophytes fractionate hydrogen isotopes (Ellsworth and Williams 2007, Lin and Sternberg 1993). However, no study has shown oxygen isotope fractionation during water uptake. By collecting xylem water and each of the plant's water sources, the proportional contribution of each water source to water uptake can be calculated with a simple mass balance equation: $\delta_{\text{xylem water}} = f * \delta_{\text{water source A}} + (1-f) * \delta_{\text{water source B}}$, where f is the fraction of water that plant used from source A. When a plant has more than two water sources, mass balance models such as IsoSource can be used to calculate the most probable proportional water source use (Phillips and Gregg 2003, Phillips and Gregg 2001).

Stable isotopes of nitrogen as a tracer of nutrient uptake

Labeling nitrogen sources with ^{15}N-enriched N provides a non-destructive method of measuring N use by individual plants, microbial communities, aquatic systems, and forests (Hofmockel et al. 2011, Handayanto and Sholihah 2010, Guak et al. 2003, Fotelli et al. 2002, Slawyk et al. 1998, Hobbie and Hobbie 2008). In experiments measuring N

uptake by plants, one or more N sources available to the plant are labeled with ^{15}N (Fotelli et al. 2002, Millard and Proe 2006, Grassi et al. 2002, Gebauer and Ehleringer 2000). Applying small amounts of ^{15}N to the soil can effectively label the N source without causing a fertilization effect, so that N uptake is not influenced by the ^{15}N addition (Fotelli et al. 2002, Millard and Proe 2006, Grassi et al. 2002, Gebauer and Ehleringer 2000). The increase in $\delta^{15}N$ of the plant is directly related to uptake of the labeled N source, considering the labeled N source has a $\delta^{15}N$ above the background plant $\delta^{15}N$. Plant uptake can then be measured by measuring the $\delta^{15}N$ of the plant or of a specific plant part. In this dissertation, I assessed N uptake by allocation of ^{15}N to leaf growth. The increase in foliar $\delta^{15}N$ over time is the rate of ^{15}N uptake from the soil. ^{15}N uptake relates to N uptake from the soil because soil N is labeled with ^{15}N.

Restriction fragment length polymorphism as a means of identifying roots to species

A combination of visual characteristics such as color and texture can be used to identify fine roots. Fine roots of different species, however, are very similar, so for many species visual characteristics are not sufficient to distinguish fine roots to species. Molecular identification techniques provide additional, valuable methods to identify the species of fine roots when identification by visual characteristics is not possible. PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis provides a reliable, fast, low-cost, and simple method that has been used successfully to identify roots to species using various DNA regions (Cavender-Bares et al. 2004, Bobowski et al. 1999, Vandenkoornhuyse et al. 2003, Brunner et al. 2001, Linder et al. 2000, Jackson et al. 1999b). In this dissertation I used the internal transcribed spacer (ITS) region, which is one of the regions that have been used successfully to identify roots to species (Linder et

al. 2000, Jackson et al. 1999b, Bobowski et al. 1999). The ITS region is a region of non-functional rDNA and is particularly useful for in very small root samples because of the high copy number of rRNA that includes the ITS region.

The process of root identification by PCR-RFLP includes DNA extraction, amplification of the ITS region by PCR, restriction enzyme digestion of the PCR product, and analysis of restriction fragments by gel electrophoresis. Roots are identified to species by comparing the banding pattern of restriction fragments of each sample to known banding patterns of each species. For samples to be identifiable by their banding pattern, all possible species in the study have to have a unique banding pattern.

DNA extraction and PCR can present problems which had to be resolved. DNA extracted from root tissue must be pure because impurities, such as polysaccharides, phenols, tannins, and salts, can interfere with PCR amplification. The DNA extraction protocol I used was specifically designed for root DNA extraction in an attempt to reduce impurities that co-extract with DNA (Brunner et al. 2001). PCR is highly useful in increasing copy number of a specific DNA region, but is very sensitive to impurities, which make PCR fail to amplify the target region or decrease drastically the number of copies of the region made. If the copy number made is too low, visualizing the banding pattern by gel electrophoresis is impossible. To increase copy number, nested PCR can be done. A nested PCR uses primers that are located slightly within ends of the PCR product, which allows the primers to adhere. The DNA source for nested PCR is the PCR product of the previous PCR that produced too few copies of the ITS region. Nested PCR can greatly increase copy number in samples that fail to amplify using traditional PCR. A potential issue can arise that would obscure the results of PCR-RFLP is hybridization of

plant species, especially the three *Quercus* species studied in this dissertation, but another study showed that Florida oaks have very low rates of hybridization (Cavender-Bares et al. 2004).

Chapter 2

Fine root density dynamics of deciduous and evergreen woody species in a dry seasonal scrub

Summary

Roots in many dry seasonal ecosystems experience two major challenges: drought conditions in the dry season and nutrient-poor soils. These conditions may select for a particular resource allocation towards root distribution in both space and time. Deciduous and evergreen species differ in leaf phenology, but it is unclear if they differ in root distribution. During the dry season fine roots experience dry conditions, and plants either maintain fine roots or shed roots situated in dry soil and re-grow them once the soil is re-wetted. Here, I tested the hypotheses that 1) wet season root density would be greater than in that of the dry season and 2) that the increase in root density from dry to wet season would be smaller in deciduous species than in evergreen species. Fine roots in each season were quantified by determining the total number of fine roots belonging to each terminal branching order. Restriction fragment length polymorphism (RFLP) was used to identify root segments to 4 species groups (*L. ferruginea, Q. geminata, Carya floridana*, and *Q. myrtifolia*/*Q. laevis*). Dry season root density in all branching orders was very low compared to that of the wet season confirming my first hypothesis. Just as high dry season root mortality appears to be an adaptation to drought, high wet season fine root production appeared to be triggered by high soil water availability. High wet season root densities in every order show that root production was capable of replenishing root branches of all orders. In both the dry and wet season, root density decreased with soil depth, similar to that of tropical forests. Fine root turnover from dry

to wet season was lowest in the deciduous *C. floridana*. It was the lowest, possibly because aboveground dormancy reduced transpiration, leaving more water available for shallow roots during the dry season, confirming my second hypothesis for this species.

Background

The wet and dry seasons of seasonal ecosystems provide distinct challenges to plant roots. For approximately six months of the year, a pronounced and consistent dry season results in water limitation, especially in the shallow soil (Sobrado 1986, Sobrado and Cuenca 1979, Murphy and Lugo 1986). The distribution of water spatially and temporally is one of the major factors affecting plant distribution (Gholz et al. 1990, Huxman et al. 2005, Darrouzet-Nardi et al. 2006). In dry seasonal forests, the distribution of available water can change dramatically with depth between dry and wet seasons (Murphy and Lugo 1986). In the wet season, shallow roots are situated in soil with high water availability, but a lack of rain in the dry season reduces water availability in that same soil layer to very low levels (Murphy and Lugo 1986). For fine roots to persist beyond the wet season, they must be able to withstand low soil matric potentials for a prolonged period.

Another challenge for fine roots is that the soil is nutrient-poor with the majority of nutrients located in the shallow layers (Myers 1990, Brown et al. 1990). Microbial decomposition releases nutrients from organic matter into the soil, but decomposition is dependent on water (Luizao et al. 1992). This fluctuation of available water creates a pulse-driven system where nutrients are available only when water is available (Grime 1994, Cui and Caldwell 1997). The principal pulse of the year is with the first rains of the wet season (Raghubanshi et al. 1990, Singh et al. 1989, Lodge et al. 1994). Low nutrient

availability and its pulsed nature in the soil create challenges for plants in when and where in the soil profile should resources be allocated to root growth and maintenance.

The distribution of nutrients and water control the distribution of roots. As the highest nutrient concentrations are in the shallow soil, not surprisingly several studies have shown that the top 50-100cm of soil has the vast majority of fine roots, and fine root density decreases with depth (Soethe et al. 2006, Vogt et al. 1996, Visalakshi 1994, Millikin and Bledsoe 1999). The type of limiting nutrient (immobile vs. mobile) and the presence of interspecific competition also affect the quantity/density of roots found in a soil layer. Immobile nutrients such as PO_4^{3-} have very low diffusion coefficients and it is estimated that 1 cm of root is necessary to extract PO_4^{3-} from 0.3 cm^3 in 10 days (Tinker and Nye 2000), so increased lateral root density is necessary to access the brief nutrient pulse. Uptake of mobile nutrients such as NO_3^- can be accomplished with relatively low root density (1 cm of root necessary to extract NO_3^- from 30 cm^3 in 10 days; (Fitter et al. 2000), unless the plants are subjected to interspecific competition where NO_3^- uptake becomes proportional to root length (Robinson et al. 1999, Hodge et al. 2002, Fitter et al. 2002). However, root distribution is not always related solely to nutrient availability alone but may be related to water uptake as well (Caldwell et al. 1996). Plants, particularly evergreens, have to have part of their roots in moist soil at least during dry periods of the year to sustain transpiration or be able to extract water under increasingly lower matric potentials. If, for example, only deep water is available during the dry season, an evergreen plant must have access to this water via a deep root system (Janos et al. 2008).

Deciduous species conspicuously differ from evergreen species in that deciduous species lose their leaves for a few months during the dry season. Clearly, deciduous species go dormant aboveground for part of the year, but it is unclear if they maintain their roots or shed them in response to the drying soil. In temperate forests, the root lifespans of deciduous trees is similar to evergreen gymnosperms and evergreen broadleaf species (Hendrick and Pregitzer 1992, Hendrick and Pregitzer 1993, Eissenstat and Yanai 1997). Unlike dry seasonal forests, the inhospitable season in temperate forests is characterized by freezing temperatures, which reduces both mortality and production in fine roots (Satomura et al. 2006, Hendrick and Pregitzer 1993). Therefore, for temperate forests, seasonality of fine root production and mortality does not appear to differ with leaf phenology. In contrast, relatively high soil temperatures and low water availability in the dry season may decrease root longevity and increase the probability that plants shed their roots instead of maintaining them (Eissenstat and Yanai 1997). These conditions could be exacerbated in evergreen species that maintain a transpiring canopy during the dry season, which adds a greater water uptake demand on their root systems than deciduous species that reduce transpiration demand by losing their leaves. If so, evergreen species may maintain leaves in the dry season at the expense of their fine roots, especially shallow fine roots.

I envision two possible root strategies during the dry season; plants either maintain their roots in the dry soil layers or shed them and regrow new roots once the soil layer is re-wetted in the wet season. Maintaining roots in dry soil can be accomplished by redistributing water from deep roots situated in a permanent water source to the roots in dry soil (Bauerle et al. 2008). For example, citrus species maintain roots in dry soil for

considerable periods of time with very low mortality (Espeleta et al. 1999, Espeleta and Eissenstat 1998, Eissenstat et al. 1999, Espeleta and Eissenstat 1998, Moreira et al. 2003). In contrast, plants adapted to dry conditions with no possibility of accessing water deeper in the soil profile tend to shed roots once the soil dries and regrow fine roots when the soil is re-wetted (Huang and Nobel 1994, Eissenstat and Yanai 1997). Species that are not adapted to drought tend to maintain fine roots in the shallow soil during dry periods (Meyer et al. 1990, Jupp and Newman 2006). Mesic species may maintain roots as soil dries because they have adapted to short drought periods, so maintaining roots during these fleeting periods of drought is beneficial. Xerophytes more readily shed roots because periods of low water availability last for extended periods (Huang and Nobel 1994).

Nutrient uptake plays a role in the seasonality of fine root production and mortality, but how is unclear. In nutrient-poor areas with short nutrient pulses, roots are expected to have long lifespans to reduce nutrient loss, which would mean that plants would maintain roots in the dry season instead of shedding them (Grime 1994, Fitter 1994). However, short lifespan and tight nutrient recycling can also reduce nutrient loss (Eissenstat and Yanai 1997). If the nutrients in fine roots could be recovered efficiently, the advantage of extending fine root lifespan would be nullified.

Studying root density through time has been accomplished in many different ways. Several past studies have focused on root diameter as a proxy for root function, but this arbitrary method ignores that roots with larger diameter have no function other than structure (Pregitzer et al. 2002, Pregitzer 2002, Pregitzer et al. 1998, Kummerow et al. 1978, King et al. 2002). Another method that has proven useful is using root branching

order where first order roots are root tips and each connecting branch is second, third, and so on (Pregitzer et al. 2002, Espeleta et al. 2009). Root branching orders are useful because each order has a specific function. First order root branches are absorptive tissue; they have the highest specific root length, highest N content, and make the largest contribution to total root length (Pregitzer et al. 2002, Guo et al. 2007, Guo et al. 2004). Second order roots are mostly absorptive tissue, but have transport function as well, meaning that first and second order roots are the most important for water and nutrient uptake. Third order root branches are primarily transport tissue and non-absorptive, except in species such as species from the family Ericaceae that have very thin roots that are absorptive until the third order (Valenzuela-Estrada et al. 2008). Fourth order roots are entirely transport tissue. By quantifying root branches by order, better estimates of absorptive tissue are possible. Also quantifying loss of roots based on root order can show the degree of root die-back during each season because it is expected that first and second order roots die first and third and fourth roots are more persistent in the soil, considering they represent a larger investment than lower order roots.

In this study I first tested the hypothesis that fine root density would change seasonally, with the wet season having a greater root density than the dry season. My second hypothesis was that, during the dry season, deciduous species would better maintain similar fine root density to the wet season, when compared to evergreen shrubs. This could be caused by low root mortality during the dry season and a lower increase in root density in the wet season. Fine roots were quantified by counting the number of live

roots present in each order for each species in each season. Roots were identified by visual characteristics or using restriction fragment length polymorphism (RFLP) based on a key of species-specific banding patterns that I developed.

Methods and Material

Sites description and study species

The site is located in a southern ridge sandhill plant community at Archbold Biological Station (ABS) in south-central Florida, USA (N27˚ 11.280'; W81˚ 20.181'; Figure 2.1). The southern ridge sandhill plant community is located along the Lake Wales Ridge, which comprises ancient beaches from the late Pliocene (Weekley et al. 2008). The soils are extremely well-drained acid quartzipsamments sands (Kalisz and Stone 1984), have very low nutrient and organic matter content (Huck 1987), and experience periodic drought (Saha et al. 2008, Menges and Gallo 1991). The site was completely burned in 1995 and 1999 and partially burned in 1991 and 2002 (ABS burn records). Frequent burns maintain low canopy cover, plant height, and less organic debris on the soil surface. The soil surface is mostly exposed sand with little organic matter layer. The woody species that dominate the southern ridge Sandhill plant community are the evergreen oaks (*Quercus myrtifolia*, *Q. inopina*, *Q. geminata, Q. chapmanii*), other evergreen species such as *Serenoa repens*, *Lyonia ferruginea, Pinus ellioti var. densa, and P. clausa,* and deciduous species such as *Carya floridana, Q. laevis*, and *Asimina obovata* (Menges 1999). The climate of the site is characterized by hot, wet summers and mild, dry winters that extend from December through April. Mean annual rainfall at

Archbold is 1345mm, with approximately 61% falling between June and September (ABS weather records 1932-2011; Figure 2.2). The yearly mean of groundwater table depth is 15m (ABS groundwater records).

Two deciduous (*Q. laevis* and *C. floridana*) and three evergreen species (*Q. myrtifolia, Q. geminata,* and *Lyonia ferruginea*) were selected because of their ubiquity in the southern ridge sandhill community at this site. Of the angiosperm species, these woody species compose the dominant woody species at the site (Menges 1999). *Carya floridana* and the *Quercus* species are ectomycorrhizal species, and *L. ferruginea* is an ericoid mycorrhizal species.

Root sampling

Fine roots were collected by extracting soil cores to a depth of 150cm and removing the fine roots. Soil cores were taken from 14 locations. Ten of the core locations were chosen because all five study species were in close proximity. Due to the relative rarity of *L. ferruginea* in the sandhill and difficulty of finding it in close proximity with the other five species, four cores were not collected close to *L. ferruginea*. At each location a soil core was collected during the dry season (January 2010), end of the dry season after deciduous species had grown new leaves (March 2010), and during the wet season (August 2010). The cores were collected within 50cm from each other at each location.

A PVC pipe with a 7cm inside diameter and a beveled end was inserted into the ground to a depth of 150cm. Soil from the core was carefully separated into three sections: 0-20, 20-50, and 50-150cm. The soil samples were packed in ice and taken to the lab. In the lab, all samples were stored at 4°C until roots were removed from the soil. Roots were cleaned of soil by placing them in a 4°C deionized water bath. Roots

segments were carefully removed from the soil to minimize breakage. All roots were scanned using a high resolution flat-bed scanner (1200 dpi resolution, 19 μm pixel size, 48-bit color, TIFF format; Epson Scanner Perfection V500 Photo, USA). All roots, except those of *L. ferruginea*, were scanned by removing the roots from the water bath, carefully patting dry, and placing on the scanner window. The roots of *L. ferruginea* were so small and soft that they had to be scanned while in water because only then they would spread out and allow differentiation of root branches. To scan the roots of *L. ferruginea* in water, a Petri dish filled with deionized water was placed on the scanner window, and the roots were placed in the Petri dish. The Petri dishes were placed on the scanner bed, and the roots were scanned at 1200dpi like the other roots. All roots were left intact while scanned unless root structure obscured the view of the root in the image. In these cases the roots were separated into smaller sections. However, the first four terminal orders were left intact, so that branching could be properly defined.

Root branch classification

In the characterization of root structure, I categorized root branches into terminal order classes based on the morphometric approach developed by Fitter (1982). All root branches with a meristematic end were considered first order roots. Second terminal order roots were those that had a junction with a first terminal order root. This method was used to define third and fourth order roots as well (Figure 2.3).

Root segment identification

After scanning, all roots were placed in 2ml microcentrifuge tubes and lyophilized. The root segments were ground using a ball mill at a frequency of $25s^{-1}$ for 3 minutes (Retsch

MM 200; Haan, Germany). To grind the samples, two 5mm stainless steel balls were placed in each 2ml microcentrifuge tube with the lyophilized root.

Root DNA was extracted using an extraction method developed by Brunner et al. (2001). The entire ITS region of the 18S–26S nuclear ribosomal DNA repeat (ITS1, ITS2, and the 5.8S ribosomal RNA gene) was amplified using the primer pair 4 and 5 described in White et al. (1990). This primer pair has been used to identify roots (Jackson et al. 1999b). This primer pair amplified a ~780bp region. Internal primer pair A and D was used. The primer pair sequences for A and D are 5'- GCT TAA ACT CAG CGG GTA GTC C – 3' and 5' – CCG CGA ATT GGT TAC AAC C – 3', respectively.

PCR amplifications were performed with a total volume of 10 μL, containing the following set of reaction reagents: 1x PCR buffer (New England Biolabs B9022S), 2.0 mM $MgCl_2$, 0.1 mM each of dATP, dCTP, dGTP and dTTP (New England Biolabs), 0.2 mM of each primer, 5% DMSO (v/v), 1-10ng of template DNA from fine roots, and 0.25 U of *Taq* DNA polymerase (New England Biolabs M0481S). DMSO increased the amplification rate in this study and has been shown to improve specificity (Miranda et al. 2010). The reaction conditions were the following: 5 min denaturation at 94°C and 40 cycles of 1 min denaturation at 94°C, 1.5 min annealing at 58°C, 2 min extension at 68°C, followed by a final extension at 68°C for 10 min.

PCR reactions that amplified DNA were identified by loading 2.5μL of PCR product on 0.8% agarose gel and running the gel at 200v for 15min. Fragment sizes were determined using a 50bp ladder. Any PCR reactions that failed to amplify sufficient product to digest were performed again with the internal primer pair A & D, and the same

PCR reaction conditions were used. Instead of extracted DNA as the DNA template, 1µL of failed PCR solution was used. These PCR products were identified by running 2.5µL of PCR product on a 0.8% agarose gel.

Restriction enzyme digestions of PCR products were performed with Taq$^{\alpha}$1 in a total volume of 50 mL containing 7.5 mL of amplified DNA, 1x NEBuffer 4 (New England Biolabs R0149) and 2 U of restriction enzyme. PCR products were digested for 8 h at 65°C. To visualize the restriction fragments, 15µL loading dye (0.2% orange G, 50% glycerol, and 1x SB buffer) was added to the 50 mL digestion reaction. A total of 20 mL was loaded on 2% agarose gels containing 1 µL Gel Red per 100ml of gel and 1x SB buffer. Gels were run at 59 V for 2 h. Gels were digitalized using Gel Doc™ XR+ System (BioRad CFW-1312M; Hercules, CA)**.** *C. floridana* formed a 3-band pattern, and *Q. geminata* and *Q. myrtifolia/Q. laevis* formed 4-band patterns that were easily distinguishable (Figure 2.4). At the site where this study was conducted, ITS region of 10 individuals of each species was digested to determine if the banding pattern was consistent for each species.

Statistical analysis

To determine if root standing crop at each depth (0-20cm, 20-50cm, and 50-150cm) increased from the dry season to the wet season, repeated-measure mixed design ANOVAS were conducted on each terminal order for each species separately. The root densities were square root transformed to meet the requirements of normality. To determine if root standing crop differed with depth during each season, one-way ANOVAs were used for each terminal order of each species during wet and dry seasons. When the data were not homogeneous, a Welch test was performed, which is a robust test

of equality of means. All root densities were transformed. Turnover was calculated as the difference between wet season fine root production (the difference between wet season and dry season root density) divided by wet season root density. Turnover was calculated for each root order separately. I used SPSS Statistics (version 19; IBM, Armonk, New York) to perform statistical tests.

Results

Root segment identification

All species could be identified either visually (*L. ferruginea*) or by RFLP (Figure 2.4), but *Q. laevis* (deciduous) and *Q. myrtifolia* (evergreen) could not be separated either visually or using RFLP. Of the numerous regions that were investigated, none had a difference in sequence between *Q. laevis* (deciduous) and *Q. myrtifolia* (evergreen). These two species were left as a single group for all analyses.

Change in root density with season

For every species, root density of each order increased from the dry season to the wet season at all depths except the fourth terminal order roots of *C. floridana* (Table 2.1; Figure 2.5). In *L. ferruginea* and *C. floridana*, root densities of all four terminal orders increased in the wet season (Games-Howell *post hoc*, $P < 0.05$), but the magnitude of the increase did not differ significantly among depths: 0-20, 20-50, and 50-150cm (Table 2.1). In *Q. geminata*, the increase in root density with first, second, and third terminal orders was greatest in the top 20cm (Games-Howell *post hoc*, $p < 0.05$). In the fourth terminal order the increase in root density was not different with depth. Root density of all terminal orders increased with depth in the *Q. myrtifolia*/*Q. laevis* complex, this

increase with depth was only significant in the first and fourth terminal orders. The root density of the second and third terminal orders showed a marginally significant increase from the dry season to the wet season (P = 0.052 and 0.054, respectively). In the first terminal order, the increase in root density from the dry to wet season was significantly greater in the top 50cm than from 50 to 150cm. For the fourth terminal order roots of the *Q. myrtifolia*/*Q. laevis* complex, the magnitude of the increase in root density in the wet season differed decreased with soil depth.

Within each season, root density was greater in the shallow soil than the deep soil for all terminal orders, except for *L. ferruginea* during the dry season (Tables 2.2-2.5; Figure 2.5). For all terminal orders, *L. ferruginea* did not differ in root density between the three depths. During the wet season, root density was highest in 0-20cm (Games-Howell *post hoc*, $p < 0.05$), and 20-50 and 50-150cm were not significantly different from each other (Table 2.2). Both *Quercus* groups (*Q. geminata* and *Q. myrtifolia/Q. laevis* complex) in the dry season and *Q. geminata* in the wet season showed the same root distribution pattern; root density was highest in the top 50 cm (Table 2.3 and 2.4; Games-Howell *post hoc*, $p < 0.05$). During the wet season *Q. myrtifolia/Q. laevis* complex differed slightly from dry season root patterns. Highest root density in the top 20 cm for every terminal order and followed by 20-50cm (Table 2.3; Games-Howell *post hoc*, $p < 0.05$). In *C. floridana* the highest root density was concentrated in the top 20cm and the root density did not change from a depth of 20 to 150cm in both the dry and wet season, except for third and fourth terminal orders in the dry season (Table 2.5; Tukey *post hoc*, $p < 0.05$).

C. floridana (deciduous) had the lowest turnover (wet season root production divided by wet season root density) in all terminal orders (Figure 2.6), while it had the lowest ^{15}N uptake rate (Chapter 4). In contrast *L. ferruginea* had the highest turnover in every terminal order (Figure 2.6), yet *L. ferruginea* had the highest ^{15}N uptake rate (Chapter 4). The *Quercus* species were intermediate between the deciduous *C. floridana* and the evergreen *L. ferruginea*.

Fine root density more than doubled from dry to wet season in all species and in all orders, except for the fourth order in *C. floridana* that was not significantly different (Figure 2.6). Fine root turnover was highest in *L. ferruginea* and the *Q. myrtifolia/Q. laevis* complex and lowest in *C. floridana*. Fine root turnover decreases with root order in *Q. geminata* and *C. floridana*. In *L. ferruginea* and the *Q. myrtifolia/Q. laevis* complex, fine root turnover was highest in first order roots, but the other three orders were all similar with root turnover in the second order being lowest in *L. ferruginea* and turnover in the third order being lowest in the *Q. myrtifolia/Q. laevis* complex.

Discussion

Change in root density with season

Root density increased drastically from the dry season to the wet season at all depths and in all root orders, confirming my first hypothesis. This increase in wet season root density represents gross fine root production, but root production and mortality likely are very similar in magnitude, meaning that most of this root production represents turnover after high dry season mortality (Hendrick and Pregitzer 1992, Lopez et al. 2001). Interestingly, this increase in wet season fine root density was similar at all depths (Table 2.1; Figure 2.5), as also was found with *Q. douglassii* in the California oak woodland (Cheng and

Bledsoe 2002). It is likely that low dry season root density represents high dry season root mortality. Considering that carbon investment and lifespan increase with root order (Guo et al. 2004, Guo et al. 2008), it was expected that fine root turnover would decrease with order. However, these species still shed more than half of all third and fourth order roots in the dry season (except fourth order roots in *C. floridana*).

In *C. floridana* and the *Quercus* species, I observed that third and fourth order roots were woody, did not have a cortex, and had a larger diameter than first and second order roots, showing that third and fourth order roots had secondary xylem and solely a transport function. This observation is corroborated in other species (Guo et al. 2004, Guo et al. 2008). In *L. ferruginea*, secondary xylem and a lack of a cortex was only observed in fourth order roots. In *Vaccinium corymbosum*, a ericoid like *L. ferruginea*, first, second, and third order roots were absorptive tissue. This distinction between absorptive and transport tissue appears to be similar in L. ferruginea (Guo et al. 2004, Guo et al. 2008, Valenzuela-Estrada et al. 2008).

They represent a much larger investment to the plant than the ephemeral first and second order roots that are absorptive tissue (Guo et al. 2004). Plants adapted to prolonged drought tend to shed roots as soon as the soil dries instead of maintaining them (Eissenstat and Yanai 1997). Shedding third and fourth order fine roots incurs a large cost when these roots are replaced in the wet season, but the construction cost associated with growing new roots in the wet season may be less than the cost of maintaining the roots through the dry season (Schoettle et al. 1994, Nobel et al. 1992, Cropper Jr and Gholz

1991). Woody species in the Florida sandhill behave in the same way as desert species that shed roots in drought conditions instead of maintaining them (Huang and Nobel 1994).

Water availability is likely the principal driver for fine root production. Several studies have shown low dry season root density compared to the wet season in ecosystems of Malaysia, Costa Rica, Panama, and Mexico (Sanford 1989, Cavelier 1992, Green et al. 2005, Sanchez-Gallen I. 1996). In Malaysia, standing crop of fine root biomass was correlated with preceding rainfall (Green et al. 2005, Rojas-Jimenez et al. 2007). In Mexico, precipitation was not significantly related to standing crop fine root biomass, but the seasonality component was strong where the wet season had higher fine root biomass than the dry season (Sanchez-Gallen I. 1996). In an irrigation experiment in Panama, fine root biomass peaked in the dry season for irrigated plots, but in control plots, fine root biomass peaked at the end of the wet season (Yavitt and Wright 2001). Soil moisture extended fine root survival through the dry season. In another study, fine root production and mortality of *Q. ilex* followed the same pattern that I observed here with high biomass during the wet winter and low root biomass during the dry summer (Lopez et al. 2001). Additionally, a study conducted in the sandhill region of Georgia, USA found that survival increased with root order, but measured little root turnover in the dry season (Espeleta et al. 2009). Contrary to my study, the Georgia sandhill study found that the dry season incurred little fine root loss. In my Florida sandhill sites, the volume of available water in the top 150 cm of soil profile in the dry season was only 43% of that of the wet season, and the volume of available water in the deep soil was

higher in the wet season. It appears that reduced water availability per unit of soil in the dry season affected all soil layers, reducing fine root density in the top 150cm of the soil profile.

Change in root density in deciduous and evergreen species

Fine root turnover was lower in the deciduous *C. floridana* for all root orders than in the evergreen species (*Q. geminata* and *L. ferruginea*) and the red oak complex (*Q. myrtifolia/Q. laevis*), confirming my second hypothesis for this species. My conclusions are limited because the second deciduous species, *Q. laevis,* was not separated from the evergreen *Q. myrtifolia*. The rate of nitrogen uptake during the dry season was lowest in *C. floridana* (Chapter 4), which means that high proportion of roots persisting through the dry season does not lead necessarily to high nutrient uptake. When the soil is dry, *C. floridana* may be like other species that down-regulate root respiration (Kosola and Eissenstat 1994, Burton et al. 1998). If so, *C. floridana* may have low root respiration and low dry season nitrogen uptake ability.

Dry season leaf out in *C. floridana* may not have increased root water stress, which may increase root mortality. In Costa Rica, newly flushed deciduous leaves in the dry season transpired very little or become fully functional until the wet season (Rojas-Jimenez et al. 2007). Root production was very low in the dry season and did not begin until the soil was re-wetted with wet season precipitation. As with other deciduous species that leaf out during the dry season, the leaves of *C. floridana* may not have been fully functional and perhaps were transpiring very little, maintaining low water uptake

demand placed on the roots until the wet season. If so, fine root mortality would be reduced and water taken up in the deep soil can be used to sustain shallow fine roots instead of fully transpiring leaves (Bauerle et al. 2008).

In contrast to the deciduous *C. floridana*, the evergreen species had higher root turnover in the dry season. *L. ferruginea* had the highest fine root turnover in the dry season that was uniform among all orders. *L. ferruginea* also had the highest nitrogen uptake rate during the dry season (Chapter 4). *L. ferruginea* only had 27% of its absorptive roots alive during the dry season, yet these roots were actively taking up nitrogen. *L. ferruginea* maintained actively transpiring leaves throughout the year and leaf turnover occurs continually. It appears that *L. ferruginea* is active aboveground and belowground with a high nitrogen uptake rate, but it a high fine root turnover rate. Possibly new roots are more active in nitrogen uptake than old roots, so high turnover rate would be beneficial to nitrogen uptake.

The *Quercus* species had a similar pattern to each other in that root density was highest at 0-20cm and declined with depth, and turnover was intermediate between *C. floridana* and *L. ferruginea*. The *Q. myrtifolia* and *Q. laevis* complex had high root turnover, but the inability to distinguish between *Q. myrtifolia* and *Q. laevis* prohibited making an intra-generic comparison of deciduous and evergreen species. Considering the similarities in root turnover between the *Q. myrtifolia/Q. laevis* complex and *L. ferruginea*, either the contribution of the deciduous *Q. laevis* is minor or it has high root turnover like the evergreen species. *Q. laevis* was less common on the study sites than the abundant *Q. myrtifolia* and may have been small proportion of total roots in the *Q. myrtifolia* and *Q. laevis* complex. Also Espeleta and Donovan (2009) found that root

production of *Q. laevis* was lower than other oak species in the study. If most of the roots in the complex belong to *Q. myrtifolia*, then the same trend occurred with *Q. myrtifolia* as with *L. ferruginea* in that the species experienced high root mortality in the dry season but still had high dry season nitrogen uptake (Chapter 4).

Fine root distribution with depth

In both the dry and wet season, the general trend of fine root distribution of all root orders in all species was that root density decreased with soil depth, except that root densities were much higher in the wet season (Figure 2.5). Course roots also followed this same pattern with most course roots in the top 50cm (Saha et al. 2010). The proportional contribution of each soil layer to the total quantity of roots was consistent for all root orders. This consistency is because the proportion of total root branches belonging to each branching order for each species remained fairly constant in both dry and wet seasons. This trend did not hold for *L. ferruginea* in the dry season because dry season root turnover was very high in all root orders and did not decrease with depth as with the other species (Figure 2.5 and 6). Fine root distribution was similar to the pattern of fine root distribution in that most roots were in the shallow soil and root density decreased with depth (Jackson et al. 1997). In particular, the proportion of roots in the shallow soil was most similar to tropical deciduous and evergreen forests, tropical savannas, and temperate coniferous forests. Interestingly, three of the biomes most similar in root distribution to what was found in this study are ecosystems that have edaphic (e.g. nutrient and water availability) and climatic conditions (e.g. wet and dry seasons) similar to the Florida scrub (Murphy and Lugo 1986, Huntley and Walker 1982, Whitmore and Burnham 1975).

Conclusions

In this study I hypothesized first that root density would increase from the dry season to the wet season and second that fine root turnover in all orders would be lowest in deciduous species. As I hypothesized, fine root density in all species and in all orders increased in the wet season. This increase in root density was likely high wet season root production to offset high dry season mortality. This means that in the dry conditions of the dry season of the Florida sandhill root death was in both absorptive (first and second order) and transport (third and fourth order) root branches. Although the majority of available water was in the deep soil (50-150cm), the deep soil had 43% less water available by volume than in the dry than in the wet season. Under these dry conditions, the study woody species shed their fine roots instead of maintaining them through the dry season. Just as high dry season root mortality appears to be an adaptation to drought, high wet season fine root production appeared to be triggered by high soil water availability. High wet season root densities in every order show that root production was capable of replenishing root branches of all orders. In both the dry and wet season, root density decreased with soil depth, similar to that of tropical forests.

Fine root turnover was lowest in the deciduous species *C. floridana*. Unfortunately, the other deciduous species *Q. laevis* could not be distinguished from the evergreen *Q. myrtifolia*, so my second hypothesis was shown to be partly true. During this same time, nitrogen uptake by *C. floridana* was the lowest of all five study species (Chapter 4). Nitrogen uptake may have been so low because while *C. floridana* was leafless, root respiration may have been down-regulated, lowing nitrogen uptake

potential. In contrast, *L. ferruginea* had high turnover and the highest nitrogen uptake rate. It appears that root density in the dry season does not reflect the uptake potential.

Table 2.1 Mixed-model analysis of repeated measures showing the effect of depth between seasons for each species.

Species	Root order	Interaction	*df*	*ms* (x 10^4)	*F*	*P*
L. ferruginea	1st	Season	1	340.67	13.5	**0.001**
		season*depth	2	24.73	1.0	0.39
		error (season)	27	25.22		
	2nd	Season	1	125.53	8.9	**0.006**
		season*depth	2	11.13	0.8	0.46
		error (season)	27	14.05		
	3rd	Season	1	70.42	10.6	**0.003**
		season*depth	2	7.43	1.1	0.34
		error (season)	27	6.66		
	4th	Season	1	31.40	10.4	**0.003**
		season*depth	2	3.91	1.3	0.29
		error (season)	27	3.02		
Q. geminata	1st	Season	1	274.05	41.4	**<0.001**
		season*depth	2	28.37	4.3	**0.021**
		error (season)	39	6.61		
	2nd	Season	1	99.75	23.1	**<0.001**
		season*depth	2	17.74	4.1	**0.024**
		error (season)	39	4.31		
	3rd	Season	1	28.21	14.5	**<0.001**
		season*depth	2	7.05	3.6	**0.036**
		error (season)	39	1.94		
	4th	Season	1	6.70	9.4	**0.004**
		season*depth	2	2.08	2.9	0.066
		error (season)	39	0.71		
Q. myrtifolia +	1st	season	1	453.77	57.8	**<0.001**
Q. laevis		season*depth	2	38.82	5.0	**0.01**
		error (season)	39	7.85		
	2nd	season	1	136.83	38.1	**<0.001**
		season*depth	2	11.48	3.2	0.052

		error (season)	39	3.59		
	3rd	season	1	46.41	36.9	**<0.001**
		season*depth	2	3.96	3.1	0.054
		error (season)	39	1.26		
	4th	season	1	11.91	33.9	**<0.001**
		season*depth	2	2.77	7.9	**0.001**
		error (season)	39	0.35		
C. floridana	1st	season	1	112.07	28.2	**<0.001**
		season*depth	2	10.43	2.6	0.086
		error (season)	39	3.98		
	2nd	season	1	26.73	19.0	**<0.001**
		season*depth	2	2.62	1.9	0.169
		error (season)	39	1.41		
	3rd	season	1	4.09	7.3	**0.01**
		season*depth	2	1.69	3.0	0.060
		error (season)	39	0.56		
	4th	season	1	0.37	2.6	0.112
		season*depth	2	0.28	2.0	0.152
		error (season)	39	0.14		

Table 2.2 One-way ANOVA showing the effect of depth on each root order for *L. ferruginea* within each season when data were homogeneous. Otherwise a Welch's test was performed (mean square was not calculated for this test).

Season	Root order	Depths	*df*	*ms* (x10^4)	*F*	*P*
Dry	1st	Between	2		2.0	0.16
		Within	16			
		Total	18			
	2nd	Between	2		2.2	0.15
		Within	15			
		Total	17			
	3rd	Between	2		1.6	0.23
		Within	16			
		Total	18			
	4th	Between	2		1.6	0.24
		Within	16			
		Total	18			
Wet	1st	Between	2	165.61	6.2	**0.006**
		Within	27	26.89		
		Total	29			
	2nd	Between	2	88.13	6.7	**0.004**
		Within	27	13.07		
		Total	29			
	3rd	Between	2	47.51	6.9	**0.004**
		Within	27	6.85		
		Total	29			
	4th	Between	2	22.99	6.8	**0.004**
		Within	27	3.38		
		Total	29			

Table 2.3 One-way ANOVA showing the effect of depth on each root order for *Q. geminata* within each season when data were homogeneous. Otherwise a Welch's test was performed (mean square was not calculated for this test).

Season	Root order	Depths	*df*	*ms* (x10^4)	*F*	*P*
Dry	1st	Between	2	47.97	5.7	**0.007**
		Within	39	8.45		
		Total	41			
	2nd	Between	2	32.88	8.6	**0.001**
		Within	39	3.81		
		Total	41			
	3rd	Between	2	21.51	10.7	**<0.001**
		Within	39	2.01		
		Total	41			
	4th	Between	2	8.07	11.6	**<0.001**
		Within	39	0.70		
		Total	41			
Wet	1st	Between	2	201.11	23.6	**<0.001**
		Within	39	8.53		
		Total	41			
	2nd	Between	2		18.3	**<0.001**
		Within	24			
		Total	26			
	3rd	Between	2		23.2	**<0.001**
		Within	24			
		Total	26			
	4th	Between	2		43.2	**<0.001**
		Within	25			
		Total	27			

Table 2.4 One-way ANOVA showing the effect of depth on each root order for the red oaks, *Q. myrtifolia* and *Q. laevis*, within each season when data were homogeneous. Otherwise a Welch's test was performed (mean square was not calculated for this test).

Season	Root order	Depths	*df*	*ms* (x10^4)	*F*	*P*
Dry	1st	Between	2	27.86	4.0	**0.027**
		Within	39	7.04		
		Total	41			
	2nd	Between	2.0		7.8	**0.003**
		Within	24			
		Total	26			
	3rd	Between	2		12.4	**<0.001**
		Within	23			
		Total	25			
	4th	Between	2			
		Within				
		Total				
Wet	1st	Between	2	194.28	9.5	**<0.001**
		Within	39	20.35		
		Total	41			
	2nd	Between	2	90.62	10.8	**<0.001**
		Within	39	8.40		
		Total	41			
	3rd	Between	2	41.91	11.9	**<0.001**
		Within	39	3.52		
		Total	41			
	4th	Between	2		15.7	**<0.001**
		Within	24			
		Total	26			

Table 2.5 One-way ANOVA showing the effect of depth on each root order for *C. floridana* within each season when data were homogeneous. Otherwise a Welch's test was performed (mean square was not calculated for this test).

Season	Root order	Depths	*df*	*ms* (x 10^4)	*F*	*P*
Dry	1st	Between	2	40.42	10.7	**<0.001**
		Within	39	3.78		
		Total	41			
	2nd	Between	2.0	29.11	21.8	**<0.001**
		Within	39	1.33		
		Total	41			
	3rd	Between	2	14.91	24.8	**<0.001**
		Within	39	0.60		
		Total	41			
	4th	Between	2	2.78	16.9	**<0.001**
		Within	39	0.16		
		Total	41			
Wet	1st	Between	2	113.74	14.9	**<0.001**
		Within	39	7.64		
		Total	41			
	2nd	Between	2	53.12	15.6	**<0.001**
		Within	39	3.42		
		Total	41			
	3rd	Between	2		16.5	**<0.001**
		Within	25			
		Total	27			
	4th	Between	2	4.91	21.3	**<0.001**
		Within	39	0.23		
		Total	41			

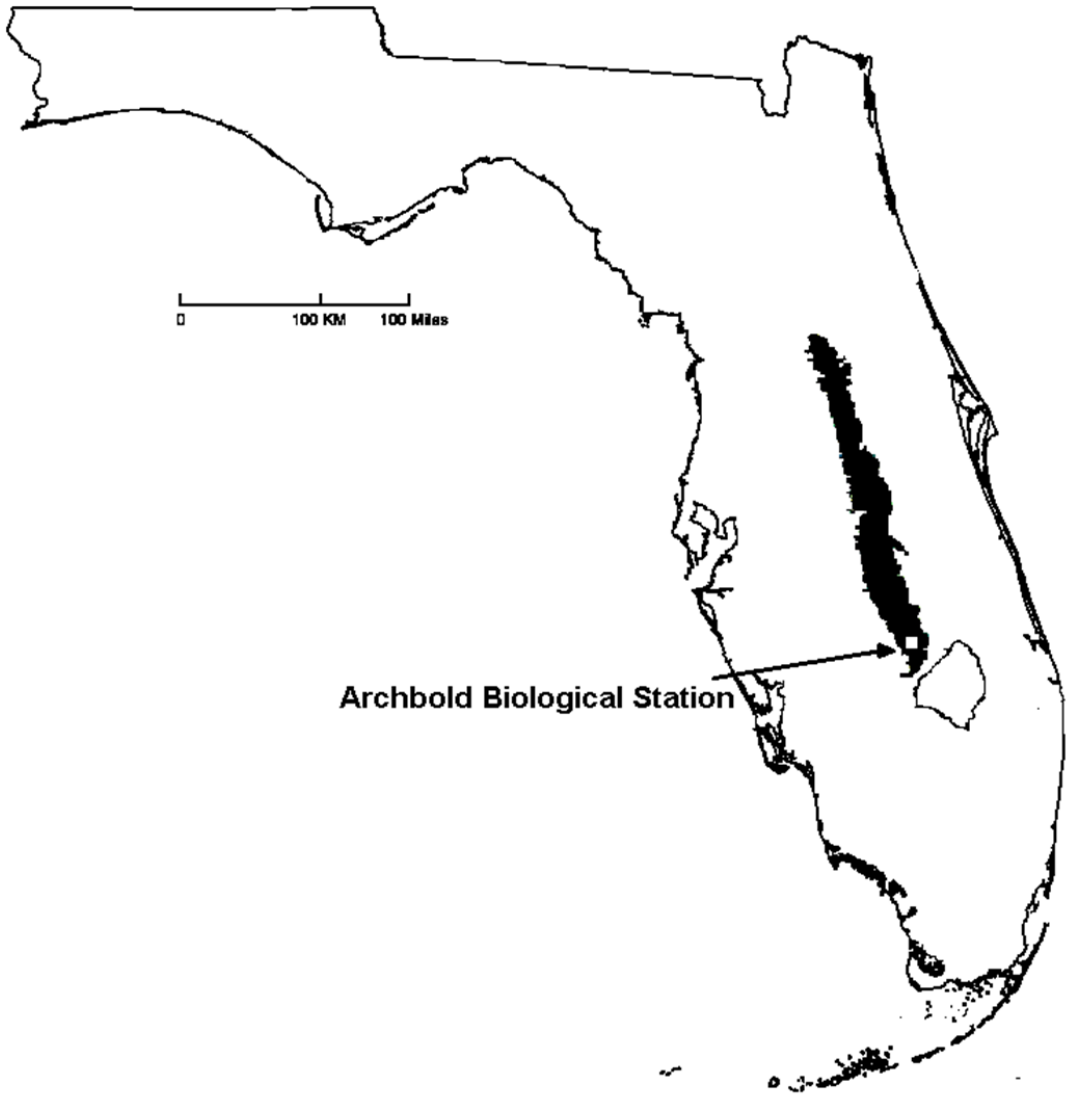


Figure 2.1 Map of Florida showing the geographical range of the Lake Wales Ridge (region highlighted in black) and the location of Archbold Biological Station. The boundary of the Lake Wales Ridge is based on Weekley et al. (2008).

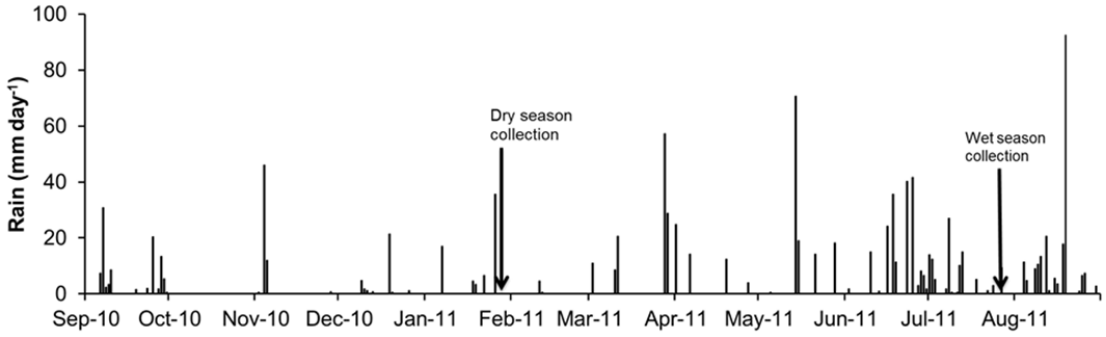


Figure 2.2 Precipitation (mm day^{-1}) at Archbold Biological Station from 1 September 2010 to 31 August 2011 (ABS weather records).

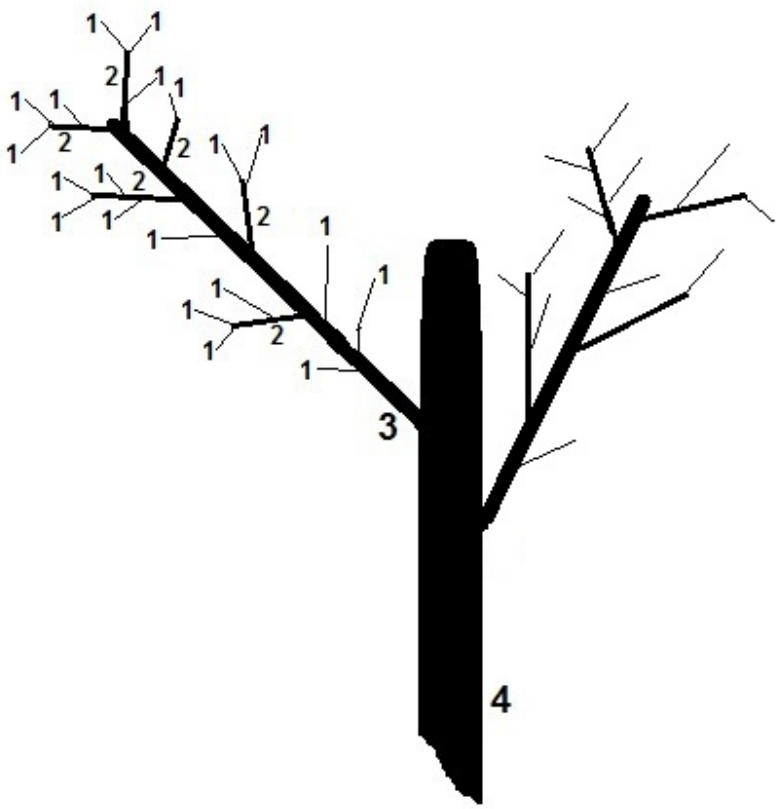


Figure 2.3 Schematic diagram of a fine root based the terminal order classification method designed by Fitter (1982). The numbers represent the terminal orders of the roots. First terminal order roots have a meristematic end. Second terminal order roots formed a junction with first order roots. First terminal order roots (roots with a meristematic tip) could arise from a higher than second terminal order root and still be considered a first terminal order root.

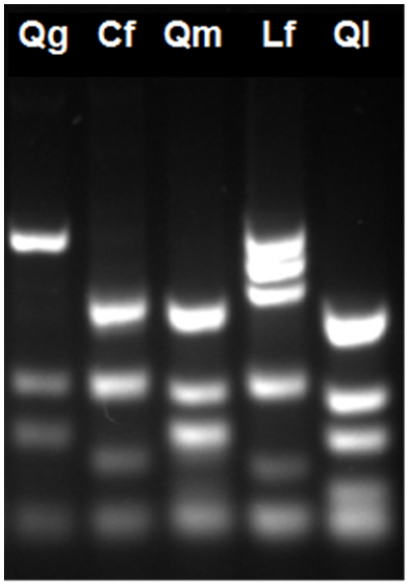


Figure 2.4 Electrophoresis gel showing the digested PCR products of the five study species: *Q. geminata* (Qg), *C. floridana* (Cf), *Q. myrtifolia* (Qm) *L. ferruginea* (Lf), and *Q. laevis* (Ql). All were easily distinguishable except *Q. myrtifolia* and *Q. laevis*, which have identical banding patterns.

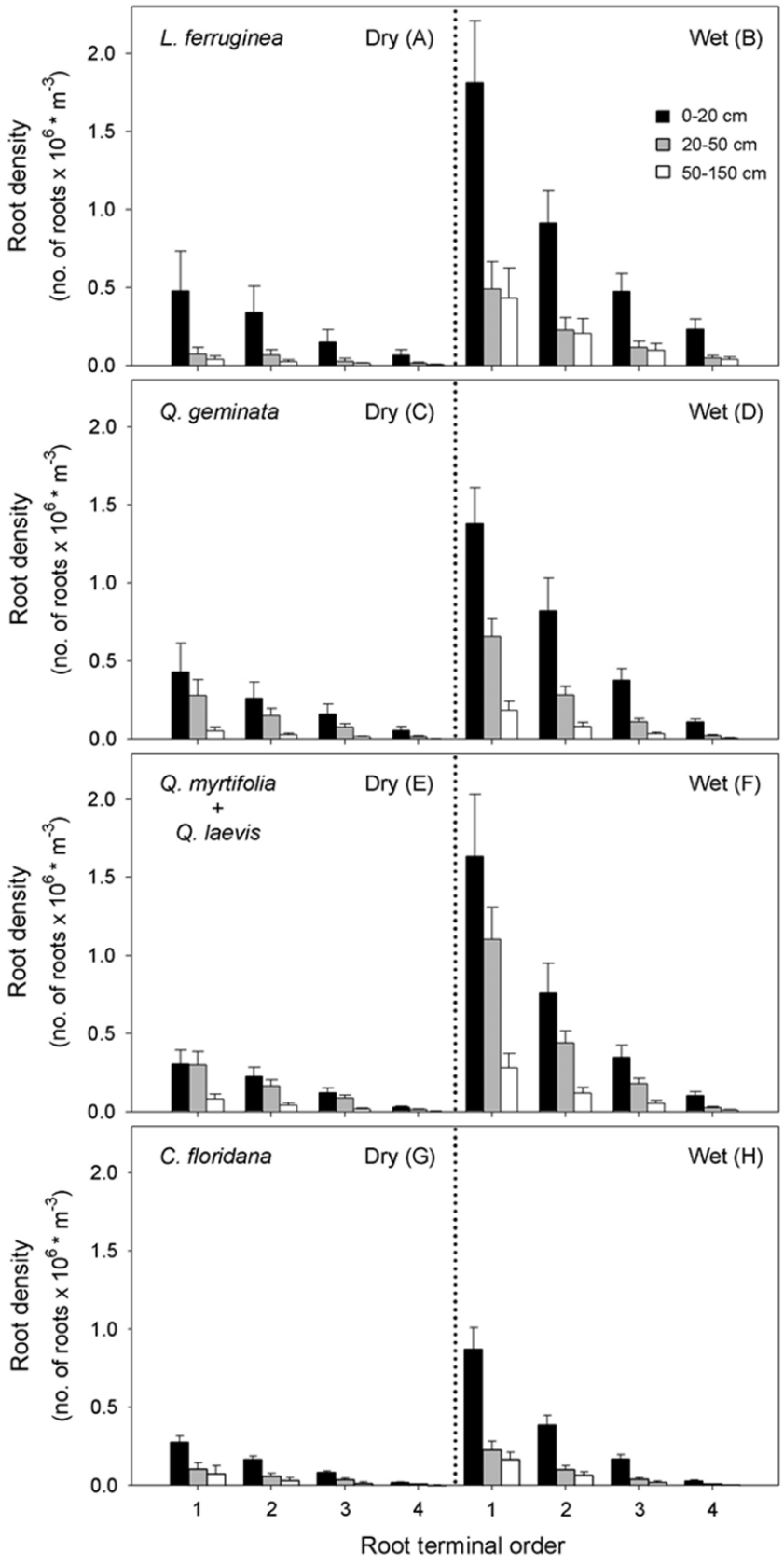

L. ferruginea
Dry (A)
Wet (B)
0-20 cm
20-50 cm
50-150 cm
Q. geminata
Dry (C)
Wet (D)
Q. myrtifolia
+
Q. laevis
Dry (E)
Wet (F)
C. floridana
Dry (G)
Wet (H)
Root density
(no. of roots x 10^6 * m^-3)
0.0
0.5
1.0
1.5
2.0
1
2
3
4
Root terminal order

Figure 2.5 Root densities (number of roots x 10^6 per m^3) by root terminal order in each season for each species at three soil depths. Each bar is the mean of 14 cores ± SE, except *L. ferruginea* (10 cores ± SE). Root densities are dependent on the number of individuals of each species surrounding the core location, which was not constant, so fine root densities cannot be compared among species.

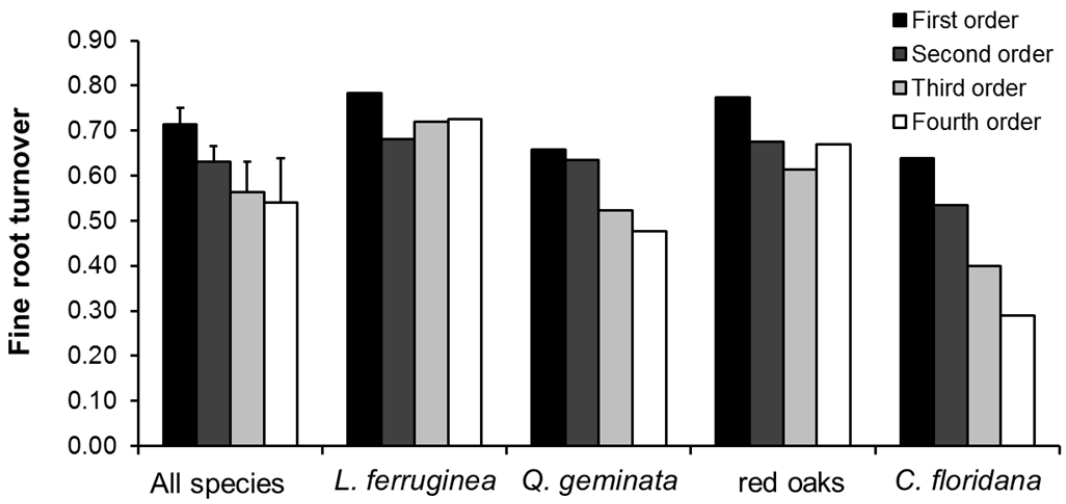


Figure 2.6 Turnover for all species and each species separately. Turnover is defined as wet season fine root production divided by wet season root density. Red oaks are *Q. myrtifolia* and *Q. laevis*. The first set of bars labeled all species is the mean turnover of all species. Fourth order of *C. floridana* did not significantly change from dry to wet season (Table 2.1).

Chapter 3

Deciduous and evergreen woody species use water sources based on water availability and root distribution in a dry seasonal scrub

Summary

Dry seasonal ecosystems are defined by a pronounced and consistent dry season, which means that plants must adapt to water limitation in the dry season and high water availability during the wet season. In the southern ridge sandhill plant community I examined the hypothesis that evergreen and deciduous woody species take up shallow soil water in the wet season. When shallow soil water is unavailable evergreen species switch to deep soil water and deciduous species lose their leaves and remain dormant. To address this hypothesis, I measured water uptake of two deciduous and three evergreen species for a period of 13 months using stable isotopes. Further, to test for possible artifacts in the stem water isotopic composition during the leafless period of deciduous species, I conducted an experiment to measure the effect of defoliation on $\delta^{18}O$ and $\delta^{2}H$ of stem water. The defoliation experiment showed that evaporation, as the result of leaflessness, enriched stem water isotopically, which explained higher $\delta^{2}H$ and $\delta^{18}O$ values of leafless deciduous species than those of evergreen shrubs during part of the dry season. When deciduous species had leaves, however, deciduous and evergreen species used proportionally the same water sources throughout the year. In the early dry season, the proportional use of each soil layer for both deciduous and evergreen species was based on its water availability and the most important source was the deep soil (50-

150cm). During the late dry season and the wet season, the use of each soil layer was based on the proportion of roots at that soil depth. Nevertheless, deep water was an important water source throughout the year.

Background

Dry seasonal ecosystems are defined by a pronounced and consistent dry season that results in water limitation, especially in the shallow soil (Sobrado 1986, Sobrado and Cuenca 1979, Murphy and Lugo 1986). Distribution of water spatially and temporally is a key factor in ecosystems that are water-limited for at least part of the year and one of the major factors affecting plant distribution (Gholz et al. 1990, Huxman et al. 2005, Darrouzet-Nardi et al. 2006). Unlike plant species such as those found in desert ecosystems that are nearly always under water limitation, plant species in dry seasonal ecosystems must be adapted to water limitation in the dry season and high water availability during the wet season (Tobin et al. 2006, Nakagawa et al. 2000).

Perennial plant species can adapt to high water availability during the wet season and low water availability and decreasing soil water potentials during the dry season by four strategies. The first two strategies rely solely on shallow water as a water source. In the first strategy, plants maintain a shallow root system that can take advantage of nutrients and water when water is available and tolerate increasingly low matric potentials as soil dries during the dry season. Xerophytes, adapted to continually dry conditions, tolerate low water availability, but can respond to precipitation events that wet the shallow soil (Smith et al. 1998). These plants would be physiological drought tolerators because they withstand desiccation and low midday water potentials while maintaining gas exchange and hydraulic conductance, and reducing the chance of

cavitation under severe water limitation (Engelbrecht and Kursar 2003, Tyree et al. 2003). In the second strategy, some species grow only during the wet season and avoid the drought conditions by remaining dormant. Many studies have hypothesized that drought-deciduous trees lose their leaves in the dry season to avoid harsh conditions of water limitation (Medina 1984, Sarmiento et al. 1985, Olivares and Medina 1992, Givnish 2002, Bowman and Prior 2005, Jackson et al. 1995, Hasselquist et al. 2010, Markesteijn and Poorter 2009).

These next two strategies incorporate deep water into plant water use. In the third strategy, some plant species maintain both shallow and deep roots and access shallow water during the wet season and access deep water during the dry season. Some studies have shown that evergreen species shift water uptake from shallow water to deep water in the dry season (Scholes and Archer 1997, Walter 1971, Jackson et al. 1995, Hasselquist et al. 2010). These trees also have been called evergreen drought delayers because they increase their access to water to delay the effects of the drought (Markesteijn and Poorter 2009, Slot and Poorter 2007, Poorter and Markesteijn 2008, Paz 2003). As the fourth strategy, trees rely predominantly on a deep, perennial water source throughout the year such as trees growing in environments where the availability of shallow water is unpredictable, while a deep water source is permanent. In arid environments, riparian trees such as willows are known often as obligate phreatophytes to avoid the sometimes abundant but fleeting and unpredictable shallow water (Snyder and Williams 2000, Dawson and Ehleringer 1991).

Deciduous and evergreen leaf phenologies, although a component of water uptake strategy, do not always result in differences in water source use and therefore may not be

the principal component of adaptations to water source use. Jackson *et al.* (1999) found that deciduous species in the Brazilian savanna took water from deeper in the soil profile than evergreen species during the dry season. In a Hawaiian seasonal forest, Stratton, Goldstein & Meinzer (2000) did not find a clear difference in depth of water uptake between deciduous and evergreen trees. Another study in Mexico found that an evergreen had the shallowest depth of water uptake, while one of the species taking water from the deep bedrock was a deciduous species (Querejeta et al. 2007). From studies on water uptake by deciduous and evergreen species, it remains unclear what is the relationship between water source use and leaf phenology. However, adaptations in root structure and function may be directly selected by edaphic factors such as soil water and nutrient availability and accessibility in space and time. These edaphic and climatic factors may be shaping leaf traits as a consequence of their selection for root traits.

In this study I examined the hypothesis that evergreen woody species utilize the third strategy mentioned above, i.e. they are drought delayers and take up shallow soil water in the wet season and take up deep soil water in the dry season when shallow soil water is no longer available. The second hypothesis that I tested was that deciduous species were drought avoiders (the second strategy mentioned above). Deciduous species take up shallow soil water during the wet season, but once the shallow soil dries in the dry season, they lose their leaves and remain dormant until shallow water becomes available again. Further, because it has been suggested previously that leaflessness may change the isotopic signature of stem water (Phillips and Ehleringer 1995) and might confound any interpretation of water source use by isotopic analysis, I conducted an experiment to measure the effect of defoliation on the isotopic identity of stem water.

With the knowledge of how defoliation might alter the isotope ratio of stem water by evaporation, I compared the changes in isotope ratio of stem water of five woody species in my field study with leaf phenology. To address my water source use hypotheses, I measured the isotopic identity of stem water of both deciduous and evergreen species as well as that of potential source water for a period of 13 months. Using an isotopic mass balance approach, I was then able to capture inter- and intra-seasonal differences in water source use.

Methods and material

Two studies were conducted. The first was a defoliation experiment to simulate deciduousness and determine if leaflessness can change the isotopic composition of hydrogen and oxygen in stem water. This first study allowed us to better interpret data from our field sampling. The second study was on water source use by deciduous and evergreen species in the field.

Defoliation experiment

The defoliation experiment was conducted in a pineland plant community in Coral Gables, Florida, USA. Five evergreen *Quercus virginiana* Mill. trees were selected during the dry season (2010). One large branch having several secondary branches on each tree (treatment branch) was completely defoliated by plucking the leaves manually. A non-defoliated branch on the same tree served as a control. Before defoliation, stem water samples were collected from both treatment and the control branches. Afterwards, stems were collected from both defoliated and non-defoliated branches of each tree 27, 34, 57, 68, and 92 days after defoliation. The stems were de-corticated, placed in 12mm

tubes, sealed with parafilm, and stored at -18˚C (Saha, Sternberg & Miralles-Wilhelm 2009). Leaf regrowth on defoliated branches was recorded each time stem samples were collected.

Site of water source use study and study species

Three sites were selected in a sandhill plant community at Archbold Biological Station (ABS) in south-central Florida, USA (Figure 3.1). The sandhill community is located on the southern ridge of the Lake Wales Ridge, which represents the ancient beaches from the late Pliocene (Watts and Hansen 1994). The soils are extremely well-drained, acid, quartzipsamments sands (Kalisz and Stone 1984), have very low nutrient and organic matter content (Huck 1987), and experience periodic drought (Saha et al. 2008, Menges and Gallo 1991). The woody species that dominate the southern ridge sandhill plant community are the evergreen oaks (*Quercus myrtifolia* Willd., *Q. inopina* Ashe, *Q. geminata* Small*, Q. chapmanii* Sarg.), other evergreen species such as *Serenoa repens* (Bartram) Small, *Lyonia ferruginea* (Walter) Nutt.*, Pinus elliottii* Engelm. var. *densa* Little & Dorman*, and P. clausa* (Chapm. ex Engelm.) Vasey ex Sarg.*,* and deciduous species such as *Carya floridana* Sarg.*, Q. laevis* Walter, and *Asimina obovata* (Willd.) Nash (Menges 1999). The climate of the site is characterized by hot, wet summers and mild, dry winters that extend from December through April. Mean annual rainfall at Archbold was 1345mm, with approximately 61% falling between June and September (ABS weather records 1932-2011; Figure 3.2). The groundwater table at Sandhill Long Unburned 2 (N27˚ 09.083’; W81˚ 21.448’) had a yearly mean of 2.3 ± 0.3 m (Archbold Biological Station, unpublished data).The well near Sandhill Recently Burned (N27˚ 11.280’; W81˚ 20.181’) and Sandhill Long Unburned 1 sites (N27˚ 11.156’; W81˚

20.084') had a median depth of 24.3 ± 0.1 m (U.S. Geological Survey). The elevation at Sandhill Recently Burned was 62 m, Sandhill Long Unburned 1 was 58 m, and Sandhill Long Unburned 2 was 44 m.

Two deciduous (*Q. laevis* and *C. floridana*) and three evergreen species (*Q. myrtifolia, Q. geminata,* and *Lyonia ferruginea*) were selected for study because of their ubiquity in the southern ridge sandhill plant community at this site. Among angiosperms, these species represent the vast majority of individual woody plants at the site (Menges 1999). In addition to their abundance in the Florida scrub, I chose both deciduous and evergreen *Quercus* because they would provide an intra-generic comparison of water source use that differs in leaf phenology.

Sampling methods

To measure water source use, I collected stems and soils in the months of May, June, August, September, November, and December of 2008 and January, March, and May of 2009. At each of the three sites, stems were collected from five individuals of each of the five species. The stems were de-corticated, stored in 12mm tubes, and the tubes were stoppered and sealed with parafilm (Saha *et al.* 2009). The tubes were kept in a dry, cool ice chest until they could be stored at -18˚C. All stems were sampled from branches that had fully suberized bark and were far from leaves to eliminate the effect of evaporative enrichment in the sapwood water (Dawson and Ehleringer 1993).

A hand auger with a 12cm diameter bucket was used to collect three 150cm deep soil cores at each site for each collection date. Soil samples were collected from each core at the following depth increments: 0-10, 10-20, 20-30, 30-50, 100-120, and 130-150 cm.

From each soil depth, approximately 50g of soil was collected and stored in screw-cap, glass culture tubes, and sealed with parafilm. The soil samples were stored in a cool, dry ice chest until they could be stored at -18˚C.

Sample preparation and stable isotope analysis

Water was extracted from stem samples by cryogenic vacuum distillation as described by Vendramini and Sternberg (2007). Water was extracted from the soil samples using a specially designed distillation line where six samples could be distilled simultaneously for a total of 12 samples per day. Soil and stem water was analyzed in a Multiflow system connected to an Isoprime mass spectrometer (Elementar, Hanau, Germany). The following modification of Prosser and Scimgeour (1995) was used to analyze the hydrogen isotope ratio of water. Each water sample (0.5ml) and a cuvette containing ~1mg of platinum black powder (Sigma-Aldrich, St. Louis, Missouri, USA) were placed in 5.9ml vials (Exetainer vials; Labco, High Wycombe, UK) and sealed with screw caps that had a pierceable rubber septum (Exetainer cap; Labco). A 10% hydrogen/helium gas mixture was injected into the vials and equilibrated with water vapor for 24 h. The isotopic analysis of the equilibrated H_2 occurred as described by Vendramini and Sternberg (2007). After hydrogen isotope analysis, a 5% CO_2/helium gas mixture was flushed through the vials and was equilibrated with water for a period of 48 h. The equilibrated CO_2 gas was analyzed to derive the oxygen isotope ratios of the water as in Vendramini and Sternberg 2007. All isotope ratios were expressed in terms of per mil (‰):

$$\delta^{2}H \text{ or } \delta^{18}O = \left[\frac{R_{sample}}{R_{standard}} - 1\right] X 1000$$

where R_{sample} and $R_{standard}$ are the $^{2}H/^{1}H$ or $^{18}O/^{16}O$ ratios of the sample and the standard, respectively. The standard used was Vienna Standard Mean Ocean Water (vSMOW), and the precision of analysis for hydrogen and oxygen isotope ratios was ±3‰ and ±0.1‰, respectively.

Water source use analysis

In the defoliation experiment, paired t-tests of $\delta^{2}H$ and $\delta^{18}O$ values of stem water were performed between the control (non-defoliated) branches and defoliated branches for each collection time. The difference between control and defoliated branches was reported as isotopic separation (Δ), which is the control stem water δ value minus the defoliated stem water δ value.

In the water source use study, IsoSource (EPA v. 1.3.1) was used to calculate the most likely proportion of water used from each soil depth (Phillips and Gregg 2003). Isotopic composition of oxygen was used to calculate the depth of water uptake because oxygen provided a more accurate and reliable tracer of water source use. Plants do not fractionate oxygen isotopes like some plants have been found to fractionate hydrogen isotopes (Lin and Sternberg 1993, Ellsworth and Williams 2007). The proportional water uptake at each depth was calculated for each individual plant collected in this study, except when deciduous study plants were leafless, for a total of 600 measurements of water source use. Soil water source use was categorized into three depth classes (0-20, 20-50, 50-150cm). The nine collections were divided into three groups of 3 collections. The first group is the early dry season (November and December 2008 and January 2009); the second group is the late dry season (May 2008 and March and May 2009); and

the third group is the wet season (June, August, and September 2008). Mean water source use for each species at each site was calculated for the early dry, late dry season, and wet seasons separately.

Expected water source use was calculated separately based on either root distribution or on water availability. Root distribution was calculated as the proportion of the total number of roots found in the top 150cm that are found in each soil layer: 0-20, 20-50, 50-150cm (Chapter 2). These proportions were calculated for the time periods and were considered the expected water source use based on root distribution (Table 3.1). Expected water source use based on water availability was calculated the proportion of plant-available water that was found in each soil depth (Table 3.1). Water availability was the difference between mean volumetric water content (VWC) of the soil when samples were collected and minimum VWC from which plants can extract water. Mean VWC was calculated for each of the three seasons. Minimum VWC was calculated from the VWC when the soil matric potential was -1.5 MP. Matric potential was calculated using the Soil-Plant-Air-Water (SPAW) model. A matric potential of -1.5 MPa was chosen as a threshold to determine when soil water was largely unavailable because, with the exception of extremely dry years, the midday water potential of woody plants at this site seldom drops below -1.5 MPa (Saha *et al.* 2008).

To measure matric potential using the SPAW model, soil moisture, percent organic matter, salinity, and soil texture were used as parameters (Saxton and Rawls 2006). Percent organic content was the difference between soil weight after drying for 24 hours at 90°C and soil weight after ashing at 600°C for four hours. The percent sand was used in the calculation was 96%. I assumed no salinity. Percent organic matter content for

the Sandhill Long Unburned 1, Sandhill Long Unburned 2, and Sandhill Recently Burned sites was 2.0, 0.85, and 0.6%, 1.55, 0.85, and 0.6%, and 1.3, 0.85, and 0.6% for depths 0-20, 20-50, and 50-150cm, respectively.

Statistical analysis

Chi-squared tests were performed to compare the observed pattern of water uptake of all species with the expected depth of water uptake based on either the distribution of available water in the soil profile or based on the root distribution (Chapter 2). Yate's correction for continuity was used for all X^2 tests (Zar 1999). Separate X^2 tests were performed for each species during the early dry, late dry season, and wet seasons. Chi-squared tests were compared using water source use of deciduous and evergreen species in each season and seasonal water source use of all species. Pairwise comparisons of water source use of all species among the three time periods were Bonferroni-corrected at $\alpha = 0.05$. Seasonal water source use was used to compare water source use based on either the distribution of available water or the root distribution with depth using X^2 tests.

Results

Defoliation experiment

Artificial defoliation of branches resulted in the isotopic enrichment of stem water. The isotopic separations ($\Delta^{18}O$ and $\Delta^{2}H$), 27 days after defoliation, were 3.6 ± 0.3 and 21 ± 4‰, respectively (Figure 3.3). Twenty-seven days after defoliating *Q. virginiana* branches, buds were swelling and some new small leaves were emerging from buds. New leaf growth after 34 days was still slight with new leaves barely emerging, and $\Delta^{18}O$ and $\Delta^{2}H$ were slightly lower than the previous week, which was 2.9 ± 0.3 and 16 ± 3‰,

respectively (Figure 3.3). On day 57, the new leaves were about two thirds of the mature leaf size, and the mean $\Delta^{18}O$ and $\Delta^{2}H$ were greater than zero, though only mean $\Delta^{18}O$ was significantly different from zero. The new leaves on the defoliated branches were approximately full size at the last two collections at 68 and 92 days, and $\Delta^{18}O$ and $\Delta^{2}H$ were not significantly different from zero (Figure 3.3). When the $\delta^{2}H$ values were regressed on the $\delta^{18}O$ values of the stem water from defoliated branches that differed significantly in $\delta^{18}O$ and $\delta^{2}H$ from those of the control branches, the slope was 5.6, which is less than the slope of 8 of meteoric waters and typical of evaporative enrichment.

Leaf phenology and stem water isotopic composition

Isotopic variation in water through time was relatively similar among species at each site, except that the deciduous species (*C. floridana* and *Q. laevis*) had higher $\delta^{18}O$ and $\delta^{2}H$ values during leafless periods of the year (December – March; Figure 3.4). *Carya floridana* dropped its leaves in late November and *Q. laevis* dropped its leaves in mid-December. Although the timing of leaf drop did not differ much between sites, the timing of deciduous leaf flush did differ among sites because of the location of individuals plants with respect to topography. Low areas were subject to lower temperatures from cold air drainage than the crest of a hill. Sandhill Long Unburned 1 and Sandhill Recently Burned, located at a high point in the Sandhill, grew new leaves in late January through early February for *Q. laevis* and late February through mid-March for *C. floridana*. Located at the base of the hill, Sandhill Long Unburned 2 did not begin to leaf out until early March for *Q. laevis* and late March to early April for *C. floridana*. Leaf lifespan depended on the species and location of the individual plants with respect to topography, and was 9.5-10.5 and 7.5-8.5 months for *Q. laevis* and *C. floridana*, respectively.

Water source use

In light of the results from our defoliation experiment, stem water δ^2H and $\delta^{18}O$ values of leafless deciduous woody plants were not included in the analysis of water source use. Water source use differed between seasons. During the early dry season water source use of all species except *L. ferruginea* was not significantly different than the expected water source use based on the distribution of available water in the soil profile, but it was different than the expected water source use based on early dry season root distribution (Table 3.2; Figure 3.5). When early dry season water source use was grouped by leaf phenology, deciduous and evergreen species did not differ in water source use (Table 3.3). For further analysis of water source use I merged deciduous and evergreen species together because they were not significantly different in water source use. During the late dry season and the wet season, water source use began to shift toward a water source pattern the same as the expected water source use based on root distribution and was significantly different than the expected water source use based on the distribution of available water (Table 3.2; Figure 3.5). As in the early dry season, water source use during the late dry season and wet season was not different between deciduous and evergreen species (Table 3.3). The late dry season and the wet season had the same pattern of water source use, which was the same as expected if water source use was based on root distribution (Table 3.3 and 3.4).

Discussion

Effect of leaflessness on stem water δ^2H and $\delta^{18}O$

The defoliation experiment revealed that evaporation as the result of leaflessness enriched stem water isotopically. Although stems were suberized and evaporation from

the stem surface is minimal (Schönherr 1982), evaporation-caused isotopic enrichment of a fixed pool of stem water over one month was enough to enrich stem water. The presence of newly formed leaves did not increase transpiration sufficiently to flush the isotopically enriched water from the sapwood. Only once the leaves were nearly maximum size, Δ^2H and $\Delta^{18}O$ decreased and became indistinguishable from zero.

Consequently this experiment explained why δ^2H and $\delta^{18}O$ values of leafless deciduous woody plants were much higher than those of evergreen shrubs at the same collection period. Except when the deciduous plants were leafless, stem water of the two deciduous and three evergreen species had similar isotopic compositions of oxygen and hydrogen. The early dry season difference in δ^2H and $\delta^{18}O$ between deciduous and evergreen species does not likely represent water uptake from an enriched soil water source because leafless deciduous stem water δ^2H and $\delta^{18}O$ values were often higher than the highest soil water δ^2H and $\delta^{18}O$ values, which were those of the soil to 10cm depth. Consequently, I did not use the stem water δ^2H and $\delta^{18}O$ values of leafless deciduous plants in the determination of water source use. Using stem water of leafless plants or plants with newly forming leaves to trace water source use is not appropriate because the stem water is enriched by evaporation and does not reflect the isotopic composition of the water source.

Water source use

Leaf phenology did not appear to influence water source use in any of the three time periods (Table 3.3). Water source use shifted between the early dry and the late dry season from predominately deep water to shallower water from the top 50cm of the soil profile (Table 3.5). Deciduous species did not lose their leaves in the early dry season or,

in other words, avoid the drought conditions because they did not have access to or take up deep soil water. The evergreen species would be considered ‘drought delayers’ by Markesteijn et al. (2009) because they delay the effects of the drought by accessing deep water as shallow water is depleted. Unlike the deciduous species, the three evergreen species have sclerophyllous leaves that extend through the dry season. The water use strategy of deciduous species was more difficult to define. On the one hand, they could be considered ‘drought avoiders’ because they lose their leaves for the first part of the dry season, but on the other hand they leafed out during the latter part of the dry season. Therefore, ‘drought delayer’ should be applied to these deciduous species as well because they accessed the same water sources proportionally as evergreen species during the late dry season (Reich and Borchert 1984, Markesteijn and Poorter 2009).

During the late dry season, deciduous and evergreen species took up water proportionally from the same depths as in the wet season, but climatically the conditions were similar to the early dry season. Deciduous species leaf-out took place during this period when the drought can be most severe and when they experience very low midday water potentials during the driest conditions (Borchert et al. 2002, Saha et al. 2008). Deciduous plants, in general, may be responding to various cues when they leaf out in the dry season, such as lower herbivory, increased temperature, and improved plant water status (Elliott et al. 2006, Murali and Sukumar 1993, Borchert et al. 2005, Williams-Linera 1997, Guswa 2010). This increase in shallow soil water use could be because the root density in the late dry season relative to the early dry season increased by 54% (Ellsworth unpublished data). Yu et al. (2007) found that increasing root density was

effective in increasing water uptake during dry conditions. In this study, an increase in root density may coincide with leaf-out in deciduous species or the beginning of the rains.

Early dry season water source use was dependent on the edaphic and climatic factors affecting soil moisture. All species, except *L. ferruginea*, used the three soil depths in proportion to the quantity of available water, meaning that most water uptake came from the deep soil (67%) and only 13% from the top 20 cm (Table 3.2, Figure 3.5). *L. ferruginea* took up more water from the deep soil than it did in the late dry season or wet season, but was intermediate in water source use between the two expected water source use scenarios based on water availability and that based on root distribution. *L. ferruginea* only had 22% of its roots in the deep soil (50-150cm) and took up 54% of its water from this layer (Chapter 2). Lower water uptake from 50-150cm in the soil profile may be because *L. ferruginea* has more limited access to the deep soil than the other species, but it is more likely that shallow soil fine roots are more active than the other species. *Lyonia ferruginea* had not only higher shallow soil water uptake but higher nitrogen uptake rate than the other species during the late dry season (Chapter 4). Considering low dry season rainfall and low water storage capacity of sandy soil, water availability in the shallow soil drops quickly. For example, in October 2009 after only one rain event of 11 mm in 13 days, the matric potential dropped below -1.5MPa. Matric potential of -1.5MPa can be used as a threshold for water uptake because predawn water potentials seldom drop below -1.5 MPa (Saha et al. 2008). In as short of a period of time as two weeks during the wet season, the top 20cm of soil became unavailable for water

uptake. Rapid soil drying in the shallow soil and low storage capacity make the deep soil the largest pool of available water. Considering that all species had access to this layer, deep soil was the principal source of water in all species.

The most important soil layer of early dry season water uptake was the deep soil (50-150cm). This compares well with other studies done in the sandhill or Florida scrub plant communities where the dry season water source use was measured (Hungate et al. 2002, Saha et al. 2008, Donovan et al. 2000). The deep soil was responsible for 54-79% of water uptake by all species, yet this layer had only 33% of the total roots in the top 150cm and a root density 6.25 times less than the root density at 0-20cm (Chapter 2). As has been shown here during the dry season and in other studies, the proportion of roots found in each soil layer does not indicate the proportional water uptake from each soil layer (Jackson et al. 1999a). For example, winter wheat had only 3% of its roots below 1m, but those roots were responsible for 20% of water uptake (Gregory et al. 1978).

During the late dry season and in the wet season, water source use was the same as was expected if water source use matched root distribution profile (Table 3.2; Figure 3.5). During the wet season, theoretically frequent rain would be more important to maintain high water availability than water storage capacity because water holding capacity of sand is lower than the water demands of transpiration and evaporation (Guswa 2010). As water was plentiful in the soil, theoretical water uptake would be at maximum rate and therefore would be limited by root distribution (Yadav et al. 2009). Not surprisingly the top 20cm contained 42.7% of roots and 43% of water uptake was from that same depth. The top 20cm has the most phosphorus of anywhere in the soil profile and has high nitrogen content relative to the rest of the soil profile (Chapter 4).

This 1:1 ratio between proportion of water uptake from a soil layer and proportion of roots in that soil layer was also found in another study of *Malus domestica* where 70% of the roots and water uptake were in the top 40cm (Green and Clothier 1999). Therefore, in this study root proliferation in the shallow soil has the essential dual function of acquiring nutrients and water, but water demands could not be met in the top 20cm or the top 50 cm alone. Based on both patterns of water source use and the strong presence of roots in the deep soil, deep water was necessary to maintain transpiration water demands.

Access to the deep soil is more than just a strategy to survive through the dry season. Proportionally the most important water source was the deep soil (50-150cm; Table 3.5). Deep water access is necessary in the wet season (29%) as well because the shallow soil (0-50cm) does appear to be adequate to meet transpiration demand. Deep roots contribute to drought tolerance by increasing the total soil volume that plants can exploit (Garwood and Sinclair 1979, Markesteijn and Poorter 2009, Slot and Poorter 2007, Poorter and Markesteijn 2008, Paz 2003). In this sandy soil, having wet season rainless periods, the ability to exploit more than the superficial soil is necessary. For example, rainless periods of the 2009 wet season reduced the calculated matric potential of the top 10 and 20cm to below -1.5MPa for 34 and 17% of the time, respectively (Figure 3.6). In the relatively wetter 2008 wet season it is likely that the shallow soil was wetter than in it was in the 2009 wet season. Nevertheless, large portions of the wet season (end of May, beginning of June, and most of September) also received little rain (Figure 3.2). In another study in a ponderosa pine-grass community, uptake from deep soil layers was only important during the dry season and represented a small proportion of annual water uptake because dry season water uptake was a small proportion to total

yearly water uptake (Eggemeyer et al. 2009). In this study deep water uptake was proportionally high in the dry season and approximately one third of water uptake during the wet season, which makes deep water an important water source throughout the year.

Conclusions

The defoliation experiment showed that when defoliated stem water is enriched isotopically, which explained higher δ^2H and $\delta^{18}O$ values of leafless deciduous species than those of evergreen species at the same collection period. The most notable difference in water source use between deciduous and evergreen species was that deciduous plants delayed the dry conditions in the early dry season by losing their leaves and remaining dormant. Leaf phenology did not influence water source use, except when deciduous plants were leafless. In the early dry season, edaphic and climatic factors resulting in low soil water availability, especially in the shallow soil, were the principal drivers determining water source use, resulting in the deep soil being the predominant dry season water source. During the late dry season and the wet season, the principal driver in determining water source use shifted from the distribution of available water to root distributions with depth. In the wet season when more water was available, water uptake was likely maximized and resulted in the pattern of water source use similar to the distribution of roots. Deep water use was an important water source throughout the year – not just in the early dry season when shallow soil (0-50cm) had low water availability. These deciduous and evergreen species are drought delayers rather than drought avoiders or drought tolerant species because the dry conditions were not avoided but delayed as much as possible by accessible deep water. Deciduous woody plants delayed the effects

of the drought by losing their leaves until the late dry season when they accessed deep water to survive. Evergreen shrubs maintained active leaves and delayed the effects of the drought during the dry season by accessing deep water.

Table 3.1 Expected proportional water source use based on the proportion of available water in the soil profile that is found in each soil layer or based root distribution with depth for each site: Sandhill Long Unburned 1 (SHLU 1), Sandhill Long Unburned 2 (SHLU 2), and Sandhill Recently Burned (SHRB).

		Soil depth (cm)		
Site	Season	0-20	20-50	50-150
Expected water source use based on water availability				
SHLU 1	Early dry	0.08	0.14	0.78
	Late dry	0.07	0.3	0.63
	Wet	0.14	0.14	0.72
SHLU 2	Early dry	0.11	0.22	0.67
	Late dry	0.14	0.34	0.52
	Wet	0.15	0.17	0.69
SHRB	Early dry	0.04	0.08	0.88
	Late dry	0.10	0.21	0.69
	Wet	0.10	0.21	0.69
Expected water source use based on root distribution				
	Early dry	0.41	0.26	0.33
	Late dry	0.42	0.30	0.28
	Wet	0.43	0.30	0.27

Table 3.2 Chi-squared (X^2) tests comparing water source use of each species to expected proportional water source use based on the proportion of available water in the soil profile that is found in each soil layer or based root distribution with depth. X^2 tests were performed separately on each species in each season.

Season	Species	df	*N*	X^2	*P*
Expected water use based on distribution of available water in the soil profile					
Early dry	*L. ferruginea*	2	45	15.21	**< 0.001**
	Q. geminata	2	44	0.22	0.82
	Q. myrtifolia	2	43	3.98	0.08
	Q. laevis	2	20	0.34	0.99
	C. floridana	2	15	0.29	0.77
Late dry	*L. ferruginea*	2	42	79.20	**< 0.001**
	Q. geminata	2	43	23.51	**< 0.001**
	Q. myrtifolia	2	44	31.98	**< 0.001**
	Q. laevis	2	43	33.16	**< 0.001**
	C. floridana	2	40	32.39	**< 0.001**
Wet	*L. ferruginea*	2	45	74.80	**< 0.001**
	Q. geminata	2	45	44.63	**< 0.001**
	Q. myrtifolia	2	44	51.67	**< 0.001**
	Q. laevis	2	43	41.22	**< 0.001**
	C. floridana	2	44	51.18	**< 0.001**
Expected water use based on root distribution with depth					
Early dry	*L. ferruginea*	2	45	10.80	0.004
	Q. geminata	2	44	34.65	**< 0.001**
	Q. myrtifolia	2	43	20.50	**< 0.001**
	Q. laevis	2	20	23.04	**< 0.001**
	C. floridana	2	15	24.57	**< 0.001**
Late dry	*L. ferruginea*	2	42	3.67	0.17
	Q. geminata	2	43	1.97	0.36
	Q. myrtifolia	2	44	0.75	0.69
	Q. laevis	2	43	0.89	0.64
	C. floridana	2	40	0.50	0.78
Wet	*L. ferruginea*	2	45	1.35	0.54
	Q. geminata	2	45	0.20	0.92
	Q. myrtifolia	2	44	0.33	0.97
	Q. laevis	2	43	0.82	0.67
	C. floridana	2	44	1.35	0.53

Table 3.3 Chi-squared (X^2) tests comparing evergreen and deciduous species and seasons. Evergreen and deciduous species were compared within each season. Pairwise comparisons were made between time periods and Bonferroni-corrected at $P < 0.05$.

Season	Comparison	df	*N*	X^2	*P*
Early dry	Evergreen vs. Deciduous	2	212	2.11	0.43
Late dry	Evergreen vs. Deciduous	2	212	0.268	0.89
Wet	Evergreen vs. Deciduous	2	221	1.352	0.52
	Early dry vs. wet season	2	387	57.62	**< 0.0001**
	Early dry vs. late dry season	2	378	61.21	**< 0.0001**
	Late dry vs. wet season	2	433	2.766	0.25

Table 3.4 Chi-squared (X^2) tests comparing water source use of all individuals of all species during each season to expected proportional water source use based on the proportion of available water in the soil profile that is found in each soil layer or based root distribution with depth. X^2 tests were performed separately on each season.

Season	df	*N*	X^2	*P*
Expected water use based on distribution of available water in the soil profile				
Early dry	2	167	2.09	0.31
Late dry	2	212	256.5	**< 0.001**
Wet	2	221	310.5	**< 0.001**
Expected water use based on root distribution with depth				
Early dry	2	167	114.4	**< 0.001**
Late dry	2	212	3.64	0.16
Wet	2	221	0.23	0.9

Table 3.5 Mean water source use ± SE for each depth. The means are the cumulative water source use of all individuals in each season.

	Proportion of water in each soil depth		
Season	0-20cm	20-50cm	50-150cm
Early dry	0.13 ± 0.01	0.21 ± 0.01	0.67 ± 0.02
Late dry	0.38 ± 0.02	0.34 ± 0.01	0.27 ± 0.02
Wet	0.43 ± 0.02	0.27 ± 0.01	0.30 ± 0.02
Annual mean	0.33 ± 0.01	0.28 ± 0.01	0.39 ± 0.01

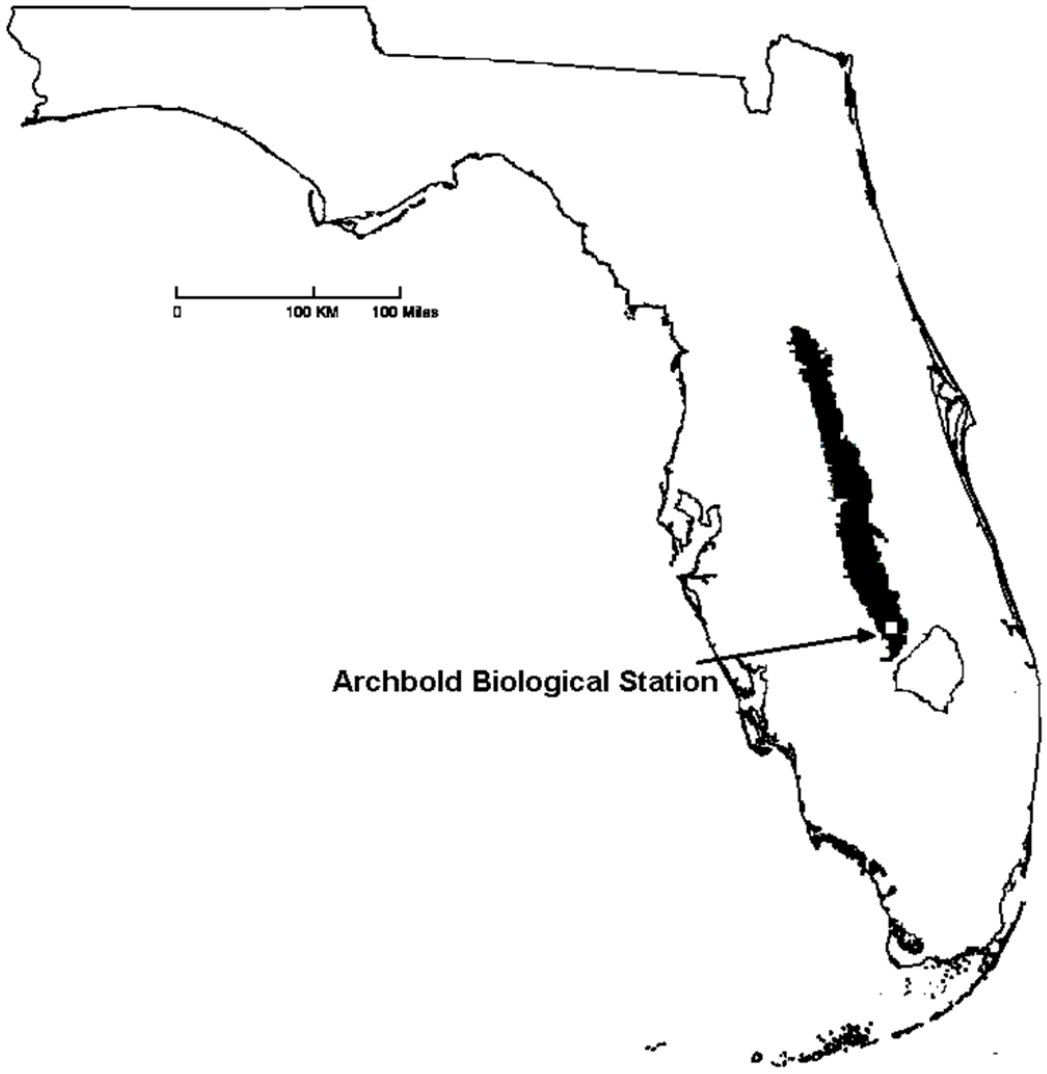


Figure 3.1 Map of Florida showing the geographical range of the Lake Wales Ridge (region highlighted in black) and the location of Archbold Biological Station. The boundary of the Lake Wales Ridge is based on Weekley et al. (2008).

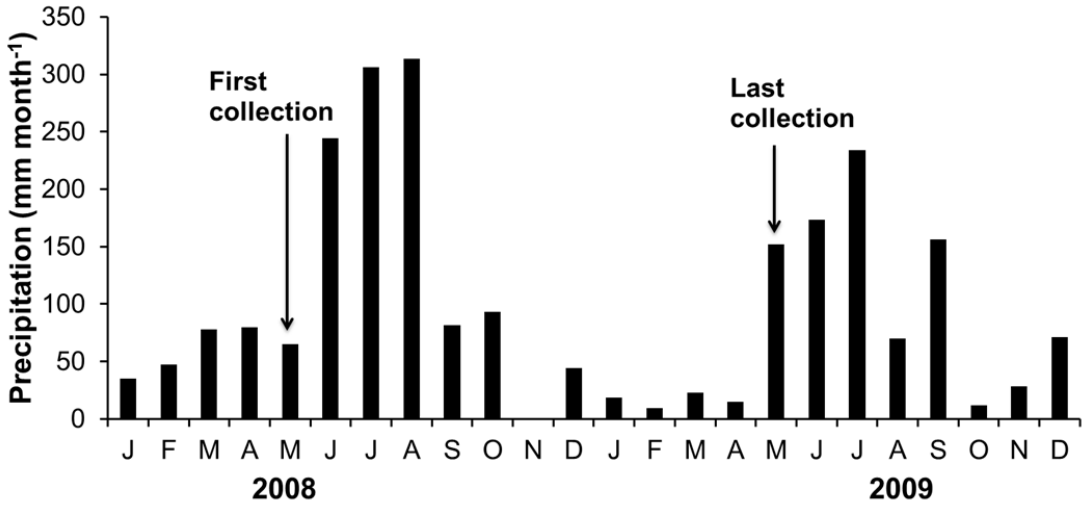


Figure 3.2 Precipitation (mm month^{-1}) at Archbold research station from 1 January 2008 to 31 December 2009 (ABS weather records).

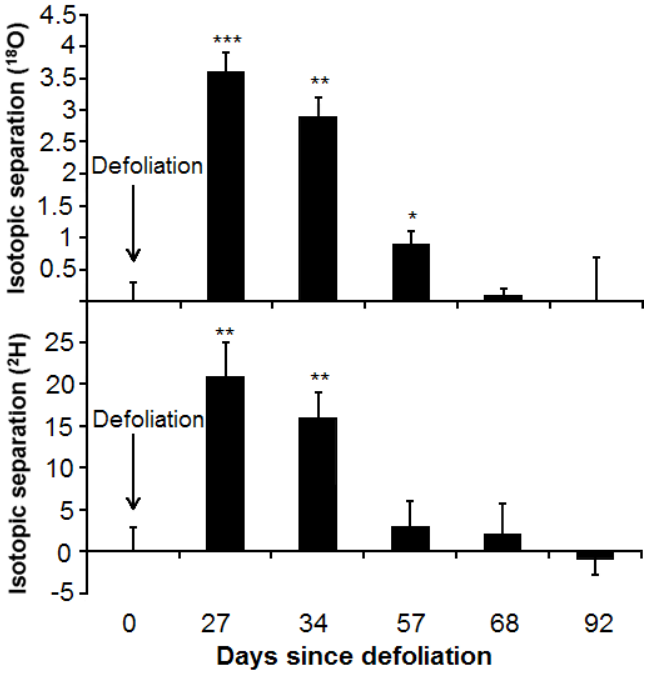


Figure 3.3 The effect of artificial defoliation of *Q. virginiana* branches on isotopic separation. Branches were defoliated on day 0. Isotopic separation is the mean of the δ values of water from defoliated stem minus those of the control stems from the same tree. Error bars are standard errors of the means. Paired t-tests were conducted to calculate if the δ values of stem water from control branches were significantly different from those of defoliated branches on the same trees. One, two or three asterisks show significance level at $P < 0.05$, 0.01, 0.001, respectively. New leaves were emerging by day 34 on defoliated branches and leaves were about two thirds of full size by day 57. By days 68 and 92 leaves were approximately maximum size.

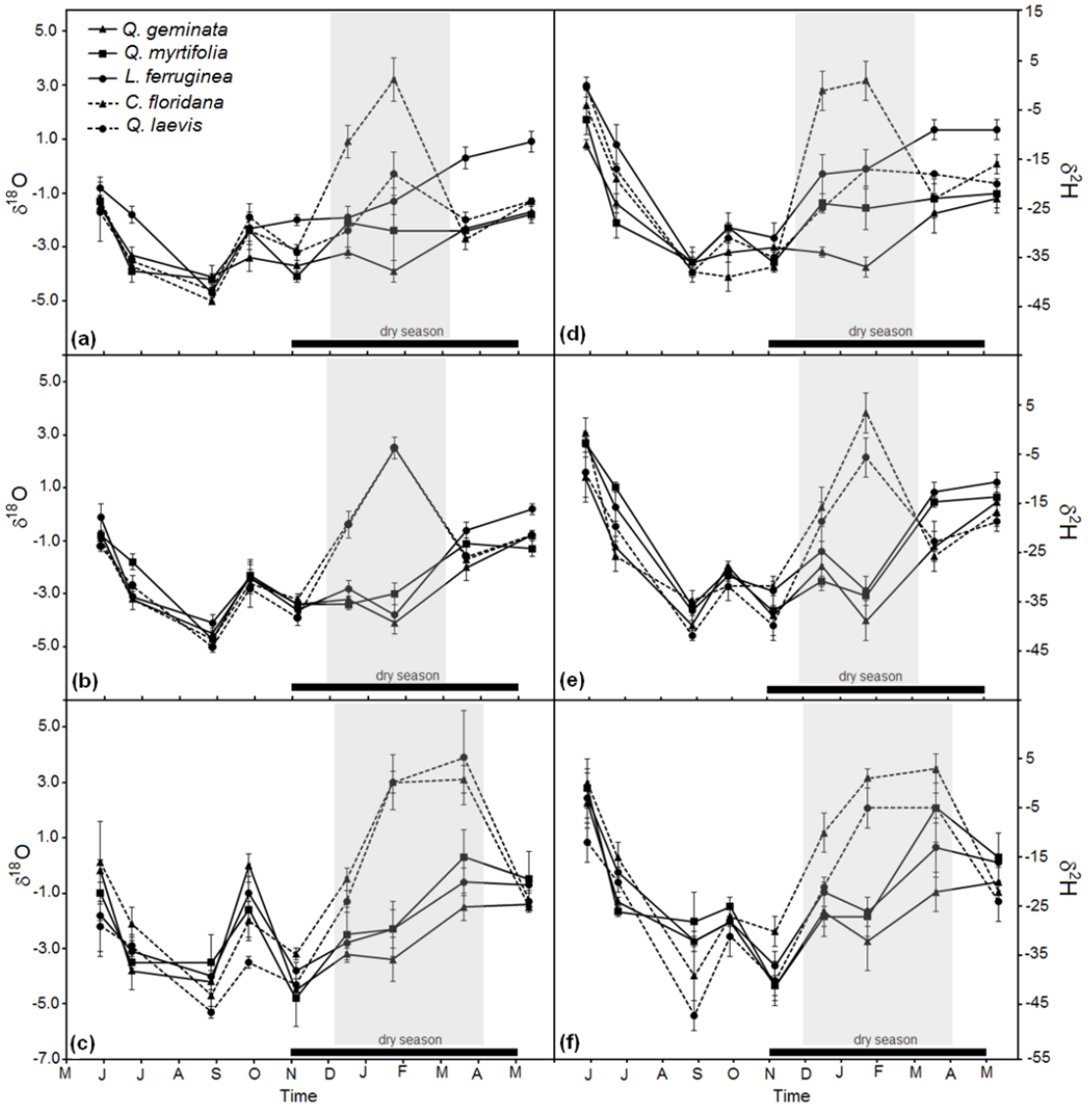
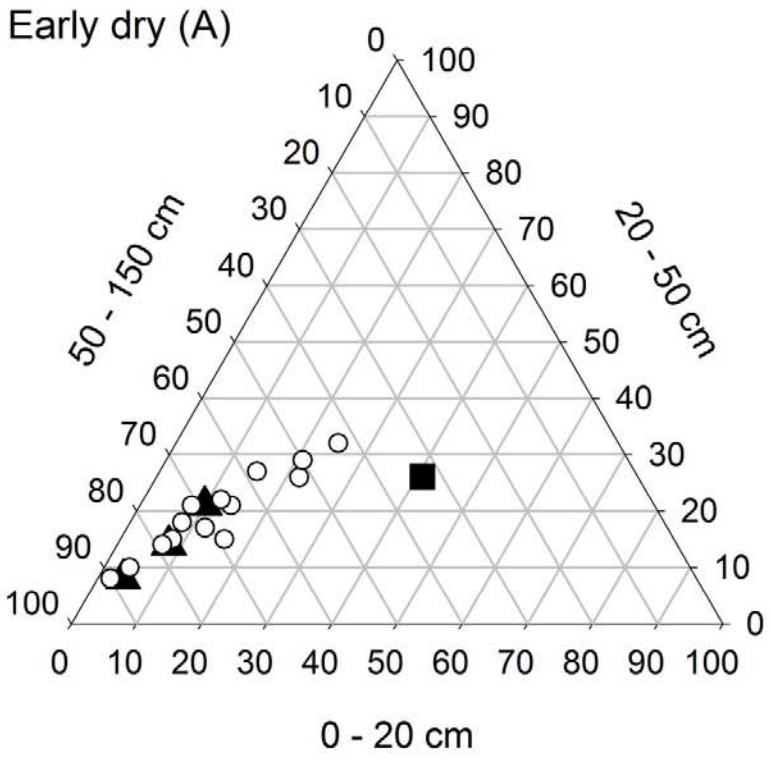
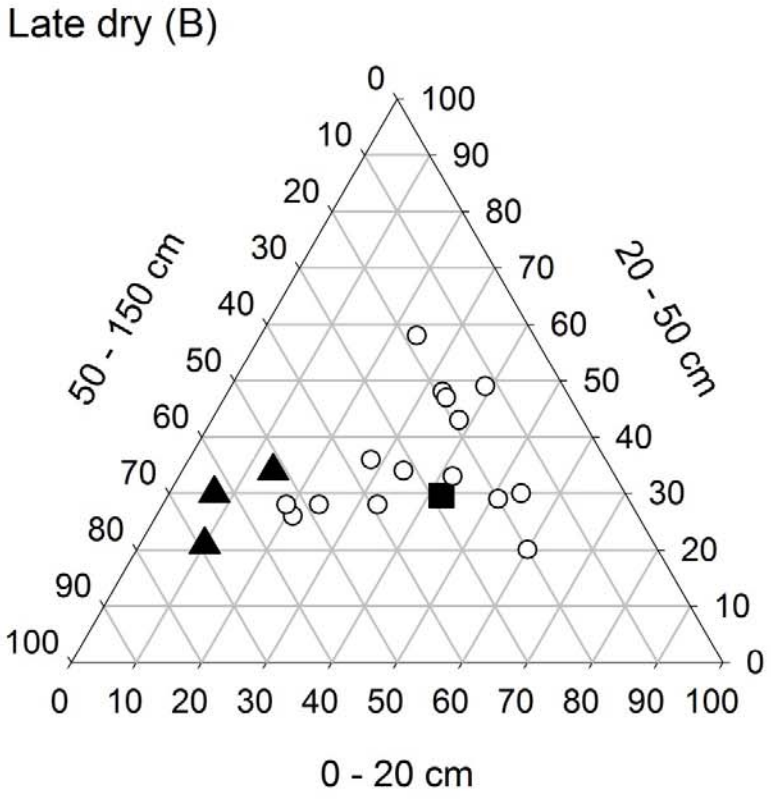


Figure 3.4 Mean $\delta^{18}O$ of stem water (a, b, c) and mean $\delta^{2}H$ of stem water (d, e, f) from May 2008 through May 2009. The three sites are (a, d) Sandhill Recently Burned, (b, e) Sandhill Long Unburned 1, and (c, f) Sandhill Long Unburned 2. Error bars represent the standard error of the mean. Dotted lines represent deciduous species and solid lines represent evergreen species. Solid bar above the x-axis represents the duration of the dry season. The shaded region of each graph represents the period of time when deciduous woody plants were leafless. Note: Leafless period is less than the entire dry season.


Early dry (A)
0
10
20
30
40
50
60
70
80
90
100
50 - 150 cm
20 - 50 cm
0 - 20 cm


Late dry (B)
0
10
20
30
40
50
60
70
80
90
100
50 - 150 cm
20 - 50 cm
0 - 20 cm

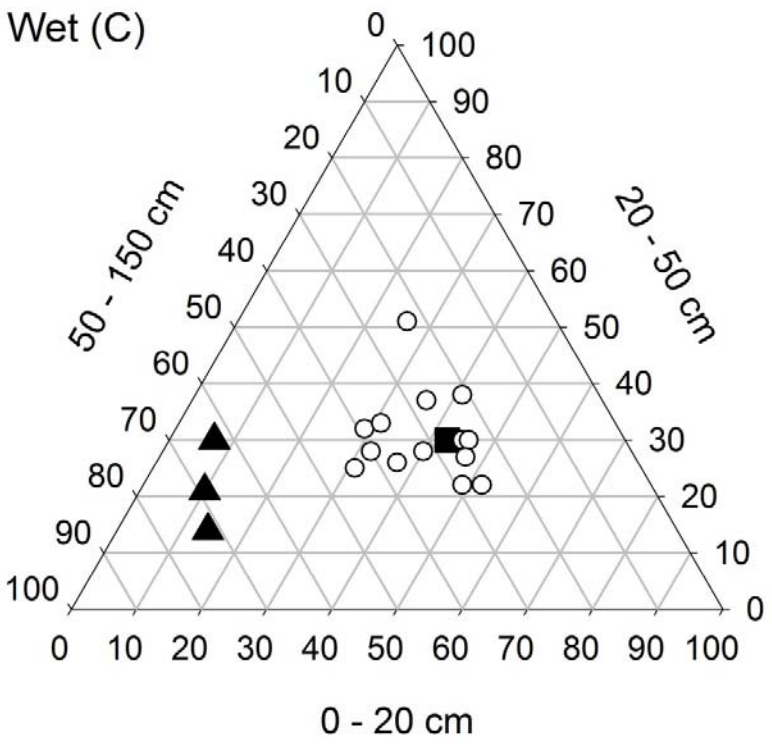


Figure 3.5 Water source use in the early dry (A), late dry (B), and the wet season (C). The axes represent the proportion of water uptake from each of the three soil depths. Large black square represents expected water source use based on proportion of fine roots located in each depth. Large black triangles represent expected water source use based on proportion of available soil water in each depth. Small open circles represent water source use of each species at each site. Water source use of the five study species was measured three different times during each of the three seasons for a total of 15 points for each season.

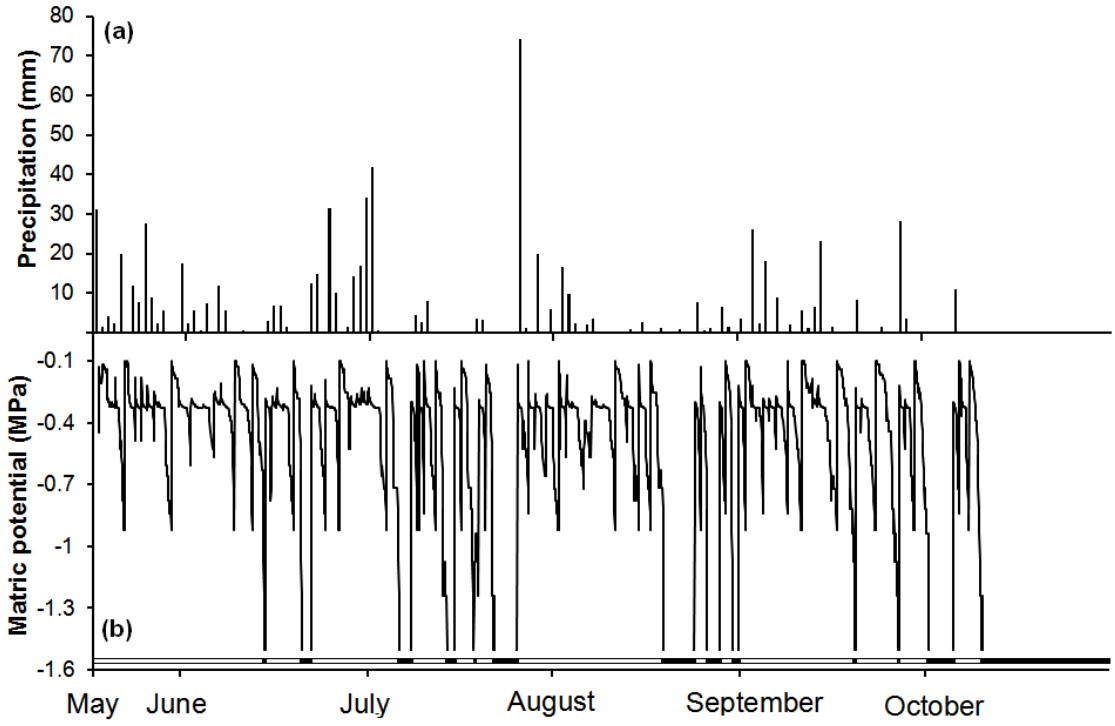


Figure 3.6 Precipitation from the 2009 wet season and matric potential at the soil depth of 10cm. (a) Graph of precipitation shows from 19 May 2009 when the wet season began through the end of October when rains of the wet season were ending. (b) Using a soil water characteristic model, matric potential was calculated from volumetric water content, organic matter content, and soil texture at a soil depth of 10cm. For clarity, all values of matric potential below the threshold of -1.5 MPa were not included in the graph. In graph B, the shaded parts of the line above the x-axis represent the periods of time that the matric potential was below -1.5 MPa, and the non-shaded parts represent times when the matric potential was above -1.5 MPa.

Chapter 4

Evergreen species have higher nitrogen uptake rates than deciduous species in a dry seasonal scrub

Summary

Dry seasonal ecosystems are defined by a pronounced and consistent dry season that results in both water and nutrient limitation. Deciduous and evergreen species have evolved different leaf phenologies, which change the temporal pattern of nutrient demands for leaf growth. In these ecosystems, coupling of nutrient uptake and deciduous leaf growth requires the presence of active roots in soil where nutrients are available during the time of leaf-out. Using ion-exchange resins to measure nutrient availability, I found that the top 10cm had the highest concentration of plant-available P in the soil profile, but nitrogen did not differ with soil depth. Concentrating root growth in the top 10cm would maximize P uptake, and N uptake from the shallow soil would be as high as anywhere in the soil profile. To measure relative N uptake for deciduous and evergreen species during the period of deciduous leaf-out, I labeled the shallow soil with ^{15}N and measured foliar $\delta^{15}N$. ^{15}N uptake rates were lowest among deciduous species. Low N uptake during leaf-out means that deciduous species may not be able to take up sufficient N to meet the demands of leaf growth and they may need to take up N throughout the wet season to compensate for N translocated to the leaves. This temporal uncoupling of nutrient demand and uptake may be why deciduous species are most common in most fertile sites in the upland Florida ecosystems.

Background

Dry seasonal ecosystems are defined by a pronounced and consistent dry season that results in both water and nutrient limitation (Sobrado 1986, Sobrado and Cuenca 1979). The soils under dry seasonal plant communities, such as the Florida scrub, are oligotrophic with the majority of nutrients located in the shallow layers (Brown et al. 1990, Myers 1990). Because of the predominant concentration of nutrients in the shallow soil layers and the temporal drying of these layers, nutrient availability is dependent on the climatic conditions that govern water availability in the shallow soil. Plant-available water and nutrients must coincide spatially and temporally for plants to be able to take up nutrients. During the dry season, the shallow soil dries and nutrient uptake is limited (Weekley et al. 2007). In the late dry/early wet season, nutrients immobilized in dry organic matter located in the shallow soil layers are mineralized by rapid microbial decomposition (Luizao et al. 1992). This nutrient pulse is characterized as a brief period during the initial weeks of the wet season (Lodge et al. 1994, Raghubanshi et al. 1990, Singh et al. 1989). Therefore, maximal nutrient uptake would occur during these nutrient pulses when both water and nutrients are most available.

The Florida sandhill plant community at Archbold Biological Station (Abrahamson et al. 1984) in south-central Florida provided an ideal place for studying the difference in nutrient uptake between deciduous and evergreen species in a nutrient-poor, seasonally dry plant community. Winter, from November through April, is a pronounced, consistent dry season similar to that of other subtropical dry seasonal ecosystems. The dry season is followed by a hot, high precipitation wet season. The soil is characterized by very low nutrient concentrations and is subject to drying rapidly during the dry season

because of its very high sand fraction (Weekley et al. 2007, Brown et al. 1990). The soil has low clay and organic matter content, so the source of most nitrogen and phosphorus is the organic matter accumulated on the soil surface (Huck 1987).

Many studies have shown that evergreen species dominate in infertile areas because greater leaf lifespan and low nutrient turnover are favored (Pornon et al. 2011, Beadle 1968, Monk 1966, Chapin 1980). Nonetheless, evergreen and deciduous perennials also co-occur while using different leaf habit strategies, which may affect nutrient and water uptake. Deciduous species leaf out quickly and rely principally on stored nutrients for leaf growth (Chapin 1983). Growing leaves with stored nitrogen require having adequate stored N and replenishing stores later in the growing season. On the other hand, evergreen species grow new leaves gradually, and nutrient uptake can be gradual, allowing for a close coupling between nutrient uptake and leaf growth (Dyckmans and Flessa 2002).

Coupling of nutrient uptake and leaf growth also depends on nutrient availability during the time period when leaf out occurs. Nutrient availability, in turn, depends on water availability in the soil layer where nutrients are found. Total extractable N and P concentrations are greatest in the shallow soil (Abrahamson and Hartnett 1990). Considering that the sandy soil found in this community has very little clay and organic matter to bind nutrients, precipitation may quickly disperse available nutrients as they percolate through the soil profile (Holtan et al. 1988). During the late dry season when deciduous species leaf out, water availability in the shallow soil is low and subsequently

nutrient availability is low, except during episodic rain events. Therefore, nutrient uptake during the dry period would be concentrated after episodic rain events before the wet season begins.

An important tool to measure nutrient availability in areas where there is a strong interplay between water and nutrient availability is the use of ion-exchange resins. Anion- and cation-exchange resins are useful in measuring nutrient availability because they bind only to ions in the soil solution (Binkley and Vitousek 1989, Binkley 1984). By effectively separating total nutrient content from plant-available nutrient content, ion-exchange resins can measure plant-available nutrients over a period of time. Resins have been successfully used in wet and dry soils (Pletsch et al. 2009, Susfalk and Johnson 2002, Giblin et al. 1994, Lajtha 1988, Gibson et al. 1985). In dry soils, resins are particularly useful because the total nutrient content of the soil is vastly different from what is available to plants, and resins function by absorbing only to nutrients that become available to plants during brief or infrequent precipitation events (Lajtha 1988). Ion-exchange resins have been used in the oak scrub (Johnson et al. 2001b), a vegetation type that often abuts sandhill on similar soils.

Another important tool to access nutrient uptake by plants in the soil is the use of isotopic labels. Several studies have measured N uptake using a labeled ^{15}N-nitrogen source (Fotelli et al. 2002, Millard and Proe 2006, Grassi et al. 2002, Gebauer and Ehleringer 2000). Adding small amounts of ^{15}N to a plant-available N source labels the N source without causing a fertilizer effect, so N uptake can be measured without being

influenced by the treatment. By labeling the soil N source, N uptake can be traced in the plant by measuring the increase in $\delta^{15}N$ of plant tissue. In this study, we assess nitrogen uptake by the allocation of the ^{15}N label to leaf growth.

Here I used the above tools to test the hypothesis that the shallow soil in this ecosystem would have more plant-available nitrogen and phosphorus than the mid to deep soil. I used resin bags placed at six depths in the top 150 cm of soil to measure N and P availability during the early dry season, late dry/early wet season) and the late wet season. I also used ^{15}N label to test whether nitrogen uptake would be higher in deciduous species compared to evergreen species because deciduous species require a greater nutrient pulse to grow new leaves. I labeled the shallow soil with ^{15}N during the time that deciduous species were leafing and measured foliar $\delta^{15}N$ throughout the year for both evergreen and deciduous species.

Methods and material

Sites of nutrient availability study, ^{15}N-labeling study, and study species

Three sites were selected in a sandhill plant community at Archbold Biological Station (ABS) in south-central Florida, USA to study nutrient availability (Figure 4.1): Sandhill Long Unburned 1 (N27˚ 09.083'; W81˚ 21.448'), Sandhill Long Unburned 2 (N27˚ 11.156'; W81˚ 20.084'), and Sandhill Recently Burned (N27˚ 11.280'; W81˚ 20.181'). One additional site was chosen to measure nutrient uptake: Red Hill site (N27˚ 11' 31.16" W81˚ 20' 7.73"). The sandhill plant community is located on the southern ridge of the Lake Wales Ridge, which comprises ancient beaches from the late Pliocene (Weekley et al. 2008). The soils are extremely well-drained acid quartzipsamments sands (Kalisz and Stone 1984), have very low nutrient and organic matter content (Huck 1987), and

experience periodic drought (Weekley et al. 2008, Menges and Gallo 1991). Recently burned and frequently burned locations have little organic matter on the soil surface. The woody species that dominate the sandhill plant community are the evergreen oaks (*Quercus myrtifolia* Willd., *Q. inopina Ashe*, *Q. geminata* Small*, Q. chapmanii* Sarg.), other evergreen species such as *Serenoa repens (Bartram) Small*, *Lyonia ferruginea* (Walter) Nutt*., Pinus elliottii* Engelm. var. *densa* Little & Dorman*, and P. clausa* (Chapm. ex Engelm.) Vasey ex Sarg*.,* and deciduous species such as *Carya floridana* Sarg*., Q. laevis* Walter, and *Asimina obovata* (Willd.) Nash (Menges 1999).

The climate of the site is characterized by hot, wet summers and mild, dry winters that extend from December through April. Mean annual rainfall at Archbold Biological Station is 1345mm, with approximately 61% falling between June and September (ABS weather records 1932-2011). The groundwater table at our sites varies between a yearly mean of approximately 2m at Sandhill Long Unburned 1 to 22m at Sandhill Recently Burned and Sandhill Long Unburned 2 (ABS groundwater records). Another sandhill site used in the ^{15}N-labeling study had a depth to groundwater of approximately 3m. The Sandhill Recently burned site was completely burned in 1995 and 1999 and partially burned in 1991 and 2002. As a result, the canopy is relatively open, and the soil surface has little organic matter accumulation. Sandhill Long Unburned 1, Sandhill Long Unburned 2, and the Redhill sites were last burned 16, 85, and 14 years ago, respectively. Plant height increased with time since the last fire, and the soil surface had more organic matter accumulation than the Sandhill recently burned site.

Two deciduous (*Q. laevis* and *C. floridana*) and three evergreen species (*Q. myrtifolia, Q. geminata,* and *Lyonia ferruginea*) were selected for study because of their

ubiquity in the sandhill community at the three study sites for nutrient availability and at the site where the nitrogen uptake experiment was conducted. Of the angiosperm species, these compose the majority of individual woody plants at the site (Menges 1999). *Carya floridana* and the *Quercus* species are ectomycorrhizal species, and *L. ferruginea* is an ericoid mycorrhizal species.

Nutrient availability

Orthophosphate, nitrate, and ammonium ion availability was measured using cation-exchange (Amberlite IR120) and anion-exchange resins (Amberlite IRA-400). Before placing the resin bags in the field, they were charged by placing them in a shaker in either 2M HCl for anion exchange resin bags or 2M NaCl for cation exchange resin bags for six hours. The volume of NaCl or HCl used was 50ml for each resin bag. Then the resin bags were rinsed three times with Nanopure water. The resin bags were kept moist in a Ziploc bag until they were placed in the field.

The resin bags were placed in all three sites: Sandhill Recently burned, Sandhill Long Unburned 1, and Sandhill Long Unburned 2. Resin bags were placed in the soil for three different seasonal periods: early dry season (December-February), late dry/early wet season (March-June), and late wet season (July-November). The late dry/early wet season period was chosen as a separate collection period from the dry and wet season because this was the period when deciduous species leafed out and evergreen species changed many of their leaves. Also during this time period the wet season precipitation begins, which may facilitate the release of a nutrient pulse from the organic layer on the soil surface as decomposition increases with the rains. Six resin bags of each cation exchange resin and anion exchange resin were placed at each site at each depth. Each

resin bag was filled with 5 g of either anion or cation exchange resin. The resin bags were placed at the depths 10, 20, 30, 50, 100, 150 cm in the soil. To place each set of resin bags, a narrow, long trowel was inserted into the wall of the pit and slightly lifted upward to make space to place an anion and a cation exchange resin bag. The resins bags at 10 cm were placed by inserting a trowel at a diagonal into the soil and again slightly lifting the soil up to insert the anion and cation exchange resin bags into the soil. In this way the resin bags were placed in the soil that minimally disturbed the soil above and below the bags. New pits were dug for each sample period.

All the resin bags were attached to a monofilament fishing line for easy removal from the soil depth. When the resin bags were removed from the soil, they were cleaned of soil by rinsing in distilled water and were placed in specimen containers marking the depth and location of the bags. The bags were stored on ice until I could extract the ions in the lab. To extract the ions from the resin bags, 50ml of 0.5M KCl was placed in each container with a resin bag. The containers were placed on a shaker for six hours. Afterward the resin bags were removed from each container, and the extracts were frozen until the time of analysis.

Analysis of nutrient availability

The quantity of orthophosphate extracted from the resin bags was determined by using the USEPA method 365.1 (USEPA 1984), which is based on the molybdate blue colorimetric approach (Fiske and Subbarow 1925). The extracts were analyzed using an Alpkem 3000 Phosphorus analyzer (Alpkem, OI Analytical, Texas, USA). The precision of analysis was ±0.1ppm.

The quantity of NO_2^-/NO_3^- and NH_4^+ extracted from the resin bags were determined using the steam distillation method and apparatus described in Bremmer and Keeney (1965). Because NO_2^-, an intermediate between NH_4^+ and NO_3^- in nitrification, is in very low quantities in well aerated soils, I only refer to the quantity of NO_2^-/NO_3^- produced as NO_3^-. The quantity of NO_3^- and NH_4^+ of each sample was calculated from the mean of 3 aliquots each measured for NO_3^- and NH_4^+. The following calculation was used to calculate the quantity of N (μg) in the form of NO_3^- or NH_4^+ that was in the extract.

$$\mu g\ N = \frac{(vol\ of\ tritrant\ for\ sample - vol\ of\ titrant\ for\ blank) N\ titrant * 14 \frac{mg}{meq} * \frac{1000 \mu g}{mg} * total\ vol\ of\ extract}{vol\ of\ extract\ used\ in\ titration},$$

(Eqn. 1)

All volumes are in milliliters. Normality was expressed as meq/ml.

^{15}N labeling study

The labeling study was divided into two subgroups. In the first subgroup ten individuals each of *Q. myrtifolia* (evergreen) and *Q. laevis* (deciduous) were chosen, in which five of each were selected as a control and the other five were selected as the '^{15}N-labeled' group. Each individual plant was selected, so that it was at least 10 m distant from any other plant used in the study. The first subgroup was labeled with ^{15}N-labeled KNO_3 on January 2009. The second subgroup consisted of *L. ferruginea* (evergreen), *Q. geminata* (evergreen), and *C. floridana* (deciduous) and labeling took place early April 2009. At this time *C. floridana* was starting to leaf out. Ten grams of 10 atomic percent ^{15}N-

labeled KNO_3 (1.38g N) was thoroughly mixed with 6L of sand. This quantity had no fertilizer effect in another study (Gebauer and Ehleringer 2000). Leaves and litter were removed from a circle with a radius of 1m around the base of each plant. I spread the sand mixed with KNO_3 uniformly over this area, and then replaced the leaves and litter over the area. No fertilizer effect was expected, but nonetheless to control for any possible fertilizer effect, the same procedure was repeated for the control group, except that 10g of non-labeled KNO_3 (1.38g N) was mixed with 6L of sand. Care was taken not to get any of the sand mixture on the leaves of the study plants.

Leaves from test and control plants were collected eight times over 130 days. Three to five leaves were collected from each plant at each collection period. Also the leaf condition (leafing out, young, mature, senescing, fully senesced) was recorded for each leaf collected. Once the leaves of the deciduous species *Q. laevis* and *C. floridana* had senesced and fallen, all the leaves were removed from around the base of each study shrub and replaced with leaves from non-labeled dead leaves. The leaves were placed at 60˚C for 72h and then ground to a fine powder.

Measuring distance of ^{15}N uptake

As part of the previously described study where the soil surface was labeled with ^{15}N around the base of each study plant, I measured the $\delta^{15}N$ of leaves from the five study species that surrounded the labeled area to determine the maximum distance that individual woody plants were taking up ^{15}N-labeled NO_3^-. The distance from the base of the main stem to the main stem of the ^{15}N-labeled woody plant along with the its species were recorded.

Foliar % C, % N, $\delta^{14}N$, $\delta^{13}C$ analysis

Ground leaf samples (5 mg) were loaded in tin cups (Elemental Micro-analysis, Milan, Italy), rolled into balls, and analyzed for % carbon and % nitrogen with an automated elemental analyzer (Euro-EA-Elemental Analyzer, Eurovector, Milan, Italy), which was connected to a continuous flow isotope ratio mass spectrometer (IsoPrime, Elementar, Germany). All isotope ratios were expressed in terms of per mil (‰):

$$\delta^{15}N = \left[\frac{R_{sample}}{R_{standard}} - 1\right] x\ 1000$$

where R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ ratios of the sample and the standard, respectively. The standard used for nitrogen was atmospheric N_2. The precision of analysis for nitrogen was ±0.1‰.

Statistical analysis

I used SPSS Statistics (version 19; IBM, Armonk, New York) to perform statistical tests. Each data set was tested for normality (Kolmogorov-Smirnov test and Shapiro-Wilk test) and homogeneity of variance (Levene's test). To compare the effect of depth on nutrient availability within each collection period, nested ANOVA was applied where site was nested within depth (Table 4.1). Nested ANOVAs also were applied to compare the effect of season on nutrient availability at each of the six depths (Table 4.2). No non-parametric test equivalent to nested ANOVA exists, so data were ranked when they were not normal even after transformation before nested ANOVA was performed. If the variation in the ANOVA was significant, a Tukey *post hoc* test (or Games-Howell *post hoc* test if the variance was not homogenous) was applied to identify the differences.

One-way ANOVAs were applied to determine differences in foliar N concentration, C:N, and N resorption efficiency. Nested ANOVAs were applied to test differences in foliar N concentration and foliar C:N with species nested within leaf phenology (deciduous and evergreen). Nitrogen resorption efficiency was calculated as the quantity of nitrogen translocated from the leaf divided by the green foliar concentration. A Kruskal-Wallis test was performed on N resorption efficiency among species. Mann-Whitney U *post hoc* tests were performed to determine significant differences between species, and the p-values were Bonferroni-corrected.

In the nitrogen uptake experiment where the soil surface was labeled with ^{15}N to measure the rate of ^{15}N uptake, relativized $\delta^{15}N$ was regressed on days since labeling. Regressions were compared to determine the rate of ^{15}N uptake after ^{15}N labeling (Armitage 1980). *Q. laevis* and *Q. myrtifolia* were labeled at the same time in January 2009 and *Q. geminata, Carya floridana*, and *Lyonia ferruginea* were labeled in April 2009. Because all of the species did not begin at the same time, foliar $\delta^{15}N$ was relativized where the largest $\delta^{15}N$ value was designated as 1 for each labeling period. The period of uptake was defined as the period that foliar $\delta^{15}N$ was increasing (the last $\delta^{15}N$ value considered was the maximum $\delta^{15}N$ value obtained for the species). The p-value for an error rate of $\alpha = 0.05$ was 0.0052 once it was corrected using Dunn-Šidák correction (Sokal and Rohlf 1995).

In the experiment to measure the distance that roots could take up ^{15}N and to measure if each species utilized soil area differently, foliar $\delta^{15}N$ of each plant was compared to the mean $\delta^{15}N$ of non-labeled individual plants of its species to determine if the foliar $\delta^{15}N$ was significantly above the background $\delta^{15}N$ using a test of significance

where single observations are compared to a sample (Sokal and Rohlf 1995). Chi-squared tests were conducted to compare the number of individuals of each species that were ^{15}N-labeled and non-labeled for distances 1-6m (Sokal and Rohlf 1995). No shrub further than 6m from the point source of ^{15}N were labeled.

Results

Phosphorus and nitrogen availability

The quantity of available orthophosphate was most variable at 10 and 20 cm (Figure 4.2 A-C). The quantity of PO_4^{3-} did not differ with depth in the early dry season, but it did in both the late dry/early wet season and the late wet season (Table 4.1). The quantity of resin-extractable orthophosphate (PO_4^{3-}) was significantly higher at 10 cm than at any other depth during the early dry season and the wet season but not during the early dry season (Tukey *post hoc*, $p < 0.05$). At 10cm the quantity of PO_4^{3-} was higher in the early dry season (45µg/bag) than in either the early dry season or the wet season (11.4 and 37.9µg/bag, respectively; Table 4.2; Tukey *post hoc* $p < 0.05$). Also during the wet season at 20cm, the quantity of PO_4^{3-} was significantly lower than that at 10cm but higher than that at 50cm (Table 4.1; Tukey *post hoc*). At 20 and 100cm PO_4^{3-} was significantly lower during the early dry season than in either the late wet season or early dry season, but the difference between them was very small (Table 4.2; Tukey *post hoc*, $p < 0.05$).

The quantities of resin-extractable nitrate (NO_3^-) and ammonium (NH_4^+) in the soil were not significantly different with depth in any collection period (Table 4.1 and Figure 4.2 D-I). However, at depths 20, 30, 50, 150 cm, the quantity of NO_3^- was significantly different between seasons, being highest in the wet season and lowest at the

early dry season (Table 4.2; Tukey *post hoc*, $p < 0.05$). There was no difference in the quantity of NH_4^+ between seasons at any depth (Table 4.2). Ammonium was less abundant than NO_3^- in the soil only during the wet season.

^{15}N uptake during the early dry season by deciduous and evergreen species

Presence of labeled-^{15}N in the leaves represented uptake in the late dry season and early dry season (Figure 4.3 and 4.4). *L. ferruginea* had the highest rate of ^{15}N uptake, being significantly higher than all other species ($P < 0.001$). *Q. geminata* and *Q. myrtifolia*, both evergreen species, were not significantly different from each other, but they were significantly lower than *L. ferruginea* and higher than *Q. laevis* ($P < 0.001$) and *C. floridana* ($P < 0.001$). The two deciduous species, *Q. laevis* and *C. floridana* had the lowest uptake compared to all other evergreen species ($P < 0.001$).

The area of uptake did not differ among the five study species when plants were within 3m of the ^{15}N-labeled point source ($X^2_{(4, N = 132)} = 3.06$, $p = 0.55$) or from 3-6m ($X^2_{(4, N = 63)} = 3.32$, $p = 0.51$). The proportion of ^{15}N-labeled deciduous shrubs did not differ from that of evergreen shrubs within 3m of the ^{15}N point source ($X^2_{(1, N = 132)} = 0.0027$, $p = 0.96$) or from 3 to 6 m from the point source ($X^2_{(1, N = 63)} = 30.0388$, $p = 0.84$).

Foliar %N, C:N, and % N resorption

Foliar N concentration was significantly different among species ($F(4, 298) = 23.4$, $p < 0.001$). The species separated into three groups: *C. floridana* (deciduous) being the highest, followed by *Q. geminata* (evergreen), *Q. myrtifolia* (evergreen), and *Q. laevis* (deciduous) as the second group, and lastly the evergreen *L. ferruginea* (Table 4.3;

Games-Howell *post hoc*, $p < 0.05$). Carbon: nitrogen ratio was similar to foliar N concentration (Table 4.3; $F(4, 298) = 34.2$, $p < 0.001$; Games-Howell *post hoc*, $p < 0.05$). The genus *Quercus* contained one deciduous species (*Q. laevis*) and two evergreen species (*Q. geminata* and *Q. myrtifolia*), but foliar N concentration and C:N were more similar to each other than either the evergreen *L. ferruginea* or the deciduous *C. floridana*. Nitrogen resorption efficiency was significantly different among the species (X^2 (4, N = 30) = 25.575, $p < 0.0001$), but evergreen and deciduous species did not have distinct reabsorption efficiencies (Table 4.3; Mann-Whitney U test, $P < 0.05$).

Discussion

Soil phosphorus and nitrogen availability

Higher orthophosphate availability (PO_4^{3-}) in the top layers of the soil profile compared to the rest of the soil profile and higher PO_4^{3-} during the early dry season and wet season are controlled by abiotic and biotic factors that affect soil phosphorus abundance and cycling. The sandhill soil is oligotrophic sand with very low clay content and phosphorus-containing minerals, so organic matter found in the shallow soil is the principal source of phosphorus in the soil profile (Myers 1990). Heavy leaching also reduces soil nutrient content even for PO_4^{3-}, albeit leaching is much lower in PO_4^{3-} than in NO_3^- and NH_4^+ because of its lower diffusion coefficient (Johnson et al. 2001a, Gholz and Fisher 1982). Mineralization of nutrients is controlled by water availability and decomposition (Singh and Singh 1991). Once the rains of the wet season began, high water availability increased decomposition and the release of PO_4^{3-} from organic matter. As a result, the soil has low levels of plant-available phosphorus with most of it in the top 10cm and highest availability is during periods of high soil moisture content.

As expected, the quantity of orthophosphate extracted from resin bags was very low throughout the soil profile and only at 10cm was PO_4^{3-} more abundant than at other depths. The quantities of N and P measured in this study compared well with other studies conducted in the Florida scrub (Johnson et al. 2001a, Schafer 2010). The quantity of resin-extractable N and P was much lower than those extracted from forests in Sweden, Wisconsin (USA) and Connecticut (USA), as would be expected considering the high soil nutrient content of these other ecosystems (Binkley and Valentine 1991, Binkley et al. 1986, Binkley and Matson 1983, Lundell 1989). Also the lowland rainforests of Costa Rica had higher available N than Florida scrub (Zou et al. 1992).

Nitrogen was available throughout the soil profile. NO_3^- was more plentiful in the wet season at several depths (20, 30, 50, 150cm), but was not constrained to the shallow soil. Large diffusion coefficients in the soil for NH_4^+ and especially NO_3^- disperse mineralized nitrogen throughout the soil profile (Lambers et al. 1998). Interestingly during the early dry season, which included the first four weeks of rain during the wet season, only P and NO_3^- (non-significant) showed a large increase in availability. Investigations in several other forests with pronounced dry and wet seasons found that the initial rains of the wet season were accompanied with a short but large nutrient pulse (Davidson et al. 1993, Raghubanshi et al. 1990, Singh et al. 1989, Luizao et al. 1992, Lodge et al. 1994). In one case, the first four weeks of the wet season released more nitrogen than the rest of the wet season (Singh et al. 1989). The first month of rains (included in the early dry season period) appears to be a period of nutrient mineralization with mineralized nutrients dispersing downward into the deep soil layers after the first month of the wet season.

Considering the N and P availability profiles, the highest overall nutrient availability is in the top 10 cm. Low phosphorus mobility in the soil and low concentration in the deep layers of the soil profile means that roots must allocate considerable root resources to the top 10 cm to exploit available phosphorus (Lambers et al. 1998). Nutrient availability, especially phosphorus, likely explains why 42% of all roots to a depth of 150cm are concentrated in the top 20 cm of soil, which represents only 13% of the total soil volume (Chapter 1).

^{15}N uptake during the early dry season by deciduous and evergreen species

Contrary to what I hypothesized but consistent with what Chapin (1983), deciduous species had lower uptake rates of ^{15}N than evergreen species during the early dry season and early wet season. Lower N uptake by deciduous shrubs could be explained if they had a broader area of shallow roots than evergreen species, so the area of N application included disproportionately fewer deciduous shallow roots than evergreen roots. If this were the case, deciduous species would have a higher proportion of labeled plants at greater distances than evergreen species. In this study, the same proportion of plants was labeled at all distances for all species, showing that the area of nutrient uptake did not differ among species.

It has been widely published that deciduousness is favored in areas of high nutrient availability, and evergreen phenology is favored in areas of low nutrient availability (Bazzaz 1979, Coley et al. 1985, Monk 1966, Kikuzawa 1991, Aerts and Van der Peijl 1993, Lloyd et al. 2009, Chapin 1980). The reason behind this hypothesis is that deciduous species have higher nutrient demands at leaf flush and higher foliar N concentration, so they require relatively fertile sites (Monk 1966, Chapin 1980). My

findings raise another possibility that deciduous species exist primarily in more fertile sites because they have lower nutrient uptake rates than evergreen plants. Deciduous species in the Florida scrub follow this pattern as they occur only in the most fertile areas: sandhill and oak-hickory scrub (yellow sands), although they are quite infertile (Menges 1999, Abrahamson et al. 1984). The sites less fertile than the yellow sands are the white sands that do not support deciduous species (Saha et al. 2008, Abrahamson et al. 1984). Deciduous species need to produce from either uptake or storage a large quantity of nitrogen and phosphorus for leaf growth. Their low nutrient uptake rate at the time of leaf out means that sites with even lower nutrient availability than the sandhill, coupled with the lower deciduous uptake rate, may not be able to sustain a nutrient pulse large enough for deciduous leafing out. It remains to be tested whether deciduous species also have a lower phosphorus uptake rate compared to evergreen species.

The low uptake rates in deciduous species indicate that the temporal pattern of nutrient uptake and leaf-out may not be coupled. Low uptake rates would require more time to take up adequate nutrients, meaning that deciduous species may take up nutrients throughout the wet season to compensate for nutrients used for new leaf growth. Most other studies have shown that N uptake in deciduous species took place mostly after leaf out (Millard et al. 2006, Guak et al. 2003, Millard et al. 2001, Dyckmans and Flessa 2002). In contrast, evergreen species in the Mediterranean and tundra relied more heavily on nitrogen uptake during leaf growth (Chapin 1983, Gray 1983, Aerts 1996, Lal et al. 2001). However, not all deciduous species relied on post leaf-out nitrogen uptake to compensate for use during leaf-out; some deciduous shrubs were actively taking up nutrients during leaf out (Millard and Grelet 2010, Frak et al. 2002, Silla and Escudero

2003). Higher reliance on current N uptake observed in the above studies may be because of high fertility at the respective study sites where current N uptake could supply adequate N for leaf growth (Silla and Escudero 2003, Chapin et al. 1990, Bloom et al. 1985). In nutrient-poor sites such as the sandhill, however, may not sustain deciduous shrubs that have low uptake rates because would not be able to compensate the high demand required for new leaf growth.

Foliar N concentration and % N resorption efficiency

Foliar N concentration and resorption efficiency indicate that greater ^{15}N uptake in evergreen species than deciduous species could not be due to lower N demand or resorption efficiency in evergreen species because foliar N concentration and resorption efficiency did not separate between deciduous and evergreen leaf phenologies. In temperate forests evergreen species have lower N concentrations and N resorption efficiencies than deciduous species (Gray 1983, Aerts 1996), but this relationship between foliar N concentration and leaf phenology was absent in this study. This lack of a clear separation in foliar N concentration by leaf phenology groups is similar to what has been found in subtropical and tropical seasonal dry forests than in temperate forests (Ares and Gleason 2007, Lal et al. 2001, Nardoto et al. 2006). Low resorption efficiency in *C. floridana* is not unprecedented because resorption efficiencies as low as 14% have been observed in South American savannah species (Nardoto et al. 2006). Contrary to studies from temperate areas that have shown a positive relationship between foliar N concentration and N resorption efficiency, *C. floridana* had the highest foliar N concentration and the lowest N resorption efficiency, probably because *C. floridana* did not have a high foliar concentration when compared to temperate species that showed this

relationship (Beadle 1968, Chapin et al. 1980, Turner and Olson 1976, Miller et al. 1976). In contrast, the opposite is true for *L. ferruginea*. *L. ferruginea* had the highest ^{15}N uptake, but it had the lowest foliar N concentration. High N uptake and low N leaf requirements are traits that would be favorable in a nitrogen-poor environment because plants would be effective in taking up the little N that is available and N demands for leaf growth would be low.

Conclusions

In this seasonally dry plant community, the top 10cm had the highest concentration of P in the soil profile, but nitrogen did not differ with soil depth. These soil P and N profiles suggest that concentrating root growth in the top 10cm would maximize P and N uptake. This observation is consistent with the root density observed in the scrub (Chapter 1). ^{15}N uptake rates from the shallow soil were highest among evergreen species. This difference in uptake rate was not related to shallow root distributions as both deciduous and evergreen species took up nitrogen proportionally from the same soil area. During the period when deciduous plants were leafing out, N uptake was very low. Low N uptake means that deciduous species may need to take up N throughout the wet season to compensate for N translocated to the leaves. Deciduous and evergreen species differed in foliar N concentration and N resorption efficiency on a species basis but not on the level of leaf phenology. This temporal uncoupling of nutrient demand and uptake may be why deciduous species are most common in fertile sites.

Table 4.1 Nested ANOVAs for ranked variables showing how quantities of PO_4^{3-}, NO_3^-, and NH_4^+ (µg bag^{-1}) changed among depths within each season. Significant effects are shown in bold.

Nutrient	Season	Parameters	*df*	*ms*	*F*	*P*
PO_4^{3-}	Early dry	Depth	5	1401	0.5	0.775
		Sites	12	2846	6.5	**<0.001**
		residual	79	440		
	Late dry/early wet	Depth	5	6485	18.8	**<0.001**
		Sites	12	328	0.5	0.90
		residual	84	631		
	Late wet	Depth	5	7133	4.9	**0.011**
		Sites	12	1447	3.3	**0.001**
		residual	84	434		
NO_3^-	Early dry	Depth	5	2879	1.0	0.45
		Sites	12	2867	6.5	**<0.001**
		residual	84	439		
	Late dry/early wet	Depth	5	3066	0.8	0.57
		Sites	12	3832	7.0	**<0.001**
		residual	92	544		
	Late wet	Depth	5	2126	0.8	0.60
		Sites	12	2834	5.3	**<0.001**
		residual	84	539		
NH_4^+	Early dry	Depth	5	4586	1.9	0.17
		Sites	12	2405	2.9	**0.002**
		residual	92	835		
	Late dry/early wet	Depth	5	473	0.1	0.98
		Sites	12	3200	5.0	**<0.001**
		residual	87	640		
	Late wet	Depth	5	1648	1.1	0.39
		Sites	12	1482	2.3	**0.01**
		residual	81	645		

Table 4.2 Nested ANOVAs showing how quantities of PO_4^{3-}, NO_3^-, and NH_4^+ (μg bag^{-1}) extracted from resin bags changed between seasons (early dry, late dry/early wet, late wet) at each depth. Significant effects are shown in bold.

Nutrient	Depth (cm)	Parameters	*df*	*ms*	*F*	*P*
PO_4^{3-}	10	season	2	2315	5.3	**0.048**
		sites	6	442	4.0	**0.003**
		residual	43	110		
	20	season	2	3378	14	**0.01**
		sites	6	246	2.9	**0.02**
		residual	43	84		
	30	season	2	1209	1.1	0.40
		sites	6	1136	23	**<0.001**
		residual	42	49		
	50	season	2	1823	4.1	0.08
		sites	6	451	6.2	**<0.001**
		residual	39	73		
	100	season	2	2411	5.2	**0.049**
		sites	6	464	5.1	**0.001**
		residual	43	92		
	150	season	2	1287	2.5	0.17
		sites	6	524	4.0	**0.003**
		residual	42	130		
NO_3^-	10	season	2	0.54	2.5	0.16
		sites	6	0.22	2.3	0.056
		residual	43	0.10		
	20	season	2	3575	8.2	**0.02**
		sites	6	439	5.4	**<0.001**
		residual	45	81		
	30	season	2	1.91	5.5	**0.04**
		sites	6	0.35	8.5	**<0.001**
		residual	45	0.04		
	50	season	2	2249	7.4	**0.02**
		sites	6	308	2.9	**0.02**
		residual	41	107		
	100	season	2	2610	4.9	0.054
		sites	6	529	6.0	**<0.001**
		residual	44	88		
	150	season	2	1.56	12	**0.01**
		sites	6	0.13	3.1	**0.01**
		residual	42	0.04		

NH_4^+	10	season	2	0.98	1.7	0.25
		sites	6	0.06	5.2	**<0.001**
		residual	42	0.11		
	20	season	2	294	0.4	0.67
		sites	6	688	4.2	**0.002**
		residual	43	163		
	30	season	2	0.31	1.2	0.36
		sites	6	0.26	2.1	0.07
		residual	43	0.12		
	50	season	2	857	1.7	0.26
		sites	6	507	3.1	**0.012**
		residual	43	162		
	100	season	2	411	0.2	0.81
		sites	6	1828	10.5	**<0.001**
		residual	45	174		
	150	season	2	0.16	0.7	0.52
		sites	6	0.23	4.6	**0.001**
		residual	42	0.05		

Table 4.3 Mean green foliar N concentration, C:N, and % N resorption efficiency (± SE). Means within a column that are preceded by the same letter are not significantly different (Games-Howell *post hoc*, $p < 0.05$ for foliar N concentration and C:N ratio and Mann-Whitney U *post hoc*, $p < 0.05$ for % nitrogen resorption efficiency).

Leaf phenology	Species	N concentration (mg g^{-1})	C:N ratio	% N resorption efficiency
Deciduous	*C. floridana*	$^{a}16.8 \pm 0.2$	$^{a}29.29 \pm 0.36$	$^{c}23.9 \pm 1.6$
Deciduous	*Q. laevis*	$^{b}13.8 \pm 0.2$	$^{b}35.03 \pm 0.6$	$^{a}56.3 \pm 2.3$
Evergreen	*Q. geminata*	$^{b}14.8 \pm 0.5$	$^{b}33.68 \pm 1.25$	$^{ab}53.7 \pm 2.7$
Evergreen	*Q. myrtifolia*	$^{b}15 \pm 0.2$	$^{b}34.47 \pm 0.43$	$^{a}60.7 \pm 1.7$
Evergreen	*L. ferruginea*	$^{c}13 \pm 0.5$	$^{c}44.3 \pm 1.36$	$^{bc}39.1 \pm 1.9$

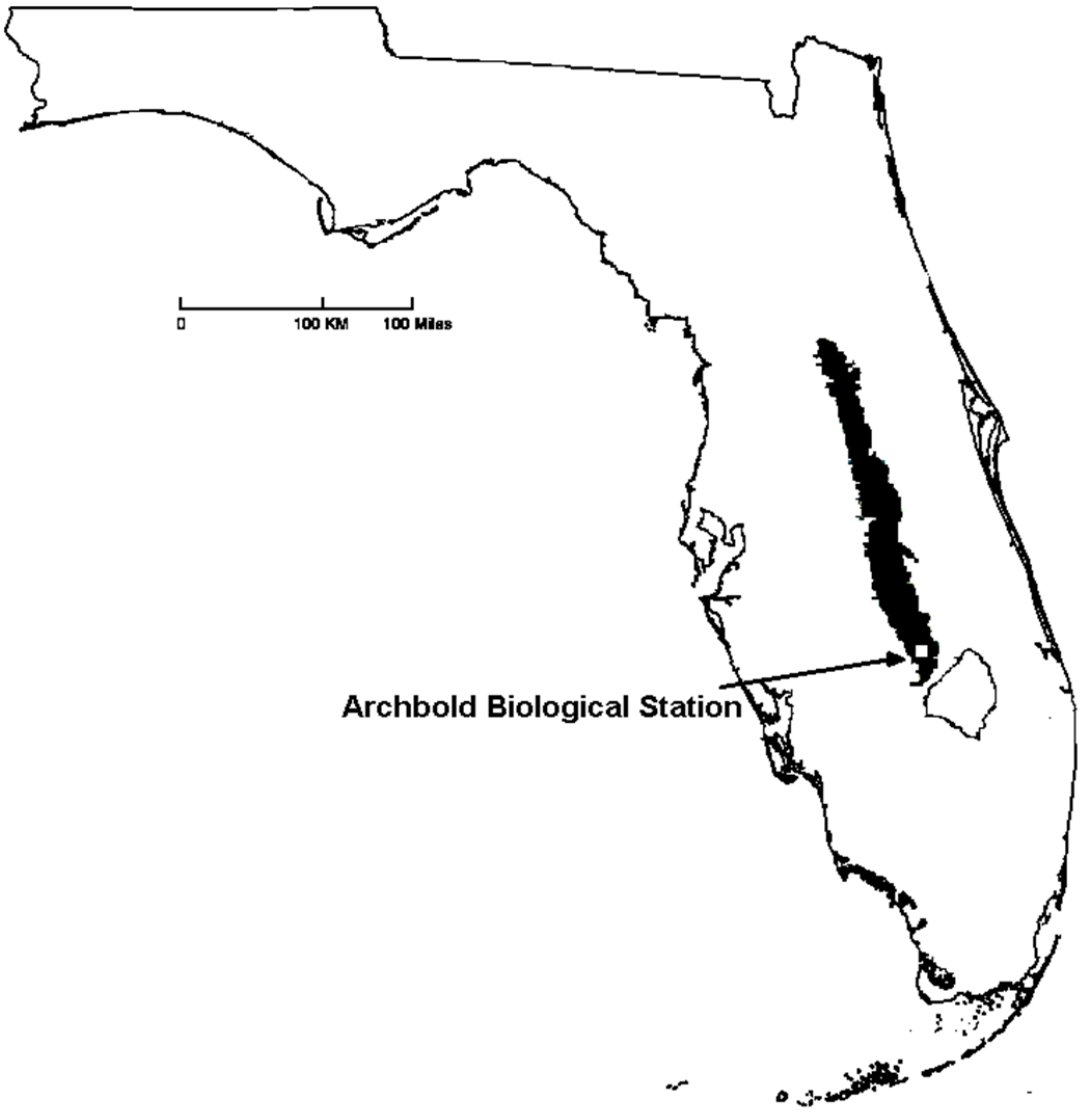


Figure 4.1 Map of Florida showing the geographical range of the Lake Wales Ridge (region highlighted in black) and the location of Archbold Biological Station. The boundary of the Lake Wales Ridge is based on Weekley et al. (2008).

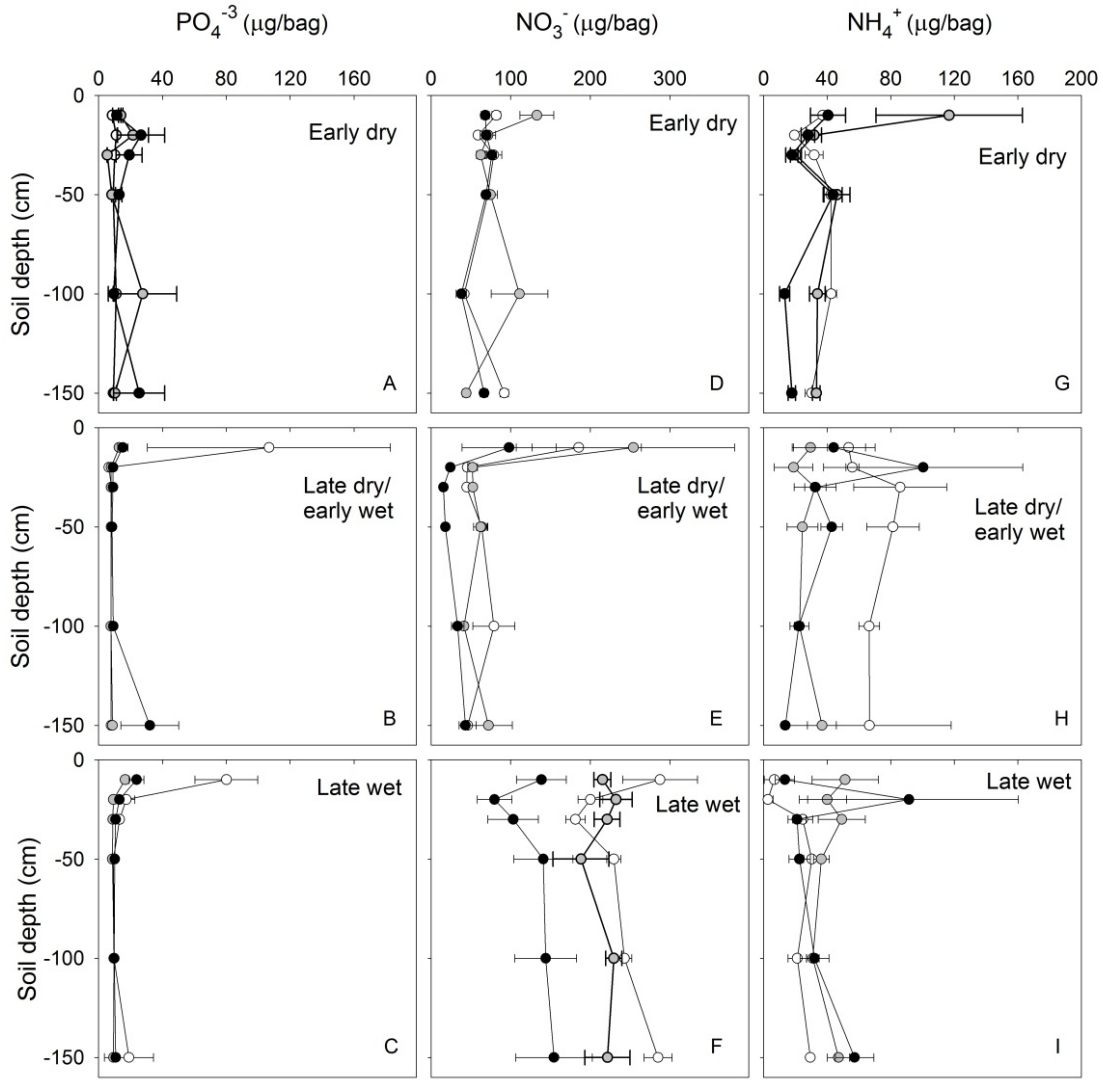


Figure 4.2 Quantity of nutrients (PO_4^{3-}, NO_3^- and NH_4^+) available during the early dry season, early dry season, and the wet season. Error bars are the standard error. Open circles represents Sandhill long unburned site 1, gray circles represent Sandhill long unburned site 2, and black circles represent Sandhill recently burned site.

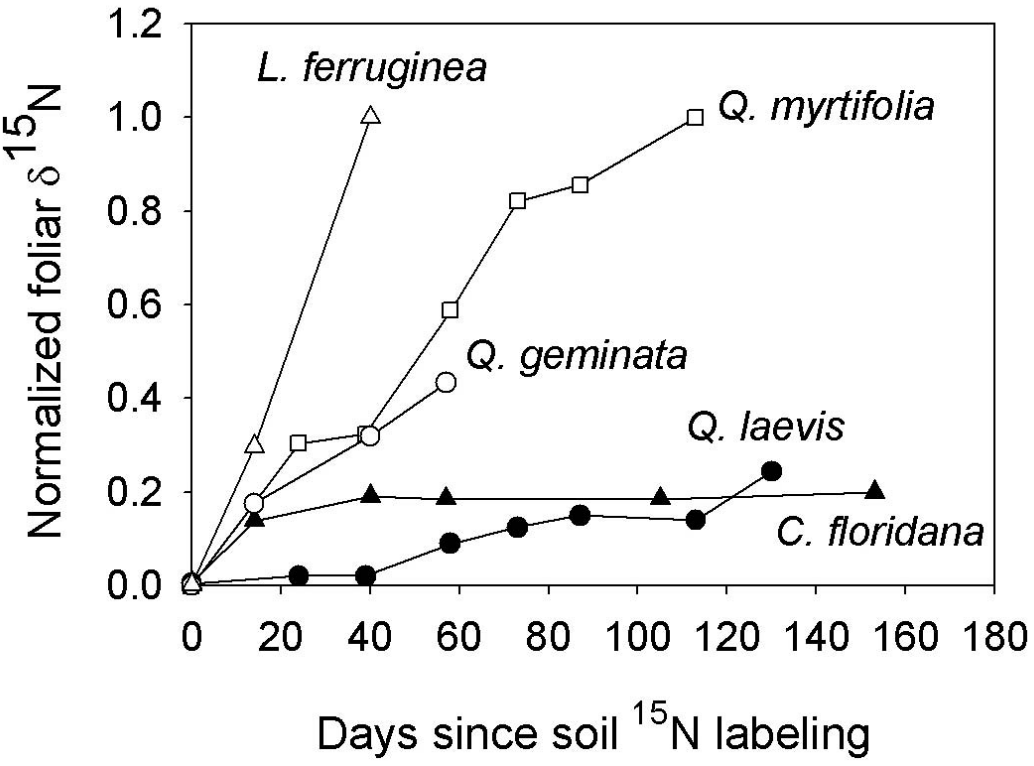


Figure 4.3 Normalized $\delta^{15}N$ representing ^{15}N uptake by the five study species. The final point in each line is the highest $\delta^{15}N$ obtained for each species. Open symbols represent evergreen species (▵ *L. ferruginea*, □ *Q. myrtifolia*, ○ *Q. geminata*). Filled symbols represent deciduous species (▲*C. floridana*, ● *Q. laevis*).

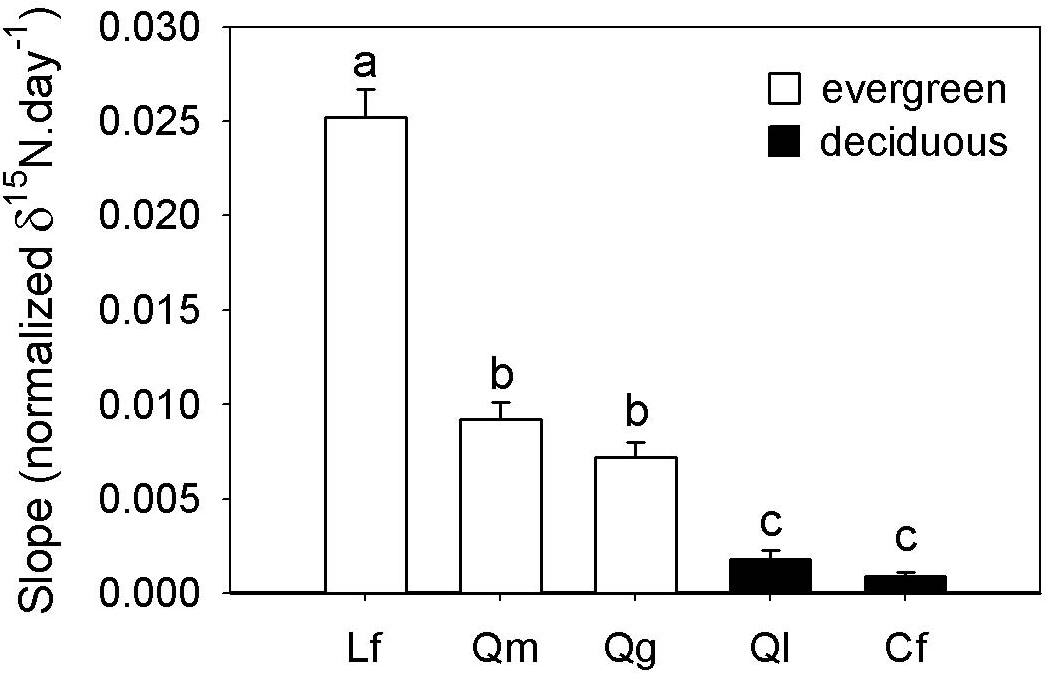


Figure 4.4 Slopes of normalized $\delta^{15}N$ over days since ^{15}N labeling (as shown in figure 4.3). Bars topped with the same letter are not significantly different.

Chapter 5

Overall conclusions

In in my dissertation research I investigated the inter-relationship between leaf phenology and root function and structure in a seasonal dry plant community. In particular, this study compared fine root structure of deciduous and evergreen species seasonally (Chapter 2) and compared fine root function of deciduous and evergreen species by measuring water (Chapter 3) and nutrient uptake (Chapter 4). Seasonality is a strong component of seasonal ecosystem, so all studies were done in a seasonal context, comparing between and within early dry, late dry, and wet seasons. Below I summarize the conclusions of each chapter and discuss how my studies advanced our understanding of evergreen and deciduous species adaptation to water and nutrient limitation in a dry seasonal ecosystem.

Seasonal fine root dynamics of deciduous and evergreen species

Fine root density increased dramatically in the wet season in all species and at all depths, except in the deciduous *Carya floridana* at 50-150cm. This increase in root density was likely high wet season root production to offset high dry season mortality. This means that these plants experience high fine root turnover from dry to wet season. High turnover was not only in absorptive roots (first and second order roots), but also in more costly roots with a solely transport function (third and fourth order roots) in all species except *C. floridana* in the fourth order. All species appear to shed roots of all orders once the soil dries, instead of maintaining them for the duration of the dry season. Shedding roots in the dry season requires high production once the soil is re-wetted to compensate for high dry season mortality. This high turnover in roots could be a disadvantageous carbon cost

unless the cost of maintaining root respiration in the dry soil is higher than initial root construction. Root respiration may be costly over the six months of dry season and appears not to be offset by any advantage to maintaining fine roots because fine root density greatly decreased. However, evergreen species nutrient uptake was less affected by fine root decline in the dry season than that of deciduous shrubs.

Fine root density increased at all depths in the wet season, showing a strong seasonality at all depths in all root orders. Water availability is likely the principal driver of fine root production. During the dry season, more water was available in the deep soil by volume, but soil water content was much lower than in the wet season. Reduced water availability per unit of soil in the dry season affected all soil layers, reducing fine root density throughout the soil profile.

Fine root turnover between dry and wet season was lowest in the deciduous species, *C. floridana*, as I hypothesized. This was the case for all fine root orders, and in the fourth order, root density did not significantly change from dry to wet season. However, roots of the second deciduous species, *Q. laevis*, could not be distinguished from the other red oak, the evergreen *Q. myrtifolia*. Lower fine root turnover means that *C. floridana* likely has lower wet season fine root construction costs, which may reduce the carbon and nutrient cost that deciduous species have at the beginning of the wet season because of growing a new canopy. The evergreen species, especially *L. ferruginea*, had high root turnover, yet they also had high nitrogen uptake in the late dry season (Chapter 4).

Defoliation and stem water enrichment

The defoliation experiment showed that evaporation, as the result of leaflessness, enriched stem water isotopically, which explained higher δ^2H and $\delta^{18}O$ values of leafless deciduous shrubs than those of evergreen shrubs at the same collection period. This phenomenon is probably universal and using stable isotopes to measure water source use is impossible when deciduous species are leafless because the isotopic signature of source water that enters stem is confounded by enrichment due to evaporation.

Seasonal water source use based on water availability and root distribution profiles

Deciduous and evergreen species did not differ in water source use during either early dry, late dry, or the wet season when both deciduous and evergreen species had leaves. I could not determine whether they differed in depth of water uptake during the period (month to month) when deciduous woody plants were leafless. Deciduous woody plants lost their leaves for 2-4 months of the dry season, depending on the site. The inability of determining water source during this period is likely unimportant because the leafless plants were taking up little if any water from their soil.

In the dry season, soil water availability as controlled by edaphic and climatic factors especially in the shallow soil, was the principal driver determining water source use, resulting in the deep soil being the predominant dry season water source. Low water availability in every depth, but principally in the shallow soil resulted in heavy use of the deep soil (50-150cm). This was the case in all species except *L. ferruginea*. *Lyonia ferruginea* was able to take up more shallow water than was expected based on water availability, but the deep water was still more than half of its water source.

During the early dry season and wet season, the principal driver in determining water source use shifted from the distribution of available water to the root distribution with depth. The total number of roots in each soil layer was highest in the top 20cm, but still one third of the roots in the top 150cm were between 50 and 150cm (Chapter 2), which accounted for 30% of water uptake. In the wet season when more water was available at all depths, water uptake was likely at maximum uptake and resulted in a pattern of water source use similar to the distribution of roots. Deep water use was an important water source throughout the year – not just in the dry season when shallow soil (0-50cm) had low water availability. Since the shallow soil layers of a sandy soil do not have high water storage; the water that is available is quickly depleted by percolation and water uptake. Even short rainless periods in the wet season can limit shallow water availability, emphasizing the need for deep water uptake.

Leaf phenology and adaptation to drought

Leaf phenology did not influence water source use, but it did influence water usage because deciduous species remained dormant for more than half of the dry season. Nonetheless, deciduous species were ‘drought delayers’ because the leafless period delayed the effects of the drought until late in the dry season. Late in the dry season deciduous species took up mostly deep water, further delaying the effects of the drought. Evergreen shrubs accessed deep water throughout the dry season to delay the effects of the drought unlike deciduous species that used a dual strategy of dormancy and access to deep water for water uptake.

Soil phosphorus and nitrogen availability

The highest PO_4^{3-} concentration was in the top 10cm of soil, and increased in the early dry season and the wet season. All other depths did not differ from each other in any season. This result was expected because PO_4^{3-} is immobile in the soil and organic matter at the soil surface is its principal source. NO_3^- and NH_4^+ did not differ with soil depth, but NO_3^- increased in the wet season. Soil P and N availability suggest that high root density near the soil surface would maximize P and N uptake. This observation is consistent with the root density observed (Chapter 2).

Nutrient uptake of deciduous and evergreen species

In the labeling experiment, ^{15}N uptake rates from the shallow soil were highest among evergreen species in the late dry season. This difference in uptake rate was not related to differences in shallow lateral root distributions as both deciduous and evergreen species took up nitrogen proportionally from the same soil area. Evergreen species had high nitrogen uptake in the late dry season, and they had higher fine root turnover as well from the dry to the wet season. During the period when deciduous species were leafing, N uptake was very low, even though the deciduous *C. floridana* had the lowest fine root turnover. Having proportionally more of its fine roots alive at the time of the labeling experiment did not translate into higher N uptake the evergreen species. Possibly little to no transpiration when the label was applied to the soil surface reduced N uptake so much that *C. floridana* took up very little of the ^{15}N label. Low N uptake at the time of leaf-out means that deciduous species may need to take up N throughout the wet season to compensate for translocated N that was used in new leaf growth.

Foliar N concentration and % N resorption efficiency

Species differed in foliar N concentration and N resorption efficiency, but these differences did not reflect differences in leaf phenology. Deciduous species leaves did not require less nitrogen for leaf construction nor did they translocate more N from the senescencing leaves, but they took up less nitrogen than evergreen species when they leafed out. This temporal uncoupling of nutrient demand and uptake may be why deciduous species are most common in more fertile sites.

Implications of this research

The purpose of these studies was to better understand the interrelationship of between leaf phenology and root structure and function. Leaf phenology is controlled by edaphic and climatic factors of a seasonal environment. From this research, edaphic and climatic factors that control nutrient and water availability are directly controlling root density and rooting depth. In water source use, it appears that these factors work independently on both leaf phenology and root function and structure because differences in leaf phenology did not lead to a difference in water source use. In nutrient uptake, leaf phenology did play a role in nutrient uptake, which may be related to differences in dry season transpiration or in fine root turnover. Fine root turnover was lowest in the deciduous species. The most important driver controlling rooting depth and distribution was water availability, while root density was controlled by nutrient availability.

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