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CONSEQUENCES OF SHRUB ENCROACHMENT: LINKING CHANGES IN CANOPY STRUCTURE TO SHIFTS IN THE RESOURCE ENVIRONMENT

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"CONSEQUENCES OF SHRUB ENCROACHMENT: LINKING CHANGES IN CANOPY STRUCTURE TO SHIFTS IN THE RESOURCE ENVIRONMENT"

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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"Believe in yourself, trust the process, change forever"

Bob Harper

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        Shrub expansion in herbaceous ecosystems is emerging as an important

ecological response to global change, especially in mesic systems where

increases in canopy biomass are greatest. Two consequences of woody

encroachment are increases in belowground resources, such as carbon and

nitrogen, and reductions in above-ground resources such as light, which affect

diversity, community trajectory, and ecosystem function. My objective was to

determine how expansion of the nitrogen-fixing shrub *Morella cerifera* affected

the resource environment across a chronosequence of shrub expansion on a

Virginia barrier island. I quantified changes in carbon (C) and (N) cycling, canopy

structure and understory light associated with *M. cerifera* expansion. Litterfall in shrub thickets exceeded litterfall for other woody communities in the same region, and due to high N concentration, resulted in a return of as much as 169 kg N ha^{-1} yr^{-1} to the soil, 70% of which was from symbiotic N fixation. Litter and soil C and N pools were 3-10 times higher in shrub thickets than in adjacent grasslands. Understory light in shrub thickets decreased to as low as 0.5% of above-canopy light. Sunflecks in shrub thickets were shorter, smaller and less intense than sunflecks in forest understories. However, relative to other shrub species such as *Elaeagnus umbellata*, *M. cerifera* was less efficient at intercepting light. Although *M. cerifera* had the highest leaf area index (LAI) of five shrub species studied, *M. cerifera* was relatively inefficient at light attenuation due to low levels of branching, steep leaf angles and a relatively shallow canopy. The shift from grassland to shrub thicket on barrier islands, and other mesic systems, results in a significant change in canopy structure that alters understory resource availability and greatly alters ecosystem function and trajectory.

CHAPTER ONE

ECOLOGICAL CONSEQUENCES OF SHRUB EXPANSION

Steven T. Brantley

Introduction

Changes in the abundance of woody vegetation, especially the expansion

of native shrubs in historically herbaceous communities, have been documented

for a range of ecosystems worldwide (Archer 1989; Goslee et al. 2003; Sturm et

al. 2005; Briggs et al. 2005; Akhalkatsi et al. 2006; Young et al. 2007). Trends in

woody encroachment have not followed traditional successional models of

disturbance and recovery and the global nature of the phenomenon suggests

that it is a state transition induced by persistent global change (Briggs et al.

2005). Evidence of a single causal factor, such as CO_2 enrichment of the

atmosphere, is weak (Archer 1995). Rather, local or regional causes such as fire

suppression and shifts in grazing pressure have been linked to woody

encroachment in many ecosystems, especially in arid and semi-arid systems of

the southwestern United States (Archer 1995). In ecosystems where there are

severe temperature limitations on plant growth, such as Arctic tundra, global

warming appears to have a major effect on initiating and maintaining trends in

woody encroachment but increased temperature does not appear to favor shrub

growth in temperate systems (Sturm et al. 2005; Knapp et al. 2007). Patterns of

shrub expansion on the Virginia barrier islands suggest that CO_2 enrichment is at least a contributing factor to global patterns of woody encroachment. These islands lack the history of grazing pressure and fire suppression characteristic of other systems, yet shrub expansion has been widespread and rapid even as sea-level has risen at a rate of ~4mm yr^{-1} (Young et al. 2007).

While a further synthesis of existing data is necessary to determine the causes of woody encroachment, there is an immediate need to determine the consequences of this change on local, regional and global processes. Differences in plant life history, morphology and tissue chemistry between woody plants and grasses drive changes in ecosystem function and community development after woody encroachment (Briggs et al. 2005). Shrubs and other woody vegetation extant in grasslands and savannas often act as ecosystem engineers by reducing soil erosion, subsidizing soil nutrient inputs by intercepting atmospheric nutrients, providing protection to understory vegetation and serving as a nutrient reservoir, especially in sandy and/or low-nutrient soils (Garcia-Moya and McKell 1970; Art et al. 1974; Joy and Young 2002). Additionally, woody encroachment often results in a substantial increase in annual net primary production (ANPP), especially in mesic systems, because of a reduction in meristem limitation associated with the shift in plant growth form (Knapp et al., 2008). Finally, changes in tissue chemistry inherent in the shift from grasses to shrubs improve litter quality and accelerate nutrient cycling (Killingbeck 1986; Briggs et al. 2005).

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While changes in plant life history and morphology after woody encroachment are consistent across ecosystems, the magnitude of the effect on ecosystem function and subsequent resource availability varies widely (Briggs et al. 2005; Knapp et al. 2008). Changes in meristem limitation associated with the shift in growth form and reduced nutrient limitation for N-fixing shrubs often results in a substantial increase in ANPP and an associated increase in leaf area index (LAI) (Knapp et al. 2008). Differences in stimulation of LAI among sites are driven by variations in mean annual precipitation (MAP) (Knapp et al. 2008). In arid and semi-arid systems, such as Sevilleta National Wildlife Refuge, NM with a mean annual precipitation (MAP) of 242 mm, LAI is likely to remain unchanged after shrub expansion (~1.5 for grasslands and shrublands at this site) because water availability limits canopy development regardless of growth form (Knapp et al. 2008). As precipitation increases, LAI of shrubs increases rapidly in relation to co-occurring grasslands because shrubs are better able to use available water to form dense canopies. In tallgrass prairie (MAP: 859 mm), expansion of *Cornus drummondii* resulted in dense patches, or 'islands', of shrubs with LAI of ~11 (Lett and Knapp 2003; Knapp et al. 2008). By comparison, LAI in mesic forest at the same latitude averages roughly half of that value (Lonsdale 1988). The high LAI within shrub islands caused an 87% reduction in available light compared to adjacent grasslands. Understory photosynthetically active radiation (PAR) was as low as 5% of incident PAR and resulted in a substantial decline in herbaceous cover and understory productivity (Lett and Knapp 2003).

Increases in LAI are naturally accompanied by a significant increase in litter production after shrub expansion. Increased quantity of litter, coupled with changes in tissue chemistry that increase litter quality, cause substantial shifts in ecosystem C and N cycling (Vitousek and Walker 1989; Briggs et al. 2005; Knapp et al. 2008). Variations in C and N cycling across ecosystems depend heavily on edaphic characteristics in addition to MAP, and sites with small pre-existing C and N pools are more responsive to shrub expansion compared to sites with well developed soil organic layers. After expansion of *Proposis glandulosa* in semi-arid plains in northern Texas (MAP: 665 mm), there was no change in surface soil C and N pools, despite substantial changes in aboveground C and N (Hughes et al. 2006). When *Proposis glandulosa* expanded in the slightly less arid (MAP: 716 mm) subtropical savanna of southern Texas, there was a significant increase in ecosystem C and N storage (McCulley et al. 2004). Note that although MAP was only slightly lower in the northern site, the north is characterized by hot, dry summers while precipitation in the more southerly site peaks in early and late summer. In Kansas, where soil organic layers are well developed, there was no change in ecosystem C or N storage after expansion of *Cornus drummondii* in tallgrass prairie despite relatively high MAP (McCarron et al. 2003). Conversely, expansion of the exotic N-fixing shrub *M. faya* on young, nutrient poor volcanic soils in Hawaii caused a 428% increase in N input into the ecosystem (Vitousek et al. 1987).

Patterns of change in ecosystem function after woody encroachment are dependent on the precipitation and edaphic characteristics which are often tightly coupled (Jackson et al. 2002; Wheeler et al. 2007; Knapp et al. 2008). However, consequences of shrub expansion have been most thoroughly documented in arid and semi-arid areas of the southwestern United States with poor soils and on well-developed soils in mesic ecosystems in the Great Plains (McCarron et al. 2003; McCulley et al. 2004; Wessman et al. 2004; Briggs et al. 2005; Hughes et al. 2006). To better understand what drives differences in ecosystem response after shrub expansion and predict future changes at local, regional and global scales there is a need to assess the consequences across the widest range of sites that represent all possible combinations of precipitation, soil characteristics and time since shrub expansion. I hypothesize that changes in ecosystem function associated with shrub expansion will be greater in mesic systems with young and/or poorly developed soils because of the large increase in leaf area after shrub expansion and the greater potential for C and N accumulation.

Background and Objectives

While changes in woody abundance have been described for a variety of systems, quantifying impacts of the phenomenon on ecosystem properties can be difficult due to the extended time-scale over which changes occur (Wessman et al. 2004). Previous work has necessarily focused on comparing adjacent grasslands and shrublands even though remnant grasslands may not accurately represent the original state of shrublands. The problem of assessing patterns of

long-term change is even more difficult when attempting to quantify the effects of shrub stand age. For instance, Wheeler et al. (2007) used stem size as a surrogate for stand age but could not account for differences in growth rates among sites. Another option is to use a space-for-time substitution, or chronosequence, where differences in location within the landscape represent time since the inception of community development (Walker and del Moral 2003). However, the relatively static landscape in most terrestrial systems does not support this approach. Rather, the occurrence of a soil chronosequence is usually limited to areas exposed by glacial retreat and coastal areas where accretion of sand has extended shorelines (Walker and del Moral 2003).

One of the best opportunities to study consequences of shrub expansion over multiple time scales is on barrier islands. Virginia's barrier islands, in particular, often experience rapid fluctuations in size and shape because of natural changes in currents that affect erosion and deposition of sand (Hayden et al. 1991). Where sand accretes, development of a soil chronosequence and subsequent colonization by dune-forming grasses is a typical outcome. However, from 1949 to 1989, Hog Island, a barrier island along the Virginia, USA coast also experienced a 400% increase in shrub cover along a chronosequence of soil development that range from 0 to ~140 years old (Young et al. 1995). While increases in shrub abundance in such systems have generally been viewed in the context of primary succession, shrub expansion on Virginia's barrier islands is not directly related to increases in upland area and shares many

characteristics with the broader global trend of woody encroachment (Young et al. 2007).

The dominant shrub on barrier islands of the southeastern United States, including Hog Island, is the nitrogen-fixing shrub *Morella cerifera* (Young 1992; Young et al. 1995)*.* One of the primary drivers of plant community composition in coastal soils is the low availability of nutrients, especially N (Art et al. 1974; Ehrenfeld 1990; Stalter and Odum 1993). Formerly known as *Myrica cerifera* (Wilbur 1994) and commonly known as wax myrtle, *M. cerifera* is well adapted to the low nutrient coastal soils of the Virginia barrier islands (Young 1992). A symbiotic association between members of Myricaceae and the actinomycete *Frankia* assures an adequate source of nitrogen (Morris et al. 1974; Vitousek and Walker 1989; Young et al. 1992). The evergreen leaf habit further facilitates nutrient conservation by allowing plants to reabsorb other nutrients, such as phosphorus, more proficiently (Monk 1966; Killingbeck 1996). These adaptations reduce nutrient stress for *M. cerifera* and, combined with high potential growth rates and bird-dispersed seeds, allow this species to form dense, nearly monospecific stands on islands that are otherwise dominated by herbaceous vegetation (Young et al. 1995; Kwit et al. 2004). Nitrogen-fixing species such as *M. cerifera* often contribute substantial nitrogen to the soil through litter because they are less proficient in resorption of nitrogen from senescing parts than non-fixers and the relatively high tissue N concentration has a substantial effect on soil N accumulation and subsequent community development (Morris and

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Eveleigh 1974; Permar and Fisher 1983; Killingbeck 1996; Uliassi and Ruess 2002). Previous estimates of soil N beneath *M. cerifera* shrub thickets and in soils without *M. cerifera* were 791 ± 195 μg/g and 321 ± 14 μg/g, respectively (Young et al. 1992). However, more work is needed to better understand the effects of shrub expansion on nutrient inputs and retention in these young coastal soils.

Expansion of *M. cerifera* thickets has also resulted in a substantial increase in community LAI on Virginia's barrier islands (MAP: 1065 mm). LAI of swales dominated by the grasses *Ammophila breviligulata* and *Spartina patens* was ~1.5 compared to an LAI of ~12.5 in adjacent shrub thickets (Steven Brantley, unpublished data; Brantley and Young 2007). The high LAI causes a substantial reduction in understory light in thickets. Understory PAR ranged from 10% of incident PAR in older sites to 0.7% in the youngest site (Brantley and Young 2007). The decrease in light availability in newly formed thickets has eliminated resident grasses and imposed severe limitations on cover and diversity of herbaceous vegetation. Although studies of shrub thickets on Hog Island and in Kansas have shown a dramatic decline in available light (Lett and Knapp 2003; Brantley and Young 2007), sampling regimes in these studies were too coarse to account for the contribution of sunflecks. Although sunflecks are an extremely heterogeneous resource, they often account for a substantial proportion of total understory PAR (Chazdon 1988; Neufeld and Young 2003). While the availability of sunflecks has been recognized as an important driver of

understory diversity in forests (Chazdon 1988; Neufeld and Young 2003), little work has been done to determine the importance of sunflecks in shrub-dominated systems. Additionally, the distribution and frequency sunflecks can vary among communities due to differences in canopy architecture, even if mean light availability is similar (Nicotra et al. 1999). Therefore, there is a need to link spatial and temporal patterns of understory PAR to canopy architecture of shrub thickets and adjacent forests to predict how shrub thickets will affect future recruitment of understory plants compared with other woody systems.

My objective is to describe the changes in resource availability and ecosystem function after shrub expansion. Specifically, I will link patterns of decreasing light availability and increasing nutrient availability in shrub thickets to changes in canopy structure and tissue composition. The study will be carried out in four parts: 1) Determine the effects of shrub expansion on the input of C and N across a chronosequence of shrub thicket development; 2). Quantify edaphic factors, including soil organic matter, N content, and soil CO_2 flux, across a chronosequence of shrub development; 3)..Quantify fine-scale spatial and temporal variability of sunflecks in shrub thickets to determine how the high LAI of *M. cerifera* thickets affects the availability of PAR compared to temperate forest; 4) Link understory light, foliage distribution and canopy architecture across a variety of shrub and tree species to link canopy structure to fine-scale light availability.

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CHAPTER TWO

SHIFTS IN LITTERFALL AND DOMINANT NITROGEN

SOURCES AFTER EXPANSION OF SHRUB THICKETS

Steven T. Brantley and Donald R. Young[1]

Abstract

Woody encroachment into herbaceous ecosystems is emerging as an important ecological response to global change. A primary concern is alterations in C and N cycling and associated variations across a variety of ecosystems. We quantified seasonal variation in litterfall and litter N concentration in *Morella cerifera* shrub thickets to assess changes in litterfall and associated N input after shrub expansion on an Atlantic Coast barrier island. We also used the natural abundance of ^{15}N to estimate the proportion of litterfall N originating from symbiotic N fixation. Litterfall for shrub thickets ranged from 8991 ± 247 to 3810 ± 399 kg ha^{-1} yr^{-1} and generally declined with increasing thicket age. Litterfall in three of the four thickets exceeded previous estimates of aboveground annual net primary production (ANPP) in adjacent grasslands by 300-400%. Leaf N concentration was also higher after shrub expansion and, coupled with low N resorption efficiency and high litterfall, resulted in a return of as much as 169 kg N ha^{-1} yr^{-1} to the soil. We estimated that ~70% of N returned to the soil was from symbiotic N fixation resulting in an ecosystem input of between 37 and 118 kg ha^{-1} yr^{-1} of atmospheric N depending on site. Considering the extensive cover of shrub thickets on Virginia barrier islands, N fixation by shrubs is likely the largest single source of N to the system. The shift from grassland to shrub thicket on barrier islands results in a substantial increase in litterfall and foliar N concentration that will likely have a major impact on the size and cycling of ecosystem C and N pools. Increasing C and N availability in these nutrient-poor

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soils is likely to permanently reduce cover of native grasses and alter community structure by favoring species with greater N requirements.

Introduction

Woody plant encroachment in historically herbaceous ecosystems has been documented for a variety of ecosystems and is emerging as a key area in the study of global change (Archer et al. 1995; Wessman et al. 2004; Briggs et al. 2005; Sturm et al. 2005). The global nature of this phenomenon has led many to argue that expansion of woody plants is linked to global phenomena such as warming or atmospheric CO_2 enrichment (Archer et al. 1995). While climate warming appears to be a key factor facilitating woody plant expansion in arctic and alpine systems (Sturm et al. 2005), Archer et al. (1995) makes a compelling case against the CO_2 enrichment hypothesis and effectively argues that regional factors, such as changes in fire regime and grazing pressure are directly linked to woody encroachment. However, on barrier islands along the Virginia, USA coast, increases in atmospheric CO_2 appear to be the only trend in global change that would favor woody expansion (Young et al. 2007). Virginia barrier islands lack the history of land management observed in arid and semi-arid systems discussed throughout Archer et al. (1995), yet have experienced rapid rates of woody encroachment in the last 60 years, even in the presence of rising sea-level (Young et al. 1995; Young et al. 2007). While a further synthesis of existing data is necessary to better determine the role of CO_2 enrichment on woody encroachment, the phenomenon does not follow traditional successional models

of disturbance and recovery and could be viewed as a state transition induced by persistent global change (Briggs et al. 2005; Young et al. 2007).

While the extent of changes in woody abundance has been described for a variety of systems (Goslee et al. 2003; Briggs et al. 2005; Sturm et al. 2005; Young et al. 2007), quantifying impacts of shrub expansion on ecosystem properties is more difficult due to spatial and temporal complexity and the extended time-scale over which shifts in vegetation occur (Wessman et al. 2004). One of the few opportunities to study long-term consequences of shrub expansion is provided by accreting shorelines on barrier islands, which result in large variations in community age over relative small spatial scales (Hayden et al. 1991). Fluctuations in island size and shape are induced by natural changes in currents that affect erosion and deposition of sand and change shoreline position, often quite rapidly (Hayden et al. 1991). Subsequent colonization by dune-forming grasses is an expected outcome of this pattern but, from 1949 to 1989, Hog Island, a barrier island along the Virginia, USA coast also experienced a 400% increase in shrub cover following expansion of the northern end of the island (Young et al. 1995). While an increase in shrub abundance in this system has generally been viewed in the context of primary succession, shrub expansion on Virginia's barrier islands is not related directly to increases in upland area and shares many characteristics with the broader global trend of woody encroachment (Young et al. 2007).

One of the primary drivers of plant community composition and primary productivity on barrier islands is availability of nutrients, especially N (Art et al. 1974; Ehrenfeld 1990; Stalter and Odum 1993). As a consequence, the dominant woody species on many barrier islands of the southeastern United States is the nitrogen-fixing shrub *Morella cerifera* (Young 1992; Young et al. 1995). Commonly known as wax myrtle, *M. cerifera* is well adapted to low nutrient coastal soils (Young 1992). A symbiotic association between members of Myricaceae (which includes the genera *Morella* and *Myrica*) and the actinomycete *Frankia* assures an adequate source of N (Morris et al. 1974; Vitousek and Walker 1989; Young et al. 1992). Furthermore, the evergreen leaf habit aides in nutrient conservation by allowing plants to retain and transport other foliar nutrients, including phosphorus, more efficiently (Monk 1966; Killingbeck 1996). These characteristics, along with high growth rates and bird-dispersed seeds, have enabled *M. cerifera* to form dense, nearly monospecific stands on islands that are otherwise dominated by herbaceous vegetation (Young et al. 1995; Kwit et al. 2004).

Changes in ecosystem function after shrub expansion, especially with regards to C and N cycling, are often quite substantial (Vitousek et al. 1987; McCarron et al. 2003; McCulley et al. 2004; Hughes et al. 2007). Shrubs and other woody vegetation in grasslands act to reduce soil erosion, subsidize nutrient inputs by intercepting atmospheric inputs and serve as a nutrient reservoir, especially in sandy and/or low-nutrient soils (Garcia-Moya and McKell

1970; Vitousek et al. 1987; Joy and Young 2002). Furthermore, shrub expansion is often accompanied by substantial changes in annual net primary production (ANPP) and changes in tissue chemistry that affect both litter quality and quantity (McCarron et al. 2003; McCulley et al. 2004; Hughes et al. 2007). For example, Briggs et al. (2005) observed a consistent trend of increased leaf N concentration when shrubs replaced grasses, especially when expanding shrubs were nitrogen-fixers. Although N conservation is an important strategy for most plants in nutrient poor soils, nitrogen-fixing species are often less proficient in resorption of N from senescing parts than other species and often contribute substantial N to the soil through litterfall (Killingbeck 1996; Sprent et al. 1978; Permar and Fischer 1983; Uliassi and Ruess 2002). Increased litter N concentration can be expected to increase rates of litter decomposition and increase N availability in soils thereby changing community dynamics (Mellilo et al. 1982; Permar and Fischer 1983; Aber et al. 1990; Ulery et al. 1995; Berg et al. 1996). Previous estimates of soil N beneath *M. cerifera* shrub thickets and in soils without *M. cerifera* were 791 ± 195 µg/g and 321 ± 14 µg/g, respectively (Young et al. 1992).

In addition to quantifying shifts in C and N cycling, it is also useful to determine principal sources of ecosystem N inputs. Previous studies have attempted to quantify atmospheric N_2 fixation by stands of nitrogen-fixing plants by scaling up from acetylene reduction assays that measure nitrogenase activity (Permar and Fischer 1983; Vitousek et al. 1987; Uliassi and Ruess 2002).

However, spatial and temporal patterns of nitrogenase activity in root nodules are complex and highly variable and attempting to extrapolate assay results to annual N_2 fixation in natural ecosystems is unreliable (Shearer and Kohl 1989; Halverson et al. 1992; Sande and Young, 1992). Shearer and Kohl (1989) and Halverson et al. (1992) suggested that measurements of N fixation using the natural abundance of ^{15}N in plant tissues are more integrative and, therefore, more accurate.

To understand the impact of woody encroachment on C and N cycling, changes in litterfall and associated N inputs must be quantified and dominant sources of N determined. Our primary objectives were to quantify variations in litterfall and litter N concentration of four *Morella cerifera* shrub thickets representing a chronosequence of shrub expansion. These data were compared to previously measured values of aboveground ANPP and foliar N of adjacent grasslands to determine how shifts in dominant growth form affect litterfall C and N inputs into the system. Furthermore, we examined seasonal trends in litterfall and litter N concentration to assess temporal variation of C and N return throughout the year. Finally, we used the natural abundance of $\delta^{15}N$ to estimate the fraction of N in *M. cerifera* tissues that originated from actinomycete-induced N fixation.

Materials and Methods

Study site-Field work was conducted from April 2004 to November 2006 on the northern end of Hog Island (37° 27' N, 75° 40' W), a barrier island located ~10

km east of the Eastern Shore of Virginia, USA. Hog Island is ~1200 ha, 10 km

long and 2.5 km across at its widest point. The island is part of the Virginia

Coast Reserve, managed by The Nature Conservancy, and is an NSF-funded

Long-Term Ecological Research site. The northern end of the island has been

accreting ~5 m yr^{-1} for ~140 years resulting in a chronosequence of progressively

older soils as one moves west across the island from the ocean shoreline

(Hayden et al. 1991; Shao et al. 1998). As the island has expanded, a series of

dense thickets, dominated by the shrub *Morella cerifera*, has developed with

thicket age increasing with soil age. Thickets now cover ~40% of the upland

area on the island (Young et al. 2007). Four thickets in order of increasing age

are the *Colonizing thicket* (8 yrs), the *Young thicket* (15 yrs), the *Mid-Island*

thicket (25 yrs), and the *Bay Side thicket* (45 yrs). Adjacent grasslands are

dominated by perennial grasses: *Spartina patens* and *Ammophila breviligulata*

(Dilustro and Day 1997).

Experimental procedure-Ten sites in each thicket were randomly selected and a

plastic litter trap, ~0.30 m^2 in area and 0.15 m deep, was placed at each site in

April 2004. Litter was collected every 6 weeks from April 2004 to May 2005;

however, the final sampling period was ~12 weeks due to logistical difficulties

associated with traveling to the island. Litter was dried at 70 °C for four days,

separated into leaf, woody and reproductive (i.e. fruits and flower parts)

components, and weighed to the nearest 0.1 g. To analyze litter N

concentration, ten leaves were selected from each thicket for each of three

collection periods (May, September and January). Additionally, five samples
each of woody and reproductive litter, taken throughout the year, were analyzed
for each thicket. All samples were ground before analysis in a Wiley mill with a
40 mesh screen. Nitrogen concentration was determined as a percentage of dry
weight using the Pregl-Dumas pure-oxygen combustion method (Perkin-Elmer
2400 elemental analyzer, Wellesley, MA, USA). Resorption efficiency of foliar N
was determined for each thicket as a percentage of fresh leaf N concentration by
comparing N per unit area of fresh leaves collected during September 2006 from
sites adjacent to litter traps and leaf litter collected during November 2006.
Nitrogen content was converted to a weight per unit area basis using values of
specific leaf area (leaf area per unit leaf weight) for fresh leaves and subsequent
litter for each site (Steven Brantley unpublished data). Leaf area was determined
as described in Yavitt and Young (1987).

Percent of N from fixation was estimated using the ^{15}N natural abundance
method as described by Schearer and Kohl (1989). In September, 2006, fresh
leaves were collected from non-nitrogen fixing species (hereafter referred to as
'non-fixers') growing within and immediately adjacent to shrub thickets. Non-
fixers were selected based on location (particularly with respect to elevation) and
rooting characteristics and included *Baccharis halimifolia* (also a shrub) and
Rubus sp. Fresh leaves were also collected from each *M. cerifera* thicket, and
from *M. cerifera* seedlings that rely primarily on N fixation (hereafter referred to
as 'fixers') due to severe soil N limitation (Young et al. 1992). Fresh leaves were

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dried at 70 °C for four days and ground in a Wiley mill with a 40 mesh screen.

Isotopic composition of N was expressed as $\delta^{15}N$ which represents the deviation

from the ratio of ^{15}N:^{14}N for atmospheric N_2. Fractional contribution of biological

N fixation (F_{bfn}) was estimated using the isotopic dilution expression:

$$F_{bfn} = (\delta^{15}N_{soil} - \delta^{15}N_{mix}) \times (\delta^{15}N_{soil} - \delta^{15}N_{atm})^{-1}$$

where $\delta^{15}N_{soil}$ is the isotopic abundance in plants that rely primarily on soil N

('non-fixers'), $\delta^{15}N_{mix}$ is the isotopic abundance in plants that use both soil and

atmospheric fixation (*M. cerifera* thickets), and $\delta^{15}N_{atm}$ is the isotopic abundance

of plants that rely primarily on symbiotic N fixation ('fixers').

Statistical Analysis-Leaf litterfall and leaf litter N concentration were analyzed

using two-way ANOVA to test for interactions between site and season. Post-

hoc comparisons (Tukey) were performed as described in Zar (1999). Data for

woody and reproductive litter N concentration, total litterfall, $\delta^{15}N$ of fresh leaves,

and $\delta^{15}N$ of litter were analyzed by ANOVA and post-hoc tests (Tukey). N

concentration of fresh leaves and leaf litter were compared with Student's t-tests

and also to verify that there was a significant difference in $\delta^{15}N$ between N fixers

and non-fixers. Total content of biologically-fixed N in leaves was estimated as

the product of litter mass, litter N concentration, and the estimated fraction of

fixed N. For all tests, p-values ≤ 0.05 were considered significant. Unless

otherwise noted, all statistics were performed in SPSS 11.5.

Results

      Total annual litterfall (i.e. leaves, woody, and reproductive litter) of *Morella cerifera* varied over two-fold among sites ($F = 50.350$, $P < 0.001$) with the Young thicket producing the most litter and the Bay Side thicket producing the least (Table 1). Leaf litterfall also varied significantly by site ($F = 54.862$, $P < 0.001$); however, there was no significant difference in leaf litterfall between the Young and Colonizing thickets. Higher total litterfall in the Young thicket was primarily due to a higher production of woody litter (Table 1). Reproductive litterfall did not vary among the three youngest thickets but was significantly lower in the Bay Side thicket ($F = 6.135$, $P = 0.002$).

      Although leaf litterfall varied by season ($F = 69.604$, $P < 0.001$), there was a significant interaction ($F = 8.221$, $P < 0.001$) between site and season (Fig. 2.1). Litterfall increased significantly for all thickets from early May to late June which coincides with leaf flush at the start of the growing season. The Mid-Island thicket had the highest leaf litterfall of the four thickets during the late spring litterfall pulse and this was the highest rate observed during the study (34.3 ± 1.7 kg ha^{-1} day^{-1}). Lowest leaf litterfall for all thickets was observed from late June to mid-August. Litterfall increased significantly beginning in mid-August and continued to increase to nearly the same rates observed during May for all sites except the Mid-Island thicket. The Mid-Island thicket experienced a small, though significant, increase in litterfall in late September but leaf litterfall in fall and winter did not approach spring levels.

Litter N concentration, averaged across all sites was 1.68 ± 0.04, 0.79 ± 0.04, and 1.49 ± 0.08 % for leaf, woody and reproductive litter, respectively (Table 1). Nitrogen concentration of woody (F = 1.811, P < 0.186) and reproductive (F = 0.846, P < 0.489) litter did not vary by site. In comparison, N concentration of leaf litter varied significantly (F = 20.837, P < 0.001) by site but did not vary by season (F = 3.251, P < 0.111) (Fig. 2.2). Estimated total N from litterfall (the sum of the product of litterfall and N concentration for all litter types) was highest for the Young thicket (169 kg ha^{-1} yr^{-1}) and lowest for the Bay Side thicket (53 kg ha^{-1} yr^{-1}) (Table 1). Averaged across all sites, 85% of total litter N was from leaf litter, 10% was from reproductive litter and 5% was from woody litter.

Mean N concentration of fresh *M. cerifera* leaves varied significantly among thickets during the growing season (F = 4.802, P = 0.022). Post-hoc tests showed that only Mid-Island (1.75 ± 0.02 %) and Bayside (1.96 ± 0.02 %) thickets differed significantly with neither of those thickets having significantly different values for leaf N than the Young (1.84 ± 0.06 %) or Colonizing thickets (1.86 ± 0.05 %). Overall resorption efficiency of *M. cerifera* for all thickets was 15%; however, this value also varied by site. The Colonizing thicket had the highest resorption efficiency with 26% and resorption declined with increasing soil age (15% in the Young thicket, 10% in the Mid-Island thicket, and 8% in the Bay Side thicket).

Overall, there was relatively little variation in $\delta^{15}N$ among species and among sites (Fig. 2.3). No significant differences were detected in $\delta^{15}N$ among *M. cerifera* thickets (F = 1.178, P = 0.362) so data were pooled across all sites before further analysis. Isotopic composition of the N-limited fixers was -1.2 ± 0.1 $\delta^{15}N$ which compares very well to values observed in other studies for seedlings grown in an N-free medium (Hurd et al. 2005). The difference in $\delta^{15}N$ between non-fixers and fixers was only 0.8. Although small, this difference was significant (t = 2.324, P = 0.036). Using the dilution expression described above with fixers and non-fixers, we estimated that ~70% of foliar N concentration was from actinomycete-induced N fixation. When the fraction of fixed foliar N is factored with total litterfall N content, at least 37 to 118 kg ha^{-1} yr^{-1} of N was fixed by *M. cerifera* thickets depending on age.

Discussion

The influence of shrub thicket expansion on litterfall and associated N input in the barrier island ecosystem was substantial. High productivity of young stands of *Morella cerifera* resulted in annual litterfall that exceeded litterfall reported for other shrub-dominated systems and temperate forests and compared with lower end of values often cited for tropical forests (Gray and Schlesinger 1981; Barbour et al. 1999; Martinez-Yrizar et al. 1999; Norby et al. 2003). By comparison, aboveground annual net primary productivity in grasslands adjacent to shrub thickets ranged from 2260 kg ha^{-1} yr^{-1} to 2740 kg ha^{-1} yr^{-1} (Dilustro and Day 1997). In our study, litterfall alone of shrub thickets

was 1.4-4.0 times greater than grassland ANPP depending on site. Nitrogen concentration of leaf litter from *M. cerifera* was also 1.6-4.6 times higher than N concentration of the two dominant grasses on the island (Dilustro and Day 1997). The coupling of high litterfall and high litter N concentration resulted in large quantities of N cycling through litterfall and explains the large differences in soil N between sites with and without *M. cerifera* previously observed by Young et al. (1992).

Our data are consistent with Uliassi and Ruess (2002), who concluded that the best predictor of ecosystem inputs of fixed N by *Alnus tenuifolia* was leaf area. The primary driver of N cycling in stands of *M. cerifera* was variation in litterfall. Although the Bay Side thicket occupies the oldest, most nitrogen-rich soils on the island (Young et al. 1992), this site consistently had the lowest litterfall of the four thickets while the two youngest thickets produced the most litter. Seasonal differences in litterfall were also observed across the chronosequence. Three of the four sites experienced two periods of increased leaf litterfall during the year: a brief spring pulse coinciding with the beginning of new leaf growth and a longer period of increased litterfall in autumn. However, the Mid-Island thicket did not show a large increase in litterfall during the autumn relative to other thickets, indicating that shrubs at this site retain more leaves throughout the winter. In spring, the Mid-Island thicket had the highest rate of litterfall even though the two younger sites had higher annual litterfall. The ability to retain more foliage through winter may be an important mechanism for nutrient

conservation in two ways. First, retention of older foliage through spring leaf-out may facilitate more efficient translocation of nutrients, such as phosphorus (Monk 1966; Killingbeck 1996), that are required for N fixation but are extremely limiting in the sandy, barrier island soils (Art et al. 1974; Ehrenfeld 1990; Young 1992). Second, abscission of large quantities of nitrogen-rich leaf litter at the beginning of the growing season may reduce N loss from the stand and supplement the N supply during the growing season when nutrients are in highest demand. Most important for this coastal system, continuous litterfall and consistently high N concentrations result in a relatively constant input of organic matter and associated N to the soil rather than a single pulse characteristic for deciduous systems (e.g. Norby et al. 2003). This may be especially significant for N cycling in sandy soils typical of coastal systems where nutrient retention is minimal (Art et al. 1974).

Our results also suggest that a large fraction of foliar N in *M. cerifera* comes from actinomycete-induced fixation of atmospheric N_2. We must qualify this statement based on the slight isotopic differences between fixers and non-fixers. Characteristics inherent to this system make it difficult to distinguish between N fixed by *M. cerifera* and N from other sources. For instance, atmospheric deposition is likely the main source of N for the system where *M. cerifera* is absent and the $\delta^{15}N$ values of nitrates and ammonium (dominant forms of N in atmospheric deposition) have been measured at -1.1 and -0.5, respectively (Russell et al. 1998); very similar to the -1.2 observed for N fixers in

this study. According to Russell et al. (1998), while $\delta^{15}N$ of DON was +5, it accounted for only 13% of total N in wet deposition which, in any event, was relatively low. Furthermore, because soils are relatively young (~5-140 yrs.), enrichment of ^{15}N often observed in better developed soils has yet to occur. Lack of variability in $\delta^{15}N$ signatures from soils is likely the reason we were unable to detect differences among thickets across the chronosequence and as a result, we pooled our site data. Generally, such small differences in $\delta^{15}N$ could be due to natural variations in N fractionation in the plants and would not be considered adequate for the model we used (Shearer and Kohl 1989); however, there were significant differences between the two end members of our model and our data followed the trend we expected.

We should also point out that our estimates are of input of fixed N through litterfall and do not reflect total N fixation because they do not account for fixed N that is incorporated into living stems or belowground structures. Nonetheless, our estimates for annual input of fixed N from litterfall are comparable to estimates of total N fixation for many other actinomycete-plant associations (Hibbs and Cromack 1990) and considerably higher than some estimates for other species within Myricaceae. For instance, Vitousek et al. (1987) estimated that *Myrica faya* contributed 18 kg ha^{-1} yr^{-1} to volcanic soils and Sprent et al. (1978) estimated N loss through litterfall in *Myrica gale* was 30 kg ha^{-1} yr^{-1} for wetlands in central Scotland. Other estimates are more comparable. Bond (1951) estimated *M. gale* fixation at 90 kg ha^{-1} yr^{-1} based on laboratory studies

and Permar and Fischer (1983) used in-field measurements to predict that stands of 100% *M. cerifera* could fix as much as 130 kg N ha^{-1} yr^{-1}. Both studies used acetylene reduction assays to estimate potential N fixation and the latter value was based on an extrapolation from 8% to 100% cover for the sites studied. Laboratory studies of nitrogenase activity in *M. cerifera* seedlings using soils from Hog Island also show very high rates of N fixation (Sande and Young 1992), but extrapolation to island shrub thickets from seedlings grown in environmental chambers would be unrealistic.

Although incorporation of fixed N into other tissues (stems and roots) was not accounted for our study, N content of leaves likely represents a majority of fixed N. While standing wood and belowground tissues represent the largest component of biomass in the system, relative N concentration of these tissues is substantially lower than photosynthetic tissues measured in our study (Conn and Day 1993; Donald Young unpublished data). Furthermore, Halverson et al. (1992) concluded that N from atmospheric fixation in legumes was preferentially directed to photosynthetic tissues while roots contained elevated levels of ^{15}N. Torrey (1978) also reported that N fixed in nodules is rapidly transported to the shoot and that fixed N is primarily returned to the soil through leaf litterfall.

Because shrub thickets now cover a large portion of the island, N fixation and subsequent litterfall in this species may be the single largest source of soil N for this system. Other sources of N for barrier islands include atmospheric deposition and fixation by free-living microbes (Ehrenfeld 1990). However,

neither of these sources is likely to approach our estimation of annual N input by litterfall within shrub thickets (Sprent and Sprent 1990; Meyers et al. 2001). Atmospheric deposition for Hog Island Bay, the shallow lagoon that separates Hog Island from the mainland, has been estimated at ~8 kg ha^{-1} yr^{-1} (Meyers et al. 2001) which is less than 22% of our estimate for shrub litterfall input at the least productive site. Currently, no estimation for free-living microbial fixation exists for Hog Island or, to our knowledge, similar systems and it is difficult to generalize based on current literature because of the wide variation in edaphic factors across the island (e.g. soil moisture, salinity, microbial diversity) (Stewart 1975; Sprent and Sprent 1990). Low P and organic matter content of the sandy soils is likely to limit N fixation by heterotrophic bacteria, and low incident light within shrub thickets and relatively low soil moisture content on dunes may to limit N fixation by cyanobacteria (Stewart 1975; Sprent and Sprent 1990; Young et al. 1992; Brantley and Young 2007).

Previous studies on the consequences of shrub encroachment have shown that effects of shrub expansion on C and N cycling, including changes in C and N storage and soil respiration, vary widely depending on precipitation and/or edaphic characteristics including soil type and size of pre-existing C and N pools (Jackson et al. 2002; Hughes et al. 2006; Wheeler et al. 2007). McCulley et al. (2004) concluded that there was an increase in both soil respiration and ecosystem C and N storage after shrub expansion in subtropical savanna. However, McCarron et al. (2003) measured a significant decrease in soil

respiration and no change in C or N storage in tallgrass prairie. Hughes et al.

(2006) also measured no change in surface soil C and N pools, despite

substantial changes in aboveground C and N. Jackson et al. (2002) concluded

that mesic systems with large soil C pools could serve as a C source after

replacement of grasses with woody vegetation because of increased soil

respiration. Although further work is needed, ecosystem responses on barrier

islands are likely to be greater than in systems with large pre-existing C and N

pools because of young, sandy soils characteristic of the islands.

We show that dense thickets of *M. cerifera* on Hog Island produce a large

quantity of N-rich litterfall that may rapidly increase C and N cycling. Increases in

litter accumulation after thicket expansion, coupled with associated long-term

increase in N inputs, will likely have irreversible effects on species composition

by contributing to reduced cover and diversity of native grasses (Day et al. 2004).

Even where shrubs have declined, thickets have been maintained by continued

shrub recruitment (Brantley and Young 2007). In the absence of major

disturbance, shrubs may be replaced by maritime forest species with higher N

requirements (Ehrenfeld 1990). Perhaps more importantly, when ecosystem N

limitation is mediated by expansion of nitrogen-fixing shrubs in nutrient poor

environments, associated increases in C sequestration may constitute an

important terrestrial sink for atmospheric CO_2 that must be accounted for in

models of global C cycling (Houghton 2003; Woodbury et al. 2007). The

dramatic shift in growth form we observed with barrier island shrub expansion

further underscores the necessity for quantification of these changes on a global

scale.

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Table 2.1 Mean litterfall, total litter nitrogen content, and estimated annual input of fixed N (all in kg ha^{-1} yr^{-1}) from *Morella cerifera* litterfall for four shrub thickets on Hog Island, VA. N concentration, as a percentage of dry weight, is shown in parentheses for each component. All means are shown with one standard error. Significant differences among thickets are noted by superscript letters.

	Bay Side	Mid-Island	Young	Colonizing
Total annual litterfall	3810^d ± 399	6466^c ± 259	8991^a ± 247	7791^b ± 325
Leaf	2732^c ± 323	5179^b ± 191	6853^a ± 274	6702^a ± 226
(%N)	(1.54^b ± 0.14)	(1.38^b ± 0.14)	(2.13^a ± 0.11)	(1.66^b ± 0.12)
Woody	667^b ± 99	477^b ± 104	1249^a ± 148	339^b ± 55
(%N)	(0.84^a ± 0.03)	(0.63^a ± 0.09)	(0.80^a ± 0.12)	(0.90^a ± 0.07)
Reproductive	411^b ± 54	810^a ± 54	888^a ± 54	749^a ± 142
(%N)	(1.33^a ± 0.18)	(1.48^a ± 0.17)	(1.45^a ± 0.16)	(1.67^a ± 0.09)
Total litterfall N content	53	87	169	127
Proportion of fixed N	0.70	0.70	0.70	0.70
Fixed N annual input	37	61	118	89

Figure Legends

Fig. 2.1 Seasonal production of leaf litter for four *Morella cerifera* thickets on Hog Island. Error bars represent ± one standard error

Fig. 2.2 Seasonal variation in N concentration, as a percentage of dry weight, for leaf litter collected from four thickets on Hog Island, VA. Thickets are represented by: BS = Bay Side thicket, MI = Mid-Island thicket, Y = Young, and C = Colonizing

Fig. 2.3 Natural abundance of ^{15}N of foliar N for multiple species from Hog Island, VA, represented as δ^{15}N or the deviation from the atmospheric ratio of ^{15}N:^{14}N. Species represented include known N-fixers, shrub thickets that rely on a combination of soil N and symbiotic N fixation, and plants that lack nitrogen-fixing symbionts
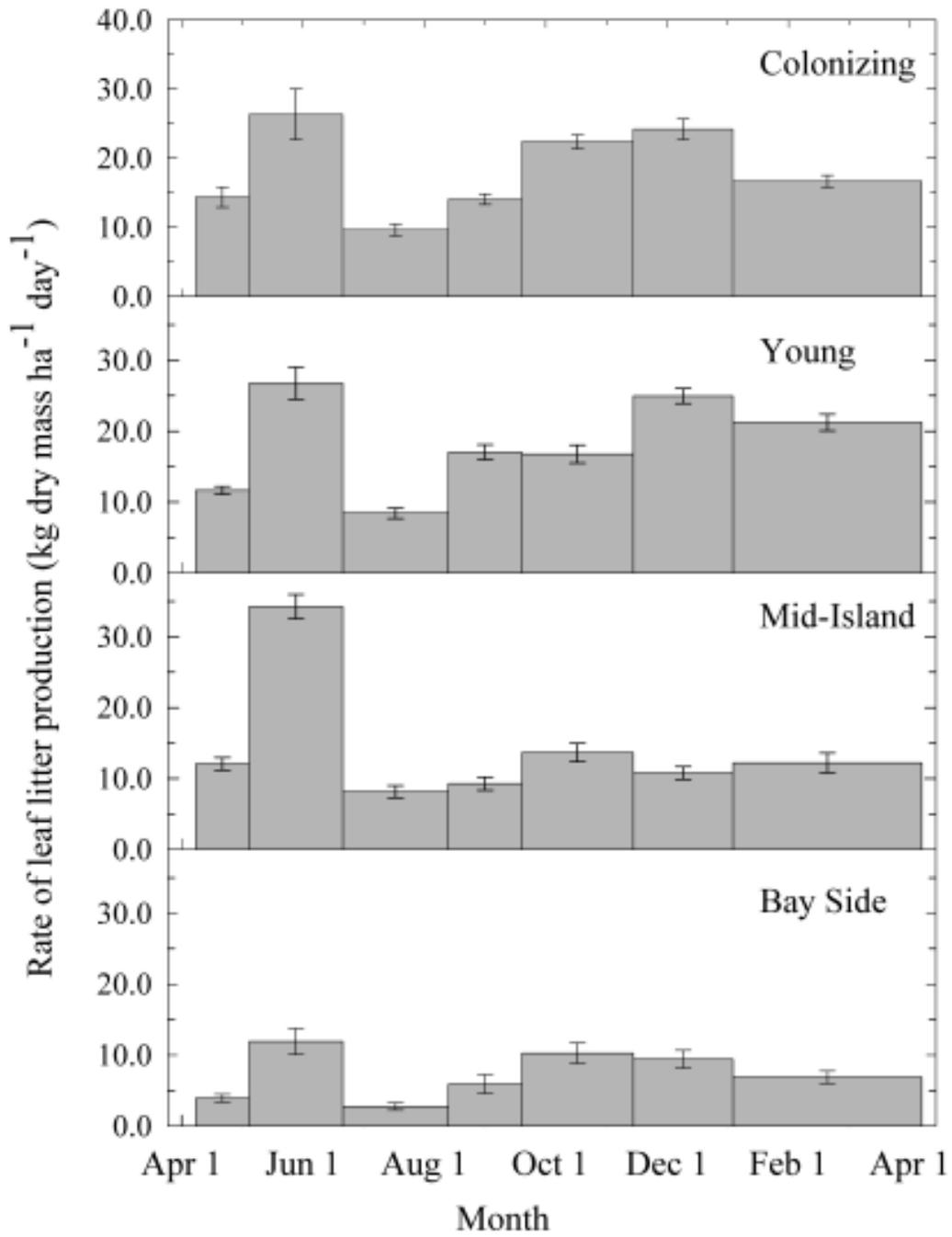
Figure 2.1
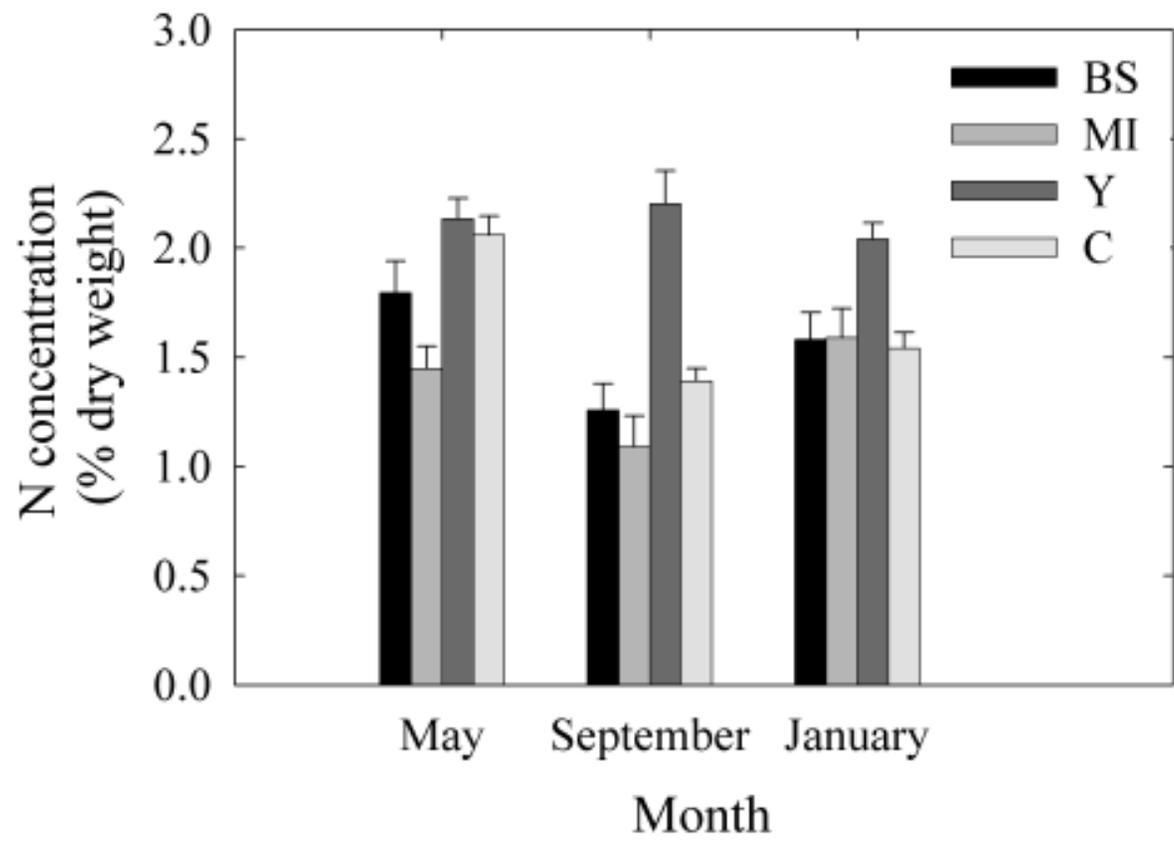
Figure 2.2
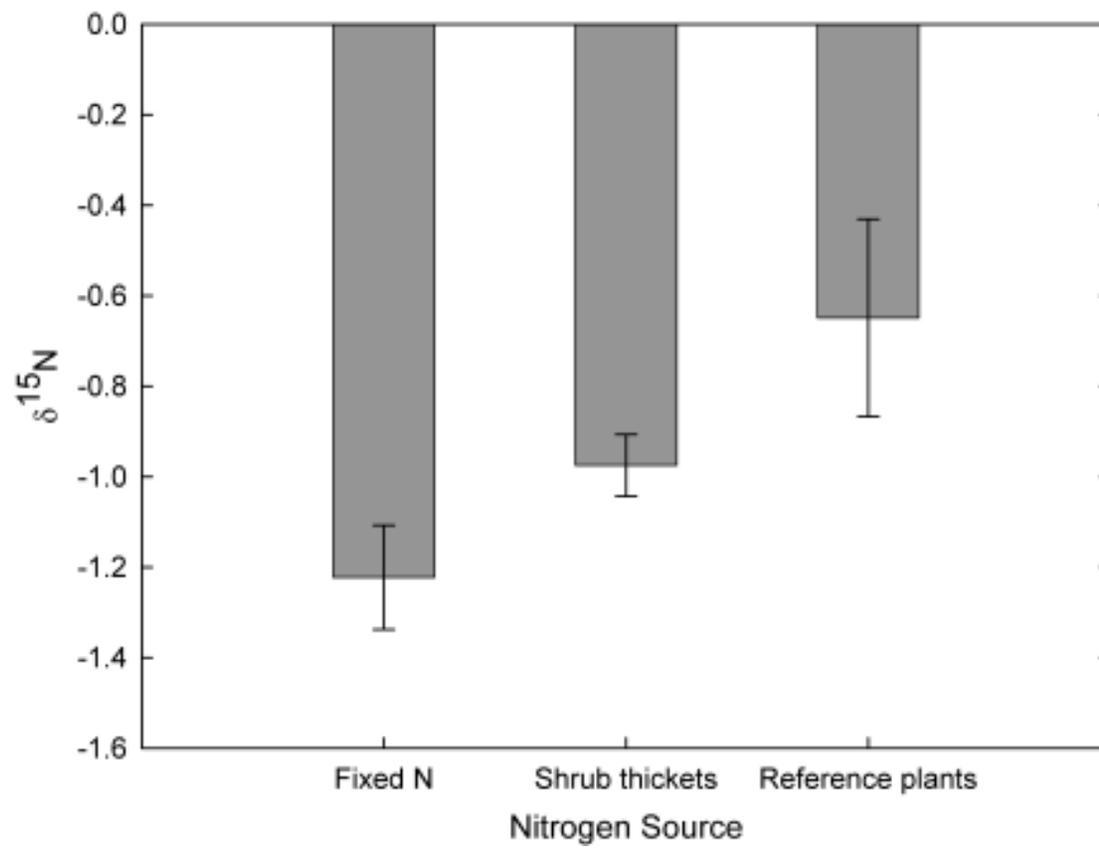
Figure 2.3

CHAPTER THREE

STIMULATION OF SOIL C AND N STORAGE ACROSS A

CHRONOSEQUENCE OF SHRUB EXPANSION IN A MESIC ENVIRONMENT

Steven T. Brantley and Donald R. Young

Abstract

Expansion of woody vegetation in grasslands is a worldwide phenomenon with implications for C and N cycling at local, regional and global scales. Although woody encroachment is often accompanied by increased annual net primary production (ANPP) and increased inputs of litter, mesic ecosystems may become sources for C after woody encroachment because of the stimulation of soil CO_2 flux that releases stored soil organic matter (SOM). Our objective was to determine if a barrier island with young, sandy soils became a source for C or if higher litterfall resulted in increased pools of soil organic carbon (SOC) and soil total nitrogen (TN) after encroachment of the nitrogen-fixing shrub *Morella cerifera*. We measured variations in litterfall in shrub thickets for four years across a chronosequence of shrub expansion. In the final year, we measured soil CO_2 flux, standing litter C and N pools and SOM, SOC and TN in shrub thickets and adjacent relic grasslands. Litterfall in shrub thickets declined with increasing age and annual variation was related to precipitation. At all sites, litterfall in shrub thickets exceeded total aboveground ANPP previously reported in adjacent grasslands by up to 400%. Heavy litterfall resulted in a dense litter layer storing an average of 809 g C m^{-2} and 35.7 g N m^{-2}. Although soil CO_2 flux was stimulated by shrub encroachment in younger sites, soil CO_2 flux did not vary between shrub thickets and grasslands at the oldest sites. Increases in CO_2 flux in shrub thickets were not enough to offset contributions of increased litterfall to SOC. SOC was 3.6-9.8 times higher beneath shrub thickets than in grassland

soils and TN was 2.5-7.7 times higher under shrub thickets. Expansion of shrub

thickets in mesic systems with low levels of soil organic matter has potential to

substantially increase C and N sequestration and storage.

Introduction

Woody encroachment in herbaceous ecosystems represents a key shift in

community structure that has potential to alter regional and global C and N

cycling (Kieft et al. 1998; Jackson et al. 2002; McCarron et al. 2003; McCulley et

al. 2004; Wessman et al. 2004; Briggs et al. 2005; Norris et al. 2007; Strand et al.

2008; Throop and Archer 2008). While woody encroachment is a worldwide

phenomenon, there is considerable uncertainty regarding the effects of woody

encroachment on global terrestrial C storage (Pacala et al. 2001; Houghton

2003; Woodbury et al. 2007). Increased annual net primary productivity (ANPP)

and associated increases in litterfall often accompany transitions from grassland

to shrubland, potentially increasing ecosystem C sequestration and SOC storage

(Kieft et al. 1998; Norris et al. 2001a; Norris et al. 2001b; McCulley et al. 2004;

Brantley and Young 2008; Knapp et al. 2008; Strand et al. 2008; Throop and

Archer 2008). However, increases in C sequestration in woody biomass and soil

organic carbon (SOC) may be offset by associated increases in soil CO_2 flux (i.e.

combined heterotrophic respiration and root respiration) resulting from increased

litterfall, increased soil moisture, and associated increases in microbial activity

that often accompany woody encroachment (Kieft et al. 1998; Norris et al. 2001b;

Jackson et al. 2002; McCarron et al. 2003; McCulley et al. 2004; Sturm et al.

2005; Brantley and Young 2008). In mesic grasslands where soils are rich in organic matter, increased respiration after woody encroachment may result in a shift in ecosystem function that changes ecosystems from C sinks to C sources despite stimulation in ANPP (Jackson et al. 2002).

Although there is often a direct link between annual precipitation and soil C storage in temperate climates, some systems with relatively high levels of precipitation (i.e. >800 mm/yr) do not contain high levels of soil organic carbon (SOC) because of age and/or land use history. For instance, Virginia, USA barrier islands are experiencing high rates of shrub expansion and are characterized by both young/infertile soils and relatively high annual precipitation (~1200 mm/yr) (Ehrenfeld 1990; Young et al. 2007). Because barrier islands are highly dynamic land forms that experience constant deposition and erosion, soils on many islands are generally young (<1000 years old) and characterized by low levels of soil organic matter (SOM), SOC and total nitrogen (TN) (Ehrenfeld 1990; Hayden et al. 1991; Young et al. 1992; Dilustro and Day 1997). Virginia barrier islands have also experienced a large increase in cover of the nitrogen-fixing shrub *Morella cerifera* in the past 40 years (Young et al. 2007). Dense thickets of *M. cerifera* are characterized by high leaf area index (LAI), high litterfall and high N-fixation levels (Brantley and Young 2007, 2008). High litterfall of thickets relative to ANPP of adjacent grasslands (Dilustro and Day 1997), coupled with low-nutrient soils will likely result in substantial increases in ecosystem C and N storage after shrub expansion (Strand et al. 2008).

In addition to stimulation in C storage directly morphological changes that affect ANPP (Knapp et al. 2008), communities where N-fixing shrubs increase in abundance often experience increased accumulation of nitrogen in soils and decreased community nutrient limitation can further enhance SOM storage (Morris et al. 1974; Permar and Fisher 1983; Vitousek and Walker 1989; Schlesinger 2000; Throop and Archer 2008). N accumulation in severely nutrient-limited systems, either through increased anthropogenic N-deposition or N-fixation, may also facilitate further expansion of woody vegetation by favoring tree recruitment (Kochy and Wilson 2005). Although N fertilization in coastal systems is associated with reduced herbaceous diversity, increased soil N may accelerate development of maritime forest, increasing standing biomass and further contributing to storage of C in coastal communities (Morris and Eveleigh 1974; Ehrenfeld 1990; Day et al. 2004).

Our goal was to describe patterns and drivers of SOC and TN sequestration across a chronosequence of shrub expansion on a barrier island. Our primary objective was to quantify SOM, SOC and TN content and relate these data to medium-term (4 years) and long-term (60 year chronosequence) variations in litterfall of expanding shrub thickets. An additional objective was to describe patterns and drivers of soil CO_2 flux as it relates to shrub expansion and soil age. These results will illustrate that broad generalizations about the effects of shrub encroachment on C and N sequestration are not reliable because of

variations in soil age and structure that interact with climate characteristics to make ecosystem responses unique.

Materials and Methods

Study site--Field work was conducted on Hog Island, a barrier island located ~10 km east of the Virginia portion of the DelMarVa peninsula, USA. Hog Island is managed by the Nature Conservancy as part of the Virginia Coast Reserve and serves as an NSF-funded Long-Term Ecological Research site. The upland portion of Hog Island is ~10 km long and 2.5 km across at its widest point with an upland area of ~750 ha. The northern end of the island (37° 27' N, 75° 40' W), has been accreting ~5 m yr^{-1} for ~140 years resulting in a chronosequence of progressively older soils as one moves west across the island from the ocean shoreline with the oldest soils <150 years old (Hayden et al. 1991; Dilustro and Day 1997; Shao et al. 1998). As the island has expanded, a series of dunes has developed running parallel to the shoreline. In the past 60 years, dense thickets of the evergreen shrub *Morella cerifera* have expanded into mesic swales that separate dune ridges. Shrub thickets now cover ~40% of the upland area on the island (Young et al. 2007) and are interspersed with dunes and relic grasslands. Four thickets in order of increasing age are *Colonizing thicket* (12 yrs), *Young thicket* (20 yrs), *Mid-Island thicket* (35 yrs), and *Bay Side thicket* (60 yrs). Adjacent grassland sites were selected to minimize effects of differences in elevation between grasslands and thickets because elevation also affects soil moisture in this system. Grasslands were also labeled *Colonizing*, *Young*, *Mid-*

Island and *Bay Side* in order of increasing age. Vegetative cover (i.e. percent

cover for grasslands and LAI for shrub thickets) generally declines with

increasing thicket age in shrub thickets and increases with age for adjacent

grasslands (Brantley and Young 2007; Steven Brantley, *personal observation*).

Precipitation data for the study period was compiled from meteorological data for

the Virginia Coast Reserve (Krovetz et al. 2008).

Litterfall-- Ten plastic litter traps, each 0.297 m^2 in area and 0.15 m deep, were

placed in each of four thickets on Hog Island in April 2004. From April 2004-April

2005, fresh litter was collected ~every 6 weeks and those data were reported in

Brantley and Young (2008). From April 2005-2008, fresh litter was collected

~every 3-4 mos. After each collection, fresh litter was dried at 70 °C for 4-5 days,

separated into leaf, woody and reproductive (i.e. fruits and flower parts)

components, and weighed to the nearest 0.1 g. Because leaf loss occurs

throughout the year, standing litter was collected before and after the growing

season (May and October) to characterize any potential seasonal variation in

standing litter mass due to seasonal differences in decomposition. A 0.033 m^2

metal cylinder was driven through the litter to the soil surface adjacent to each

litter trap and all litter within the cylinder was collected to the bare soil/humus

layer. Standing litter was dried at 70 °C for four days, sifted in a 2mm sieve to

remove soil particles, and weighed to the nearest 0.1 g.

Soil organic matter --Soil samples were collected in concert with standing litter

collections during October. Additional soil samples were taken at 10 sites in relic

grasslands adjacent to each thicket for a total of 80 samples. After removal of standing litter from each site, the top 10 cm of soil was collected. Soil was dried to a constant weight at 105°C and sifted through a 2 mm sieve to remove large litter particles and fine roots. Bulk density was determined for the <2 mm size fraction as sample mass divided by sample volume. Further fractionation of litter and soil was not considered necessary due to large soil particle size and lack of soil horizon development. Soil organic matter (SOM) was determined for each site using mass loss on ignition. Soil sub-samples (10.00 ± 0.01 g) were placed in aluminum trays and heated in a muffle furnace at 450°C for 4 hr. Samples were weighed again and percentage of mass lost was determined.

Elemental analysis--Standing litter C, standing litter N, SOC and soil TN concentration were determined using a Perkin-Elmer 2400 elemental analyzer (Perkin-Elmer, Waltham, MA, USA). Standing litter collected at the end of the growing season was sub-sampled after weighing and ground in a Wiley mill to pass through a 40-mesh screen. SOC and N content was quantified for 40 sites (4 thickets and 4 grasslands, 5 samples each). Although soil acid treatment is often used before elemental analysis to remove inorganic carbonates and prevent overestimates of SOC, such treatment can result in a reduction in organic C and N at acid concentrations as low as 0.1 M, and loss is greater at higher acid concentrations often recommended (i.e. 6M) (Midwood and Boutton 1998; Harris et al. 2001). Because SOC and TN are already extremely low for island soils, no acid treatments were conducted because of potential for C and N

loss. Rather, eight soil samples from across the island (one per site), that had been treated for LOI and were thus free of organic C, were used as negative controls. All negative controls contained no measurable SOC, returning values of $\leq 0.03\%$ C by weight, which is within the margin of error for the instrument.

Soil CO_2 flux--24 polyvinyl chloride (PVC) soil collars, 10-12 cm deep were placed in soils across the island chronosequence (four grassland sites and four thicket sites, each with three collars). Collars were driven into soils 6-10 cm and no more than 2 cm of the collar extended above the soil surface. For each sample, a custom PVC soil chamber (Davidson *et al*. 2002) with a volume of 2108 cm^3 covering an area of 211.2 cm^2 was mated to each soil collar and connected to a Li-Cor 6200 closed-flow gas exchange system (Li-Cor Biosciences, Lincoln, NE). After scrubbing ambient CO_2 from the chamber, CO_2 concentration was recorded every 30 sec until chamber CO_2 exceeded ambient atmospheric CO_2 (3-7 minutes depending on respiration rates). The change in CO_2 was converted to a flux measurement ($\mu mol\ m^{-2}\ s^{-1}$). Sampling was repeated three times during the year (July, October, and January) to represent variations in soil temperature (T) effects. During each measurement, soil T (at 10 cm depth) and surface soil gravimetric water content (GWC) were also measured. GWC was determined by collecting the top 10 cm of soil, measuring wet mass, drying the sample at 105° C to a constant mass and then calculating the percentage of water from initial and final masses.

Statistical analysis--Analysis of variance (ANOVA) was performed to detect

significant differences in shrub thicket standing litter, SOM, SOC and TN among

sites. ANOVA and Post-hoc tests (Tukey) were also performed for soil

respiration among sites for each sampling period. A two-way ANOVA was

performed on both total annual litterfall and annual leaf litterfall (including annual

production reported in Brantley and Young 2008) to detect significant differences

between thickets and year as well as interactions between the two. Linear

regression was used to quantify relationships between SOM and SOC and/or soil

N. Multiple linear regression was performed to determine which soil metric

(SOM, GWC and soil T) best predicted soil CO_2 flux for each season and

throughout the year. All means are reported with one standard error. All

statistics were performed in SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Litterfall—Mean annual litterfall for all sites was 733 ± 33 g m^{-2}, with associated C

and N inputs of 351 ± 16 g C m^{-2} and 6.9 ± 0.3 g N m^{-2}. Leaf litter varied

significantly among thickets (F = 70.56, p <0.001) and among years (F = 32.65, p

<0.001) and there was a significant interaction between thicket and year (F =

2.46, p = 0.012) (Fig.1). Leaf litterfall declined with increasing thicket age while

annual leaf litterfall for all sites was related to annual and summer (June, July

and August) precipitation (Fig. 1, Fig. 2). Results for total litterfall were different.

Total litterfall varied by year (F = 30.36, p < 0.001) and by thicket (F = 72.53, p <

0.001) but interaction between year and thicket was not significant (F = 1.76, p =

0.080). There was no relationship between total litterfall and annual precipitation but leaf litterfall and both annual precipitation and precipitation from June-August for the previous year were related (Fig. 2). Mean standing litter mass was 1668 ± 43 g m^{-2} and did not vary among thickets or seasons and there was no significant interaction (all p > 0.05) (Table 1). Mean C and N concentration in standing litter was 48.5 % and 2.14 %, respectively and did not vary among thickets (p > 0.05) (Table 1). Standing litter C and N pools were estimated at 809 g C m^{-2} and 35.7 g N m^{-2}.

Soil CO$_2$ flux--Soil CO$_2$ flux varied by site for July (F = 6.30, p = 0.001), October (F = 4.35, p = 0.007) and January (F = 2.73, p = 0.046) (Fig. 3). When data were pooled by community type (i.e. shrub thickets or grassland), mean soil CO$_2$ flux (µmol m^{-2} s^{-1}) was significantly higher in shrub thickets than grasslands during July (5.5 ± 1.1 and 2.0 ± 0.5, respectively), October (3.3 ± 0.6 and 0.7 ± 0.3, respectively) and January (0.8 ± 0.2 and 0.1 ± 0.1, respectively). The *Colonizing* and *Mid-Island* thickets were significantly higher than adjacent grassland sites during July and October; however, in the oldest sites there was no significant difference between shrub thickets and grasslands during any sampling period. Soil CO$_2$ was highest for the oldest site in grasslands but not in shrub thickets (Fig. 3). When all seasonal measurements were pooled, the principle driver of soil CO$_2$ flux was soil T, however the predictive value was relatively poor (r^2 = 0.17, F = 13.83, p < 0.001). Separating pooled seasonal measurements by community improved the relationship of CO$_2$ flux and soil T (Fig. 4). During July,

there was a strong negative relationship between soil T and CO_2 flux (r^2 = 0.62, F = 31.53, p = < 0.001) because warmer soils also tended to contain substantially less moisture, which was the primary driver of soil CO_2 flux during summer (r^2 = 0.52, F = 20.32, p < 0.001) (Fig. 4). Soil moisture was also the best predictor of soil CO_2 flux during October but predictive value was poor (r^2 = 0.17, F = 4.45, p = 0.046). Soil CO_2 flux was primarily related to soil temperature during January (r^2 = 0.21, F = 6.00, p = 0.023). SOM did not predict soil CO_2 flux during any period (all p > 0.05).

Soil C and N pools—SOM, SOC, and TN varied significantly by site (F = 9.13, p < 0.001; F = 5.485, p < 0.001 and F = 4.643, p = 0.001, respectively) and generally increased with age in both shrub thickets and grasslands (Fig. 5). Comparing each thicket to the adjacent grassland, SOM (as a percentage of dry weight) was 1.9 to 9.5 times higher under shrub thickets compared with adjacent in grasslands. Concentrations of SOC ranged from 0.08% in the youngest grassland to 2.01% in the oldest shrub site and SOC concentration was 3.6 to 9.8 times higher under shrub thickets than in adjacent grasslands. Concentrations of TN followed a similar pattern and ranged from <0.01% in the youngest grassland to 0.11% in the oldest shrub thicket. Total N concentrations were 2 to 12.5 times higher under shrub thickets than adjacent grasslands. Soil bulk density did not vary significantly by site or by community when site data were pooled. Mean soil bulk density for all sites was 1.09 ± 0.07 g cm^{-3}. Total

SOC pools ranged from 80 to 2190 g m^{-2} while TN pools ranged from 22 to 334 g m^{-2}, both highest in older sites in either grassland or shrub thicket (Fig. 5).

Discussion

Encroachment of shrubs in mesic grasslands often substantially stimulates ANPP and aboveground C storage (Strand et al. 2008; Knapp et al. 2008), but potential for higher ANPP to increase C sequestration may be mitigated by resultant release of C from rich organic soils often found in mesic grasslands (Jackson et al. 2002). However, in our study increases in litterfall resulting from shrub expansion led to a substantial stimulation in soil C storage relative to adjacent grasslands. Because of logistical limitations, we were not able to measure hourly or daily variations in soil CO_2 flux. Although our soil CO_2 flux measurements are useful for comparison between shrub thickets and grasslands, it would not be appropriate to scale-up our flux readings to estimate annual flux (McCulley et al. 2004). However, increases in soil organic matter indicated that stimulation in soil CO_2 flux from shrub encroachment did not offset increases in C inputs from litterfall and we observed a significant increase in SOC pools after shrub expansion. Unlike other mesic sites with large soil pools of C antecedent to woody encroachment, even the oldest soils in our study sites are likely well below any potential threshold of C storage and enhanced SOC accumulation is likely to continue (Schlesinger 1990; Lichter 1998; Post and Kwon 2000)

Shrub thicket expansion also greatly increased soil N and the N-rich litter layer contributed significantly to overall N pools. Standing litter in shrub thickets had N concentrations 2-4 times higher than standing biomass in grasslands (Dilustro and Day 1997). Increased N availability can enhance C sequestration through stimulation of SOM storage in some soils (Schlesinger 2000; Oren et al. 2001). Accumulation of N beneath shrub thickets will also favor future growth of species with lower nutrient use efficiencies than native grasses, including maritime forest species that could sequester additional C in biomass (Ehrenfeld 1990; Vitousek 2002). Because of sandy soils, there was some question as to whether high rates of N-fixation observed in Brantley and Young (2008) would lead to substantial changes in N pools. Dudley et al. (1996) found that N-fixing plants had little or no effect on growth of neighboring plants and attributed this to rapid leaching of fixed N through sandy soils. Leaching of N in these soils has not been measured but Lajtha et al. (1995) demonstrated that sandy coastal soils have relatively low N retention efficiencies. We did not report rates of N accumulation because we lack specific thicket age data (i.e. the exact year of shrub establishment) for each site and we would have had to account for the SOM accumulation in grasslands prior to shrub establishment. Considering the large annual inputs of C and N from litter, it appears that large amounts of C and N are being leached, especially in the two youngest sites. However, rates of N accumulation appear higher than systems with comparable soil texture and vegetation history (Lichter 1998). Accompanying increases in SOM, and a dense

layer of fine roots that has developed between the litter layer and mineral soil in many older sites (*personal observation*) may have enhanced N retention by limiting water filtration rates, a primary driver of N leaching (Lajtha *et al*., 1995).

Annual variation in leaf litterfall was dependent on thicket age and also varied with precipitation during the previous year. Annual litterfall in shrub thickets was substantially higher than annual litterfall in forests with the same temperature and precipitation regimes (Lonsdale 1988) even after a drought year (2007), indicating that high litterfall previously measured for these shrub thickets (Brantley and Young 2008) is robust with respect to variations in rainfall. Total litterfall was also related to thicket age but relatively high rates woody litterfall in the Young thicket, probably a result of self thinning, reduced the relationship between total litterfall and climate. While thicket age affected litterfall, standing litter mass and associated C and N pools did not vary with thicket age. This suggests that higher litterfall rates in younger sites are coupled with high decomposition rates but decomposition rates in these communities have not yet been evaluated. Although differences in standing litter N content among thickets were not significant, there was a trend towards higher N concentration in younger thickets and this could have contributed to more rapid decomposition of litter.

Soil CO_2 flux also varied by site but there was no discernable pattern in variation among shrub thickets. Soil CO_2 flux increased significantly in the oldest grassland site. Most importantly, we observed no difference in soil CO_2 flux between grasslands and shrub thickets at the oldest sites. Stimulation in soil

CO_2 flux with shrub expansion may be limited to younger sites where cover of grasses is lowest but LAI of adjacent shrub thickets is highest (Brantley and Young 2007). Known drivers of soil CO_2 flux across a variety of habitats include temperature, soil moisture, litterfall and root biomass (e.g. McCarron *et al*. 2003; Hibbard *et al*. 2005; Rodeghiero and Cescatti 2005) and all of these factors except root biomass are directly related to canopy cover. We found a positive relationship between soil T and CO_2 flux when all seasonal data were pooled; however, in July when respiration rates were highest, variation in soil CO_2 flux was dependent on soil moisture, and there was a negative relationship between temperature and soil CO_2 flux. This apparent anomaly was due to the strong negative relationship between soil T and soil moisture because of differences in vegetative cover that affected soil moisture retention but suppressed soil T. This pattern changed during January when soil CO_2 flux was higher under shrub thickets and was positively related with soil T. While some grassland soils were below 0° C, soil T in adjacent thickets was 5-10° C warmer, likely due to insulating properties of a dense canopy and associated litter (Sturm *et al*. 2005; Brantley and Young 2007).

Much of our current understanding about consequences of shrub encroachment is based on studies in arid and semi-arid systems (Asner *et al*. 2003; Wessman *et al*. 2004; Wheeler *et al*. 2007; Strand *et al*. 2008; Throop and Archer 2008). Many of the mesic systems that have been studied also have a history of soil development that has created soils that are rich in organic matter

(McCarron *et al*. 2003; Briggs *et al*. 2005). Mesic ecosystems with young and/or infertile soils may be an underestimated sink for C, especially when the system has experienced significant encroachment of woody vegetation. This potential sink may be even larger if soil nitrogen accumulation is accelerated through symbiotic nitrogen fixation. Although barrier islands are comparatively small in area compared to the large tracts of forest that drive terrestrial C cycles, they have potential to sequester relatively large amounts of C. Other communities that combine a mesic climate, a community with intrinsically high ANPP, and young and/or infertile soils need to be identified to help answer remaining uncertainties about global C budgets.

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Table 1. Standing litter mass, C and N concentration (mean ± one standard error), and estimates of total C and N pools in standing litter beneath *Morella cerifera* shrub thickets. Litter mass, C concentration and N concentration did not vary significantly by site.

Thicket	Litter mass (g m^{-2})	C concentration (% dry mass)	Total C (g m^{-2})	N concentration (% dry mass)	Total N (g m^{-2})
Bay Side	1605 ± 95	47.7 ± 1.0	766	2.02 ± 0.09	32.4
Mid-Island	1697 ± 53	49.1 ± 0.5	833	2.10 ± 0.08	35.6
Young	1665 ± 108	49.8 ± 0.3	829	2.16 ± 0.07	36.0
Colonizing	1695 ± 86	46.5 ± 2.0	784	2.30 ± 0.13	39.0
All sites	1668 ± 43	48.5 ± 0.5	809	2.14 ± 0.05	35.7

Figure Legends

Figure 3.1. Annual total litterfall (leaf, woody and reproductive) (A) and annual leaf litterfall (B) from 2004-2007 in *Morella cerifera* shrub thickets on a Virginia barrier island (mean ± one standard error). Sites in order of increasing age are *colonizing, young, mid-island* and *bay side.* Data for 2004 is presented in more detail in Brantley and Young (2008).

Figure 3.2. Relationship of mean leaf litterfall to annual and summer (June through August) precipitation for *Morella cerifera* shrub thickets on a Virginia barrier island.

Figure 3.3. Seasonal variation in soil CO_2 flux (mean + one standard error) among four *Morella cerifera* shrub thickets and four adjacent grasslands on a Virginia barrier island. Significant differences among sites for July and October are noted with letters. There were no significant differences among sites during January. Sites, in order of increasing age, are *colonizing grassland/thicket* (CG/CT), *young grassland/thicket* (YG/YT), *mid-island grassland/thicket* (MG/MT) and *bay side grassland/thicket* (BG/BT).

Figure 3.4. Relationship of soil CO_2 flux to gravimetric water content during July (A) and to soil temperature for pooled seasonal data (B). Results of simple linear regression are shown for pooled site data in A. Results of simple linear regression for pooled seasonal data for each community in are shown in B. Grassland sites are represented by open symbols and a dashed line in B. Thickets are represented by dark symbols and a solid line in B.

Figure 3.5. Variation in soil organic matter (SOM), soil organic carbon (SOC) and total nitrogen (TN) in the top 10 cm of soil across a chronosequence of shrub expansion on a barrier island. SOC and TN were calculated from SOC and TN concentrations and soil bulk density of 1.09 g/cm^3. Significant differences among sites are noted with letters. Sites, in order of increasing age, are *colonizing grassland/thicket* (CG/CT), *young grassland/thicket* (YG/YT), *mid-island grassland/thicket* (MG/MT) and *bay side grassland/thicket* (BG/BT).
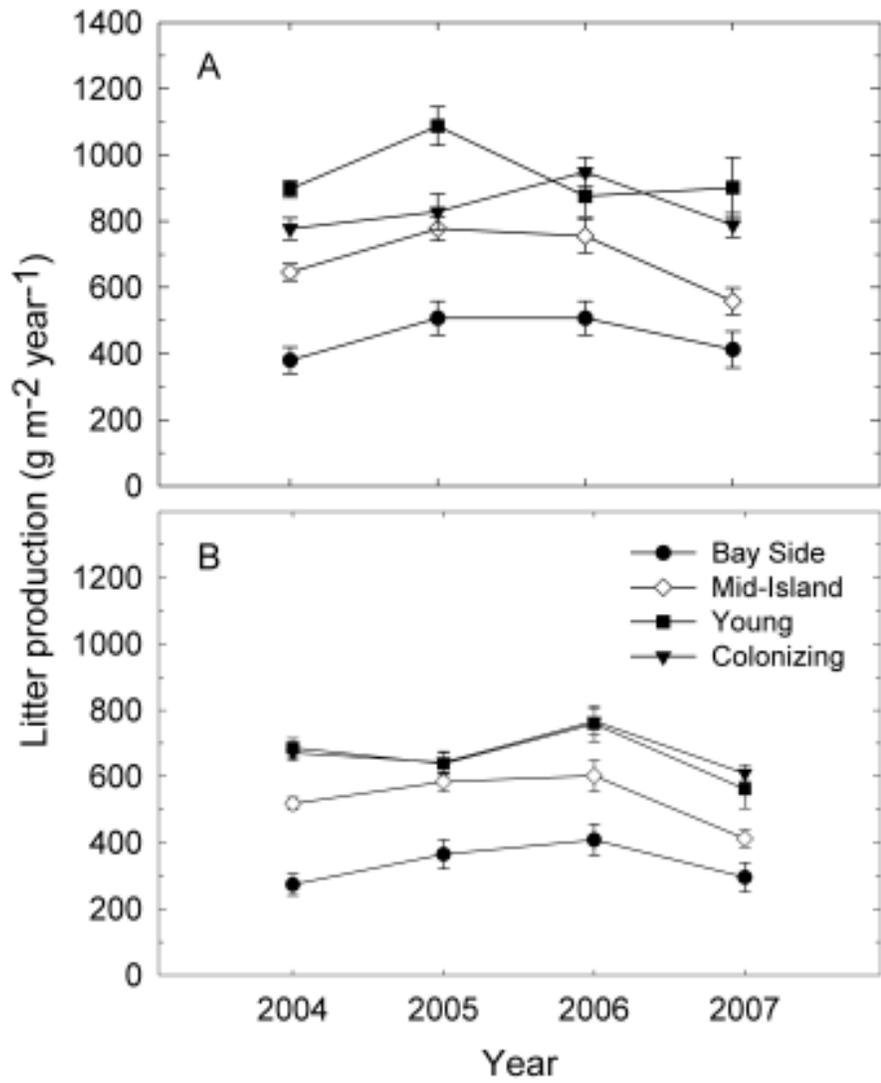
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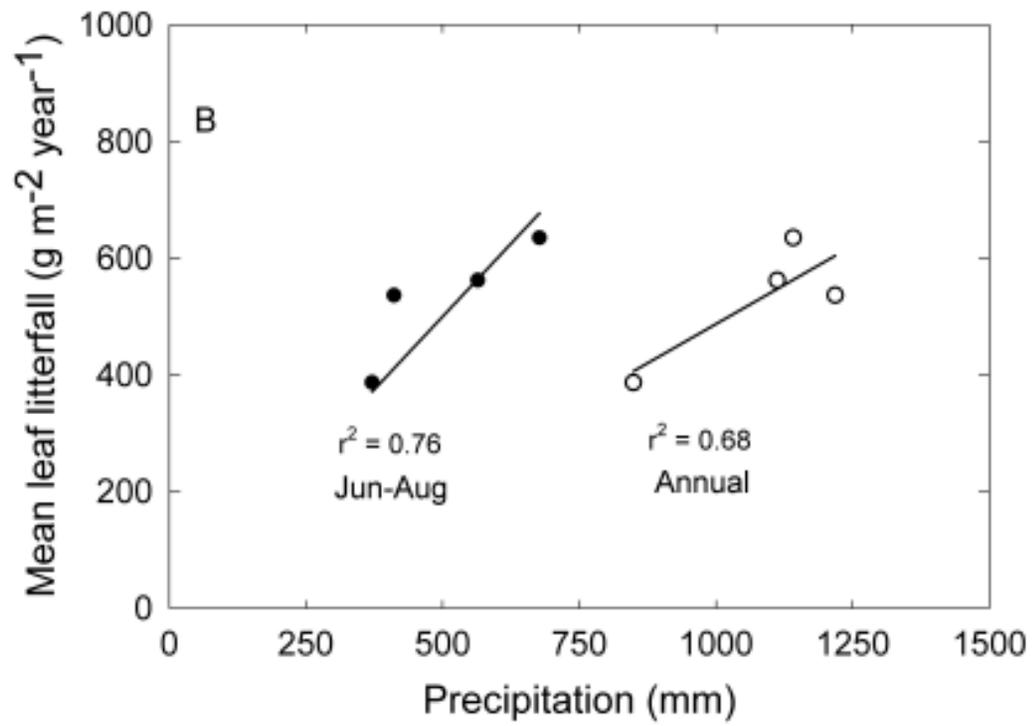
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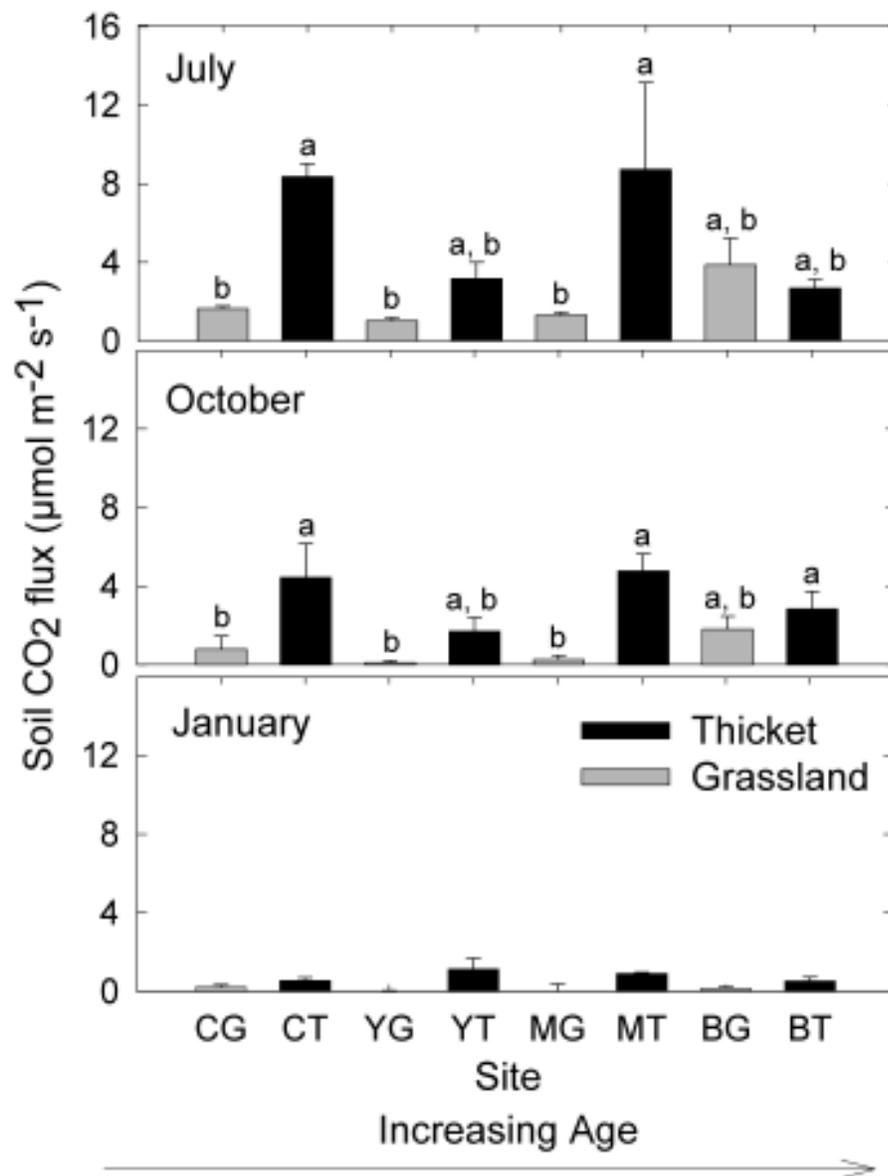
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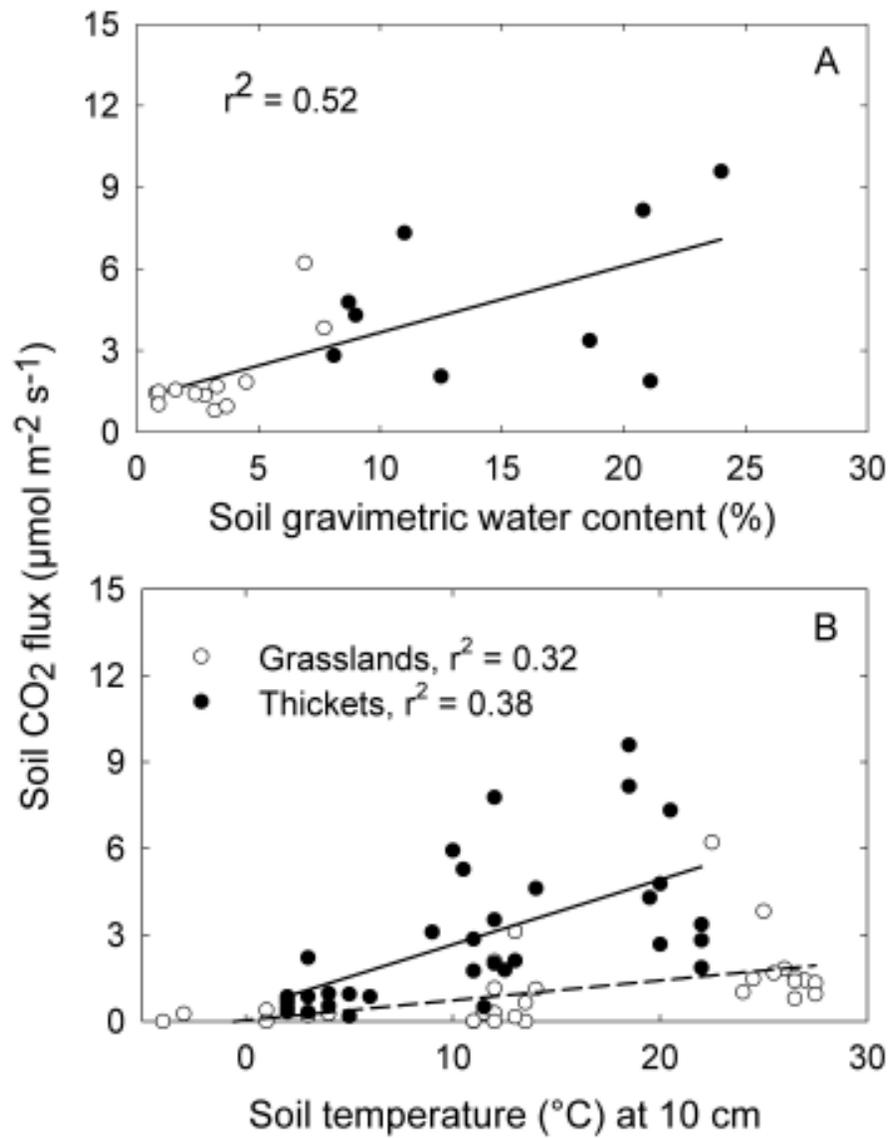
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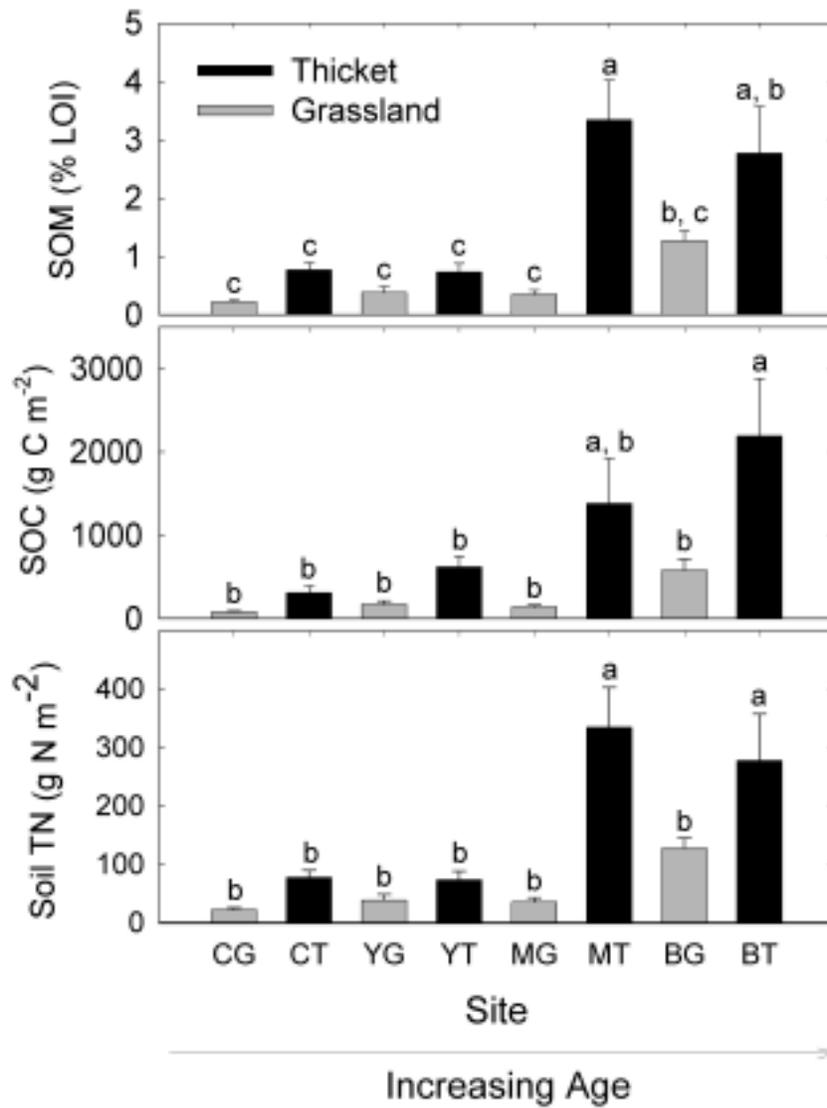
Figure 3.5

CHAPTER FOUR

CONTRIBUTION OF SUNFLECKS IS MINIMAL IN EXPANDING SHRUB

THICKETS COMPARED TO TEMPERATE FOREST

Steven T. Brantley and Donald R. Young[1]

Abstract

Ecological consequences of shrub encroachment are emerging as a key issue in the study of global change. In mesic grasslands, shrub encroachment can result in a five-fold increase in ecosystem leaf area index (LAI) and a concurrent reduction in understory light and herbaceous diversity. LAI and light attenuation are often higher for shrub thickets than for forest communities in the same region, yet little is known about the contribution of sunflecks in shrub-dominated systems. Our objective was to compare fine-scale spatial and temporal dynamics of understory light in shrub thickets to the light environment in typical forest communities. We used an array of quantum sensors to examine variation in diffuse and direct light and determine the relative contribution of sunflecks during mid-day in *Morella cerifera* shrub thickets, a 30 yr-old abandoned *Pinus taeda* plantation and a mature, second-growth deciduous forest. Instantaneous photosynthetic photon flux density (PPFD) was measured at 1 s intervals at five sites in each community during mid-day. In summer, understory light during mid-day in shrub thickets was ~0.8% of above-canopy light, compared to 1.9% and 5.4% in pine and deciduous forests, respectively. During summer, PPFD was uncorrelated between sensors as close as 0.075 m in shrub thickets compared to 0.175 m and 0.900 m in pine and deciduous forests, respectively, indicating that sunflecks in shrub thickets were generally small compared to sunflecks in the two forests. Sunflecks in shrub thickets were generally short (all <30 s) and relatively low in intensity (<150 μmol m^{-2} s^{-1}) and

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contributed only 5% of understory light during mid-day. Sunflecks were longer

(up to 6 min) and more intense (up to 350 µmol m^{-2} s^{-1}) in the two forest

communities and contributed 31% and 22% of understory light during mid-day in

pine and deciduous forest, respectively. The combination of high LAI and

relatively short-stature of *M. cerifera* shrub thickets produces a dense canopy

that reduces both diffuse light and the occurrence of sunflecks. The lack of

sunflecks may limit the number of microsites with a favorable light environment

and contribute to the reduction in understory cover and diversity within the shrub

thickets.

Introduction

Encroachment of native woody vegetation, especially shrubs, into

historically herbaceous communities has been observed in a variety of

ecosystems worldwide (Archer 1989; Briggs et al. 2002; Goslee 2003; Sturm et

al. 2005; Akhalkatsi et al. 2006; Young et al. 2007). Shifts in dominant growth

form result in a variety of changes to ecosystem structure and function including

changes in nutrient cycling (Jackson et al. 2002, Wessman et al. 2004; Briggs et

al. 2005; Wheeler et al. 2007; Brantley and Young 2008) and increased

competition for aboveground resources, especially light (Lett and Knapp 2003;

Brantley and Young 2007). Changes in ecosystem function are strongly linked to

a fundamental shift in foliar chemistry and canopy structure that accompanies

conversion of grassland to shrubland (Briggs et al. 2005; Knapp et al. 2007;

Brantley and Young 2007; Brantley and Young 2008). Changes at the stand

level include increased canopy height and increased leaf area index (LAI), especially in mesic systems (Knapp et al. 2007; Brantley and Young 2007). In regions with mean annual precipitation (MAP) approaching 1000 mm or more, LAI of shrub thickets not only exceeds that in adjacent grasslands, but it also surpasses forest communities in the same region (Brantley and Young 2007; Knapp et al. 2007). Such shifts in LAI result in a substantial reduction in understory light availability and an accompanying reduction in understory cover and diversity (Lett and Knapp 2003; Brantley and Young 2007). For instance, within dense 'islands' of the shrub *Cornus drummondii* in tallgrass prairie in Kansas, USA, available light was reduced to ~5% of above-canopy photosynthetic photon flux density (PPFD) (Lett and Knapp 2003). In shrub thickets of *Morella cerifera*, or wax myrtle, on the Virginia, USA coast, understory light was reduced to as low as 0.7% of above-canopy light during peak LAI (Brantley and Young 2007).

Although a substantial reduction in total light has been observed when shrub thickets establish in mesic systems, fine-scale spatial and temporal dynamics of understory light in shrub-dominated systems have not been investigated. Lett and Knapp (2003) used a ceptometer, which averages PPFD values along a 1 m long linear axis, to describe average understory light. Estimates of understory light from Brantley and Young (2007) were calculated by integrating hourly values based on readings taken every 5 min. These studies provide a valuable comparison of light availability in shrub thickets relative to

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adjacent grasslands; however, the relatively coarse spatial and/or temporal

sampling does not account for fine-scale dynamics of understory light. PPFD

can vary by an order of magnitude within a few centimeters and total daily PPFD

may be completely uncorrelated over distances of less than 1 m (Chazdon 1988;

Baldocchi and Collineau 1994). Such high heterogeneity requires

characterization of finer spatial (e.g. leaf level) and temporal (i.e. seconds to

minutes) scales to fully understand the role of light in community processes

because the availability of microsites with a favorable light environment drives

many key ecological and physiological processes such as seed germination, C

gain and energy balance (Young and Smith 1979, Gross 1982; Chazdon 1988;

Neufeld and Young 2003).

Heterogeneity of understory light is driven by spatial and temporal

variation in the occurrence of brief periods of direct radiation penetrating the

canopy that have alternatively been referred to as sunflecks and sunpatches

(e.g. Young and Smith 1979; Chazdon 1988; Pearcy and Chazdon 1991). Smith

et al. (1989) distinguished between sunflecks and sunpatches, as well as gaps

and clearings, based on optical properties of canopy light penetration as

determined by gap size and canopy height. Generally, sunflecks tend to be

shorter (<10 min), smaller and less intense than sunpatches (10-60 min) (Smith

et al. 1989). Both sunflecks and sunpatches result from an interaction between

solar position and openings in the canopy (Chazdon 1988; Smith et al. 1989);

therefore, canopy structural characteristics, especially foliage density and

distribution, determine the size, duration and intensity of direct light reaching the understory. According to Smith et al. (1989), gap size and distance from the opening to the incident surface determine the quantity of radiant energy that an individual sunfleck contributes to the understory environment. Using this model, one could assume that sunfleck activity would vary substantially between forests and short-stature communities such as shrub thickets.

Although sunflecks generally last only a few seconds to a few minutes, they often contribute substantially to total understory light and have been linked to species distribution and potential C gain in low light environments (Young and Smith 1979; Gross 1982; Pearcy et al. 1994; Pearcy et al. 1997; Neufeld and Young 2003). The importance of sunflecks, a key resource that drives understory ecophysiology, has been described for a variety of forest ecosystems (Hutchinson and Matt 1977; Chazdon and Fletcher 1984; Ustin et al. 1984; Koizumi and Oshima 1993; Horton and Neufeld 1998) but little work has been done in shrub-dominated systems. Lei et al. (2006) showed a reduction in the occurrence and contribution of sunflecks beneath *Rhododendron maximum* shrub thickets, but those sites were in the understory of a *Quercus*-dominated second-growth forest. The high LAI values for mesic shrub-dominated systems and the relatively short canopy of shrubs increases foliage density and reduces the size of canopy openings. Furthermore, Knapp et al. (2007) proposed that the high LAI values observed in mesic shrub thickets can only be maintained through

the "dense and even display of foliage" which would reduce the frequency of large openings because of more efficient space-filling.

Our primary objective was to quantify the fine-scale distribution of understory light in expanding thickets of the broadleaf, evergreen shrub *Morella cerifera* and compare spatial and temporal dynamics of diffuse light and sunflecks in thickets to the light regimes of two temperate forests. A secondary objective was to compare estimates of understory light in shrub thickets from short, intensive sampling of instantaneous PPFD to previous coarser, but longer duration sampling of integrated PPFD. We hypothesize that understory light in shrub thickets is dominated by low diffuse light and that sunflecks are short, small, relatively low in intensity, and contribute relatively little to total available light. We further hypothesize that the relative lack of sunflecks will result in similar estimates for both the coarse and fine-scale sampling regimes.

Materials and Methods

Study site— Shrub thickets were located on the northern end of Hog Island, VA, a barrier island located approximately 10 km east of the Eastern Shore of Virginia, USA. The island is managed by The Nature Conservancy and is part of the Virginia Coast Reserve Long-Term Ecological Research (LTER) site. Mesic sites are currently dominated by dense, nearly monospecific thickets of the actinorhizal shrub *Morella cerifera* (L.) Small (Myricaceae) and are noteworthy for a near-absence of herbaceous vegetation compared to adjacent grasslands (Table 1). Two forest sites were chosen as reference sites and are typical of

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forest communities found in the region. Forest sites include an abandoned pine plantation dominated by *Pinus taeda* and a stand of mature, second-growth deciduous forest dominated by a mix of *Quercus* spp. and *Acer rubrum* (Table 1). The two forest sites, henceforth referred to as *pine* and *deciduous*, are located at the Virginia Commonwealth University Rice Center for Environmental Life Sciences, Charles City County, Virginia, USA. Subcanopy (> 1 m tall but < 10 cm dbh) and herbaceous (<1 m tall) species richness in each community was quantified by counting all species in five 10 m diameter circular plots (Table 1). Stand size was determined using Arc-GIS 9.2 and Digital Ortho Quarter Quads. *Light measurements*—Understory light, measured as instantaneous PPFD, was quantified using an array of eight (thicket) or nine (forest) quantum sensors and three data loggers (190S and Li-1400, respectively, Li-Cor, Lincoln, NE). In shrub thickets, the ninth sensor was used to measure incident PPFD in an adjacent open area. Due to the lack of an adequate clearing at the forest sites, above-canopy PPFD was measured in an open area immediately before and after each sampling period and average above-canopy PPFD for the sampling period was interpolated from those readings. Preliminary sampling was conducted to determine the optimum distance between sensors to adequately describe spatial heterogeneity and the minimum time necessary to accurately quantify spatial heterogeneity and measure the longest sunflecks in each community. Preliminary work included measurement of PPFD every 1 s for ~80 min during mid-day in each community during late spring after full leaf-out.

During three separate sampling periods in each community, sensors were placed at either 1 m, 0.10 m or 0.025 m intervals in each site. From these data, we determined that graduated spacing (from 0.025 m to 5 m) between sensors would be able to capture spatial heterogeneity across the broadest range of distance. These data also showed that most sunflecks were relatively short (< 5 minutes) and that variation in spatial heterogeneity due to sunflecks at a given site could be adequately described with sampling periods as short as 10 min when sampling at 1 s intervals. Based on the aforementioned definition by Smith et al. (1989), who defined sunflecks as periods of direct light lasting less than 10 min; we considered this to be an appropriate scale for sampling sunflecks during mid-day, although we did allow for some additional time to detect longer periods of direct light.

For all subsequent samples, PPFD was measured every 1 s for ~15 min (n ≥ 900). Individual sensors were arranged linearly at graduated distances from 0.025 m to 2 m (thickets) or 5 m (forests). This sampling regime was repeated at ≥ 5 sites in each community located at random distances along a linear transect bisecting the stand. Sites were located at least 30 m apart to avoid replication beneath the same canopy space and all sites were located at least 5 m (thicket) or 30 m (forest) from the edge of the stand to reduce edge effects. All readings were taken on cloudless days within two hours of solar noon to minimize effects of changing solar elevation and sky conditions. Summer measurements were taken from late June to early September and winter measurements were taken

from late January to late February. All field work was conducted from July 2006 to September 2007.

Data Analysis--Although sunflecks have generally been defined based on arbitrary values (e.g. 50 or 100 µmol m^{-2} s^{-1} PPFD), thresholds between the intensity of direct and indirect light varies substantially among communities. For instance, a sunfleck in the understory of tropical forest has a lower PPFD than diffuse light in a temperate forest (Chazdon 1988). Thus, we defined a sunfleck differently for each community based on a visual analysis of raw data. For each sample, we plotted PPFD against time to estimate the upper threshold for background diffuse light for each community. For all subsequent analyses, we defined a sunfleck as any PPFD value, or series of values, that exceeded 25 µmol m^{-2} s^{-1} for shrub thickets, 50 µmol m^{-2} s^{-1} for pine forest, and 100 µmol m^{-2} s^{-1} for deciduous forest. These thresholds were used for both summer and winter. Although the intensity of diffuse light in the understory increased as LAI decreased after the growing season, the increase in diffuse light in the two evergreen communities was slight. In the deciduous forest, the increase in diffuse light was greater, but we maintained 100 µmol m^{-2} s^{-1} as the threshold value for a sunfleck because there was still substantial light attenuation by branches and boles.

For each community, we determined average light attenuation (i.e. understory PPFD as a percentage of above-canopy PPFD), frequency distribution of instantaneous light readings (based on frequency classes with

either 25 or 100 µmol m^{-2} s^{-1} increments depending on site and season) and temporal characteristics of sunflecks, including sunfleck duration. We also determined the maximum intensity of each sunfleck and related this to duration using linear regression analysis after log-log transformation to correct for heteroscedacity. The relative contribution of different sunfleck lengths and total contribution of sunflecks to overall mid-day understory light were also determined as the sum of all PPFD readings that exceeded the threshold for sunflecks minus the contribution of diffuse light. Preliminary data were included in these analyses where appropriate. Spatial heterogeneity of understory light on the horizontal plane was quantified by creating correlagrams relating the Pearson correlation coefficient of simultaneous readings from any two sensors to distance between sensors (Baldocchi and Collineau 1994).

Results

Understory light, as a percentage of above-canopy PPFD, during mid-day in summer was 5.4% for deciduous forest, 1.9% for the pine forest, and 0.8% for shrub thickets. In winter, understory light increased in all communities to 39.0 %, 5.1% and 2.2% of above-canopy light for deciduous, pine, and shrub thickets, respectively, but both evergreen communities were still deeply shaded relative to the deciduous site. The frequency distributions for individual PPFD values during summer were highly skewed for the shrub and pine sites with most values at the lower end of the range (e.g. < 50 µmol m^{-2} s^{-1}) (Fig. 4.1). Frequency distribution in the deciduous forest was also skewed toward lower PPFD values but the most

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frequent readings were in the 75-100 µmol m^{-2} s^{-1} range and no values below 25 µmol m^{-2} s^{-1} were recorded (Fig. 4.1). Overall, 96%, 85%, and 68% of values were below the established threshold for sunflecks in shrub thickets, pine forest and deciduous forest, respectively. Frequency distributions were very similar in summer and winter for both pine and thicket communities, but there was a slight shift toward higher PPFD (Fig. 4.2). In winter, frequency of PPFD in the deciduous forest showed a large shift toward higher values and had a bimodal distribution with peaks around 150 and 1000 µmol m^{-2} s^{-1} with each peak representing areas of bright diffuse light and direct light.

Average diffuse PPFD for the three communities was 76, 24, and 13 µmol m^{-2} s^{-1} for deciduous, pine and shrub communities, respectively during summer. Sunflecks were a major component of total understory light for the forest communities, contributing 22% and 31% for deciduous and pine forest, respectively (Fig. 4.3). In shrub thickets, sunflecks contributed only 5% of total understory light. The relative contribution of direct light during winter increased substantially for all three communities to 82%, 47% and 29% for deciduous, pine and shrub communities, respectively (Fig. 4.3). Temporal dynamics of sunflecks had a strong effect on the relative contribution of direct light in each community. There was a strong, positive relationship between sunfleck duration and maximum PPFD for all three communities with longer sunflecks generally characterized by more intense light (Fig. 4.4). As a result, while short sunflecks (e.g. < 30 s) were most common in all three communities (Fig. 4.4), longer, more

intense sunflecks accounted for a larger proportion of overall understory light in both forest communities (Fig. 4.5). In shrub thickets, no sunflecks longer than 30 s were observed and the maximum PPFD observed during summer was 149 μmol m^{-2} s^{-1}. By contrast, the maximum duration of any sunfleck in pine forest was > 6 min and the maximum PPFD recorded during that period was 268 μmol m^{-2} s^{-1}. A similar result was observed in deciduous forest where maximum sunfleck duration was ~3.5 min and maximum PPFD during that period was 323 μmol m^{-2} s^{-1}. Maximum PPFD for any sunfleck during summer was 498 μmol m^{-2} s^{-1} (~27% of above-canopy light) recorded in the deciduous forest.

Fine-scale spatial patterns of understory light also demonstrated differences among communities (Fig. 4.6). Mean correlation coefficients between the closest sensors (0.025 m) were 0.28 ± 0.09, 0.65 ± 0.07 and 0.95 ± 0.01 in shrub, pine and deciduous communities, respectively during summer. The distance where correlation coefficients between sensor pairs averaged ≤ 0, henceforth referred to as d_0, was 0.075 m for shrub thickets. For pine and deciduous forest, d_0 was 0.175 m and 0.900 m, respectively. During winter, d_0 for pine forest increased to 0.500 m showing that individual sunflecks were larger while d_0 for deciduous forest decreased to 0.300 m. Spatial heterogeneity in shrub thickets also increased during winter. Sensor pairs had an average correlation coefficient of 0.01 at 0.025 m and -0.05 at 0.050 m indicating that average sunfleck size had been reduced.

Discussion

The lack of sunflecks in shrub thickets is likely a key factor limiting diversity and cover of vegetation within the thicket understory. The low diffuse light we observed demonstrates that sunflecks should be an important resource for understory plants in all three communities, especially in the pine forest and shrub thickets where understory light was generally <30 μmol m^{-2} s^{-1}, even at mid-day (Larcher 2001; Neufeld and Young 2003). Diffuse light in the pine forest was comparable to that in shrub thickets at some sites and lower than that reported for *Pinus taeda* in other studies (e.g. Sinclair and Knoerr 1982; Sampson and Allen 1998). All light measurements reported here were taken at the forest floor, where the dense understory of deciduous shrubs and saplings under the pine canopy also contributed to light attenuation. Diffuse light in the deciduous forest exceeded the light requirements for most shade-tolerant plants and shade-adapted leaves (Larcher 2001), perhaps reducing the importance of sunflecks for C gain in that community, but large sunpatches could still be an important driver of species distribution due to species-specific responses related to energy balance and/or water relations (Young and Smith 1979). Sunflecks are probably a key resource in the pine forest where they contribute substantially to total light at mid-day, despite very low diffuse light.

Spatial and temporal dynamics of sunflecks are tightly coupled and distinguishing among the effects of sunfleck size, intensity and duration is difficult because the three parameters are inherently correlated (Smith et al. 1989).

Although intensity and duration are relatively easy to measure and compare, quantifying and representing variations in patch size among communities is difficult. An adequate description of larger sunflecks may be possible through direct observation and measurement of visible patches. However, this type of sampling would not account for brief sunflecks, or for areas that exceed background diffuse light but are not easily observed as direct light due to penumbra effects (Smith et al. 1989). Additionally, sunfleck size and shape are often irregular and heterogeneous through time because solar path and sky conditions change daily (Chazdon 1988). Analysis of spatial heterogeneity provides a means to describe spatial characteristics of sunflecks and serves as a surrogate for sunfleck size because it represents what Baldocchi and Collineau (1994) termed the "integral length of scale."

Spatial heterogeneity varied among communities for summer and winter. Shrub thickets had both the shortest duration sunflecks and the shortest d_0, indicating that most sunflecks were small. Although sunfleck frequency increased in winter, d_0 decreased for shrub thickets indicating that average sunfleck size decreased. This response may result from two factors. First, solar elevation is much lower in winter (e.g. ~35 v. 76 degrees for this location) which increases the effective canopy depth since light must penetrate the canopy at an angle. Secondly, the ~50% reduction in LAI during winter (Steven Brantley, unpublished data) may have resulted in the formation of additional, but smaller, canopy openings which would reduce the average sunfleck size. In summer, the

few sunflecks that were present were likely the result of comparatively fewer, larger canopy openings. The pine forest showed a different seasonal trend with d_0 increasing in winter. The small d_0 in summer showed that most sunflecks were relatively small. Although the longest sunflecks were observed in the pine forest, most sunflecks in this community were < 60 s. However, the few sunflecks that were > 60 s contributed substantially more to understory PAR at these sites during mid-day because of their length and increased intensity and are likely a key factor in maintaining understory diversity based on the values observed for diffuse light. The increased d_0 in winter indicated that the average size of canopy openings increased. This effect is attributed to leaf loss by deciduous shrubs and saplings in the understory rather than a change in LAI of the pine canopy. LAI in *Pinus taeda* canopies declines by as much as half in winter (Sampson et al. 2003), but this would not result in larger patches as evidenced by the results from the shrub thickets. Montgomery (2004) found that heterogeneity of understory light was affected by complex interactions of canopy, subcanopy, and herbaceous vegetation rather than being directly affected by the forest canopy.   Our results in the pine forest support Montgomery's conclusion.

The deciduous forest had the longest d_0 of the three communities during summer and the majority of direct beam radiation came from larger sunflecks lasting from 30-120 s. In the deciduous forest, d_0 decreased during winter despite a large increase in total light after leaf fall. In this case, seasonal differences in spatial heterogeneity were caused by a functional reverse in the

patches that determine spatial heterogeneity—i.e. areas of shade against a background of direct light in winter acted as patches in the same way that sunflecks act as patches against low diffuse light in summer. Therefore, d_0 would increase during leaf fall as sunflecks became larger until some threshold where direct light becomes dominant in the understory and d_0 is then driven by patches of shade from interception by the remaining, leafless canopy. Differences in understory vegetation between the two forest communities were not accounted for in this study; however, the presence of understory trees and shrubs in both forests affected both diffuse light and sunfleck activity. In a Costa Rican rainforest understory, shrub and sapling density, but not tree density or basal area, had a significant effect on light at the forest floor (Montgomery and Chazdon 2001). In our study, a more deliberate selection of sites based on variations in understory structure might have reduced variation within communities and helped explain differences in seasonal variation between forests.

As with any study attempting to quantify understory light, adequate spatial and temporal sampling frequency is essential if useful data are to be collected (Baldocchi and Collineau 1994). The primary limitation on sample size in the current study was the memory of the data-loggers which limited total sampling time to ~80 min per day when sampling at 1 s intervals with three sensors attached to each data-logger. The data presented here represent ~350,000 individual PPFD values, yet there are still gaps in some areas of the analyses

that would have benefited from additional sampling, especially in the forests where sunfleck activity was more variable. Previous estimates of light in the same shrub thickets (Brantley and Young 2007) were based on ~2500 PPFD values measured over two weeks. Despite differences in sampling frequency and periods sampled (i.e. mid-day v. all day), estimated understory light in shrub thickets was comparable to values (0.8% v. 0.7%) reported in Brantley and Young (2007), indicating that little information was overlooked in the former study because of the coarse sampling regime. This contrasts with the findings of Chazdon and Fletcher (1984) who determined that significant errors in total understory PPFD estimates can occur if sampling intervals are too coarse to detect brief sunflecks. The similarity in results between the two shrub thicket studies was due to the relative lack of sunfleck activity observed in the latter and these results have little relevance to forest communities unless they also lack significant sunfleck activity. In the two forest sites, a coarser sampling regime for understory light would have overlooked significant sunfleck activity and underestimated total light.

The reduced occurrence of sunflecks in dense shrub thickets relative to temperate forests may help explain the lack of diversity in the thicket understory because diffuse light is too low to support further recruitment and growth of herbaceous vegetation extant on the island. Other factors that could inhibit recruitment in this community, such as allelopathy, have also been observed (Tolliver et al. 1995). However, significant increases in diversity beneath larger

canopy gaps suggest that these factors are less inimical when light limitation is removed or reduced (Crawford and Young 1998). Further work is needed to determine whether other shrub-dominated systems exhibit the same lack of sunfleck activity observed in *M. cerifera* thickets or if variations in canopy structure among thickets of different species result in concurrent variations in understory light and associated community structure. Few authors have compared sunfleck dynamics across communities to link stand-level sunfleck dynamics to understory diversity and even less work has focused on understory light beneath shrub thickets. Showing a direct link between canopy structure, light availability and understory diversity is difficult, but such work might uncover novel links between canopy structure and community structure for a relatively underrepresented growth form. More importantly, it would help predict the magnitude and direction of shifts in community structure that accompany shrub encroachment and aide in management of ecosystems where shrub cover has increased.

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Table 4.1. Summary of site characteristics for three woody communities in Virginia, USA.

Stand characteristics	Shrub thicket	Pine forest	Deciduous forest
Lat./Lon.	37° 26' 50" N, 75° 39' 40" W	37° 19' 50" N, 77° 11' 50" W	37° 19' 50" N, 77° 12' 05" W
Size (ha)	15	36	27
Age (yrs)	12[a]	29[b]	80-150 [b]
Dominant canopy species	*Morella cerifera*	*Pinus taeda* [c]	*Quercus* spp./*Acer rubrum* [c]
Subcanopy richness (mean ± S.E. spp./plot)	0	4.8 ± 1.7	9.5 ± 1.0
Herbaceous richness (mean ± S.E. spp./plot)	0.4 ± 0.2	9.5 ± 2.5	4.5 ± 0.7

Sources: *a* Young et al., 2007; *b* Gaston, 2002; *c* Donald R. Young, unpublished data

Figure Legends

Figure 4.1. Frequency histograms for instantaneous PPFD measurements for three temperate woody plant communities in Virginia, USA during summer. Readings were taken every 1 s for ≥15 min during mid-day. Distributions represent pooled data from sensors at five sites in each community divided into 25 μmol m^{-2} s^{-1} classes.

Figure 4.2. Frequency histograms for instantaneous PPFD measurements for three temperate woody plant communities in Virginia, USA during winter. Readings were taken every 1s for ≥15min. during mid-day in summer. Distributions represent pooled data from sensors at five sites in each community divided into 25 or 100 μmol m^{-2} s^{-1} classes. Note that the scales for instantaneous PPFD differ between the top two figures and the bottom figure.

Figure 4.3. Total PAR and relative contribution of sunflecks and diffuse light for three temperate woody plant communities in Virginia, USA. Sunflecks were defined as any reading, or series of readings, that exceeded thresholds of 25, 50 and 100 μmol m^{-2} s^{-1} for shrub thickets, pine forest, and deciduous forest, respectively.

Figure 4.4. Relationship of sunfleck duration (t) and maximum PPFD (Q_{max}) for three temperate woody plant communities in Virginia, USA. Values for r^2 represent results of linear regression analysis after log-log transformation to correct for heteroscedacity; $p < 0.001$ for all three regressions.

Figure 4.5. Relative contribution of sunflecks of different durations for three

temperate woody plant communities in Virginia, USA during summer. The inset

in the top figure shows the same data at a smaller scale on the x-axis.

 Figure 4.6. Spatial heterogeneity of PPFD measured across a linear transect for

three temperate woody communities during summer (left) and winter (right).

Data represent mean correlation coefficients ± 1 standard error. Note that,

although measurements were taken to 2 m (shrub) or 5 m (forest), only the first 1
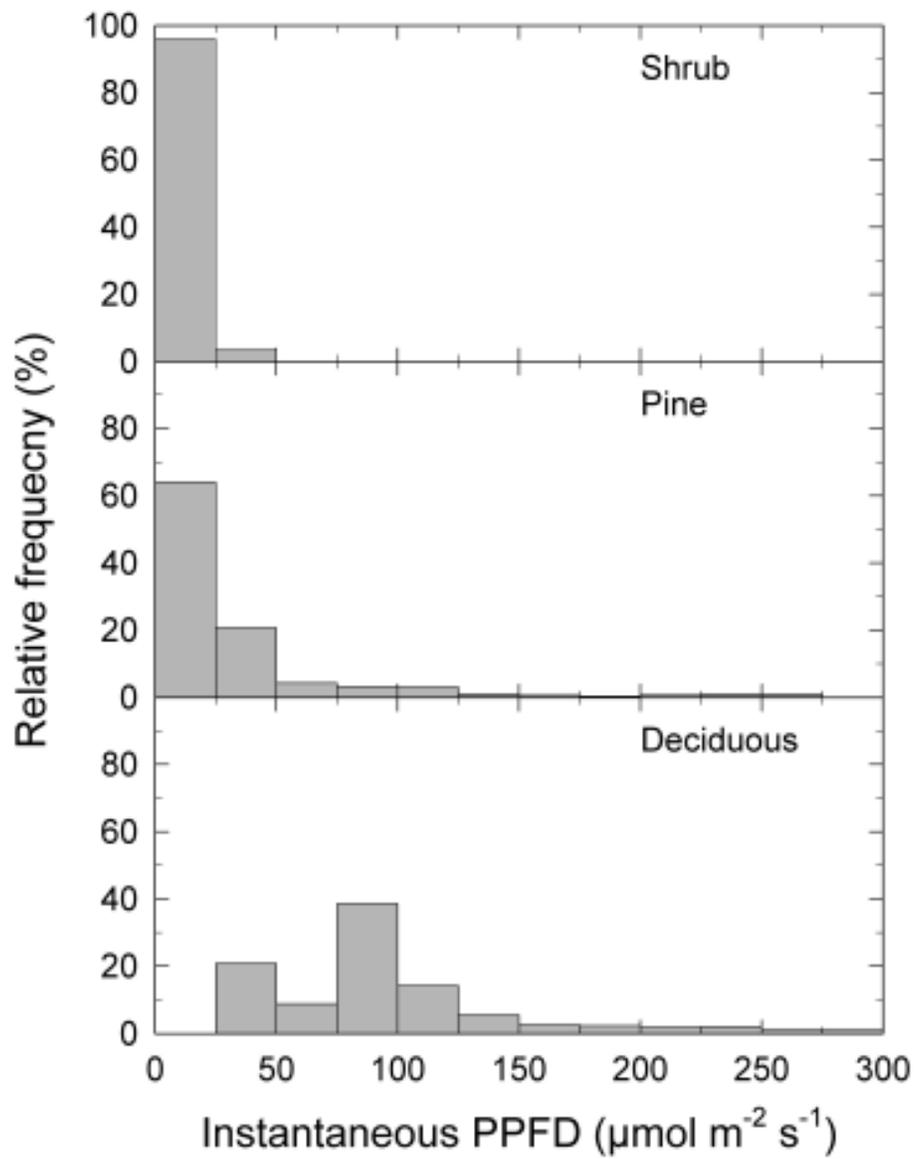
m is presented because of the low values beyond that distance.

Figure 4.1
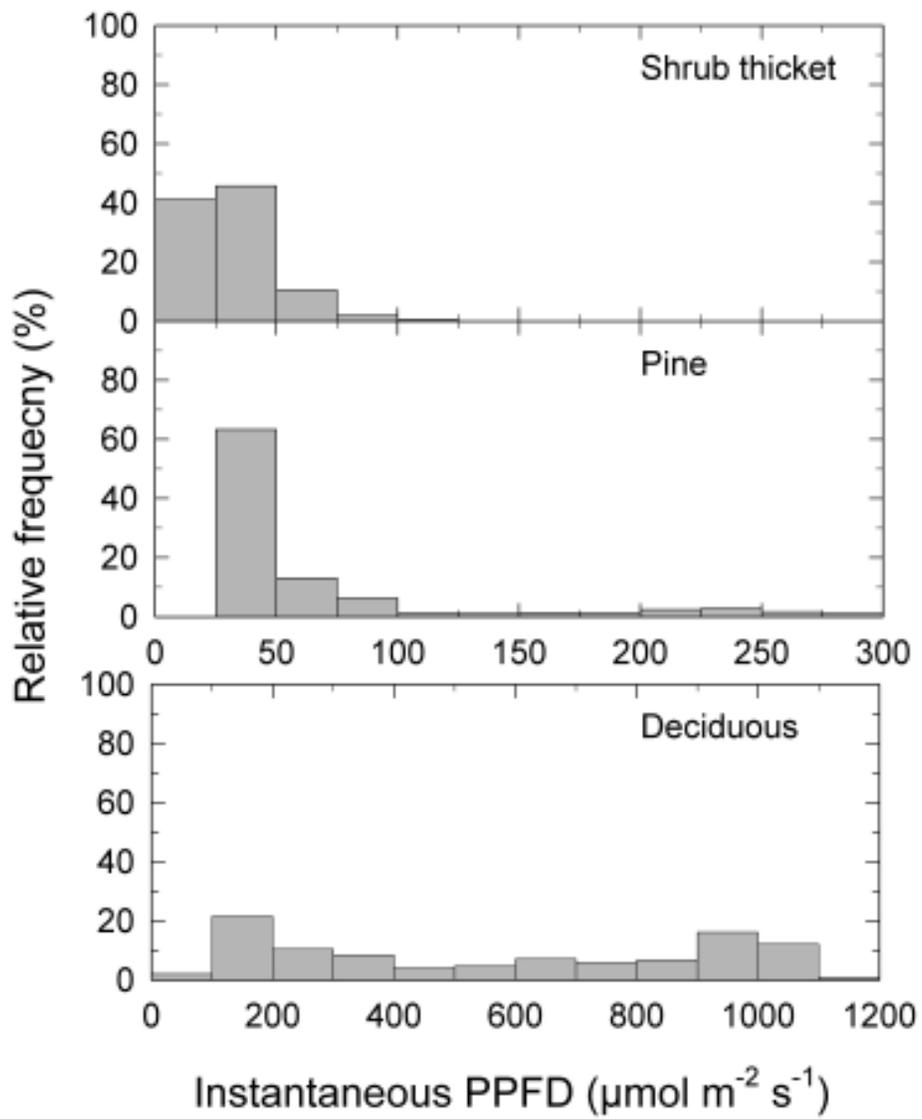
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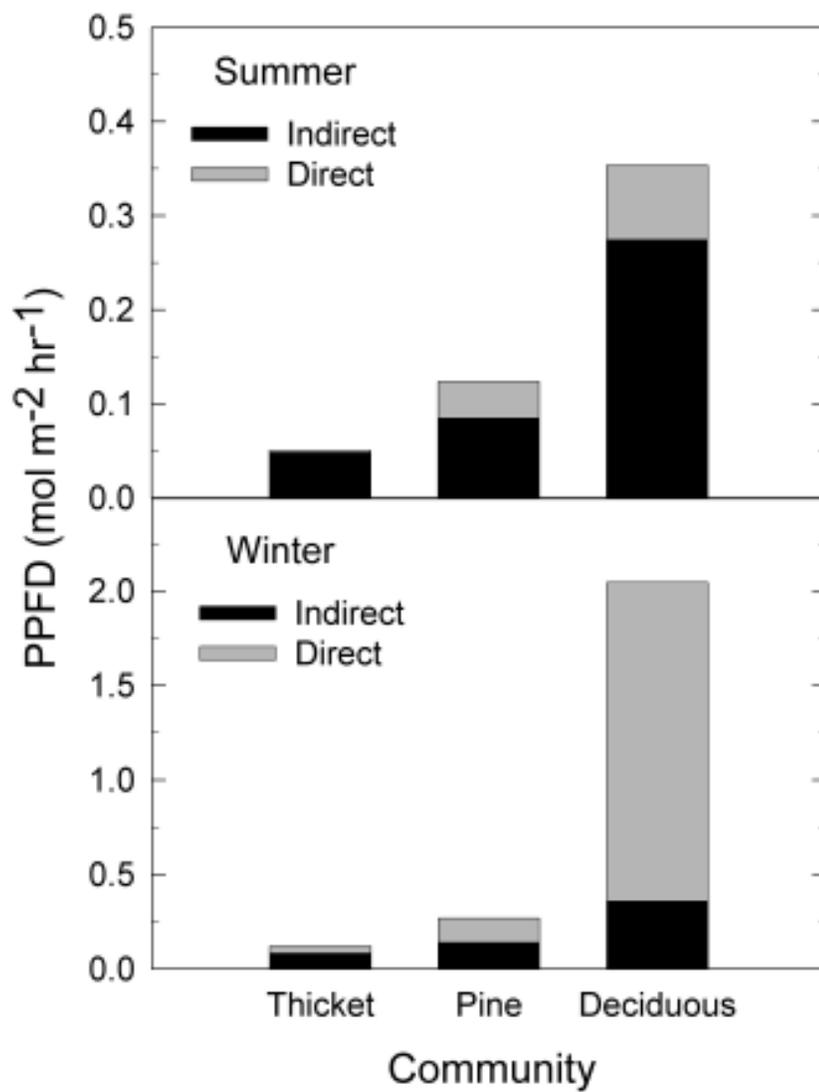
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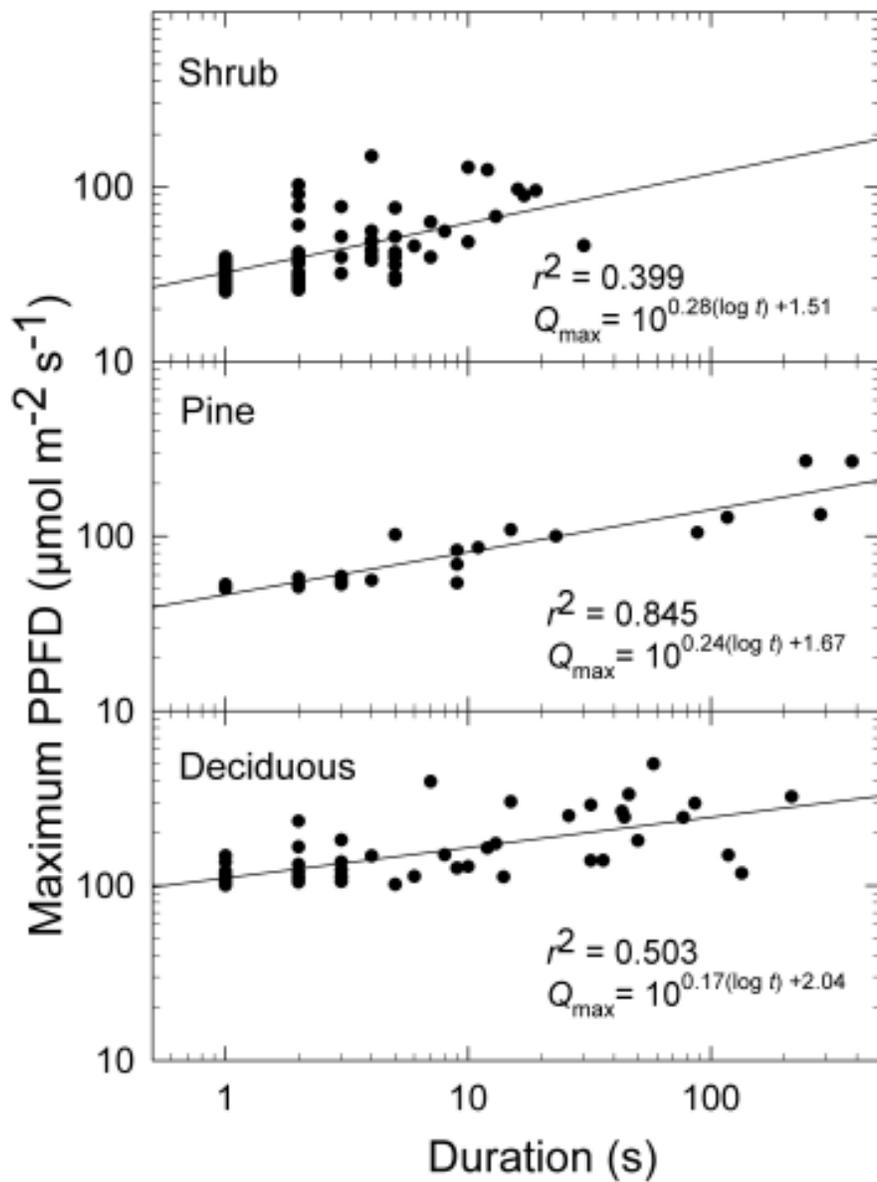
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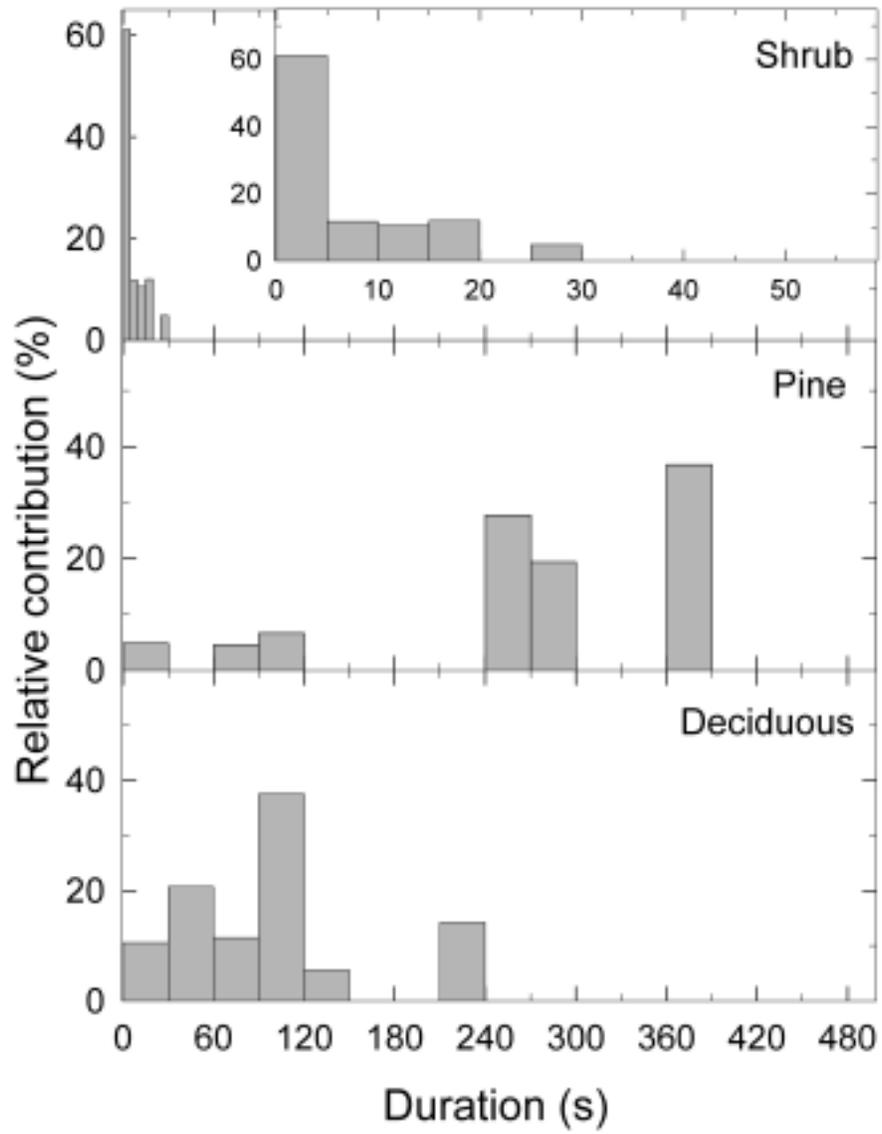
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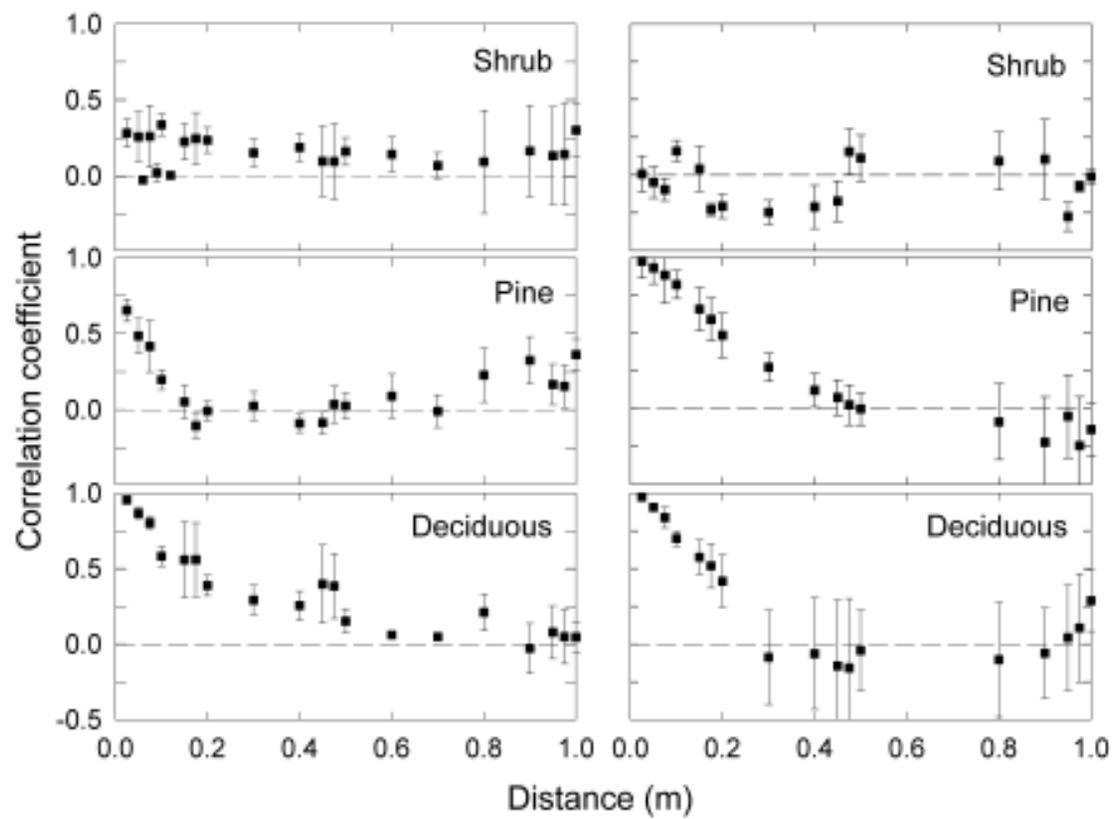
Figure 4.6

CHAPTER FIVE

LINKING LIGHT ATTENUATION, SUNFLECKS AND

CANOPY ARCHITECTURE IN MESIC SHRUB THICKETS

Steven T. Brantley and Donald R. Young[1]

Abstract

Expansion of shrubs into grasslands is often accompanied by a substantial reduction in understory light and an associated reduction of shade-intolerant species. Light in shrub thickets is reduced to levels below that found in adjacent forests; however, effects of specific architectural characteristics on light attenuation and penetration of sunflecks in shrub thickets are unknown. Our objective was to determine what characteristics of canopy architecture most influence understory light in five communities dominated by monospecific shrub thickets.   We quantified understory light and canopy architecture for a variety of native and introduced shrub species that have a history of expansion and we used stepwise multiple regression to determine which canopy characteristics best predicted total light attenuation and relative contribution of sunflecks. Measurements included leaf angle and azimuth, branch bifurcation ratio, leaf area index (LAI), canopy depth and leaf area density (LAD) as well as photosynthetic photon flux density (PPFD) measured every 1 s during mid-day. The best predictor of light attenuation and relative contribution of sunflecks for all species was canopy depth, or the vertical distance from the bottommost leaf to the top of the canopy.   Leaf and plant-level measurements were highly species-dependent and several fine-scale characteristics were correlated with canopy depth. Although LAI for the invasive shrub *Elaeagnus umbellata* was lower than LAI for *Myrica cerifera* or *Rhododendron maximum*, understory light and contribution of sunflecks in *E. umbellata* thickets was lowest for the five species

examined here. *E. umbellata* thickets exhibited the greatest canopy depth of the five communities and this species had branch bifurcation ratios far higher than the other species while also displaying horizontal leaf angles and leaf azimuths with a directional bias towards the south. The potential of shrubs to intercept light and alter aboveground resource gradients is highly dependent on fine-scale, species-specific variations in leaf display and vertical distribution of leaves in the canopy.

Introduction

Expansion of both native and non-native woody plants, especially shrubs, has been identified as an important component of global change in the past century (Archer 1989; Wessman et al. 2004; Briggs et al. 2005; Sturm et al. 2006; Knapp et al. 2008). Changes in ecosystem services that accompany woody plant expansion include altered ecosystem hydrology, increased rates of nutrient cycling and storage, and changes in herbaceous diversity and cover (Wessman et al. 2004; Briggs et al. 2005; Knapp et al. 2008; Brantley and Young 2008). Woody encroachment may also result in a substantial increase in annual net primary production (ANPP), especially in mesic systems where reduced meristem limitation after shrub encroachment results in a substantial increase in LAI (Lett and Knapp 2003; Brantley and Young 2007; Knapp et al. 2008). For example, in tallgrass prairie in Kansas, expansion of *Cornus drummondii* has resulted in dense patches of shrubs with LAI of ~11 (Lett and Knapp 2003) and on the Virginia coast, LAI of *Morella cerifera* exceeded 12 in recently established

thickets (Brantley and Young 2007). By comparison, LAI in temperate deciduous forest typically reaches a maximum of ~6 (Bolstad et al. 2000; Norby et al. 2003). On the Virginia coast, the high LAI has resulted in <1% of available light reaching the understory in newly established shrub thickets and the very low understory light has been associated with a substantial decline in herbaceous cover and diversity in the understory (Brantley and Young 2007; Brantley and Young 2009)

Knapp et al. (2008) proposed that mesic shrub thickets could maintain high leaf area through a "dense and even" display of leaves. In forests, canopy architecture, which includes variation in leaf display, is a major factor influencing interception of radiation by a stand with a given LAI (Teh et al. 2000); however, studies comparing canopy architecture among shrub species and linking canopy architecture to understory light beneath shrubs have not been published. Monsi and Saki (1953) modified the Beer-Lambert law to explain differences in light attenuation based on LAI and an extinction coefficient, termed k. While this model of light attenuation is useful to make rapid estimates of LAI, it has several limitations. The model is most accurate with even sky conditions, which can be problematic under all but the heaviest cloud cover (Rosenberg 1974). Furthermore, k values can vary substantially with canopy architecture and are usually either estimated based on previous literature or estimated empirically, which requires *a priori* knowledge of LAI through direct measurement (Gower et al. 1999; Brantley and Young 2007). In addition, accuracy of the Beer-Lambert

equation in predicting LAI depends heavily on stand characteristics that can vary with site and age, even for the same species (Brantley and Young 2007).

The Beer-Lambert equation also does not account for the influence of brief periods of direct light, or sunflecks. The ecological importance of sunflecks to understory communities is well-established (see Chazdon 1988; Neufeld and Young 2003). Sunflecks create a heterogeneous environment in forest understories that is important in maintaining understory diversity, but heterogeneity varies with canopy architecture (Ustin et al. 1984; Montgomery and Chazdon 2001; Montgomery 2004; Lei et al. 2006; Brantley and Young 2009). Brantley and Young (2009) concluded that sunfleck frequency, length and intensity were all reduced in shrub thickets compared to temperate forests, although diffuse light was often similar. They hypothesized that differences in heterogeneity of understory light among communities was related to stand-level differences in canopy architecture, such as stem density and leaf area density (LAD), that affect distribution and size of canopy gaps. Differences in canopy height alone could also be associated with the relative lack of sunflecks in shrub thickets compared to forests. Smith et al. (1989) demonstrated that size and maximum intensity of sunflecks were related to both gap size and distance from the gap to the incident surface. Shrub height alone should reduce the size, length and intensity of sunflecks at the surface even if LAI and other canopy architectural characteristics, such as leaf display, are similar.

Differences in architecture below the stand level (i.e. plant and leaf-level characteristics) also affect the efficiency of canopy radiation capture (Teh et al. 2000). Growth characteristics of terminal shoots and biomass allocation (i.e. leaf v. support structure) are important in optimizing light capture because these traits affect distribution of leaves in canopy space thereby increasing light interception while preventing self-shading (Nicola and Pickett 1983; Takenaka, Takahashi and Kohyama 2001; Kitijima et al., 2005). While a variety of shoot morphologies can efficiently capture light, branching strategies at the plant level that optimize light capture through leaf dispersion can be effectively summarized and compared using branch bifurcation ratios (Whitney 1976; Kempf and Pickett 1981; Valladares et al. 2002). Lower bifurcation ratios are associated with a nonrandom monolayer canopy characteristic of low light environments, whereas higher bifurcation ratios are common in woody plants exposed to high light resulting in a multilayered canopy (Whitney 1976; Steingraeber et al. 1979). At the leaf level, foliage orientation (i.e. leaf angle/azimuth) is also important in preventing self-shading and affects daily whole-canopy radiation capture through optimal interception of light earlier and later in the day (Sands 1995; Drouet and Moulia 1997; Wirth et al. 2001; Niienments and Fleck 2002; Falster and Westoby 2003).

Our objective was to compare understory light and canopy architecture in five species of shrubs with a history of expansion. Our primary goal was to determine what characteristics of canopy architecture drive attenuation of diffuse

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understory light and penetration of sunflecks. We hypothesized that LAD would

be the best predictor of understory light availability because it reduces the size of

gaps, thus inhibiting penetration of diffuse light and sunflecks. A second goal

was to compare canopy architectural characteristics across three levels of

organization within the canopy (leaf, plant and stand-level) and determine which

characteristics are related to high LAI values previously observed in mesic shrub

thickets. We further hypothesized that leaf angles would be positively correlated

with LAI and LAD. Species with dense canopies should display vertically

oriented leaves to maintain a favorable light environment for each leaf and avoid

self-shading. Our results should provide valuable insight into how shrubs are

able to maximize light capture for carbon gain and exclude shade-intolerant

grassland species through competition.

Materials and methods

Study sites--Field work was conducted in five communities dominated by shrub

species forming dense monospecific thickets. General site descriptions are

provided in Table 1. Each species has a history of expansion or invasion and all

have the potential to substantially alter ecosystem structure and function

(Petranka and McPherson 1979; Beier et al. 2005; Baer et al. 2006; Owens et al.

2007; Young et al. 2007). Two communities were located on Hog Island; a

barrier island located ~10 km east of the Delmarva Peninsula, Virginia, USA.

The island is part of the Virginia Coast Reserve, an NSF-funded Long-Term

Ecological Research site owned by the Nature Conservancy. Thickets of *Morella*

cerifera (L.) Small (Myricaceae), a native, evergreen, nitrogen-fixing shrub, are located in mesic interdunal swales in the upland portion of the island (Young et al. 1995). Stands of *Iva frutescens* L. (Asteraceae), a native, salt-tolerant shrub, occur at the upland edge of salt marsh (Young et al. 1994). Stands of *Rhus copallina* L. (Anacardiaceae)*,* a native shrub or small tree are located in an old field at the Eastern Shore National Wildlife Refuge, VA and developed after a prescribed burn (personal observation). Thickets of *Elaeagnus umbellata* Thunb. (Elaeagnaceae)*,* an invasive, nitrogen-fixing shrub that forms dense thickets in open fields and along roadsides and forest edges (Baer et al. 2006) are located at Fort A.P. Hill, VA. *Rhododendron maximum* L. (Ericaceae), a native, evergreen shrub, forms dense thickets in mountain forest understories and around the edge of high-elevation grassy balds (Schafale and Weakley 1990). Sites are located adjacent to grassy balds along Wilburn Ridge in the Mount Rogers National Recreation Area, Jefferson National Forest, VA. Sampling of each species, except *M. cerifera*, was done over 3-4 consecutive days. All measurements for *M. cerifera* were taken over a two week period. All sampling was done during the summer of 2008.

Stand level measurements—Canopy depth (D), stem density (d_s), stem basal area, leaf area index (LAI), leaf area density (LAD) and light (PPFD) were quantified for six plots in each community. Depending on canopy height, stem size classes and stem density, plot sizes varied from 1 x 1 m (*I. frutescens*) to 5 x 5 m (*M. cerifera*, *E. umbellata*, and *R. maximum*). Canopy height and height to

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the canopy bottom were measured using a telescoping pole marked in 0.1 m

increments. Mean canopy depth (D) for each plot was measured as the mean

difference between canopy height and height to the bottommost leaf along 5

vertical transects in each plot. Stem density and basal area were measured at

0.7 m height for all species. LAI was estimated using allometric models

developed for each species relating stem diameter and leaf mass and converted

to leaf area using specific leaf area (SLA). LAD was calculated as LAI*D^{-1} and is

expressed as m^2 leaf area*m^{-3} canopy space (Campbell and Norman 1989).

Leaf and plant level measurements--For each species, leaf angle (θ) and leaf

azimuth (*A*) were measured for 200 canopy leaves. Leaf angle was measured to

the nearest 5° using a clinometer. Leaf azimuth was measured as the direction

that the leaf surface was facing to the nearest 5° using a lensatic compass.

Specific leaf area was measured for 20 leaves for each species (Brantley and

Young 2007). Plant bifurcation ratios were measured for 20 stems exhibiting

terminal shoots exposed to full sunlight. Branch bifurcation ratios were then

calculated using the equation:

$$R_b = \frac{N-1}{N-N_1}$$

where *N* is the total number of branches of all branch orders on the stem and N_1

is the total number of first order branches (Steingraeber et al. 1989). Only live

branches were included in the measurements.

Light measurements -- Understory photosynthetic photon flux density (PPFD)

was sampled in each plot using three Li-Cor 190S quantum sensors attached to

an LI-1400 data logger (Li-Cor Biosciences, Lincoln, Nebraska, USA). Sensors were placed at ground level and spaced 0.5-1.0 m apart. For each sample, PPFD was measured and logged every 1 s for ~1 hour during mid-day (within 2 hours of solar noon). Above-canopy PPFD was measured immediately before and after each sampling period and a mean above-canopy PPFD value was interpolated from those readings. For each sampling period, total understory PPFD (Q_b) was calculated as the percentage of above-canopy light. Sampling occurred on cloudless days when possible so that sunfleck characteristics could be included in the analyses. For a given PPFD value, understory PPFD was considered to come from a sunfleck if it exceeded a predetermined threshold that represented the maximum value for diffuse PPFD. Thresholds between diffuse PPFD and sunflecks were estimated individually for each plot by visually analyzing a time-series of each sample and assigning a threshold value for maximum diffuse light at the nearest 25 µmol m^{-2} s^{-1} increment. Thresholds ranged from 25 µmol m^{-2} s^{-1} in five of the *M. cerifera* plots to 375 µmol m^{-2} s^{-1} in two plots of *R. copallina.* Cumulative PPFD from sunflecks (mol m^{-2} hr^{-1}) was then calculated for each sensor as the difference between total PPFD during the sampling period and total diffuse PPFD (i.e. all values below the threshold for a sunfleck) and averaged across sensors for each plot (see also Brantley and Young 2009).

Statistical analyses— Differences among species for each parameter were analyzed using ANOVA for all individual characteristics of canopy architecture

and light except leaf angle and leaf azimuth. Leaf angles for three species

exhibited non-normal distributions and were analyzed using non-parametric

(Kruskal-Wallis) as well as parametric tests to detect significant differences. To

describe leaf azimuth, A and length of the mean vector (z, ranging from 0 to 1)

were calculated based on Zar (1999). Significance of z in relation to a uniform

distribution ($z = 0$) was tested using the Rayleigh test (Zar 1999). A and z were

then combined into a common metric, termed here A_s for southern leaf bias, with

a range of 0 to 1 by scaling A to equal 1 at 180° (i.e. facing due south), 0.5 at 90°

or 270° and 0 at 0° and multiplying A by z. All parameters were entered into a

stepwise multiple linear regression to determine which, if any, characteristics of

canopy architecture (independent variables) could predict each component of

understory light detailed in the preceding paragraph (dependent variables).

Independent variables were then analyzed using a cross-correlation matrix to

detect interactions between canopy structural characteristics within and among

levels of canopy organization. When appropriate, a curve-fit estimation was used

to describe relationships between individual independent and dependent

variables. All statistics were performed in SPSS 15.0 (SPSS Inc., Chicago, IL,

USA) unless otherwise noted.

Results

For all samples except those for *R. maximum*, above-canopy light ranged

from 1710 to 1970 µmol m^{-2} s^{-1}. Above-canopy light during our sampling of *R.*

maximum ranged between 340 and 365 µmol m^{-2} s^{-1} and we were not able to

include sunfleck data for *R. maximum* because of persistent cloud cover. Total understory light, Q_b, expressed as a percentage of above-canopy PPFD, varied significantly by species (F = 12.048, p < 0.001) (Fig. 5.1). Thresholds between sunflecks and diffuse light, estimated individually for each plot, were generally similar within species with some notable variation for species with lower LAI. For example, thresholds for *M. cerifera* plots were either 25 or 50 µmol m^{-2} s^{-1} but thresholds for plots of *R. copallina* varied from 100 to 375 µmol m^{-2} s^{-1}. The relationship between total understory light and understory light from sunflecks was strong and positive (r^2 = 0.90, p < 0.001) (Fig. 5.2). Sites with more frequent and longer sunflecks tended to have higher levels of understory PPFD from both sunflecks and diffuse PPFD.

Stem leaf mass was predicted (all r^2 ≥ 0.90, p ≤ 0.001) by stem diameter for each species, demonstrating that the allometric models were a reliable means of estimating LAI (also see Brantley and Young, 2007 for *M. cerifera* results). LAI across all plots ranged from 0.4 (*I. frutescens*) to 14.0 (*M. cerifera*). Mean LAI varied significantly (F = 14.721, p <0.001) by species from 0.9 to 10.0 (*I. frutescens* and *M. cerifera* respectively) (Fig. 5.3). Canopy depth (D) also varied significantly by species (F = 22.442, p < 0.001) (Fig. 5.3) and was more correlated with leaf and plant-level characteristics than with other stand-level characteristics (Table 2). Leaf area density, a function of LAI and D, also varied significantly among species (F = 4.817, p = 0.006) (Fig. 5.3). Variations in LAD

among all plots were better predicted by LAI (r^2 = 0.33, p = 0.002) than by D (r^2 = 0.06, p = 0.23) (data not shown).

Plant and leaf-level variations were also substantial among species. Bifurcation ratios (R_b) varied significantly among species (F = 24.538, p < 0.001). Mean R_b for *E. umbellata* was 13.3, more than twice the value for the next species, *I. frutescens*, and more than four times higher than the other species (Fig. 5.4). Leaf angle (θ) exhibited bimodal distributions for two species, *M. cerifera* and *I. frutescens*, while distribution of θ was skewed, though to differing degrees, toward higher angles for other species (Fig. 5.5). Leaf angle varied significantly (p < 0.001) according to both parametric and non-parametric tests and mean leaf angles ranged from 27.8 ± 1.5 (*E. umbellata*) to 42.8 ± 1.6 (*M. cerifera*) (Fig. 5.5). Leaf *A* also varied among species (Fig. 5.6). Leaf *A* for two species, *M. cerifera* and *R. copallina,* was calculated but the distribution did not deviate from random (p = 0.056 and 0.966, respectively). Of the remaining species, *I. frutescens* (p = 0.004) displayed a mean *A* of ~300° and *z* of 0.13 (on a 0 to 1 scale). *E. umbellata* (p < 0.001) and *R. maximum* (p < 0.001) both demonstrated a south-facing bias with a mean *A* of ~170° and the greatest *z* values (i.e. directionality was greatest) at 0.27 and 0.28, respectively.

Multiple regression analysis indicated that the best model to predict total understory light included variables D and LAI (R^2 = 0.53, F = 13.714, p < 0.001). However, LAI and canopy depth were collinear (r = 0.43, p = 0.027). Removing LAI from the model resulted in a linear model with D as the single independent

variable (r^2 = 0.42). The predictive power of D was improved using a non-linear model (Fig. 5.7). A curve-fit analysis indicated that Q_b could be best related to D through a power function (r^2 = 0.58) given by the equation:

$$Q_b = 7.54(D)^{-1.30}$$

Results were similar when understory PPFD from sunflecks was analyzed by multiple regression but contribution of sunflecks was slightly more difficult to predict. A linear model that included only D had an r^2 of 0.41. No other independent variable, including LAI, was significant in predicting PPFD from sunflecks in the multiple regression analysis. The predictive power of D was not improved substantially by using a non-linear model. For example, a power function as used previously resulted in an r^2 of 0.39 (Fig. 5.7). LAD was a poor predictor of total understory PPFD (r^2 = 0.007, F = 0.187, p = 0.669) or PPFD from sunflecks (r^2 = 0.001, F = 0.063, p = 0.805) and was not included in any of the models.

A correlation matrix of the independent variables revealed several significant relationships across levels of canopy architecture. Perhaps most important in the context of the above relationships, there were significant correlations ($p < 0.01$) between D, R_b, θ and A_s. (Table 2). For example, there was a negative correlation between D and θ (r = -0.519, p = 0.005) indicating that lower leaf angles were associated with deeper canopies. Deeper canopies also had higher levels of branching (r = 0.633, p < 0.001) and leaf azimuths with greater bias towards a southerly direction (r = 0.502, p = 0.008). Additional

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significant correlations were often the result of interdependence due to methodology and such relationships were omitted from consideration. For instance, LAI and basal area were highly correlated because both LAI and basal area values were calculated from the same stem diameter measurements.

Discussion

Interception of light by shrub thicket canopies is dependent on vertical distribution of leaves in the canopy as well as fine-scale, species-specific variations in leaf display. For the five shrub species, total understory light and light from sunflecks within thickets were significantly related, demonstrating that there are commonalities between the mechanisms that drive attenuation of diffuse light and penetration of sunflecks. This contrasts with Brantley and Young (2009) who suggested that penetration of diffuse light and sunflecks might each be driven independently, at least in part, by different canopy architectural characteristics, although that study compared one species of shrub to two forest stands. Specific stand level characteristics remain the best predictors of understory light, however we failed to support our hypothesis that LAD would best predict light attenuation and the contribution of sunflecks. LAD was a poor predictor of understory PPFD either from diffuse PPFD or from sunflecks. It appears that effects of high LAI on light attenuation are mediated by clumping of leaves on branches (see discussion of bifurcation ratios) which creates an overall high LAD value for the stand but does not necessarily reduce the number or size of canopy gaps.

Our results further revealed that canopy depth was the single best stand-level structural parameter to predict total light attenuation and penetration of sunflecks. LAI was also a significant predictor of total understory light (but not sunflecks); however, LAI and canopy depth were collinear. A positive relationship between LAI and D seems intuitive, as a canopy with greater depth would necessarily require a higher LAI, at least for small LAI values. The conclusion that canopy depth would greatly influence light attenuation is not novel. Smith et al. (1989) postulated that likelihood of a sunfleck penetrating the canopy and reaching the ground would decline as canopy depth increased. According to Smith et al. (1989), likelihood of a sunfleck being rapidly eclipsed as solar position changed would increase as canopy depth increased. The same principle may apply to indirect light as well. Although canopy depth predicted some variation in sunfleck availability, diffuse light was actually more predictable.

As mean leaf angle was not significantly correlated with either LAI or LAD, we also failed to support our second hypothesis. Early successional woody species, or any species exposed to full sunlight, often exhibit vertically oriented leaf and shoot angles that support a uniform distribution of leaves and improve efficiency of light capture for the whole canopy (Kitijima et al. 2005). Plants exposed to high light may also exhibit canopies with both erect and horizontal leaf angles with few leaves oriented at intermediate angles (de Wit 1965, as cited in Teh et al. 2000). Two of the species in our study, *M. cerifera* and *I. frutescens*, displayed a bimodal distribution of leaf angles that would result from this type of

leaf display. The bimodal distribution again appears unrelated to LAI, however, as these two species had the highest and lowest LAI values of the species examined. What our results indicate is that vertically oriented foliage is not a requisite canopy characteristic for maximizing light interception. Both LAI and mean leaf angle for *E. umbellata* were significantly lower than for *M. cerifera* but understory light levels were similar.

Elaeagnus umbellata appears to be more structurally efficient at interception of sunlight through a combination of structural characteristics including high bifurcation ratios, leaf azimuths biased towards the south and horizontal leaf angles which allow greater light attenuation at mid-day. Although some tree species vary in bifurcation ratios between sunlit and shaded sites (Steingraeber et al. 1979), bifurcation ratios have generally been considered a fixed value for a given species based on genetic potential (Whitney 1976). Therefore, our observed variation among species may be typical as all sites were exposed to full sun. Branch length and angle also affect leaf distribution by minimizing leaf overlap and these characteristics also vary among species (Kempf and Pickett 1981). Although we did not measure these characteristics, they are generally linked to bifurcation ratios (Whitney 1976). The two species with highest bifurcation ratios, *E. umbellata* and *I. frutescens*, both exhibited erect asymmetrical branching as described by Whitney (1976) while the other species demonstrated branching by symmetrical dichotomy (personal observation) resulting in lower bifurcation ratios.

One of our more curious findings was that the mean leaf azimuth for *I. frutescens* was toward the west-north-west at ~300°. Typically, leaf azimuths vary with respect to either optimizing photosynthesis, modulating energy balance or some combination of both (Sands 1995; Valladares and Pugnaire 1999; Falster and Westoby 2003; Kitajima et al. 2005). Our results for *R. maximum* and *E. umbellata* indicate that increasing capture of solar radiation may be most important for influencing leaf azimuths because they each demonstrated a southern bias. Under this assumption, one would expect that any non-uniform distribution would either reflect a bias toward the general direction of the sun or, in the case of full sun exposure and moderate temperature, orthogonal to the sun to reduce radiation stress. While a 300° leaf azimuth, typified by *I. frutescens*, would certainly mitigate exposure to excess sunlight, leaf azimuth may also be influenced by other environmental factors, such as the prevailing wind observed in open sites on Hog Island. Effects of wind on leaf azimuths is poorly understood but Elmore et al. (2005) demonstrated that leaf azimuths of *Zea mays* could be changed experimentally by placing windbreaks against the prevailing wind direction during leaf development. Leaves of *I. frutescens* were arranged approximately perpendicularly to prevailing wind direction during the growing season (data not shown) indicating that other stresses, such as resistance to leaf abrasion, may also influence leaf azimuths in this community. Of the two Hog Island species, *I. frutescens* leaf azimuth exhibited a greater

directional bias and these thickets were located in a much more exposed site than the *M. cerifera* thickets.

The shrub growth form is efficient at exploiting horizontal space by altering fine-scale leaf display through variations in branch length and branch angle that depend on sun exposure (Kempf and Pickett 1981). These characteristics, coupled with variations in leaf angle and azimuth, not only improve the overall plant carbon budget by maximizing light capture but they also improve competitive dominance through high attenuation of light (Sands 1995; Kitajima et al. 2005). For the species we examined, *E. umbellata* had the highest branch bifurcation ratios and lowest leaf angles and also exhibited leaf azimuths with a directional bias towards the south. These characteristics explain why total understory light and total contribution of sunflecks in those thickets was the lowest for the five species examined here even though LAI for *E. umbellata* was substantially lower than for *M. cerifera* or *R. maximum*.

Based on our results, the potential of shrubs to alter aboveground resource gradients and community structure is dependent on fine-scale, species-specific characteristics of canopy architecture that affect distribution of leaves in the canopy and enhance efficiency of light interception. The order of magnitude variation in LAI values was somewhat surprising considering the similarity in climatic factors that existed among the communities examined; nonetheless, the range of LAI values we observed represents the range of LAI values observed for shrub-dominated systems across North America (Knapp et al. 2008) and these

results may be applicable to more arid systems. Our results may be confounded by extreme heterogeneity and complexity of canopy architecture and understory light at fine scales. However, to our knowledge, this is the first field study to link canopy depth directly to understory light availability in shrub thickets. Furthermore, the fact that differences can be significant within such a narrow range of variation in canopy depth among these communities demonstrates the importance of this mechanism for light attenuation. Three of the species we studied, *M. cerifera*, *E. umbellata* and especially *R. maximum*, are also common understory species. Whether these results would be comparable for these shrub species in forest understories would need further study. Understanding the link between leaf, plant, and stand-level architectural characteristics of shrub canopies could provide valuable new insight into specific traits that facilitate woody expansion and control interactions between woody vegetation and grasses.

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Table 5.1. Site, habitat and climate summary for five mesic shrub communities in Virginia, USA. All species

except *E. umbellata*, an invasive, are native to Virginia.

Species	Location	Elevation (m above mean sea level)	Habitat type	Mean annual temperature[a] (°C)	Mean annual precipitation[a] (mm)
Morella cerifera	37.449° N, 75.667° W	1-3	Interdunal swale	14.2	1065
Iva frutescens	37.452° N, 75.673° W	<2	High salt marsh	14.2	1065
Rhus copallina	37.134° N, 75.960° W	4	Old field	14.7	1035
Elaeagnus umbellata	38.093° N, 77.335° W	64	Old field	13.6	1167
Rhododendron maximum	36.652° N, 81.511° W	1530	Grassy Bald	11.9	1212

[a]Source: National Climatic Data Center, Asheville, NC

Table 5.2. Correlation matrix for canopy characteristics for five shrub and small tree species that form monotypic stands. Correlations with † are related methodologically. Correlations with ** are significant at p = 0.05.

	LAI (Leaf area index)	D (Canopy depth)	LAD (Leaf area density)	R_b (Bifurcation ratio)	Θ (Mean leaf angle)	A_s (Southern leaf bias)
LAI	--					
D	0.426**	--				
LAD	$0.577^\dagger$	$-0.237^\dagger$	--			
R_b	-0.103	0.633**	-0.368	--		
θ	-0.056	-0.519**	0.083	-0.685**	--	
A_s	0.315	0.502**	0.242	0.584**	-0.581**	--

Figure Legends

Figure 5.1. Total understory light (PPFD) and total light from sunflecks (mean ± 1 standard error) for five mesic shrub communities (total light from sunflecks for *R. maximum* could not be determined because of persistent cloud cover). Significant differences between species are noted with lowercase letters.

Figure 5.2. Relationship of PPFD from sunflecks to total understory PPFD for 27 plots located in mesic shrub thickets. Coefficient of determination (r^2) was determined by least squares regression.

Figure 5.3. Stand-level canopy characteristics (mean + 1 standard error) for five mesic shrub communities. Bar height in B represents canopy height while the black area represents space filled by foliage (canopy depth). Significant differences among species are noted with lowercase letters.

Figure 5.4. Branch bifurcation ratios (mean ± 1 standard error) for five mesic shrub species. Significant differences among species are noted with lowercase letters.

Figure 5.5. Frequency distributions of leaf angles for five mesic shrub species. Distributions are divided into 15° classes. Mean leaf angle (± 1 standard error) for each species is shown in parentheses. All leaves were located on terminal shoots in the outermost canopy.

Figure 5.6. Radial distribution of leaf surface azimuths for five mesic shrub species shown in relation to cardinal direction. Leaf azimuths of *M. cerifera* and

R. copallina did not deviate from a random radial distribution (p > 0.05). Mean

azimuths for other species are represented with arrows.

Figure 5.7. Curve estimation for the relationship of total understory PPFD (top)

and total PPFD from sunflecks (bottom) among 27 plots (23 for bottom figure)

representing five (four for bottom figure) shrub species. Curves, equations and r^2

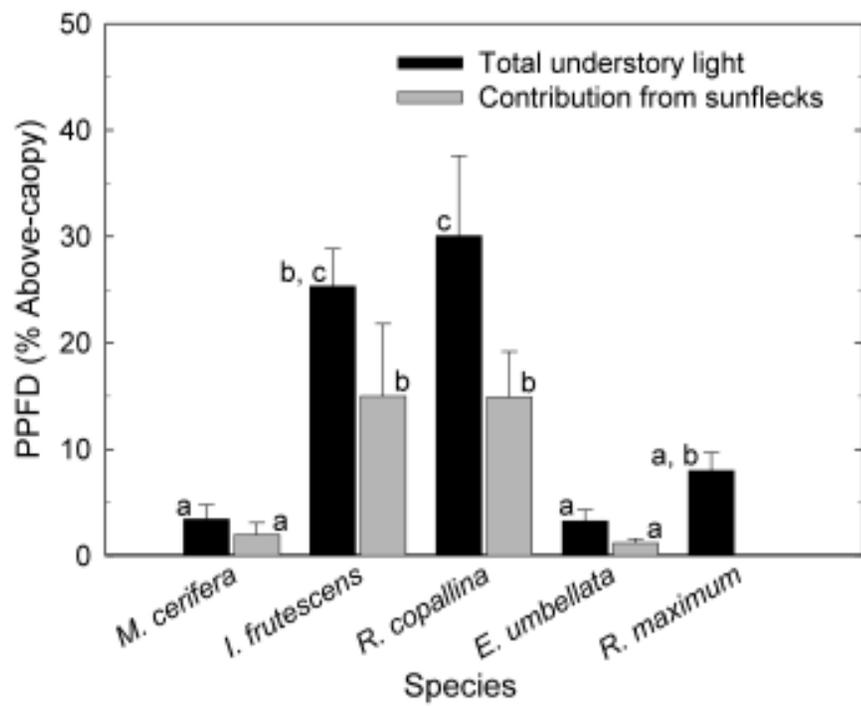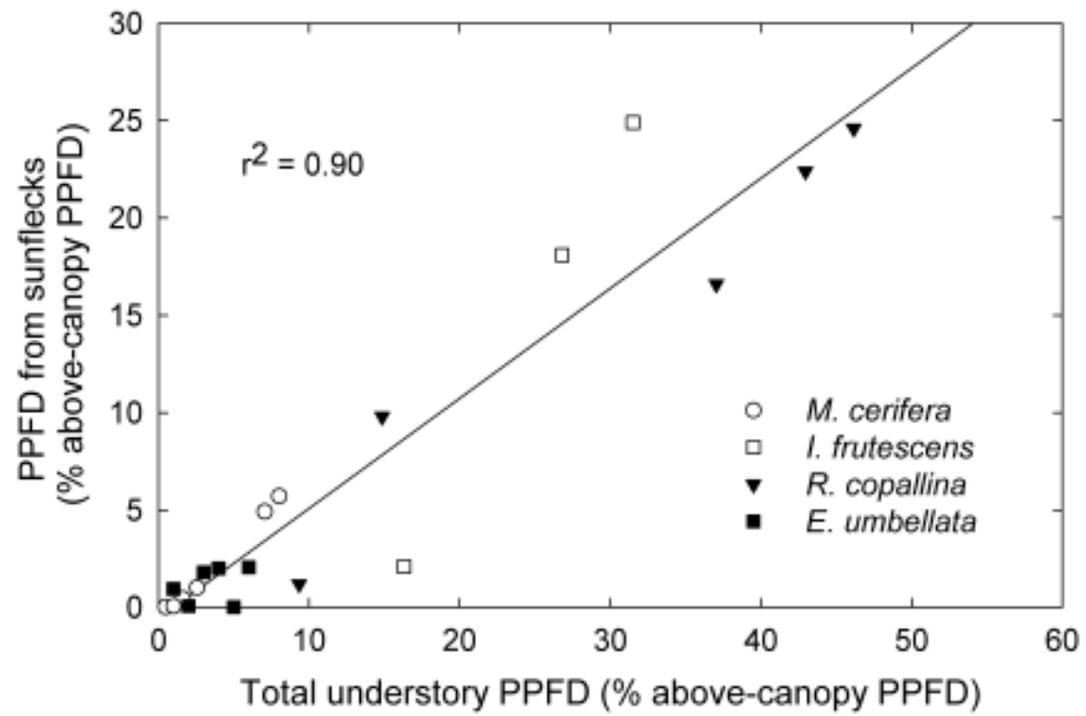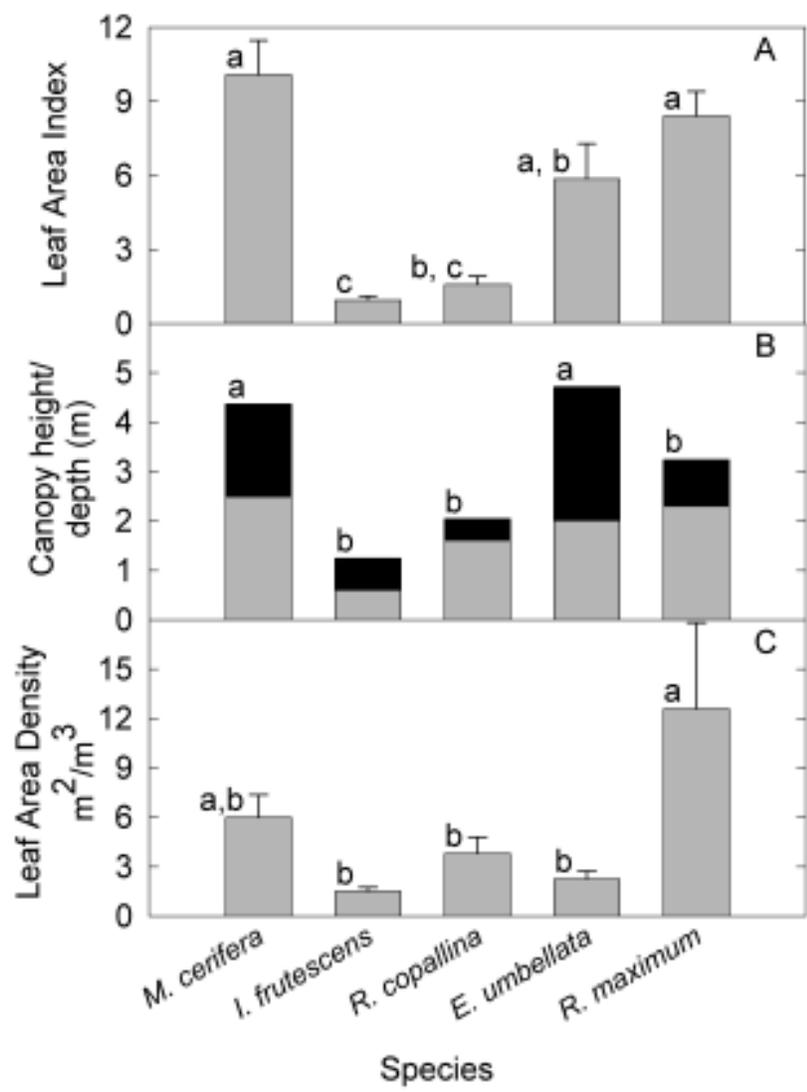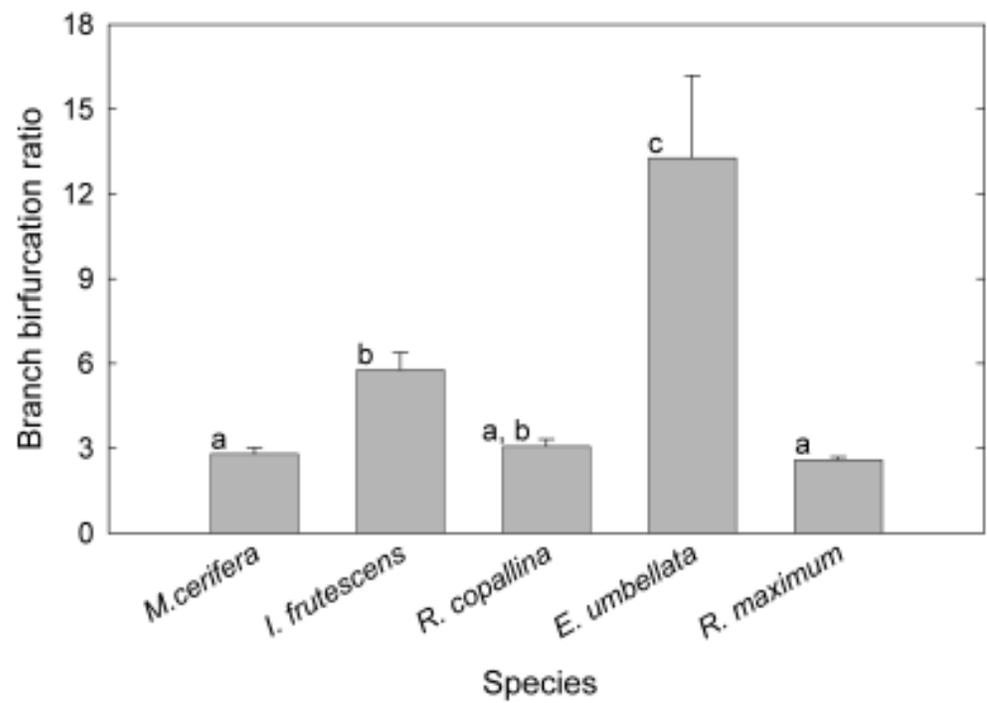values represent the best-fit relationship for a power function.

Figure 5.1

Figure 5.2

Figure 5.3

Figure 5.4

Figure 5.5

N

W — E

S

---- *M. cerifera*
— *R. copallina*

N

W — E

S

I. frutescens

N

W — E

S

R. maximum

N

W — E

S

E. umbellata
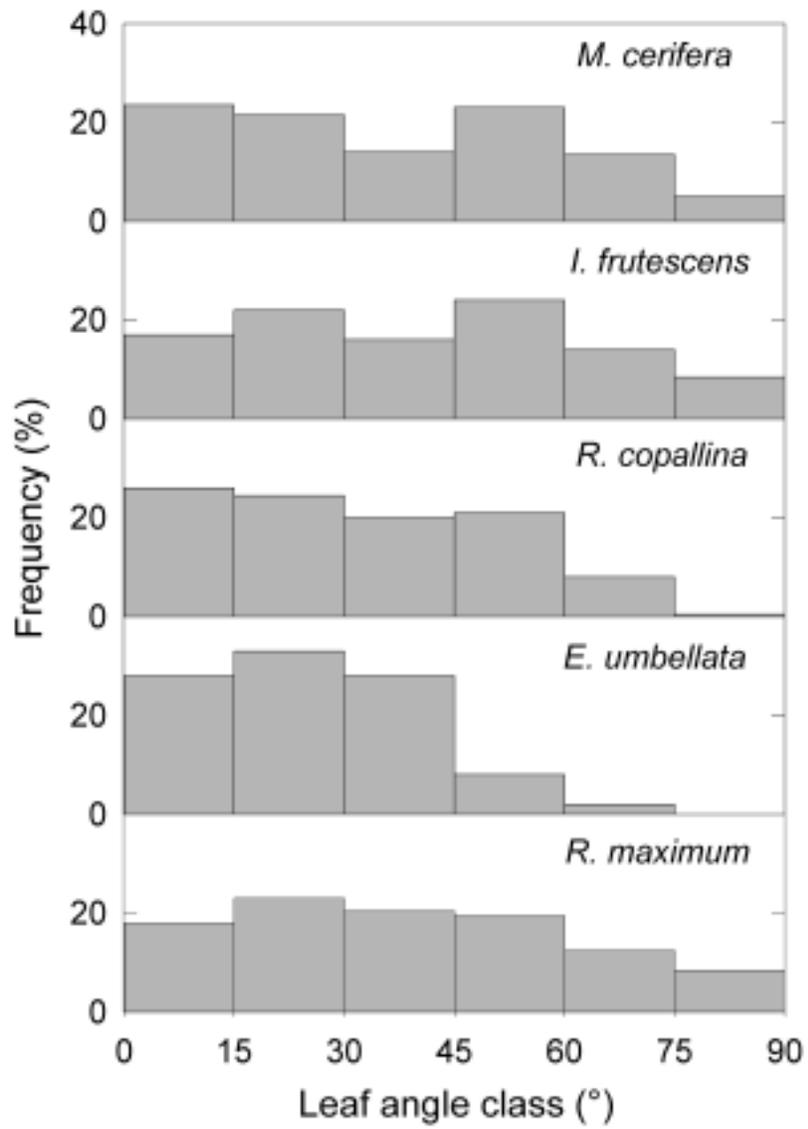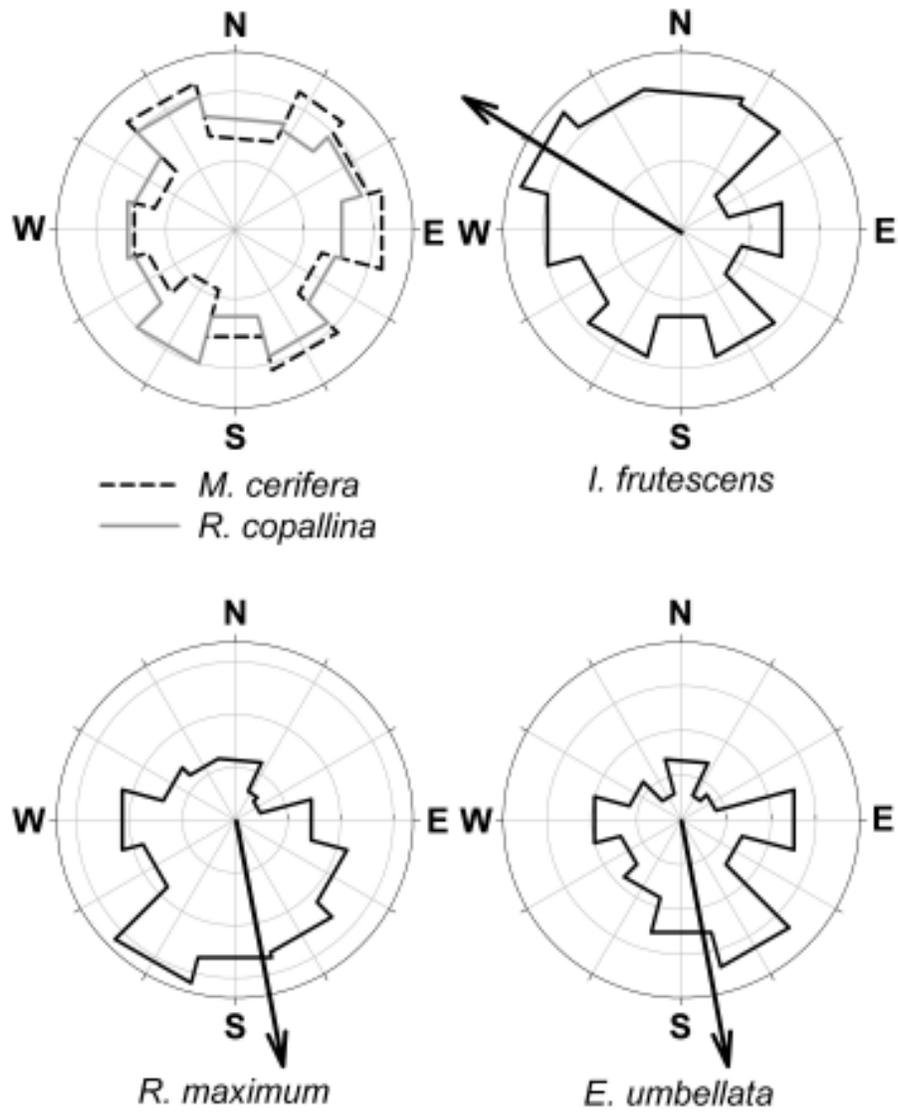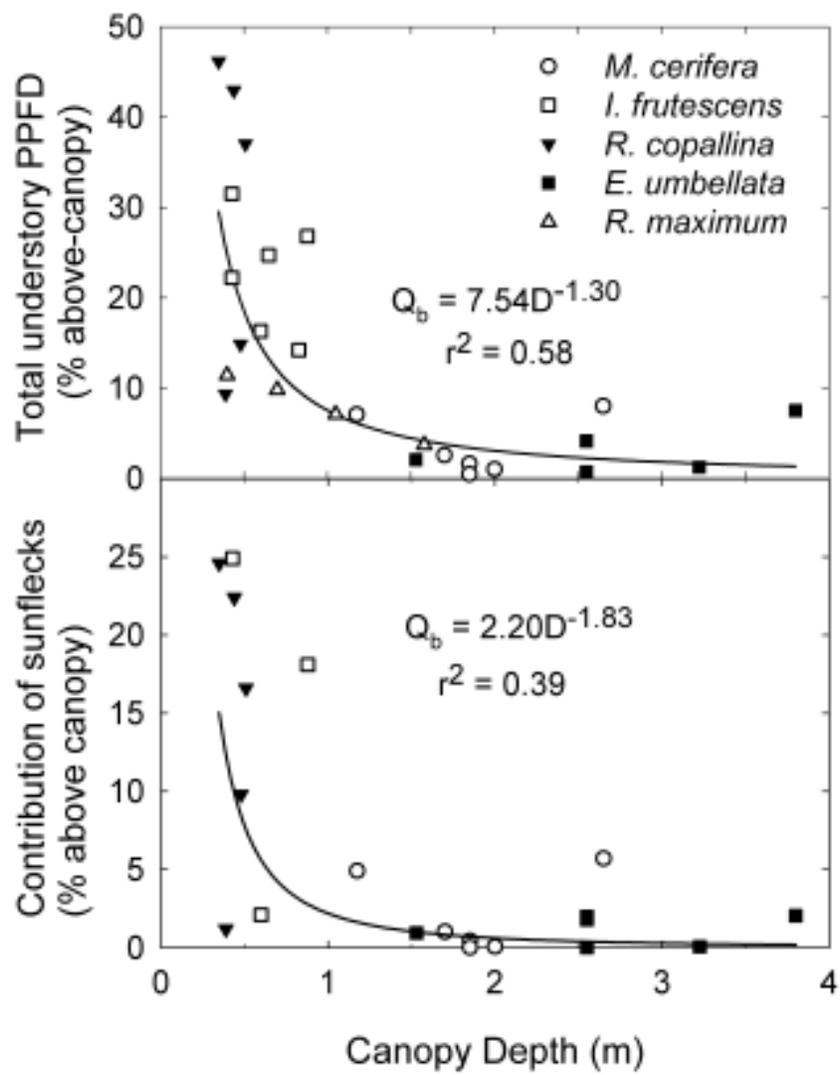
Figure 5.6

Figure 5.7

VITA

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