

ABSTRACT

Title of Dissertation: PLANT-INSECT INTERACTIONS IN A SHIFTING COASTAL ECOSYSTEM: AVICENNIA GERMINANS AND ITS ASSOCIATED ARTHROPODS

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The climate's role in determining where species occur is increasingly well understood, but our ability to predict how biotic interactions both influence and respond to species' range shifts remains poor. This is particularly important when considering climate-change-driven range shifts in habitat-forming species like mangroves, given their impact on ecosystem structure and function. In this dissertation, I consider the arthropods associated with the black mangrove, *Avicennia germinans*, to explore whether patterns of arthropod diversity affect the rate of a plant's range expansion, and, in turn, how a range-expanding plant alters arthropod communities in habitats where it is invading. Among arthropods with the potential to influence plants' range dynamics, pollinators can directly affect plant reproduction and ability to spread into new territory. Breeding system experiments reveal that *A. germinans* relies on pollinators for full fruit set, and surveys along the Florida coast show a substantial northward decline in the overall frequency of pollinator visits to *A.*

germinans flowers. However, the decline in abundance of some common pollinator taxa is partly offset by an increase in the frequency of other highly effective taxa. Furthermore, range-edge *A. germinans* produce more flowers than southern individuals, contributing to high range-edge fecundity and enabling range expansion. As a woody plant with nectar-producing flowers, *A. germinans* is a novel resource for arthropods in the salt marshes where it is encroaching. To understand arthropod community assembly on these frontier mangroves, and how mangrove presence affects marsh arthropod community composition, I compare arthropod communities in these adjacent vegetation types. Arthropods form distinct communities on mangroves and marsh vegetation, with at least one *A. germinans* specialist already present in this range-edge population. However, neither mangrove proximity nor the abundance of mangrove flowers appears to influence salt marsh arthropod community structure, indicating that mangrove encroachment may lead to a net increase in arthropod diversity in coastal regions by increasing habitat heterogeneity. In sum, plants that rely on pollinators can avoid range-edge reproductive failure by attracting a diverse group of pollinating taxa, and range-expanding plants can rapidly alter invaded communities by shaping diversity at very local scales.

PLANT-INSECT INTERACTIONS IN A SHIFTING COASTAL ECOSYSTEM:
AVICENNIA GERMINANS AND ITS ASSOCIATED ARTHROPODS

by

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Field ecologists like to joke that their profession requires little more than proficiency with Ziplock bags and Sharpies. But it's a rare field research project that doesn't also require an enormous number of person-hours and, in cases where the field sites are far from home, an institution willing to accommodate said Ziplock- and Sharpie-toting persons. For me, those institutions were the Smithsonian Marine Station in Fort Pierce, FL, where Station Managers Hugh Reichardt and Sherry Reed were endlessly accommodating, and the Guana-Tolomato-Matanzas National Estuarine Research Reserve, whose Research Director, Nikki Dix, offered generous logistical and material support. I am also grateful for the Florida State Parks staff, who welcomed my ragtag research operation. This work also would not have been possible without the small army of undergraduate assistants who contributed – in particular, Micah Miles, Elizabeth Stieber, Jenny Schefski, and Jessica Chui, who toughed it out in challenging field conditions. The cast and crew of the Gruner lab have taught me so much, and helped immeasurably in getting this research from the hair-brained-idea stage to completion. Finally, it is no exaggeration to say that this would not have been possible without my personal secretary, cheerleader, drill sergeant, field assistant, and sounding board, Sean.

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Table of Contents

Acknowledgements.....	ii
Table of Contents.....	iii
List of Tables	v
List of Figures.....	vi
Chapter 1: Overview	1
Conceptual background	1
Study system.....	2
Outline of the dissertation.....	4
Chapter 2: Pollinator-dependence in <i>Avicennia germinans</i> , the black mangrove	6
Abstract.....	6
Introduction.....	7
Materials and Methods.....	10
Field site description.....	10
Experiment 1: Reproductive strategy.....	11
Experiment 2: Pollinator exclusion.....	14
Monitoring for windborne pollen.....	15
Results.....	16
Experiment 1: Reproductive strategy.....	16
Experiment 2: Pollinator exclusion.....	18
Monitoring for windborne pollen.....	18
Discussion.....	19
Chapter 3: Major turnover in pollinator community composition does not impact fecundity of a range-shifting plant.....	23
Abstract.....	23
Introduction.....	24
Materials and Methods.....	29
Study system	29
Floral visitor identity and frequency.....	31
Floral visitor identity and frequency - Analysis	32
Pollinator effectiveness.....	34
Pollinator effectiveness - Analysis.....	36
Flower and propagule production	37
Flower and propagule production - Analysis.....	38
Results.....	40
Floral visitor identity and frequency.....	40
Pollinator effectiveness.....	43
Flower and propagule production	44
Discussion	47
Pollinators and pollen limitation at the range edge.....	48
Reproductive allocation at the range edge	50
Conclusion	52
Chapter 4: In a shifting ecotone, mangrove encroachment alters arthropod communities at fine scales	53

Abstract.....	53
Introduction.....	54
Materials and Methods.....	60
Study system.....	60
Experiment.....	60
Analysis.....	62
Results.....	67
Do mangroves host a distinct community?.....	67
Does mangrove proximity affect marsh arthropods?.....	72
Do mangrove flowers alter community composition?.....	73
Discussion.....	76
Appendices.....	81
Appendix A.....	81
Appendix B.....	83
Appendix C.....	86
Bibliography.....	90

List of Tables

Table 3.1: Taxonomic categories assigned to *A. germinans* floral visitors, and total number of visitors of each taxon observed, across sites and years, over the course of the study. 32

Table 4.1: Models of marsh arthropod abundance, richness, rarefied richness, and Shannon diversity as a function of the type of transect (near vs far from the focal mangrove) and the PCA axes representing variation in marsh plant composition... 73

List of Figures

Figure 1.1: A bumble bee visits <i>A. germinans</i> flowers, whose abundant nectar earned this species the title of ‘honey mangrove’ among Florida’s beekeepers.	4
Figure 2.1: Sites where pollinator exclusion experiments were conducted.	11
Figure 2.2: Variation among treatments and sites in fruit set.	17
Figure 2.3: Propagule production (measured as number of propagules produced by an inflorescence).....	19
Figure 3.1: (a) The frequency of all insect visits to <i>A. germinans</i> flowers declined from south to north. (b) The probability of at least one insect visiting <i>A. germinans</i> flowers during an observation period declined at higher latitudes.	41
Figure 3.2: (a) Random slope estimates, representing taxon-specific relationships between visitation frequency and latitude. (b) Nonmetric multidimensional scaling (NMDS) of floral visitors observed at each site and date.	42
Figure 3.3: (a) The overall pollinator effectiveness of each observation, a measure of both pollinator quantity and quality, generally declined at northern sites. (b) Pollen deposition on <i>A. germinans</i> stigmas was unrelated to latitude.	44
Figure 3.4: (a) <i>A. germinans</i> flowering increased with latitude, but (b) fecundity showed no relationship with latitude. (c) Consequently, the fruit set rate declined slightly at northern sites.	45
Figure 3.5: (a) The density of reproductive <i>A. germinans</i> (those with at least one inflorescence/infructescence at the time of survey) increased with latitude, (b) as did propagule mass.....	47
Figure 4.1: (a) Abundances of the 12 most common arthropod orders, summed across all samples collected from marsh vegetation far from mangroves (“Far Marsh”), marsh vegetation near mangroves (“Near Marsh”), and directly from mangroves. (b) Hemiptera alone, further subdivided into morphospecies (the 10 most common of which are shown).	68
Figure 4.2: (a) Raw arthropod richness (at the order level) was significantly higher in marsh than in mangroves. (b) However, Shannon diversity was not significantly different between the two vegetation types.	70
Figure 4.3: Principal Coordinates Analysis (PCoA) on (a) arthropod order abundances, and (b) Hemiptera only, split into morphospecies..	71
Figure 4.4: (a) Random slope estimates for mangrove arthropods, representing order-specific relationships between abundance and the number of mangrove inflorescences present. On mangroves, Diptera (b) and Araneae (c) were the only two orders whose abundance was associated with the number of inflorescences. Lines show model-estimated relationships.	74
Figure 4.5: (a) Random slope estimates for marsh Hemiptera, representing morphospecies-specific relationships between abundance and the number of mangrove inflorescences nearby (at the order level, marsh arthropods did not show any relationship between abundance and flowers). Marsh Heteroptera (b) and a	

cicadellid morphospecies (c) had the strongest responses to nearby mangrove
inflorescences. Lines show model-estimated relationships. 75

Chapter 1: Overview

Conceptual background

Climate change has repeatedly been linked to species distributional shifts around the world, in the terrestrial, freshwater, and marine realms (Parmesan 2006, Chen et al. 2011, Poloczanska et al. 2013). In general, these shifts reflect predicted movements – typically upward in elevation or latitude – given models of species’ physiological tolerances and projections of future climatic conditions (Parmesan and Yohe 2003). However, species are not all shifting their distributional limits at the same rates, or even the same directions, a fact that will lead to a degree of “reshuffling” of local ecological communities (Williams and Jackson 2007, Loarie et al. 2009, Urban et al. 2012). As a result, climate change is expected to alter networks of biotic interactions, with some species losing interaction partners as their distributions grow apart, and others gaining new interaction partners (Tylianakis and Morris 2017). Consequently, the composition of local ecological communities, their functioning, and the ecosystem services they provide are extraordinarily challenging to predict.

For most species, we lack the ability to predict two important aspects of climate-driven range shifts: 1) the ways in which biotic interactions will inhibit or facilitate species’ range dynamics, and 2) the biotic interactions that rapidly shifting species will take part in, and thus the ecological role they will play, in the novel systems into which they spread. This dissertation illustrates some of the traits and

circumstances that facilitate a species' rapid response to climate change, and thus contributes to our ability to predict future ecosystem structure and function.

Study system

As a flowering plant with well-described range dynamics and distributional limits, *Avicennia germinans* (L.) L. (Acanthaceae), black mangrove, is an ideal organism with which to answer questions surrounding range shifts and biotic interactions. Like the approximately 60 other species of mangroves found worldwide, *A. germinans* is a tree adapted to the salty conditions of the intertidal zone, with a primarily tropical distribution (Tomlinson 1986, Duke 1995). Mangroves – a polyphyletic group – have evolved a variety of physiological, chemical, and anatomical traits that confer tolerance to salinity and flooding (Tomlinson 1986, Krauss et al. 2008, Feller et al. 2010). *A. germinans* achieves this through, among other mechanisms, salt excretion glands on its leaves and aerial roots ('pneumatophores') containing aerenchyma that promotes efficient gas exchange (Dawes 1981, Borg and Schönenberger 2011). Traits like these allow mangroves to dominate in settings that few other plants can tolerate, making mangroves critical providers of habitat and ecosystem services along tropical and subtropical coastlines around the world (Tomlinson 1986, Barbier et al. 2011, Mcleod et al. 2011, Kelleway et al. 2017).

Their tolerance of salinity and inundation notwithstanding, mangroves are believed to be generally intolerant of cold conditions (Stuart et al. 2007, Cavanaugh et al. 2015, Osland et al. 2015). Previous research has revealed a tight link between *A. germinans*' thermal tolerance and its range dynamics in the southeastern U.S. (Stuart

et al. 2007, Cavanaugh et al. 2014, 2015, Osland et al. 2017). Over the past two centuries, *A. germinans* and the region's two other (less cold-tolerant) mangrove species have repeatedly shifted their northern range edge across approximately 3° of latitude (~28-31°N), roughly in sync with the occurrence of extreme cold events in the region (Cavanaugh et al. 2019). Given its physiological constraints and climate forecasts that predict a decline in hard freezes, *A. germinans* is expected to permanently dominate coastlines where it currently co-occurs with temperate salt marsh, effectively shifting the mangrove-marsh ecotone northward (Cavanaugh et al. 2019).

At its northernmost range margin, *A. germinans* forms single-species stands within salt marsh – a biologically simplified arrangement that lends itself to observational study and experimentation. Even where *A. germinans* does not occur in monospecific stands, it provides physical substrate in a dynamic coastal setting and thereby facilitates many other taxa – both benthic marine organisms and terrestrial species (Barbier et al. 2011, Kelleway et al. 2017, Armitage et al. 2020). It attracts a wide range of taxa to its flowers (Figure 1.1), although the degree to which it relies on pollinators is unknown, and no previous effort has been made to identify its most important pollinators in the southeastern U.S. In sum, *A. germinans* is an important foundation species in the coastal regions where it occurs, facilitating a diverse community of associated organisms.

In this dissertation, I consider the arthropods associated with the black mangrove, *A. germinans*, to explore whether patterns of arthropod diversity affect the



*Figure 1.1: A bumble bee visits *A. germinans* flowers, whose abundant nectar earned this species the title of 'honey mangrove' among Florida's beekeepers.*

rate of a plant's range expansion, and, in turn, how a range-expanding plant alters arthropod communities in habitats where it is invading.

Outline of the dissertation

I begin, in Chapter 2, by establishing baseline biological information regarding *A. germinans*' breeding system – specifically, the degree to which it depends on pollinators. Obligate mutualisms are thought to present a barrier to range shifts, in that the mutualist partner may not be present beyond the range edge. To determine the degree to which *A. germinans* depends on pollinator mutualists, I monitored for windborne pollen and experimentally manipulated *A. germinans* flowers to either exclude pollinators or prevent spontaneous self-pollination.

Having established that *A. germinans* does rely on pollinators to achieve maximum fecundity, Chapter 3 examines whether pollination or reproductive failure

constrain *A. germinans*' northward range expansion. To assess variation in the pollination environment, I monitored pollinators visiting *A. germinans* populations along a latitudinal gradient, from south-central Florida to the northern range edge. I also measured the relative effectiveness of different pollinator taxa, to quantify each population's pollination environment. Finally, I measured flower production, propagule production, and propagule mass to assess inter-population variation in allocation to reproduction. These approaches reveal how a range-shifting pollinator-dependent plant can avoid pollen limitation at its expanding range margin, and how it also maintains high reproductive output near its physiological limit.

Finally, Chapter 4 pivots to consider how arthropod communities respond to *A. germinans* encroachment into salt marshes. Range shifts of dominant, habitat-forming species like *A. germinans* result in large structural, chemical, and phenological changes for local taxa within the ecotone. I sampled arthropod communities in range-edge *A. germinans* and in adjacent salt marsh at varying distances, to test the hypothesis that mangrove encroachment facilitates a new arthropod community and has effects that extend into surrounding marsh habitat. I also manipulated the number of *A. germinans* flowers to specifically test the influence of the novel floral resources on the structure and differentiation of arthropod communities in neighboring vegetation. Understanding how resident communities respond to the introduction of a new foundation species, and how communities vary across the patchy habitat of an ecotone, will make it easier to predict the effects of distributional shifts on regional biodiversity.

Chapter 2: Pollinator-dependence in *Avicennia germinans*, the black mangrove

Abstract

Avicennia germinans (black mangrove) is a cold-intolerant species undergoing a northward range shift in the southeastern U.S., as winter temperatures warm. This species' ability to track the changing climatic conditions may depend, however, on its breeding system. If *A. germinans* can maintain adequate fruit set without the assistance of pollinators – thanks to either wind pollination or spontaneous self-pollination – then its range expansion will not be constrained by pollen limitation at its range edge. Alternatively, if *A. germinans* relies on pollinators for full fruit set, then its ability to shift its distribution northward could be slowed if it reaches areas of low pollinator abundance. To determine the degree to which *A. germinans* depends on pollinators, we assessed the likelihood of wind pollination in *A. germinans* by monitoring for airborne pollen, and we tested *A. germinans*' ability to set fruit when pollinators were excluded and when within-flower selfing was prevented. We found no *A. germinans* pollen on pollen traps immediately adjacent to flowering trees, suggesting that this species is rarely, if ever, wind-pollinated. We also found significant declines in fruit set when pollinators were excluded from flowers, and no decline in fruit set when within-flower selfing was prevented. These results indicate that although *A. germinans* can reproduce to a limited extent without the assistance of pollinators, this species depends on pollinators to avoid pollen limitation.

Introduction

Avicennia germinans (L.) L. (black mangrove) is a common, sometimes dominant member of intertidal vegetation in tropical and subtropical regions of the Americas and West Africa (Tomlinson 1986). It is a culturally, economically, and ecologically important species, contributing to ecosystem services like carbon storage, wave attenuation, and nursery habitat for various fisheries (Barbier et al. 2011, Doughty et al. 2015, Kelleway et al. 2017). Despite its prominence in coastal settings, *A. germinans*' pollination system is not well understood. Its flowers are known to be attractive to animals – there are several records of floral visitors (Sánchez-Núñez and Mancera-Pineda 2012, Landry 2013a), and, at least historically, Floridian beekeepers have obtained high yields from hives in *A. germinans* stands (Pellett 1919) – but the degree to which it relies on pollinators is unknown. Given that this species may be undergoing climate-driven range expansion in the southeastern US (Cavanaugh et al. 2014), and given reproduction's critical role in plants' range dynamics, understanding these aspects of *A. germinans*' pollination biology is important for making predictions about the rate of its range shift and its future distribution.

If *A. germinans* were wind-pollinated or were capable of spontaneous autogamy (unassisted pollination of flowers by their own pollen), then this species would not require pollinators to ensure reproduction. And indeed, molecular methods have revealed that *A. germinans* does self-pollinate – sometimes quite frequently (Nettel-Hernanz et al. 2013). However, it is unknown whether this selfing occurs is spontaneous. In other words, selfing may occur either via spontaneous autogamy, via

pollinator-mediated fertilization of flowers with their own pollen or pollen from neighboring flowers on the same plant (geitonogamy), or via both mechanisms (Goodwillie et al. 2005). Furthermore, *A. germinans* may not be capable of spontaneous autogamy at all, since its flowers are protandrous (its anthers mature before the stigmas) (Daniel 2016) – a reproductive strategy that is thought to help plants avoid self-pollination (Lloyd and Webb 1986). De Lima Nadia et al. (2013) found that a sister-species, *A. schaueriana* Moldenke, that is also protandrous, is incapable of spontaneous autogamy, and it therefore relies on a vector for self-pollination. Two other sister-species – *A. officinalis* L. and *A. marina* (Forssk.) Vierh. – have recently been shown to suffer reduced fruit set (though not outright reproductive failure) in the absence of pollinators (Chakraborti et al. 2019). If *A. germinans* cannot spontaneously self, or can only do so to a limited extent, then pollinators may be essential for maximum reproduction. However, this has not been tested.

One additional means by which *A. germinans* can avoid dependence on pollinators is wind pollination. *A. germinans* is usually assumed to be exclusively animal-pollinated (Tomlinson 1986), but no test for windborne pollen has ever been reported. Some aspects of *A. germinans*' floral morphology – the showy corollas, copious nectar production, and moderate pollen production – are considered atypical for a wind-pollinated plant (Faegri and van der Pijl 1979). Additionally, the attractiveness of *A. germinans*' flowers to insects – it has variously been described as having a generalized pollination system (Sánchez-Núñez and Mancera-Pineda 2012), and as being bee-pollinated (Nettel-Hernanz et al. 2013) – seems to have led

observers to assume that wind pollination is unimportant. But *A. germinans* possesses several characteristics common in wind-pollinated species (Culley et al. 2002), such as a high pollen:ovule ratio (measured as $3,351 \pm 881$ SD, *unpublished data*) and a relatively small pollen size (Bertrand 1983). Wind pollination could help ensure reproduction in settings with low pollinator availability – a situation that individuals at the leading range edge could encounter. That being said, *Avicennia* pollen is scarce in the fossil record (Duke 1995), suggesting that it infrequently becomes airborne.

The details of *A. germinans*' pollination biology have important implications for its range dynamics. If *A. germinans* can self only with the assistance of pollinators, then its ability to shift its distribution may depend on pollinator availability (Moeller et al. 2012). In settings like the salt marshes of the southeastern US – the habitat into which mangroves are encroaching – pollinator availability may be low, due to the dominance of wind-pollinated grasses (Steffan-Dewenter and Tschardt 1999, Hegland and Boeke 2006). If this is the case, *A. germinans*' rate of range expansion may rely on its ability to attract pollinators from neighboring upland habitat. On the other hand, if *A. germinans* is capable of spontaneous autogamy or wind pollination, and therefore does not rely on pollinators for reproduction, then its range dynamics may not directly depend on pollinator availability. However, even if *A. germinans* is capable of some reproduction in the absence of pollinators, its breeding system and the genetic structure of its populations may still be influenced by the abundance and identity of pollinators in a given environment (Loveless and Hamrick 1984).

This study's objective is to describe the pollination biology of *A. germinans*. Specifically, we sought to identify its pollen vector or vectors – i.e. insects and/or wind – and to assess its ability to spontaneously self-pollinate. Ultimately, we wanted to determine if *A. germinans* is capable of setting fruit in the absence of pollinators. If *A. germinans* relies even partially on pollinators, its ability to track the changing climate of the Southeastern U.S. may depend on the insect communities of the region's coastal zone.

Materials and Methods

Field site description

Pollinator exclusion experiments were carried out during the summers of 2014 and 2015 at several sites spanning approximately 320km of Florida's Atlantic coast (Figure 2.1). The northern sites (Anastasia State Park and the Guana-Tolomato-Matanzas NERR) contain some of the northernmost mangroves in Florida, having scattered, sometimes very dense, monospecific stands of short *A. germinans* surrounded by salt marsh vegetation (mainly *Sporobolus alterniflora* (Loisel.) P.M. Peterson & Saarela and *Batis maritima* L.). Heading south, sites are increasingly mangrove-dominated, with taller trees and increased abundance of Florida's other two mangrove species (*Rhizophora mangle* L. and *Laguncularia racemosa* (L.) C.F. Gaertn.).

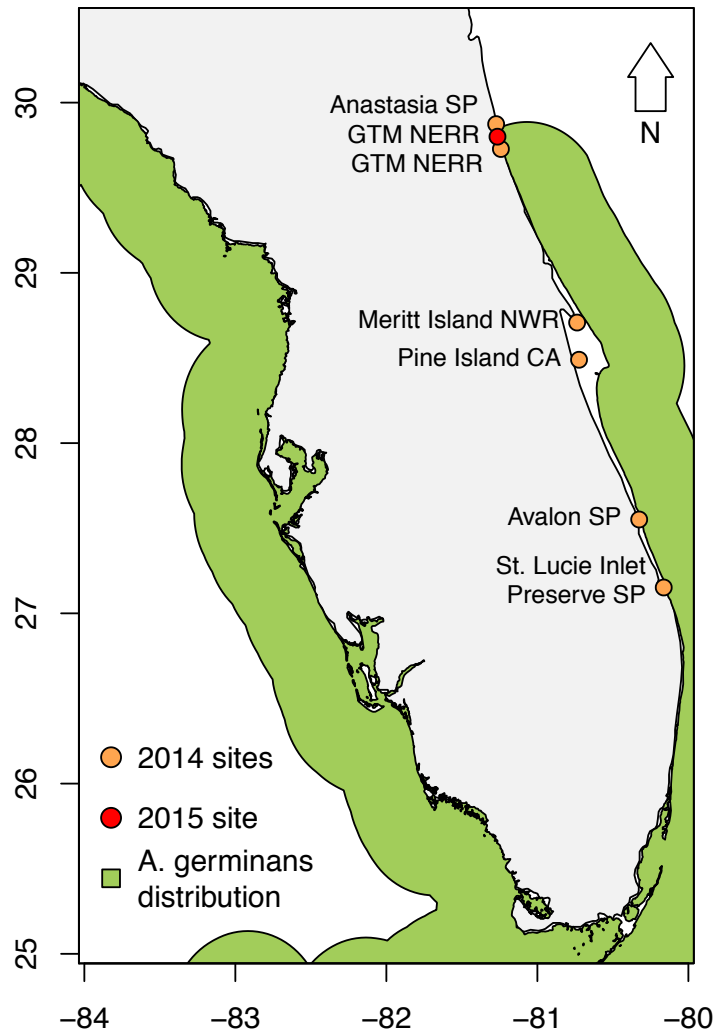


Figure 2.1: Sites where pollinator exclusion experiments were conducted, in 2014 (orange) and 2015 (red). All sites were located on the lagoon side of barrier islands. See text for individual site descriptions.

Experiment 1: Reproductive strategy

To assess *A. germinans*' ability to self without pollinators, the contribution of within-flower selfing to fruit set, and inter-site variation in *A. germinans*' breeding system, we conducted an experiment to test reproductive strategy across six sites in 2014 (Figure 2.1). At each site, we haphazardly selected 12-21 *A. germinans*

individuals, with most trees at least 10m apart. In June-July (depending on site), each tree received all of the following treatments: anther removal, pollinator exclusion, pollinator exclusion plus hand-pollination with self pollen, and unmanipulated natural pollination. Up to 20 nearly-opened flowers, each on a different inflorescence, were selected within each tree. Each flower was haphazardly assigned to one of the treatment levels, such that all treatments were replicated up to five times within a tree. In order to identify each replicate at the end of the experiment, the calyx of each focal flower was marked with white correction fluid, and the pedicel of its inflorescence was tagged with flagging tape.

The first manipulative treatment – anther removal – effectively left flowers unable to self-pollinate. Therefore, this treatment’s fruit set, when compared to that of the unmanipulated natural-pollination treatment, would reveal the degree to which within-flower selfing boosts *A. germinans*’ fruit set. This treatment was applied by using dissecting scissors to cut out all four anthers from focal flowers, prior to pollen release; in total, 449 flowers received this treatment across all six sites.

For the pollinator exclusion treatment, inflorescences containing the focal flowers were covered with a 7.5x10cm fine-mesh (0.05 mm) bag prior to anthesis, to ensure the flowers had not been pollinated prior to the treatment. The bags remained in place for 5-8 days, by which point the anthers and corollas had abscised. In total, 462 flowers received this treatment across all six sites.

To partly account for the confounding effect that increased temperatures inside the pollinator exclusion bags had on fruit set (Figure A.1), we included a hand-pollination treatment, in which replicates were bagged as in the pollinator-exclusion

treatment, but also received supplemental pollen (applied with a brush from another open flower on the same tree). This allowed us to directly compare the fruit set of bagged flowers – for which spontaneous autogamy was the only means of pollination – to the maximal fruit set of bagged flowers that received abundant pollen. This treatment also gave some indication of the extent of fruit set decline due to heat/humidity within the pollinator exclusion bags: if there were no such negative effects, then fruit set of this treatment would be expected to be as high or higher than fruit set in the unmanipulated natural-pollination treatment. Across all six sites, 309 flowers received this treatment.

Five to seven weeks after applying the treatments, we covered any developing focal propagules with large-mesh (~1.0 cm) bags, to catch the mature propagules as they dropped (which occurred 4-5 months later). In the fall, we recorded the presence or absence of a propagule from each focal flower.

In R version 3.5.0, we modeled fruit set (presence/absence) using a binomial generalized linear mixed model (glmer function in the lme4 package), with treatment, site, and a treatment×site interaction as fixed effects, and tree as a random intercept (Bates et al. 2015, R Core Team 2018). We used a bootstrap approach to test the significance of the treatment, site, and interaction predictors (Halekoh and Højsgaard 2014), and used post hoc Tukey testing (Hothorn et al. 2017) to assess differences in treatment effects site-by-site. Finally, we calculated the model's marginal and conditional R^2 , for fixed effects and both random and fixed effects, respectively, with the r.squaredGLMM function in the MuMIn package (Bartoń 2018).

Experiment 2: Pollinator exclusion

The second pollinator exclusion experiment, in 2015, was conducted at a single site (Figure 2.1) and was designed to better account for treatment effects that were revealed in the first experiment, by using pollinator exclusion bags with wider mesh. In early June, before flowers had opened, 20 *A. germinans* were selected, with all trees at least 10m from one another. We selected six inflorescences on each tree, randomly assigning three to a pollinator exclusion treatment and three as unmanipulated natural pollination (ultimately n=45 and n=54, respectively, after some replicates were lost over the course of the experiment). The pollination exclusion treatment here was the same as in the first experiment in 2014, except for the size of the mesh in the exclusion bags. Inflorescences assigned to the pollinator exclusion treatment were covered with large-mesh (~1.0 cm) plastic bags, which were meant to exclude most large insect pollinators (however, we did observe one Crabronid wasp leaving one of the bags partway through the experiment, indicating that the bags were not impenetrable to large insects). Inflorescences assigned to the natural pollination treatment were left unbagged and were marked with flagging. In late August, once flowering had finished, bags were placed over the control inflorescences, as well, to catch propagules as they dropped. We collected all bags in early November, 2015, and counted both the number of propagules inside and the number of initial floral buds on the inflorescence.

The number of propagules was modeled using a Poisson generalized linear mixed model, with treatment as a fixed effect, tree as a random intercept, and total

floral buds as an offset, in R (glmer function in the lme4 package) (Bates et al. 2015, R Core Team 2018).

Monitoring for windborne pollen

Monitoring for windborne *A. germinans* pollen was conducted in 2015 at the same range-edge site as the second pollinator-exclusion experiment. During peak flowering, we selected five 1-2m tall *A. germinans* that were at least 1.5m away from any other *A. germinans*. At each tree, we installed five 1m-tall stakes: one near the tree's main trunk, and one in each of the four cardinal directions approximately 0.5m from the tree's periphery. At 8:30am, sticky traps – Vaseline-coated microscope slides – were hung on each stake facing the focal tree, with the stake near the tree's main trunk receiving four sticky traps, each facing outward. The sticky traps were left in place for 24hrs to collect pollen. After collecting the traps, we covered their adhesive surfaces with glass slip covers, and inspected them at 100X magnification using a compound microscope. To provide a reference search image, we intentionally applied *A. germinans* pollen to one unused sticky trap. Two points were randomly selected on each sticky trap's adhesive surface, and the area inside the field of view (approximately 2.5mm²), centered on these points, was examined to record any *A. germinans* pollen present. The rest of the slides were also scanned to check for any missed clumps of pollen.

Results

Experiment 1: Reproductive strategy

In 2014, there was substantial variation in fruit set among sites, with the highest natural rates of fruit set observed at the northernmost site (Anastasia) and at one of the southernmost sites (Avalon) (Figure 2.2). Of 466 natural-pollination flowers across all sites, 130 had initiated propagule development by the end of the experiment, amounting to an overall fruit set rate of 0.28 propagules/flower. The full model, with treatment, site, and treatment×site fixed predictors and tree ID as a random intercept, explained a moderate amount of variation in fruit set (marginal $R^2=0.34$, model conditional $R^2=0.39$).

We detected a significant interaction between treatment and site (bootstrap $p = 0.001$), such that treatment effects varied among sites (Figure 2.2, Figure A.2). In general, anther removal did not decrease fruit set; in four out of six sites, flowers that had their anthers removed set fruit at a rate similar to those exposed to natural levels of pollination. (Two sites – Pine Island and Merritt Island – saw an increase in fruit set for flowers without anthers, relative to natural-pollination flowers.)

Additionally, bagging to exclude pollinators tended to decrease fruit set; in another four of the six sites, flowers that were bagged and received no supplemental pollen set fruit at a significantly lower rate than flowers exposed to natural levels of pollination (Figure 2.2, Figure A.2). The remaining two sites (Merritt Island and the GTM NERR) showed no difference in fruit set between bagged and natural-pollination flowers; notably, these two sites were those with the lowest rates of natural fruit set.

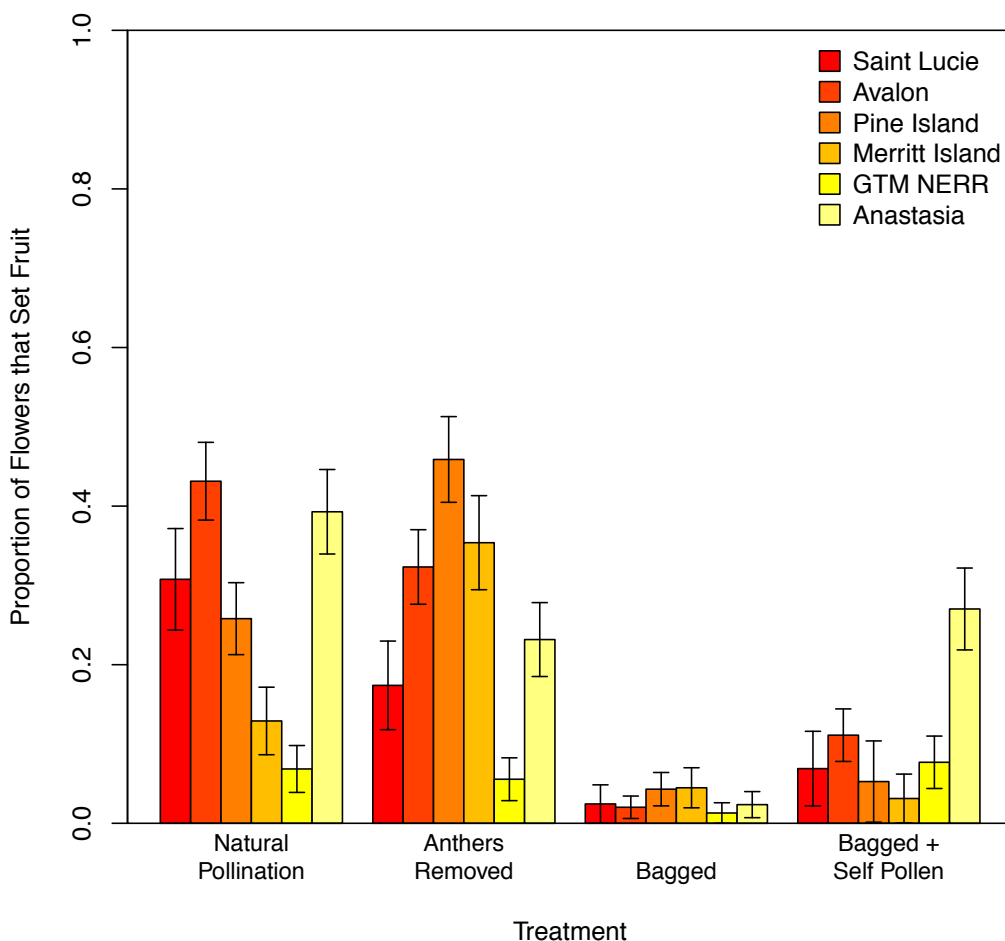


Figure 2.2: Variation among treatments and sites in fruit set, in the first reproductive strategy experiment in 2014. Y-axis values are calculated as the number of propagules that began to develop divided by the number of flowers assigned to this treatment and site. Sites are arranged within each treatment from southernmost on the left (red), to northernmost on the right (yellow). Bars show standard errors. For significant differences among treatments within each site, see Figure A.2.

Flowers that received supplemental self-pollen and were bagged mostly displayed similar levels of fruit set to bagged flowers, except for one site (Anastasia), where supplemental pollen increased fruit set relative to bagged flowers, restoring it to a level similar to that of natural-pollination flowers (Figure 2.2, Figure A.2). At three additional sites (Saint Lucie, Avalon, and the GTM NERR), bagged flowers that

received supplemental pollen showed slightly higher rates of fruit set, relative to flowers that were bagged and received no additional pollen; however, the differences in fruit set at these sites were not significant. In addition, compared to natural-pollination flowers, flowers that received supplemental pollen tended to display a reduced fruit set rate – although this difference was only statistically significant at one site (Avalon).

Experiment 2: Pollinator exclusion

The 2015 experiment, involving a single pollinator exclusion treatment and larger exclusion mesh, also showed a negative effect of pollinator exclusion on propagule production (Figure 2.3). Control inflorescences had a mean propagule production rate of 0.25 propagules/floral bud (± 0.14 SD), whereas bagged inflorescences produced propagules at a rate of 0.13 propagules/floral bud (± 0.13 SD) (model marginal $R^2=0.21$, model conditional $R^2=0.46$).

Monitoring for windborne pollen

In our monitoring for windborne *A. germinans* pollen, we did not observe a single *A. germinans* pollen grain across all 40 sticky traps associated with our five focal trees.

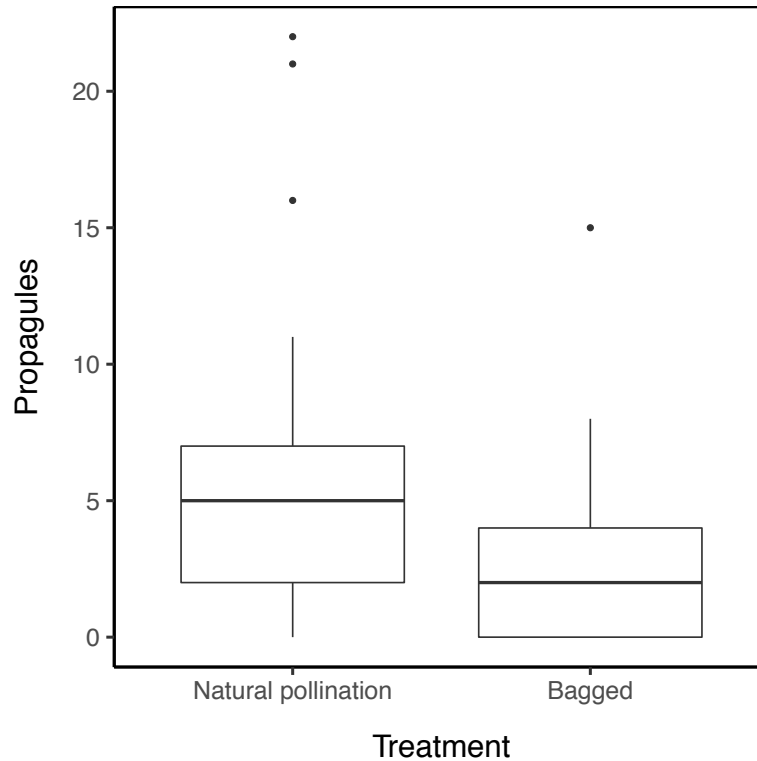


Figure 2.3: Propagule production (measured as number of propagules produced by an inflorescence), from the second pollinator exclusion experiment in 2015. Bagged inflorescences produced significantly fewer propagules per floral bud than unmanipulated inflorescences that received natural levels of pollination (bootstrap $p = 0.005$).

Discussion

Avicennia germinans displays a moderately high level of pollinator dependence, and does not appear to rely on wind for pollination. Here, we have shown that while *A. germinans* is capable of spontaneous self-pollination, its fruit set declines substantially in the absence of pollinators. When pollinators were largely excluded from flowers, fruit set dropped by nearly half (Figure 2.3). Additionally, flowers that were entirely inaccessible to pollinators may have been pollen-limited, as evidenced by the tendency for fruit set to increase when supplemental pollen was

supplied (although this increase was only statistically significant at one site) (Figure 2.2). Furthermore, flowers prevented from spontaneously self-pollinating – those whose anthers were removed – did not experience a decrease in fruit set rate, indicating that within-flower selfing is negligible, or that it can be entirely made up for by other pollen vectors. Finally, we found no evidence for windborne *A. germinans* pollen, suggesting that anemophily is not an important means of pollination in this species. Together, these findings point to reliance on pollinators for full fruit set in *A. germinans*, and to the potential for pollinator availability to influence this species' range expansion.

This result is somewhat counterintuitive, given *A. germinans*' life history. An effective colonizer, *A. germinans* might benefit from the reproductive assurance that spontaneous autogamy provides, especially during the early stages of colonization, when density of individuals is low (Baker 1955). Indeed, plants that predominantly self-pollinate have been found to have larger range sizes than those that are predominantly outcrossing, presumably due to their ability to establish in new settings, (Grossenbacher et al. 2015), and *A. germinans*' range is certainly very large (Tomlinson 1986). On the other hand, in the presence of inbreeding depression, selfing can cause a decline in fecundity and/or offspring fitness that is large enough to offset the benefits of reproductive assurance (Hargreaves and Eckert 2014). Perennial plants, like *A. germinans*, can escape this tradeoff between inbreeding depression and reproductive uncertainty by avoiding self-pollination (via mechanisms like herkogamy and dichogamy) and simply waiting to reproduce another season, if pollination in a given season is low (Stebbins 1970, Morgan et al. 1997). The

magnitude of inbreeding depression has not been tested in this species; it may be considerable, given our finding that *A. germinans* rarely spontaneously self-pollinates, despite the presumed benefits of doing so. Alternatively, the ability to achieve full fruit set via spontaneous self-pollination may not have evolved in this species thanks to a highly generalized pollination system and an adequate supply of pollinators wherever *A. germinans* establishes.

While we are confident that *A. germinans* is dependent on pollinators for full fruit set, it is important to emphasize that the magnitude of *A. germinans*' reliance on pollinators is difficult to derive from this study. The tradeoff between floral damage from heat/humidity (with narrow-mesh exclusion bags) and incomplete pollinator exclusion (with wide-mesh exclusion bags) means that our first experiment, using narrow-mesh bags, overestimated the strength of pollinator dependence, while our second experiment, using wide-mesh bags, underestimated it. Furthermore, meta-analysis has revealed that experiments like these, where just a fraction of each individual plant's flowers are involved, tend to overestimate the strength of pollen limitation, on average (Knight et al. 2006). This may occur if plants allocate resources away from flowers with low pollen receipt (e.g. flowers experimentally subjected to pollinator exclusion). Whether or not *A. germinans* does this is unknown. Our experiments somewhat avoided this issue by applying treatments to entire inflorescences; trees are less likely to allocate resources among distant branch tips than among adjacent pedicels (Knight et al. 2006).

A. germinans' dependence on pollinators means that pollinator availability can influence its ability to shift its distribution in sync with the changing climate of the

southeastern US. In this region, *A. germinans*' rate of range expansion may depend on how well it attracts pollinators from neighboring upland habitat, given a potential scarcity of pollinators in the salt marsh habitats into which mangroves are encroaching. In theory, this will be challenging for the northernmost colonizing individuals, which are small and isolated (Kunin 1993). However, our results reveal a complex picture regarding the relationship between reproductive success and proximity to the range edge: a general negative trend in fruit set with increasing latitude was erased by very high fruit set seen at the northernmost population (Figure 2.2). This suggests that *A. germinans*' ability to attract pollinators near its range edge will be site-specific, and that its continued encroachment northward will not necessarily be pollinator-limited. Future research, with this species and others, should test the importance of pollinator dependence and availability for plants near their range limits, as they respond to climate change.

Chapter 3: Major turnover in pollinator community composition does not impact fecundity of a range-shifting plant

Abstract

Plants' ability to reproduce near their geographic range edge can determine whether distributions will shift in response to changing climatic conditions. However, range-edge reproduction can be a challenge, particularly for species that rely on pollinators. The pollinator assemblage at a plant's range edge can differ in abundance and identity from assemblages within the geographic core of the plant's distribution, potentially causing pollen limitation at the range edges. Additionally, for plants whose range limits are defined by environmental tolerances, abiotic conditions at the range edge can be stressful, affecting allocation to reproductive output. Animal-pollinated plants that have shifted their distributions have overcome these multiple barriers, but the mechanisms by which they have done so is poorly understood. In this study, I examined plant-pollinator interactions hypothesized to impact reproduction as populations of the black mangrove, *Avicennia germinans*, expand poleward in eastern coastal Florida, USA. I identified and counted pollinators visiting *A. germinans* populations varying in proximity to the geographic range edge, evaluated the relationship between local pollinator assemblages and pollen deposition (a measure of pollen limitation), and measured *A. germinans* flower and propagule production. I found that despite a 72% decline in pollinator abundance at the range edge, species turnover resulted in a highly effective assemblage of pollinator taxa there; consequently, pollen deposition remained high at the range edge. I also observed

elevated flower production in northern populations, which, combined with the maintenance of pollinator effectiveness there, contributed to high per-tree reproductive output at the range edge. Furthermore, mean propagule mass in northern populations was 18% larger than propagules sampled from the southernmost populations. Together, these findings suggest that range-edge *A. germinans* are able to rapidly encroach on surrounding salt marsh, and corroborate research that points to rapid expansion of mangrove cover in the region. These results show that the climatic constraints that set this species' range limit do not necessarily limit range-edge allocation to reproduction, and that pollinator identity, rather than sheer abundance, can support plant reproductive success at an expanding range edge.

Introduction

In response to climate change, the geographic distributions of many taxa are shifting to track changing environmental conditions (Parmesan 2006, Lenoir et al. 2008, Chen et al. 2011). For plants (and other sessile organisms), range shifts depend, in part, on the ability to reproduce at or relatively near the leading range edge, so that propagules can disperse into newly suitable habitat. However, a number of abiotic and biotic conditions can constrain a plant's ability to produce viable seeds near the edges of its geographic range. Reproductive failure can be as important as mortality in setting plants' range limits (Pigott 1992, Gaston 2009). For plants subject to changing climatic conditions, reproductive failure at the range edge can result in a delayed range shift, or in no shift at all (Clark et al. 2001, Alexander et al. 2018, Brown et al. 2018).

Multiple ecological mechanisms can decrease plant fecundity, or the number of viable seeds produced per individual, near geographic range edges. For species whose range limits are set by environmental tolerances, particularly climatic ones, conditions at the range edge can be generally stressful across all life stages, leading to reduced plant vigor and allocation to reproduction (Reinartz 1984, Chiariello and Gulmon 1991, García et al. 2000, Gaston 2009). While some plants can react to stressful conditions by increasing reproductive effort (to compensate for early losses to reproductive components or for reduced adult survival), allocation to reproduction generally declines as resources become limiting – particularly for perennial plants (Chiariello and Gulmon 1991). But reduced fecundity due to a decline in overall vigor is not the only reason that physical conditions can limit range-edge fecundity; different developmental stages can respond differently to abiotic conditions, leading to situations in which, for example, adults can persist but are unable to reproduce. For instance, Pigott and Huntley (1981) observed prohibitively slow pollen-tube growth in otherwise apparently healthy populations of *Tilia cordata* near its northern range edge, which they attributed to cold spring temperatures there. Weis and Hermanutz (1993) found that a population of *Betula glandulosa* near the species' northern range edge produced relatively few staminate and pistillate flowers, and little pollen per catkin, leading to a complete failure to set seed; they speculated that winter freezes or the short growing season could be responsible. More recently, Brown et al. (2018) demonstrated that despite an increasingly favorable climate for adult trees at the forest-tundra ecotone in arctic regions, an interaction between decreased tree density, growing degree days, and precipitation results in reduced viable seed production

there, constraining trees' ability to colonize the tundra, and potentially creating a lag in the northward shift of the arctic treeline. Additional studies have found range-edge reproductive failure in plants whose reproductive phenology is incompatible with earlier winters or later springs there (Chuine and Beaubien 2001, Tremblay et al. 2002, Griffith and Watson 2006, Morin et al. 2007, Chuine 2010). In sum, harsh abiotic conditions at a plant's range edge can cause reproductive failure there, even if individuals are still capable of establishment and vegetative growth.

In addition to abiotic conditions, biotic factors at a plant's range edge can create barriers to reproduction. While negative biotic interactions, particularly competition, have received growing research attention as the causes of species' range limits, there is increasing recognition of the role that positive species interactions can play in setting species' range limits (Gaston 2003, Sexton et al. 2009, HilleRisLambers et al. 2013, Svenning et al. 2014). For example, absence of mutualists beyond a plant's range edge can prevent local establishment (Parker 1997, 2001, Nuñez et al. 2009, Moeller et al. 2012, Afkhami et al. 2014). Plants that rely on pollinators for full fruit set – i.e. most flowering plants (Ollerton et al. 2011) – face this biotic barrier to range-edge reproduction. Pollinator communities often vary in composition throughout the distribution of a given plant species, potentially creating spatial gradients of pollen limitation (Herrera 1988, Horvitz and Schemske 1990, Gómez et al. 2010). If a plant and its pollinators are similarly limited by environmental conditions – e.g. aridity, soil conditions, or temperature – then pollinator availability can decline (and pollen limitation can rise) toward the plant's range edge (Stone and Jenkins 2008, Moeller et al. 2012). Alternatively, pollinator

visitation can decline at a plant's range edge if the habitat beyond contains few floral resources, and therefore supports few pollinators (Chalcoff et al. 2012). Finally, pollination can decline if a plant's density, height, and/or floral display are reduced at the range edge, thereby reducing its attractiveness to pollinators (Kunin 1993, Brody and Mitchell 1997, Donnelly et al. 1998, Hegland and Boeke 2006).

If changes in the abundance or identity of pollinators result in increased pollen limitation of a plant species near its range edge, plant performance can decline there, affecting range dynamics. How often these gradients of pollen limitation and geographical ranges align is unknown; while increased pollen limitation due to changes in pollinator abundance and identity can limit plant performance at some range edges and invasion fronts (e.g. Hopkins 1914, Parker 1997, Chalcoff et al. 2012, Moeller et al. 2012, Rivest and Vellend 2018), this pattern has not emerged in other systems (e.g. Stanton 1987, Busch 2005, Traveset and Richardson 2014, Hargreaves et al. 2015). This inconsistency has led to disagreement concerning the importance of pollination as a factor influencing plants' range dynamics (Traveset and Richardson 2014, Hargreaves et al. 2015).

Despite these potential abiotic and biotic barriers to range-edge reproduction, animal-pollinated plants have successfully shifted their range edges in response to climatic changes, both past and present. *Avicennia germinans* (L.) L. (Acanthaceae) (black mangrove) in the southeastern U.S. is one such species responding to contemporary climate change. A freeze-intolerant tree that depends on pollinators for full fruit set, *A. germinans* has expanded rapidly northward in the Gulf of Mexico and northern Florida over the past three decades, in tandem with a decline in the

frequency of hard freezes over that period (Tomlinson 1986, Osland et al. 2013, Cavanaugh et al. 2014). Recent work has reconstructed a longer history of mangrove extent in the region, revealing a highly dynamic range edge that has oscillated between mangrove dominance and salt marsh dominance for at least the last 150 years, mirroring decadal-scale fluctuations in the region's climate (Rodriguez et al. 2016, Cavanaugh et al. 2019). These repeated and rapid re-expansions of the *A. germinans* range edge point to this species' ability to quickly respond to changing climatic conditions. However, this research has not addressed range-edge fecundity and reproduction, and particularly the pollination ecology, that could contribute to geographic range expansion and population growth beyond historical range limits.

In this study, I address the question of how *A. germinans* pollination, reproductive effort, and ultimately fecundity vary along a geographical gradient from the range interior to the leading range edge, testing the hypothesis that barriers to reproduction are more severe toward this species' northern periphery. Specifically, I hypothesized that the quality of the pollination environment and allocation to reproduction decline from the core of the distribution toward the northern range edge. I expected that the abundance and overall effectiveness of pollinators would decline in range-edge populations, where mangrove stands are surrounded by a matrix of largely wind-pollinated salt marsh species. I investigated variation in local pollination environments by quantifying a) the relationship between proximity to the range edge and the abundance and identity of floral visitors; b) the degree to which different floral visitors vary in their effectiveness as pollinators; and c) variation in stigmatic pollen deposition and fruit set rates, measures of pollen limitation. Additionally,

considering that northern populations of *A. germinans* are subject to colder temperatures and a shorter growing season (conditions that affect their leaf and vascular morphology [Cook-Patton et al. 2015]), I predicted that flowering and propagule size would decline in range-edge populations, due to reduced allocation to reproduction.

Materials and Methods

Study system

Avicennia germinans is an intertidal tree or shrub that bears its bisexual flowers on terminal panicles of spikes. Floral corollas are white, ~1cm in diameter, and open in pairs within each spike. Flowers contain four stamens and a gynoecium with four ovules and a single style and bilobed stigma. The flowers are protandrous, with anthers that release pollen prior to the opening of the stigmatic lobes (Borg and Schönenberger 2011, Daniel 2016). *A. germinans* is self-compatible (Nettel-Hernanz et al. 2013), but in previous work we demonstrate that it relies on pollinators for full fruit set. Typically, flowers produce at most a single seed that, as with other mangrove taxa, germinates while still attached to the parent plant, giving rise to a dispersive seedling known as a ‘propagule’ (Tomlinson 1986). Although reproduction occurs year-round in the tropics (Daniel 2016), in subtropical regions *A. germinans* flower synchronously May-August and release their propagules September-November (*unpublished data*).

Data were collected over the course of three years at eleven sites containing *A. germinans* populations in eastern Florida (Table B.1, Figure B.1). These sites span the

full extent of Florida's eastern mangrove-marsh ecotone – an approximately 200-km coastal zone where salt marsh in the north transitions to mangroves in the south – and extend southward into a region that has been continuously mangrove-dominated for several thousand years (Scholl 1964).

The southernmost sites (latitudes 27.1-27.9°N) contain mixed stands of all three Floridian mangrove species [the two others being *Rhizophora mangle* L. (Rhizophoraceae) and *Laguncularia racemosa* (L.) C.F. Gaertn. (Combretaceae)], in varying stages of regeneration following impoundment for mosquito control in the first half of the 20th century. Farther north, in the southern reaches of the mangrove-marsh ecotone (latitudes 28.5-29.1°N), many sites are also recovering from impoundment or other human alteration, but here are characterized by mixed-mangrove stands edging waterways, with salt marsh vegetation [primarily *Distichlis spicata* (L.) Greene (Poaceae), *Spartina alterniflora* Loisel. (Poaceae), *Batis maritima* L. (Bataceae), and *Salicornia* sp. L. (Amaranthaceae)] landward. At the northern end of the study region (latitudes 29.6-29.9°N), sites contain some of Florida's northernmost mangroves (Cavanaugh et al. 2019). Here, clusters of short (generally <3m tall) mangroves – almost exclusively *A. germinans* – are embedded in a salt marsh matrix consisting primarily of *S. alterniflora*, *B. maritima*, and *Salicornia* sp. Most of these northern sites fall within the Guana-Tolomato-Matanzas National Estuarine Research Reserve (GTM).

Sampled mangroves varied in their proximity to upland vegetation, ranging from <10m to over 300m distant, depending on the site and specific location. Upland vegetation consists of mixed hardwood hammock, oak scrub, and high marsh, the

latter containing abundant *Borrchia frutescens* (L) DC (Asteraceae) and *Limonium carolinianum* (Walter) Britton (Plumbaginaceae), which we observed co-flowering with *A. germinans*.

Floral visitor identity and frequency

The identity and frequency of *A. germinans*' floral visitors were assessed during the 2013, 2014, and 2015 flowering seasons. All 11 sites were monitored at least once over these three years, though only seven were monitored in any given year (Table B.1), with each site visited repeatedly within a flowering season. Across all sites in a given year, visitation data were collected over 105.5hrs, 67hrs, and 33.5hrs in 2013, 2014, and 2015, respectively.

A. germinans flowers were monitored for floral visitors during 15-minute observation periods, which we conducted in fair weather from mid-morning to mid-day. Focal *A. germinans* were selected haphazardly; trees were at least 10m apart and contained at least 10 open flowers. We conducted a single observation period per day at each focal tree (i.e. no tree was monitored twice on the same date). During each observation period, the observer selected 4-10 open flowers in close proximity to one another, and recorded the identity of each floral visitor and the number of focal flowers visited by each individual insect. Insects were typically identified to family, with the exception of the Apidae which were identified to genus or species. A complete list of the 29 taxonomic labels used in floral monitoring, as well as the study-wide abundance of each taxon, is available in Table 3.1. Altogether, 2,127 individual insects were observed visiting *A. germinans* flowers.

Table 3.1: Taxonomic categories assigned to *A. germinans* floral visitors, and total number of visitors of each taxon observed, across sites and years, over the course of the study.

Taxon	# Visitors
Hymenoptera	
<i>Apis mellifera</i>	1,037
<i>Melissodes</i>	143
<i>Bombus</i>	109
<i>Xylocopa</i>	19
<i>Triepeolus</i>	1
<i>Halictidae</i>	10
<i>Megachilidae</i>	8
<i>Vespidae</i>	59
<i>Crabronidae</i>	28
<i>Pompilidae</i>	15
<i>Sphecidae</i>	7
<i>Formicidae</i>	275
Lepidoptera	
<i>Pieridae</i>	40
<i>Hesperiidae</i>	11
<i>Nymphalidae</i>	8
<i>Lycaenidae</i>	2
<i>Noctuidae</i>	1
Diptera	
<i>Syrphidae</i>	306
<i>Muscidae</i>	6
<i>Uliidiidae</i>	3
<i>Calliphoridae</i>	2
<i>Stratiomyidae</i>	2
<i>Anthomyiidae</i>	2
<i>Dolichopodidae</i>	1
Unk. Diptera	11
Coleoptera	
<i>Oedemeridae</i>	16
<i>Scarabaeidae</i>	3
<i>Coccinellidae</i>	1
Orthoptera	
Unk. Orthoptera	1

During each observation period, the observer recorded the focal tree's height (to the nearest 0.5m) and estimated the total number of open flowers on the tree.

Floral visitor identity and frequency - Analysis

These, and all subsequent analyses, were performed in R version 3.5.0 (R Core Team 2018). Floral visitation was modeled three separate ways: as total visits across all taxa (to explain overall trends in insect abundance), as a binary

presence/absence variable across all taxa (a form that may be more relevant for *A. germinans*, for which the difference between the presence and absence of floral visitors is more important than the difference between few and many floral visitors), and as taxon-specific visitation (to explore changes in the floral visitor assemblage). To model total visits across all taxa, I used a generalized linear mixed model (GLMM) with a negative binomial error distribution, to account for overdispersion in the data (*lme4* package [Bates et al. 2015]). Latitude (here, and in all modeling in this study, centered on its mean), year, a latitude×year interaction, the number of flowers open on the focal tree, and tree height were included as fixed effects; date was included as a random effect (multiple observation periods were conducted on the same date). In addition, the number of flowers watched during the observation period was included as an offset. I used a bootstrapping method (*pbkrtest* package [Halekoh and Højsgaard 2014]) to test the significance of each fixed effect in the model. Marginal and conditional R^2 – measures of variance explained by fixed effects and variance explained by both fixed and random effects, respectively – were calculated following Nakagawa and Schielzeth (2016).

Floral visitor presence/absence was modeled similarly, but used a GLMM with a binomial error distribution (*lme4* package [Bates et al. 2015]). For the response variable, all non-zero visit totals were converted to 1. Once again, I used bootstrapping to test the significance of each fixed effect (*pbkrtest* package [Halekoh and Højsgaard 2014]), and used the *MuMIn* package (Bartoń 2018) to calculate marginal and conditional R^2 .

To assess changes in the floral visitor assemblage across the latitudinal gradient of the study sites, I constructed a third GLMM with a random slope term that allowed the effect of latitude to vary among taxa. I subset the raw visitation data to the 12 most abundant taxa (representing 97% of all flower visits), and used a negative binomial error distribution (*glmmADMB* package [Fournier et al. 2012]). In addition to the by-taxon random slope and intercept terms, the model included random terms for date and observation period (as individuals observed during the same observation period might behave similarly), a single fixed effect for latitude, and an offset for the number of flowers watched during the observation period. I then plotted the model-estimated random slopes to assess differences among taxa in their variation with latitude. Finally, I performed a non-metric multidimensional scaling (NMDS) on visitation rates (*vegan* package [Oksanen et al. 2018]), using just the ten most abundant taxa and summing across observation periods made at the same site and on the same date (to avoid problems associated with low abundance per sample). The NMDS used Bray-Curtis dissimilarities.

Pollinator effectiveness

To approximate the relative importance of individual taxa as pollinators of *A. germinans*, we captured floral visitors and measured the size of their pollen loads. Using hand nets, we collected individual insects representing 11 of the 12 most frequently observed floral visitor taxa, directly from *A. germinans* flowers (one frequent taxon – Pompilidae – was not encountered during this time). We collected as close to 10 individuals per taxon as possible (see sample sizes in Figure B.2). We quickly immobilized the insects in coolers with ice packs, and later transferred them

to a freezer to kill them. They were subsequently pinned and inspected using a dissecting microscope.

In the lab, each insect was swabbed with a $\sim 2\text{mm}^3$ cube of fuchsin jelly for up to 10 minutes to sample its pollen load. In the case of *A. mellifera* and *Bombus*, hydrated corbicular pollen was avoided, as it is thought to be unlikely to contribute to pollination (Thorpe 2000). The fuchsin jelly was then transferred to a microscope slide, where the number of *Avicennia* and non-*Avicennia* pollen grains could be counted using a compound microscope at 100X and 400X magnification.

Using these pollen load data and the visitation data described above, I created an index of pollinator effectiveness, which incorporates aspects of floral visitor quantity and quality during each observation period (Herrera 1987). I defined the effectiveness index as the visitation rate of each taxon during an observation period (i.e. the “quantity”) multiplied by each taxon’s respective mean pollen load (i.e. the “quality”), summed across all taxa recorded during the observation period. This resulted in an index of overall pollinator effectiveness, for every observation period in our dataset.

Finally, to assess pollen deposition, we collected *A. germinans* stigmas from six sites in 2015 (Table B.1). Each site was visited twice – once in the early flowering season and once in the mid-flowering season. During each site visit, up to 12 trees were haphazardly selected, and we collected up to eight flowers with mature stigmas from throughout the tree canopy. Mature stigmas could be identified by their spread lobes, which open on approximately day 3 of anthesis (*personal observation*); flowers are syncarpous, with a single stigma per flower. In total, we collected 768 stigmas

from all six sites. In the lab, each stigma was removed from its flower and mounted in fuchsin jelly on a microscope slide, which we inspected at 100X and 400X magnification on a compound microscope. Given the sometimes-large number of pollen grains present, we counted the number of *A. germinans* pollen grains three times on each stigma, and used the average of these three counts for analysis.

Pollinator effectiveness - Analysis

To test for variation among taxa in the size of their pollen loads, I used the *MASS* package (Ripley 2018) to construct a generalized linear model (GLM) with a single fixed effect of taxon. To account for overdispersion, I used a negative binomial error distribution. This model was compared to a GLM with only an intercept term, using a likelihood ratio test.

To assess the degree to which observation-level pollinator effectiveness varies with latitude, I built a GLMM with a negative binomial error distribution (to account for overdispersion in the data), with latitude as the sole fixed effect, observation date as a random effect, and the number of flowers on the focal tree as an offset (*lme4* package [Bates et al. 2015]). I used bootstrapping (*pbkrtest* package [Halekoh and Højsgaard 2014]) to test the significance of latitude, and calculated marginal and conditional R^2 following Nakagawa and Schielzeth (2016).

Similarly, pollen deposition was modeled with a negative binomial GLMM, to address overdispersion in the dataset (*lme4* package [Bates et al. 2015]). The response variable was the average of the three tallies of *A. germinans* pollen on each stigma. As predictors, I included a single fixed effect (latitude) and single random effect (tree ID). Again, I used bootstrapping (*pbkrtest* package [Halekoh and Højsgaard 2014])

to test the significance of latitude, and calculated marginal and conditional R^2 following Nakagawa and Schielzeth (2016).

Flower and propagule production

Flower production per tree was measured during each observation period for floral visitors, as described above. In late October-early November of 2014 and 2015, when propagules were maturing on parent trees, we established transects at multiple sites to measure the densities of reproductive *A. germinans* trees and fecundity (propagule production per tree). In each year, transects were established at six sites (though not the same set of sites both years; see Table B.1). At each site, we identified three areas of high *A. germinans* density and laid out one 20m transect in each area (resulting in three transects per site). We recorded the number of reproductive *A. germinans* over 0.5m tall that occurred within 1m of the transect tape, on either side.

To measure propagule production per tree, or fecundity, we randomly selected five reproductive trees along each transect (or in close proximity to the transect, if fewer than five were immediately adjacent) and estimated the number of propagules present by counting the number on a representative portion of the tree and extrapolating to the entire canopy. Because different sites were in different stages of propagule drop, I then corrected these estimates to account for propagules that had already fallen. I did this using infructescences at each site that had been covered in mesh bags prior to the onset of propagule drop (three bags per tree on up to 30 haphazardly selected *A. germinans* per site); these bags collected falling propagules, and allowed us to calculate the fraction of total propagules that remained attached to

the pedicel. By multiplying our original propagule counts by the inverse of this fraction, I could estimate the total number of propagules originally produced by the trees.

Fruit set rates, defined as the number of mature propagules produced per floral bud, were also measured using these same bags. Once each bag was collected, we counted the number of propagules (both abscised and still attached to the pedicel) as well as the total number of floral bud scars. Fruit set rate was calculated as the total number of propagules produced by a given inflorescence, divided by the number of floral bud scars.

In 2015, propagules were collected from seven sites (Table B.1) and weighed. To avoid bias resulting from earlier phenology at the northern sites, we collected only mature propagules that had dropped from their pedicels. To do this, we haphazardly selected 13-22 *A. germinans* at each site in late summer, once flowering had ended; we installed large-mesh (~1.0cm) bags over three inflorescences, each on a separate branch. In late October/early November, we returned to the sites, collected any mature propagules that had dropped from the pedicels into the bags, and weighed them to the nearest 0.001g. In total, we collected 326 propagules.

Flower and propagule production - Analysis

Flower production, reproductive tree density, and propagule production all showed signs of overdispersion, so were modeled with negative binomial error distributions. Flower production and reproductive tree density were analyzed using GLMs (*MASS* package [Ripley 2018]) with a single predictor: latitude. I used likelihood ratio tests to compare these models to ones without latitude as a predictor,

and calculated McFadden's pseudo- R^2 using the *rsq* package (Zhang 2018). I modeled propagule production in two ways, in order to 1) test latitude as a predictor of *A. germinans* fecundity (which involved modeling propagule production as a function of latitude) and 2) to explore inter-site differences in fecundity (which involved modeling propagule production as a function of site, instead). Both models were GLMMs (*lme4* package [Bates et al. 2015]), with transect as a random effect. For both, I used bootstrapping (*pbkrtest* package [Halekoh and Højsgaard 2014]) to test the significance of latitude, and calculated marginal and conditional R^2 following Nakagawa and Schielzeth (2016).

Fruit set rate was modeled using a binomial GLMM (*lme4* package [Bates et al. 2015]), with the total number of original floral buds set as a weight, or number of trials. Latitude was the sole fixed effect; tree ID was included as a random predictor, as was an observation-level random effect, to account for overdispersion. I tested the significance of latitude using bootstrapping (*pbkrtest* package [Halekoh and Højsgaard 2014]) and used the *MuMIn* package (Bartoń 2018) to calculate marginal and conditional R^2 .

Finally, propagule mass was similarly modeled with a single fixed effect of latitude and a random effect of tree ID, using a GLM with Gaussian errors (*lme4* package [Bates et al. 2015]). Once again, I tested the significance of latitude using bootstrapping (*pbkrtest* package [Halekoh and Højsgaard 2014]) and used the *MuMIn* package (Bartoń 2018) to calculate marginal and conditional R^2 .

Results

Floral visitor identity and frequency

Across all taxa observed visiting *A. germinans* flowers, total visitation rates declined slightly, but significantly, as latitude increased (parametric bootstrapping: $p = 0.03$) (Figure 3.1). Neither the number of flowers on the focal tree, tree height, year, nor the latitude \times year interaction were significant predictors of total visitation rate (parametric bootstrapping: $p = 0.16, 0.07, 0.68,$ and $0.29,$ respectively); the complete model had low explanatory power ($R^2_m = 0.04, R^2_c = 0.15$). However, a model in which floral visitation was treated as presence/absence (binomial) performed considerably better ($R^2_m = 0.08, R^2_c = 0.26$), and revealed a marked northward decline in the proportion of observations that recorded at least one floral visitor (latitude parametric bootstrapping: $p = 0.01$) (Figure 3.1). (Again, the number of flowers on the focal tree, tree height, and year were not significant predictors [parametric bootstrapping: $p = 0.44, 0.32,$ and $0.52,$ respectively].)

Although overall floral visitation rates declined at higher latitudes, the relationship between visitation rate and latitude varied among insect taxa. Estimates of the taxon-specific random slopes revealed that some taxa – like pierid butterflies, ants, and honey bees (*Apis mellifera* [Hymenoptera: Apidae], the most abundant floral visitor observed [Table 3.1]) – did indeed visit *A. germinans* flowers less frequently at northern sites. However, visitation by other taxa – primarily *Xylocopa* bees, *Bombus* bees, and syrphid flies – was positively associated with latitude (Figure 3.2). Positions of taxa in the NMDS (stress = 0.18) reflected the results of the random slopes model, with southern sites clustering near *A. mellifera* and northern sites

falling closer to *Xylocopa* (Hymenoptera: Apidae), *Bombus* (Hymenoptera: Apidae), and Syrphidae (Diptera) (Figure 3.2). In sum, the composition of the *A. germinans* floral visitor assemblage shifted along a north-south geographical gradient.

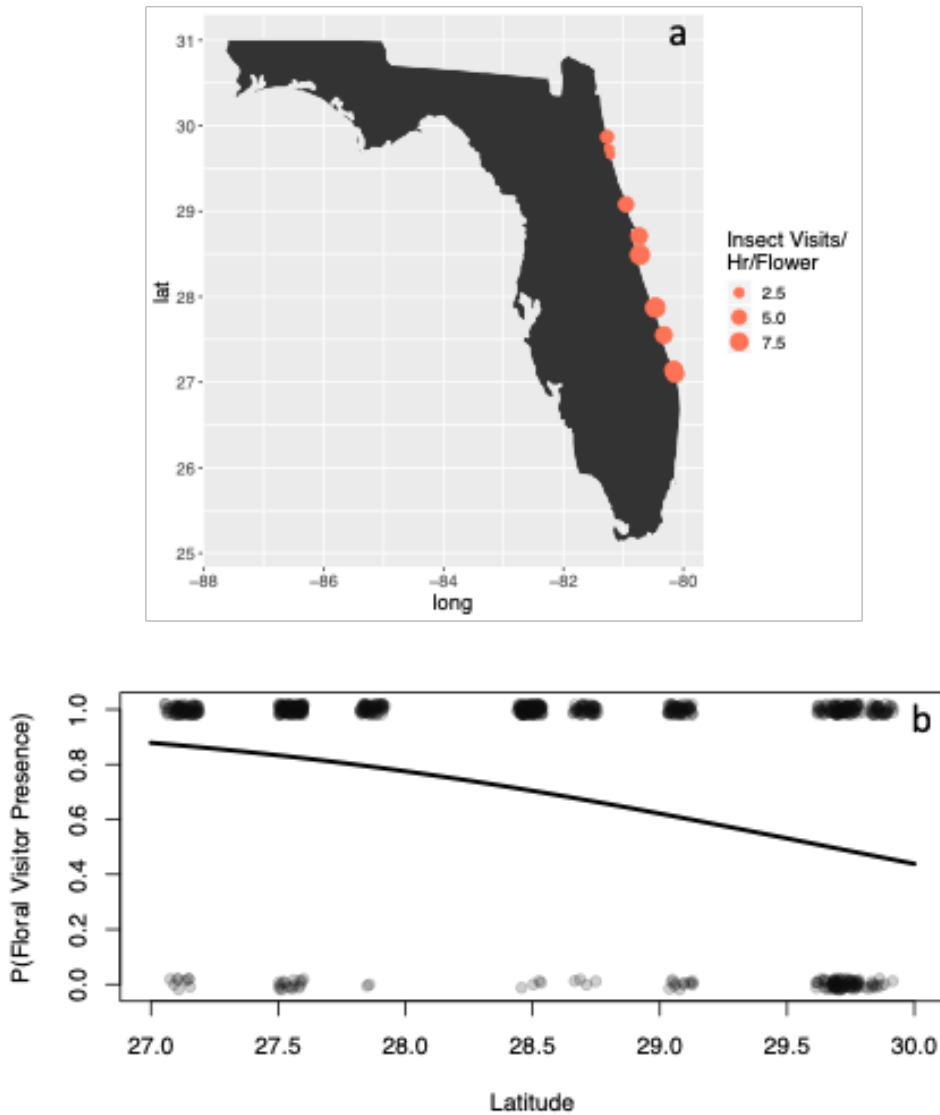


Figure 3.1: (a) The frequency of all insect visits to *A. germinans* flowers declined from south to north. Points show sampling locations; point size indicates mean visitation rate, across all taxa. (b) The probability of at least one insect visiting *A. germinans* flowers during an observation period declined at higher latitudes. Line shows model-estimated relationship. Points are jittered to better display the frequencies of presences and absences.

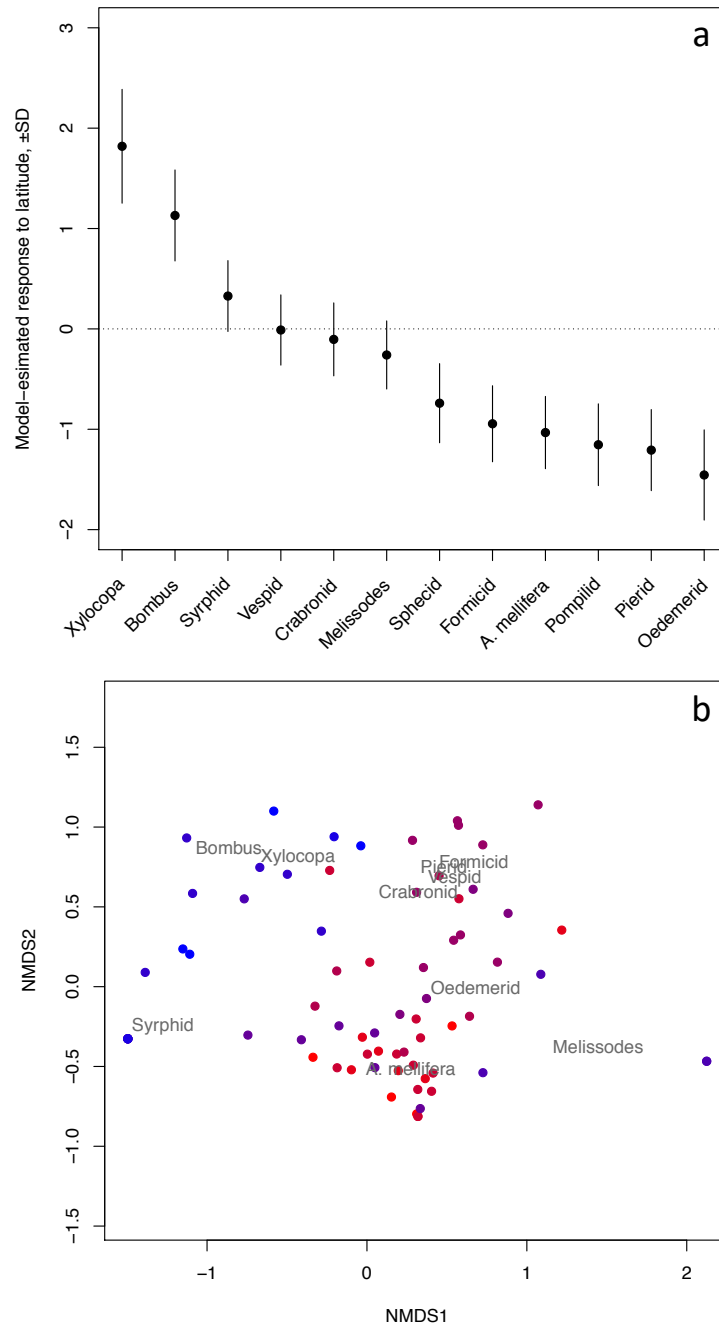


Figure 3.2: (a) Random slope estimates, representing taxon-specific relationships between visitation frequency and latitude. Points falling near the dashed line at 0 indicate taxa whose visitation rate varied little with latitude; points above/below the line represent taxa that increased/decreased in visitation frequency with latitude, respectively. Bars show SD. (b) Nonmetric multidimensional scaling (NMDS) of floral visitors observed at each site and date. Points are color-coded to indicate site latitude, with red representing southern sites and blue representing northern sites.

Pollinator effectiveness

Pollen load size varied significantly among flower-visiting taxa (parametric bootstrapping: $p < 0.001$), with large-bodied bees and wasps – e.g. *Xylocopa*, *Melissodes*, *A. mellifera*, and crabronids – carrying the largest amounts of *A. germinans* pollen on their bodies (Figure B.2). Ants (Formicidae) and pierid butterflies carried the smallest pollen loads, each with a median of 0 *A. germinans* pollen grains collected from the individuals sampled.

The GLMM of pollinator effectiveness – my index combining pollen load and visitation data – revealed a significant decline in pollinator effectiveness at northern sites (parametric bootstrapping: $p = 0.01$) (Figure 3.3). This decline was probably driven by the large drop in the proportion of observation periods that recorded at least one floral visitor (Figure 3.1). While model-predicted pollinator effectiveness was lower closer to the *A. germinans* range edge, variation in pollinator effectiveness among observations was very large at the four northernmost sites; indeed, some of the highest-recorded indices of pollinator effectiveness were recorded during observation periods at range-edge sites (Figure 3.3).

Pollen deposition, however, was unrelated to latitude (parametric bootstrapping: $p = 0.14$) (Figure 3.3). We observed substantial intra-site variation in the amount of *A. germinans* pollen deposited on floral stigmas, with a median of 11 pollen grains and an interquartile range of 3-34 pollen grains.

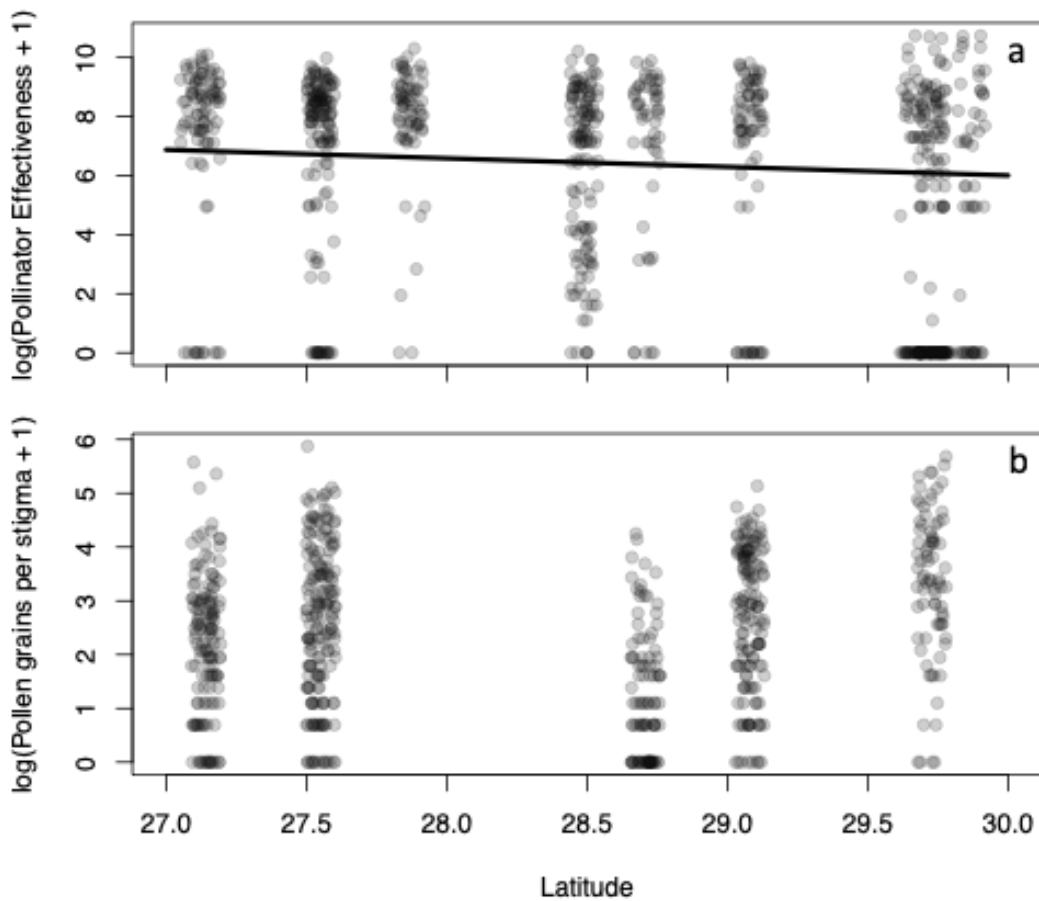


Figure 3.3: (a) The overall pollinator effectiveness of each observation, a measure of both pollinator quantity and quality, generally declined at northern sites ($R^2_m < 0.01$, $R^2_c = 0.02$). However, the increased relative abundance of some highly-effective taxa at higher latitudes led to some of the largest-recorded pollinator effectiveness indices there (note the greater spread of values to the right). (b) Pollen deposition on *A. germinans* stigmas was unrelated to latitude (model $R^2_m < 0.01$, $R^2_c = 0.27$).

Flower and propagule production

Flower production per tree was significantly higher at northern sites ($\chi^2 = 14.2$, $p < 0.001$), rising from a median of 150 flowers per tree at the southernmost site

to 380 flowers per tree at the northernmost (Figure 3.4). However, fecundity – measured as the number of propagules produced per tree – did not vary consistently

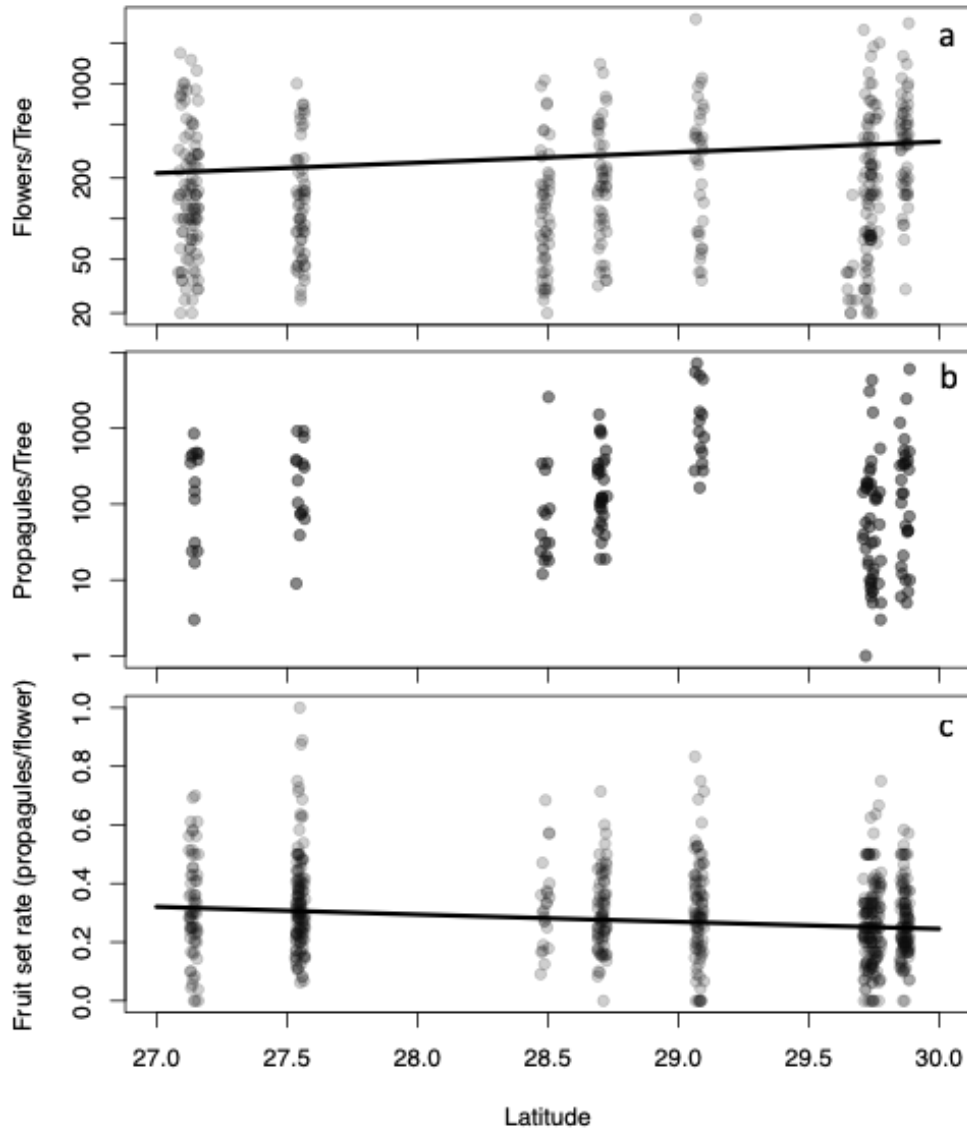


Figure 3.4: (a) *A. germinans* flowering increased with latitude, but (b) fecundity showed no relationship with latitude. (c) Consequently, the fruit set rate declined slightly at northern sites. Note the logarithmic scale of the Y-axes in (a) and (b). Lines show model-estimated relationships.

with latitude (parametric bootstrapping: $p = 0.86$, $R^2_m < 0.001$, $R^2_c = 0.29$), but did vary significantly among sites (parametric bootstrapping: $p = 0.010$, $R^2_m = 0.22$, $R^2_c = 0.30$) (Figure 3.4). Fruit set rate (the rate at which flowers develop into propagules), therefore, declined slightly with latitude (parametric bootstrapping: $p = 0.01$, $R^2_m < 0.01$, $R^2_c = 0.08$), from 0.31 at the southernmost site to 0.26 for *A. germinans* nearest the range edge (Figure 3.4).

The density of reproductive *A. germinans* increased significantly with latitude ($\chi^2(1) = 14.32$, $p = 0.0002$), growing from 2.5 ± 1.0 (mean \pm SD) trees per transect at the southernmost site sampled to 21.2 ± 14.3 trees per transect at the northernmost site (Figure 3.5). Substantial variation in reproductive tree density remained unexplained by the model, however (McFadden's pseudo- $R^2 = 0.04$). Propagule mass was also significantly larger at higher latitudes, increasing by 0.210g (± 0.075 g SE) with every northward degree of latitude (parametric bootstrapping: $p = 0.010$, $R^2_m = 0.02$, $R^2_c = 0.16$) (Figure 3.5).

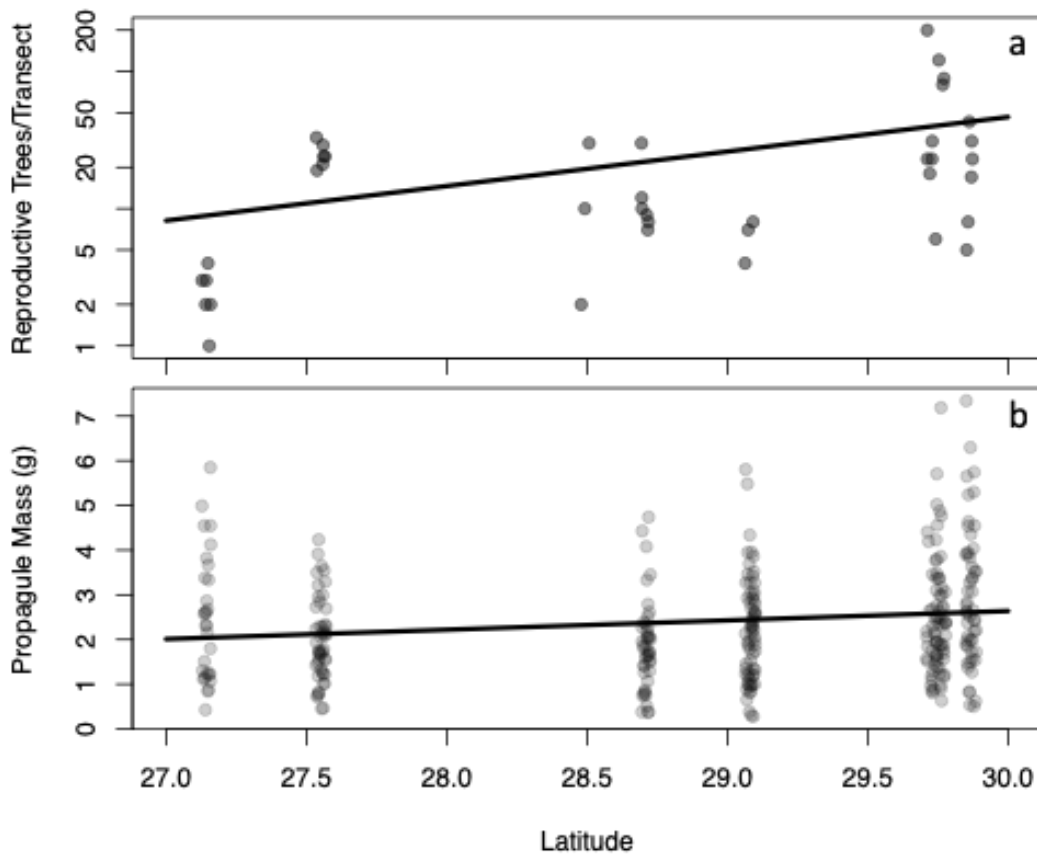


Figure 3.5: (a) The density of reproductive *A. germinans* (those with at least one inflorescence/infructescence at the time of survey) increased with latitude, (b) as did propagule mass. Note the logarithmic scale of the Y-axis in (a). Lines show model-estimated relationships.

Discussion

Contrary to expectations, *A. germinans* fecundity remained high in populations near the leading range edge, due in part to substantial allocation to flower and propagule production and to changes in the pollinator assemblage there. Overall, floral visitation declined toward the range margin, but this decline was partly offset by an increase in the frequency of taxa that carry large amounts of *A. germinans*

pollen, such as *Xylocopa* and *Bombus* bees, at more northern latitudes. Consequently, the amount of pollen deposited on *A. germinans* stigmas did not decline toward the range margin, suggesting that pollen limitation does not act as a barrier to range-edge reproduction. Incidentally, fruit set rates did decline in northern populations of *A. germinans*, but this may be due to the effect of increased flowering there, and to the considerable resource allocation required to scale up propagule production. *A. germinans* propagules are large structures – especially at the range edge, where propagule size was on average 18% larger than at the southernmost site – and may be too resource-intensive to scale proportionally with increased flower production. In combination with the high density of reproductive trees in northern populations, these findings point to *A. germinans*' ability to produce large numbers of propagules at its expanding range edge, which may contribute to its continued northward range shift.

Pollinators and pollen limitation at the range edge

This study found that a latitudinal shift in pollinator composition, which resulted in a small but sufficient pollinator assemblage in the northernmost sites, rescued range-edge *A. germinans* from pollen limitation. Few previous studies have documented variation in pollinator assemblages with respect to a plant's range edge, and its consequences for plant reproduction. Chalcoff et al. (2012) found a decline in pollen receipt and fruit set toward a Patagonian shrub's dry range limit, which they attributed to a decline in abundance of the shrub's most effective pollinator, hummingbirds. Similarly, Moeller et al. (2012) observed a decline in effective bee pollinators at an annual plant's arid range edge, leading to reduced fruit set there. On the other hand, Hargreaves et al. (2015) documented high abundances of an annual

alpine plant's most effective pollinators (*Bombus* bees) at its altitudinal range limit, contributing to a constant seed set across an altitudinal gradient. Likewise, in the case of an alpine herbaceous perennial, Theobald et al. (2016) observed large differences in the pollinator assemblage among sites, but no consistent altitudinal trends in pollinator frequency or identity; consequently, pollen limitation was unrelated to proximity to the range edge

Other studies have found that the expected positive relationship between pollinator abundance and pollen receipt by plants can be erased by spatial changes in resource availability or in the co-flowering plant assemblage. For example, Stone and Jenkins (2008) found decreased pollinator visitation near the altitudinal range limit of a tropical shrub, but no real differences in pollen limitation between low- and high-altitude sites. They hypothesized that stressful conditions at the high-altitude sites, and perhaps increased self-compatibility there, made high-altitude plants less responsive to a changing pollination environment. In contrast, Castilla et al. (2011) observed an increase in fruit set at a Mediterranean shrub's range edge, which they attributed to fewer co-flowering plants and decreased competition for pollinators there, as well as to turnover in the pollinator assemblage.

In sum, when considering these studies collectively, there emerges no consistent relationship between pollinator abundance, reproductive success, and proximity to a plant's range edge. Some of this variation may be explained by idiosyncrasies in resource limitation or co-flowering plants, as mentioned above. Additional variation can be attributed to differences in the factors controlling plants' range limits and the identity and abundances of their pollinators. For example, in

some settings, a single environmental gradient – e.g. precipitation – can directly shape both the distribution of a plant and the abundance of its pollinators (Chalcoff et al. 2012, Moeller et al. 2012). That is unlikely to be the case in this study system, given the increases in some pollinator taxa toward the *A. germinans* periphery, and the occurrence of *Apis mellifera* well north of the *A. germinans* range limit. Rather, in this study, regional changes in land use and apicultural practice may play an important role in determining the pollinator assemblage for a given *A. germinans* population. Alternatively, the mosaic nature of the mangrove-marsh ecotone, and the presence of mangroves within or adjacent to large areas of salt marsh, might reduce their apparency to pollinators (Kunin 1993) or might only attract pollinators that can energetically afford to forage in a patchy landscape (Essenberg 2013). Taken together, our findings and those of other researchers suggest that successfully predicting the effects of pollinators and pollen limitation on plants' range shifts requires an understanding of both the drivers of variation in the pollinator assemblage, and the setting into which the plant is spreading.

Reproductive allocation at the range edge

Instead of declining toward the range edge, *A. germinans* fecundity was maintained even at the northernmost populations studied. This is partly due to turnover in the pollinator community and the preservation of range-edge pollinator effectiveness, as described above. It is also partly attributable to an increase in flowering – contrary to my expectations, flowering was greater in populations near the range edge, with the northernmost trees producing over twice as many flowers as the southernmost, despite being substantially smaller in size. Increased allocation to

reproduction was not restricted to flowers; propagules produced by these northern trees were significantly larger than propagules from populations closer to the range center.

There are several reasons why *A. germinans* might increase allocation to reproduction near its range edge. Similarly to how some plant species have a “suicidal” strategy of compensating for herbivore damage by increasing reproductive output (Trumble et al. 1993), *A. germinans* might respond to cold stress at northern sites by allocating more resources to flowering and propagule growth. Alternatively, range-edge *A. germinans* may, somewhat counterintuitively, experience superior growing conditions in northern Florida, despite infrequent die-offs due to hard freezes (Cavanaugh et al. 2019). Competitively superior to some dominant salt marsh plants (Kangas and Lugo 1990), and released from competition with other mangrove species for space, water, and light, northern populations of *A. germinans* may be under less physical stress, which translates into greater reproductive output (Chiariello and Gulmon 1991). Another explanation for the increased allocation to reproduction could lie in the range-edge trees’ young-to-intermediate age, if *A. germinans* is a species with a life history strategy that promotes early reproduction. (However, most plants are thought to show a positive relationship between age/size and fecundity [Greene and Johnson 1994].) Finally, selection or the founder effect could be acting on range-edge populations to produce a phenotype that reproduces early and often (Phillips et al. 2010, Dangremond and Feller 2016). Regardless of its cause, *A. germinans*’ strong allocation to reproduction and high fecundity at its range edge has important implications for its continued northward range shift.

Conclusion

This study demonstrates that the climatic constraints setting the *A. germinans* range margin do not necessarily limit range-edge allocation to reproduction, and that pollinator identity, rather than sheer abundance, can be more important for maintaining the pollination environment at an expanding range edge. These results are consistent with research showing recent, rapid increases in mangrove cover in northern Florida, coinciding with a decrease in the frequency of hard freezes (Cavanaugh et al. 2014, 2019). Of course, there is more to range expansion than maintaining fecundity; dispersal, establishment, germination, and seedling survival and growth are also critical for range shifts (Angert et al. 2011). Given *A. germinans*' capacity for long-distance dispersal (Dodd et al. 2002), reproduction close to the leading range edge may not be essential for sustained northward expansion. Still, local reproduction will be important for the growth of frontier populations around new colonizers, which can, in turn, promote further dispersal (Shigesada et al. 1995). The fate of range-edge propagules and the origins of range-edge colonizers – from range-edge populations, or from populations closer to the interior of the distribution – is worthy of further study. So, too, is the underlying cause of spatial variation in pollinator abundance and identity, in order to better predict future *A. germinans* spread and the range shifts of other pollinator-dependent plants.

Chapter 4: In a shifting ecotone, mangrove encroachment alters arthropod communities at fine scales

Abstract

As climate change alters plants' distributional limits, new ecotone regions will form where relatively homogeneous habitat previously existed. Understanding the impact of ecotone shifts on local arthropod communities is of particular importance, given their diversity and their critical roles in ecosystem function. Here, I study the mechanisms behind and scale at which arthropod communities respond to expansion by *Avicennia germinans*, the black mangrove, into salt marshes of the southeastern U.S. I hypothesized that advancing trees would host a novel arthropod assemblage relative to salt marsh, and that this effect on community composition would spill over into salt marsh adjacent to invading trees, mediated by a novel plant trait introduced by *A. germinans* to the system: nectar-producing flowers. I found a stark divide between mangrove- and marsh-associated arthropod communities, even at sub-meter scales. Arthropod abundance in salt marsh vegetation was over six times greater than in mangrove vegetation, and salt marsh rarefied richness was over twice as high as that in mangroves. Each vegetation type – marsh and mangroves – contained arthropod taxa not present in the other. Remarkably, *A. germinans*' presence did not impact the arthropod community of neighboring salt marsh; arthropod assemblages in marsh vegetation near mangroves were no different from those farther away. Experimental manipulation of *A. germinans* flowers had little effect on arthropod communities, indicating that mechanisms other than floral traits drive the observed

changes in arthropod community composition. In sum, the addition of mangroves to a salt marsh results in a patchy mosaic of habitat types, with discrete arthropod communities that mirror the spatial heterogeneity of the vegetation at very fine spatial scales. These findings provide support for the idea that the addition of a functionally distinct foundation species increases diversity by increasing habitat heterogeneity, and suggest that mangrove encroachment on salt marsh will drive large changes in coastal arthropod communities.

Introduction

As climate change drives the redistribution of species on Earth, the locations of ecotones – regions where neighboring biomes meet and intermix – will also change (Parmesan 2006, Harsch et al. 2009, Poloczanska et al. 2013). In some cases, one or both neighboring biomes are defined by the presence of a foundation species – i.e. one that is abundant, common, and that forms habitat for other organisms (Ellison et al. 2005). While some ecotones can be abrupt transition zones, many are diffuse, with patches of both foundation species co-occurring and creating a heterogeneous landscape (Harsch et al. 2009, Harsch and Bader 2011). This heterogeneity can play an important role in structuring the community of associated organisms that inhabits the ecotone; high diversity of plant traits, for example, has been linked to greater faunal diversity (Siemann et al. 1998, Tschardt et al. 2008, Angelini and Silliman 2014). Indeed, ecotones can host higher levels of diversity than either of their two neighboring habitats on their own (Ward et al. 1999, Kark and van Rensburg 2006, Kark 2013). Given the unprecedented movements of species distributions due to climate change, understanding how shifting ecotones host biodiversity, and how this

is mediated by the traits of the new foundation species being introduced, is important for determining how communities in these regions will be affected. This study considers these questions within a shifting mangrove-salt marsh ecotone and its associated arthropod communities.

When a new plant species is introduced to an ecosystem, the consequences for local fauna depend on several factors. In relatively equilibrated systems, i.e. those that are not undergoing a response to a recent large perturbation, arthropod diversity is typically positively related to plant diversity (Novotny et al. 2006, Forister et al. 2015). But studies of biological invasion find that invasive plants – especially woody species – tend to reduce arthropod abundance and richness, on average (van Hengstum et al. 2014, Litt et al. 2014). Potentially solving the paradox, Angelini and Silliman (2014) proposed that the effect of an additional foundation species – i.e. one that is abundant, common, and that forms habitat for other organisms (Ellison et al. 2005) – on faunal diversity depends on both the degree to which the new species alters habitat availability and the degree of functional difference from the foundation species already present. For example, the introduction of a short, insect-pollinated forb to a grassland would be expected to alter the diversity of the insect community, but not necessarily its overall abundance (provided the new arrival does not displace the preexisting grasses). Conversely, the establishment of a new, tall grass species would provide more, but functionally similar, habitat, resulting in a larger but compositionally similar insect community. Thus, the nature of a community's response to a novel foundation species will hinge on the traits of the new species, relative to the resident one(s).

In a patchy landscape, the effects of a novel foundation species will also depend on the spatial scale at which organisms respond to it (Johnston and Gruner 2018). A newly established foundation species can have a very localized effect on the faunal community, or it can have a spatially diffuse effect that extends into the surrounding landscape. The community response will be highly localized if organisms can detect and respond to changes in foundation species at fine spatial scales. On the other hand, the influence of encroaching foundation species can extend into the surroundings if its presence modifies how organisms perceive neighboring species. For example, the identity of a plant's neighbors can influence its apparency or attractiveness to herbivores, pollinators, and natural enemies (i.e. associational effects; Hambäck et al. 2014). Spillover can also occur, whereby mobile species move from one habitat type into an adjacent one (Rand et al. 2006, Stobart et al. 2009). Regardless of the mechanism causing it, the influence of an encroaching foundation species on the community that surrounds it should decay with distance (Morlon et al. 2008), meaning that the adjacent community will be more strongly affected than communities farther away.

The mangrove-salt marsh ecotone of the southeastern U.S. is an ideal system for addressing questions regarding shifting ecotones and their effects on local diversity. This ecotone is diffuse and broad, spanning the Gulf Coast and over 200km of the Atlantic coast in Florida. Transitioning from mangroves at southern latitudes to salt marsh in the north, this is a zone between two ecologically, economically, and culturally important biomes (Tomlinson 1986, Barbier et al. 2011, Doughty et al. 2015, Kelleway et al. 2017). As winters in the region have warmed in recent decades,

the ecotone has shifted northward substantially. The three mangrove species found here – *Rhizophora mangle* L. (Rhizophoraceae), *Laguncularia racemosa* (L.) C.F. Gaertn. (Combretaceae), and *Avicennia germinans* (L.) L. (Acanthaceae) – are cold-intolerant trees that have undergone a dramatic increase in cover at their northern range limit, encroaching on salt marsh (Osland et al. 2013, Cavanaugh et al. 2019). The species best at surviving moderate freezes is *A. germinans* (hereafter ‘*Avicennia*’), which occurs the farthest north, forming monospecific stands of short trees within the salt marshes of northern Florida (Cavanaugh et al. 2015). The northward shift of the ecotone, therefore, is marked by the introduction of *Avicennia* to previously mangrove-free salt marsh.

Avicennia and salt marsh vegetation possess different physical traits that could contribute to differences in their associated arthropod communities. Because grasses tend to retain senesced leaves for a considerable period of time (Bouchard and Lefeuvre 2000), salt marshes support large detritivore assemblages composed of snails, crustaceans, and insects – notably, flies (Davis and Gray 1966, Currin et al. 1995, Pennings and Bertness 2001). Insects are the predominant herbivores in salt marshes (Pennings and Bertness 2001); in addition to katydids and grasshoppers, specialist sap-feeding Hemiptera are often abundant (Davis and Gray 1966, Pfeiffer and Wiegert 1981, Bertness et al. 1987), and are preyed on by spiders and some Hemiptera (Davis and Gray 1966, Döbel and Denno 1994). External free-living herbivores are less abundant on *Avicennia*, which has foliage that is chemically and structurally well-defended (Farnsworth and Ellison 1991, Feller et al. 2007, 2010). However, specialist leaf-galling psyllids can cause significant damage to leaves, and

crickets are occasional folivores (Feller et al. 2007). *Avicennia* has also been documented as a host for external-feeding lepidopteran caterpillars, leaf-mining flies, and xylem-feeding beetles (Farnsworth and Ellison 1991, Cannicci et al. 2008). Several species of spider have been recorded on *Avicennia*, probably none of them specialists (Morrisey et al. 2010).

Morphological and phenological differences in *Avicennia* and salt marsh plants' flowers also likely drive differences in their associated arthropod communities. *Spartina alterniflora* Loisel. (Poaceae), the dominant plant in temperate North American salt marshes, is a wind-pollinated grass that flowers in late summer/fall at subtropical latitudes (Crosby et al. 2015). *Avicennia*, on the other hand, blooms in late spring/summer (*personal observation*), producing large displays of showy white flowers that attract a broad array of insect taxa, especially Hymenoptera and Diptera (Sánchez-Núñez and Mancera-Pineda 2012, Landry 2013b). While specialized salt marsh herbivores are probably unable to consume most *Avicennia* tissues, this may not be true when it comes to *Avicennia* flowers, which produce easily-accessible nectar and pollen. These flowers are a novel resource in the grass-dominated salt marsh, where nectar is scarce and pollen is not widely available in the summer months. Flowers and extrafloral nectaries are known to structure arthropod communities, both by attracting pollinators and by attracting predatory taxa that exert top-down control on lower trophic levels (Inouye and Taylor Jr. 1979, Yano 1994, Romero and Vasconcellos-Neto 2004, Rudgers and Gardener 2004). Sometimes nectar has complex community-wide effects, as when pollinating and predatory taxa interact (LeVan et al. 2014), or when herbivores are also attracted to

the plant (Adler and Bronstein 2004). If floral availability attracts marsh arthropods into mangrove vegetation, then the communities found on mangroves and in marsh vegetation will appear more blended when mangroves are in bloom. Alternatively, flowers could amplify the differences between mangrove- and marsh-associated arthropod communities, by attracting mobile taxa that occur only on mangroves.

Given the structural, chemical, and phenological differences between mangroves and salt marsh, I predicted that mangrove encroachment would drive an increase in arthropod diversity within the ecotone. Specifically, (1) I compared arthropod community composition on young frontier mangroves to that of surrounding salt marsh vegetation, to test the hypothesis that mangroves host a novel arthropod community (rather than a subset of the marsh community), thereby increasing ecotone biodiversity. (2) I also predicted that salt marsh arthropod communities immediately adjacent to mangroves would differ from those farther away, as would be expected if associational effects or spillover were to occur. Finally, (3) I experimentally manipulated *Avicennia* flowering in order to test if mangrove flowers amplify compositional differences between mangrove- and marsh-associated arthropods. By quantifying changes in community structure across fine spatial scales, this study illustrates how arthropod communities respond to the encroachment of a novel foundation species.

Materials and Methods

Study system

On the Atlantic coast of Florida, the mangrove-salt marsh ecotone extends from approximately 28°N to 30°N. Toward the southern end of the ecotone, mixed stands of Florida's three mangrove species co-occur with scattered patches of salt marsh, while at the northern edge of the ecotone, only scattered stands of *Avicennia* are embedded in extensive salt marsh. This study was conducted at one site in the northern ecotone where salt marsh dominated by *Spartina alterniflora*, as well as *Salicornia* sp. L. (Amaranthaceae) and *Batis maritima* L. (Bataceae) is being colonized by *Avicennia* (Figure C.1). Mangrove cover in this area has fluctuated over time, with mangroves periodically extirpated from the region by hard freezes; historical imagery suggests that the area has seen rapid mangrove expansion in the last 20 years (Cavanaugh et al. 2019).

Experiment

In June of 2015, we selected 30 young focal *Avicennia* at the study site that were similar in size (~1.5m tall), distance to other mangroves, and distance to open marsh. All focal *Avicennia* were separated from each other (but not necessarily from other mangroves) by at least 10m. To establish variation in flower number, we randomly assigned each *Avicennia* to one of the following categories: no inflorescence removal, partial inflorescence removal, or complete inflorescence removal. (Note that inflorescences contain 1-2 open flowers at any given time; thus, inflorescence number, and not flower number, are included in all analyses.) We used

hand clippers to either remove all inflorescences (complete removal), roughly half of the inflorescences (partial removal), or a similar number of non-flowering branch tips (no removal) to simulate any effects that damage alone might have on the arthropod community. Once this process was completed, we counted the number of inflorescences remaining on each focal *Avicennia*.

The following day, all focal trees were vacuum sampled using a modified Craftsman blower-vac and nylon paint strainer bags as collection bags. Each tree was sampled for 30 seconds, with the vacuum operator thoroughly sampling the entire tree. All collected material and specimens were bagged and immediately put into a cooler with ice; on return to the lab several hours later, all samples were immediately moved to the freezer. Following a four-day period (to give marsh-associated arthropods enough time to recolonize areas disturbed during the mangrove vacuum sampling), the marsh surrounding each focal *Avicennia* was also vacuum sampled. Marsh was sampled along two 5m x 1m transects that circled each focal mangrove, one transect at 1.5m (“near”) and one at 5m (“far”) from the focal mangrove’s main stem. Each sample had a duration of 30 seconds (Figure C.2). Together, each focal *Avicennia* and its two associated marsh transects were termed a ‘plot’.

Sampling of all plots was repeated twice more over the summer: once in early/mid-July, and once in late July/early August. Each time, mangroves were sampled first, and surrounding marsh transects were sampled several days later. Because marsh transects were not permanently staked, the sampled transects did not always fall precisely in the same place.

Finally, to capture marsh heterogeneity and any consistent difference in vegetation characteristics between near and far transects that might be an important influence on the arthropod community, we measured the relative cover of marsh plant species surrounding each focal mangrove. We established circular transects at 1.5m and 5m from each focal *A. germinans*, and placed a 40cm x 40cm quadrat every meter along these transects. Within each quadrat, we estimated percent cover of each plant species present, as well as the cover of bare ground (percent cover was constrained to add to 100%). For subsequent analysis, the percent cover of each species was later averaged across all quadrats within a transect.

Arthropods were stored in ethanol and sorted to the order level, with the exception of ants, which, being functionally distinct and having unique life histories, were split from the rest of the Hymenoptera. Given that Hemiptera were a large portion of all collected arthropods, that abundances of different hemipteran taxa appeared to vary substantially with vegetation type, and that at least one hemipteran *Avicennia* specialist (Hemiptera: Psyllidae: *Telmapsylla*) was present, we further sorted Hemiptera to morphospecies. As a result, this generated two datasets – order-level abundances, and hemipteran morphospecies-level abundances – which were analyzed separately.

Analysis

All analyses were performed in R version 3.5.0 (R Core Team 2018). For each sample, we calculated: 1) its total arthropod abundance; 2) raw arthropod richness, at both the order level and hemipteran morphospecies level; 3) individual-based rarefied richness, at both the order level and hemipteran morphospecies level (mangrove and

marsh samples were rarefied separately, given large differences in abundance) (Oksanen et al. 2018); and 4) Shannon diversity, at both the order level and hemipteran morphospecies level (Oksanen et al. 2018).

To simplify marsh vegetation heterogeneity for modeling purposes, I used Principal Components Analysis (PCA) to reduce species cover dimensionality to two principal axes (which together explained 73% of the variation in plant species cover among marsh transects). Positive values of the first axis reflect dominance by *S. alterniflora* over marsh forbs; positive values of the second axis indicate sparser vegetation and more bare ground (Table C.1).

Do mangroves host a distinct community?

To determine if *Avicennia* host a unique community that differs in either identity or abundance from that found in the surrounding salt marsh vegetation, I first compared lists of arthropod taxa found in mangrove and marsh samples, to determine if any taxa were uniquely associated with one vegetation type. Then, for order-level arthropod abundances, I used mixed models (containing a random effect for mangrove/transect ID, to account for repeated measures) to compare mangrove and marsh samples in terms of their total abundance (Poisson), richness (Poisson), and Shannon diversity (Gaussian), testing the importance of vegetation type with parametric bootstrapping (Halekoh and Højsgaard 2014, Bates et al. 2015). Rarefied richness of the two vegetation types was not compared, as the very low abundances in mangrove samples relative to those in marsh forced the marsh rarefaction curves to become too truncated.

Because multivariate dispersion differed between marsh and mangrove samples ($F = 107.4, p = 0.0001$) (Oksanen et al. 2018), and PERMANOVA and other similar multivariate tests assume equal dispersion among groups (Anderson and Walsh 2013), I opted to use a Generalized Linear Mixed Model (GLMM) approach to quantify community differences between marsh and mangrove vegetation. For each dataset (arthropod order and hemipteran morphospecies abundances), I built a negative binomial GLMM with a random slope term that allowed the effect of vegetation type to vary among arthropod orders or hemipteran morphospecies, depending on the response being modeled (Fournier et al. 2012). This model structure allows for the easy identification of taxa driving a community response to a predictor. These GLMMs also included random effects for plot ID and for mangrove/transect ID, to account for blocking and repeated sampling. For each model, a likelihood ratio test was used to determine if the random slope term had significant explanatory power. To visualize the community differences between the two vegetation types, I performed Principal Coordinates Analyses (PCoA) on arthropod order and Hemiptera morphospecies abundances, excluding rare taxa, averaging across the three repeated samples of each mangrove and transect, and calculating Bray-Curtis dissimilarities between samples (Oksanen et al. 2018).

Then, for both order-level and Hemiptera-only datasets, I quantified the degree to which mangrove community composition is nested within marsh communities, using the NODF metric (Almeida-Neto et al. 2008). For each dataset, I summed across all three repeated samples of each mangrove/marsh transect to generate a sample×species matrix, and converted the matrix to presence/absence; I

then sorted the matrix rows (samples) by vegetation type, with those from mangroves at the bottom and those from marsh at the top. Columns (arthropod orders or Hemiptera morphospecies, depending on the dataset being used) were sorted by decreasing frequency of occurrence. Using the *vegan* package (Oksanen et al. 2018), I simulated 1,000 null matrices that maintained richness within a sample but reshuffled the taxa found within each sample, based on their known occurrence frequencies.

Does mangrove proximity affect marsh arthropods?

To assess differences between marsh arthropod communities found near mangroves versus those farther away from mangroves, I repeated many of the analyses described above, replacing vegetation type with marsh transect as a predictor (and omitting mangrove samples from the analysis). Total arthropod abundance, order richness, rarefied richness, and Shannon diversity were compared using GLMMs, with transect location and the first two marsh vegetation PCA axes as fixed effects, and transect ID and plot as random effects (Bates et al. 2015). I used parametric bootstrapping to test the significance of each fixed effect in these models (Halekoh and Højsgaard 2014). Marginal and conditional R^2 – measures of variance explained by fixed effects and variance explained by both fixed and random effects, respectively – were calculated following Nakagawa and Schielzeth (2016).

To quantify taxon-level differences in community composition between near and far marsh transects, I built a negative binomial GLMM with a random slope term that allowed the effect of marsh transect to vary among arthropod taxa (Bates et al. 2015). I constructed two such models: one for order-level abundances, and one for

hemipteran morphospecies. These GLMMs also included random effects for plot ID and for transect ID, to account for blocking and repeated sampling, and they included the first two marsh vegetation PCA axes as fixed effects, as well.

Do mangrove flowers alter community composition?

The effects of mangrove flowers on community composition were first quantified separately for mangrove and marsh samples. For each vegetation type and each dataset (abundance by order and by hemipteran morphospecies), I constructed a negative binomial GLMM with a fixed effect for the number of mangrove inflorescences in the plot, and a random slope term that allowed the effect of inflorescence number to vary among arthropod taxa (Fournier et al. 2012). In addition to the single fixed effect of inflorescence number, and the by-taxon random slope and intercept terms, the models for mangrove communities included a random effect for mangrove ID, to account for repeated sampling; marsh community models included all of these predictors plus a random effect for plot, to account for the spatial proximity of the two transects within each plot. For each model, a likelihood ratio test was used to determine if the random slope term had significant explanatory power. If it did, I then plotted the model-estimated random slopes to assess differences among taxa in their variation with inflorescence number.

Finally, to determine whether *Avicennia* flowers drive differences between *Avicennia*- and marsh-associated communities, I calculated dissimilarities between each pair of mangrove and near-marsh samples within a plot, and modeled these dissimilarities as a function of the number of mangrove inflorescences present. This was done separately for order abundances and for hemipteran morphospecies

abundances. I removed rare taxa from each dataset (those found fewer than five times over the course of the study) and calculated Bray-Curtis dissimilarities on Wisconsin-standardized square-root-transformed abundances. Because Bray-Curtis dissimilarity is analogous to a proportion and varies between 0 and 1, I constructed a binomial GLMM for each dataset (Bates et al. 2015), modeling dissimilarity between a mangrove community and its nearby marsh community as a function of mangrove inflorescence number, with mangrove/marsh transect ID as a random effect. For both models, I used bootstrap parametric testing to calculate the significance of inflorescences as a predictor of sample dissimilarities (Halekoh and Højsgaard 2014).

Results

Altogether, we collected 58,282 arthropods across all samples over the course of the experiment, with representatives from 17 taxonomic orders, 10 of which were insect orders (including ants as their own group). At the order level, the arthropod communities of both mangrove and marsh vegetation were highly uneven, and were dominated by Diptera and Hemiptera (Figure 4.1). We further subdivided the Hemiptera into 17 morphospecies: 10 cicadellids, two delphacids, two flatids, Heteroptera (mainly Miridae), *Telmapsylla* (a psyllid specialized on *Avicennia*), and non-*Telmapsylla* Sternorrhyncha (mainly Aphididae).

Do mangroves host a distinct community?

Each vegetation type contained arthropod orders not found in the other. Amphipods, isopods, collembola, and mantids were orders unique to the marsh

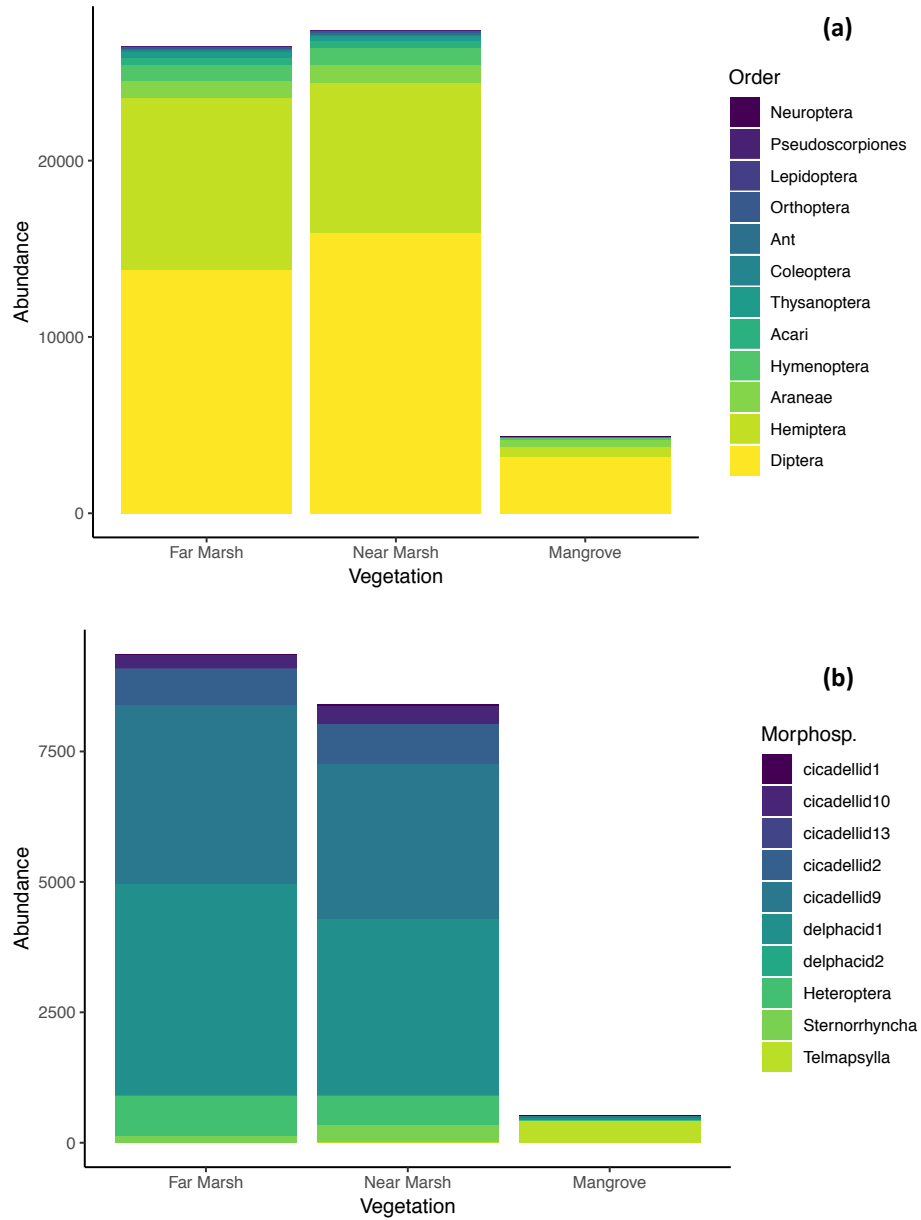


Figure 4.1: **(a)** Abundances of the 12 most common arthropod orders, summed across all samples collected from marsh vegetation far from mangroves (“Far Marsh”), marsh vegetation near mangroves (“Near Marsh”), and directly from mangroves. **(b)** Hemiptera alone, further subdivided into morphospecies (the 10 most common of which are shown). *Telmapsysylla* is an *Avicennia* specialist.

vegetation; however, these were all rare, with three or fewer individuals found across all marsh samples. Neuroptera, on the other hand, were solely collected from mangroves (all 10 individuals belonged to a single species, the mantidfly *Climaciella*

brunnea [Say in Keating, 1824]). Unique hemipteran morphospecies were also present in each vegetation type, with marsh containing six morphospecies that were entirely absent in mangroves, while mangroves contained three morphospecies absent in marsh. Notably, the specialist *Avicennia* psyllid, *Telmapsylla*, was found in both vegetation types: of 430 *Telmapsylla* collected in the study, 12 of them were recovered from marsh vegetation.

Total arthropod abundance was significantly higher in marsh vegetation than in mangroves (parametric bootstrapping: $p = 0.0011$) (Figure 4.1), with an average of 327 individuals collected per marsh vacuum sample versus 49 individuals per mangrove sample. Predictably, therefore, raw richness at the order level was also significantly higher in marsh vegetation (parametric bootstrapping: $p = 0.0010$) (Figure 4.2). However, Shannon diversity at the order level did not differ significantly among vegetation types (Figure 4.2), probably because marsh communities were so uneven (as can be seen in Figure 4.1).

When considering the abundances of different arthropod orders, a model that allowed taxa to randomly vary in their response to vegetation type significantly improved model fit over one that did not (random slope LRT: Δ deviance = 229.2, $p < 0.00001$), indicating that not all orders responded similarly to vegetation type. While every order except Neuroptera was more abundant in marsh vegetation than on mangroves, the model identified Hemiptera, Diptera, and Hymenoptera as responding more positively to marsh vegetation than other taxa (estimated random slope coefficients \pm SD: 1.7 ± 0.45 , 0.77 ± 0.31 , and 0.55 ± 0.30 , respectively). Hemipteran morphospecies also differed in their response to vegetation type (random slope LRT:

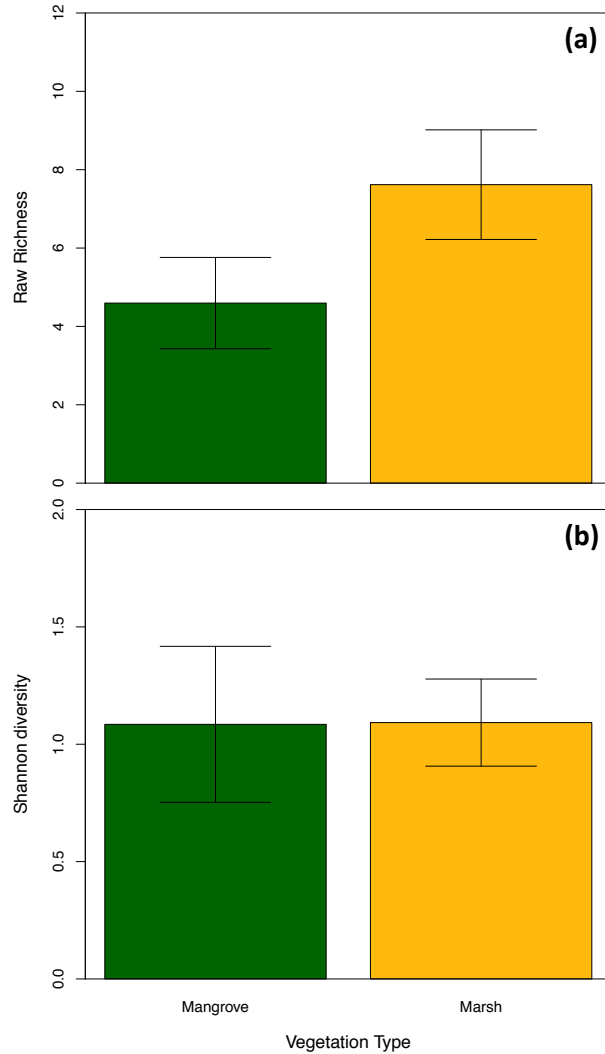


Figure 4.2: **(a)** Raw arthropod richness (at the order level) was significantly higher in marsh than in mangroves. **(b)** However, Shannon diversity was not significantly different between the two vegetation types, indicating that marsh arthropod communities were more strongly dominated by a few groups.

Δ deviance = 88.8, $p < 0.00001$). A PCoA of Wisconsin-standardized order abundances explained 44% of the variation with the first two axes, and indicated substantial differentiation between mangrove and marsh communities (Figure 4.3). Similarly, a PCoA of hemipteran morphospecies abundances explained 72% of variation in the first two axes, with much of the variation being attributable to

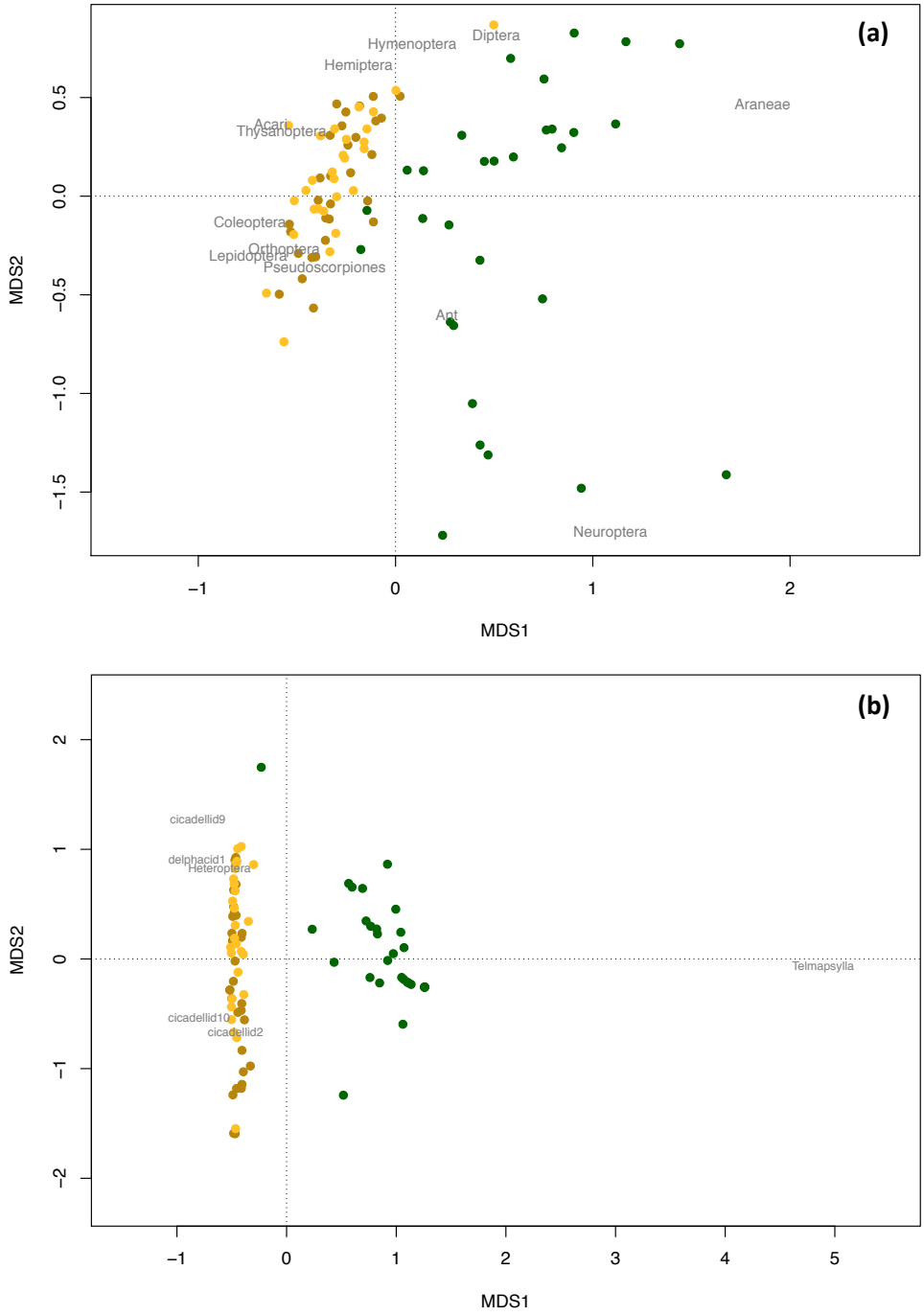


Figure 4.3: Principal Coordinates Analysis (PCoA) on **(a)** arthropod order abundances, and **(b)** Hemiptera only, split into morphospecies. Samples collected from mangroves are shown in dark green; samples from marsh vegetation near/far from the mangroves are shown in dark/light yellow, respectively.

dominance by *Telmapsylla* (in the case of mangroves) versus dominance by Auchenorrhyncha and Heteroptera (in the case of marsh vegetation) (Figure 4.3).

At the order level, the relatively low-richness mangrove samples appeared to be nested within high-richness marsh samples, such that arthropod orders represented in mangrove communities were subsets of those present in marsh ($p = 0.0010$). However, nestedness of mangrove hemipteran morphospecies within marsh Hemiptera was not evident ($p = 0.26$), suggesting that the pooling of taxa within orders obscures some mangrove-marsh differentiation, and that nestedness disappears when finer taxonomic resolution is used.

Does mangrove proximity affect marsh arthropods?

Marsh transects near mangroves largely shared the same arthropod taxa as those far from mangroves (Figure 4.1); all orders or hemipteran morphospecies not common to both transect types were so rare ($n=1-2$) as to be unlikely or impossible to be found in both based on rarity alone. Neither total arthropod abundance, raw richness, rarefied richness, nor Shannon diversity differed significantly between marsh transects near and far from mangroves (Table 4.1). Rather, abundance, richness, and diversity were better explained by the composition of the marsh vegetation (Table 4.1). Furthermore, neither arthropod orders nor hemipteran morphospecies significantly varied in their response to transect type (random slope LRT Δ deviance = 18.0, $p = 0.36$; and Δ deviance = 3.1, $p = 0.21$, respectively); random slope estimates were approximately 0 across all taxa. Confirming this result, the PCoA's of order and hemipteran morphospecies abundances both revealed little differentiation between far marsh and near marsh transects (Figure 4.3).

Table 4.1: Models of marsh arthropod abundance, richness, rarefied richness, and Shannon diversity as a function of the type of transect (near vs far from the focal mangrove) and the two PCA axes representing variation in marsh plant composition. Random effect estimates are not shown. Marginal R^2 is given for each model. P -values were calculated using parametric bootstrapping.

Response (R_m^2)	Fixed Effect	Coefficient Est.	SE	p
Total arth. abundance (0.22)	Transect (Near)	-0.034	0.072	0.66
	PCA axis 1	0.13	0.027	0.0012
	PCA axis 2	0.09	0.037	0.024
Order richness (0.03)	Transect (Near)	0.010	0.064	0.86
	PCA axis 1	-0.018	0.019	0.32
	PCA axis 2	-0.050	0.026	0.052
Rarefied order richness (0.24)	Transect (Near)	-0.042	0.13	0.83
	PCA axis 1	-0.23	0.064	0.0012
	PCA axis 2	-0.37	0.084	0.0012
Shannon diversity (0.21)	Transect (Near)	-0.00017	0.028	0.97
	PCA axis 1	-0.025	0.011	0.033
	PCA axis 2	-0.068	0.014	0.001

Do mangrove flowers alter community composition?

On *Avicennia*, arthropod community composition was significantly affected by the number of mangrove inflorescences present, with the effect of inflorescences varying among arthropod taxa (random slope LRT: Δ deviance = 49.8, $p < 0.00001$). However, order-specific random slope estimates revealed that only two orders showed a truly significant response to inflorescences: Diptera and Hemiptera, both of which increased in abundance with mangrove flowering (Figure 4.4). (Orthoptera, which also appeared to show a positive response to flowering on mangroves, were too uncommon for the effect to be meaningful.) In contrast, the composition of

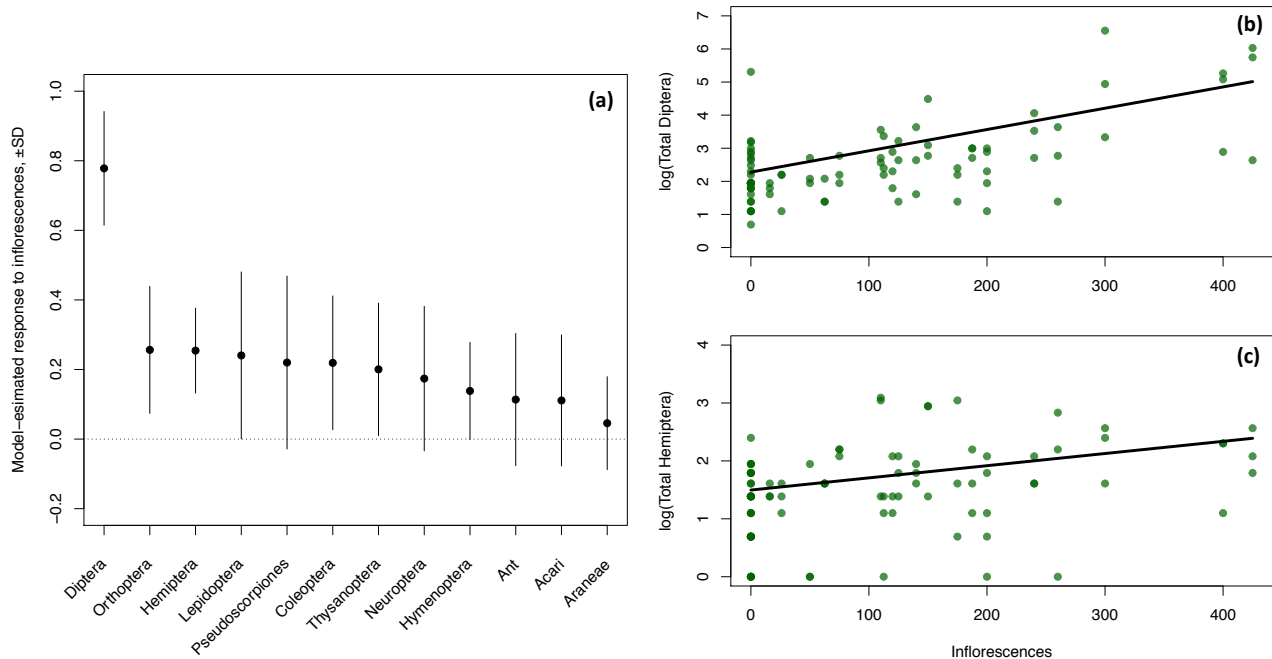


Figure 4.4: **(a)** Random slope estimates for mangrove arthropods, representing order-specific relationships between abundance and the number of mangrove inflorescences present. Points falling near the dashed line at 0 are orders whose abundance did not vary with inflorescence number; points well above/below 0 (e.g. Diptera) are orders whose abundance increased/decreased with mangrove inflorescences, respectively. On mangroves, Diptera **(b)** and Hemiptera **(c)** were the only two orders whose abundance was meaningfully associated with the number of inflorescences. Lines show model-estimated relationships.

hemipteran morphospecies found on mangroves was unaffected by the number of inflorescences present (random slope LRT: Δ deviance ≈ 0 , $p \approx 1$), with random slope estimates close to 0 for all morphospecies.

Marsh arthropod community composition showed the opposite pattern with respect to mangrove flowers, with no response to the number of nearby mangrove inflorescences at the order level (random slope LRT: Δ deviance = 2.56, $p = 0.11$), but with significant differences among hemipteran morphospecies in their response

(random slope LRT: Δ deviance = 11.2, $p = 0.001$). In particular, Heteroptera were more abundant in marsh surrounding heavily-flowering *Avicennia*, whereas two of the cicadellid morphospecies and Sternorrhyncha (predominantly aphids) were slightly less common in marsh when mangrove flowers were in the vicinity (Figure 4.5).

However, despite these effects of mangrove flowers on arthropod community composition, the Bray-Curtis dissimilarities between mangrove-associated arthropod communities and nearby (1.5m) marsh-associated communities were unrelated to the

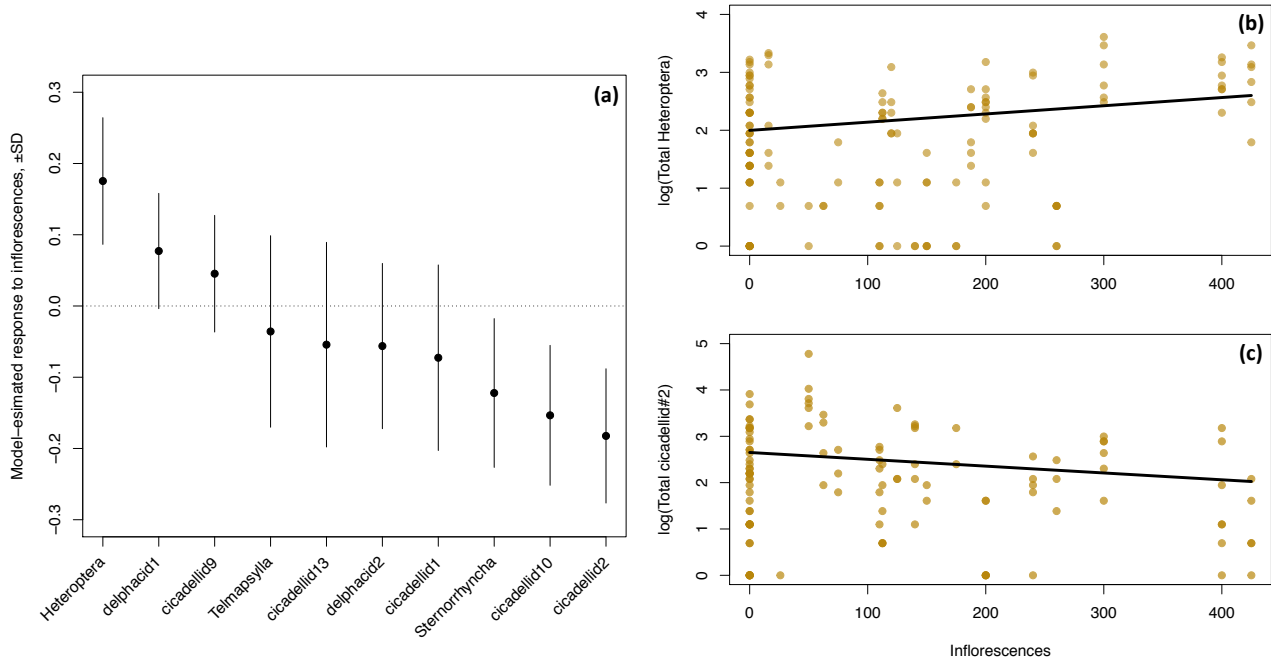


Figure 4.5: **(a)** Random slope estimates for marsh Hemiptera, representing morphospecies-specific relationships between abundance and the number of mangrove inflorescences nearby (at the order level, marsh arthropods did not show any relationship between abundance and flowers). Points falling near the dashed line at 0 are morphospecies whose abundance did not vary with inflorescence number; points well above/below 0 are morphospecies whose abundance increased/decreased with mangrove inflorescences, respectively. Marsh Heteroptera **(b)** and a cicadellid morphospecies **(c)** had the strongest responses to nearby mangrove inflorescences. Lines show model-estimated relationships.

number of mangrove inflorescences present (order-based dissimilarities: bootstrap $p = 0.16$; hemipteran morphospecies-based dissimilarities: bootstrap $p = 0.46$). This indicates that the flowering responses seen in Diptera and Hemiptera on mangroves, and in several hemipteran morphospecies in marsh vegetation, did not change how dissimilar overall mangrove community composition and marsh community composition were from one another (Figure C.3).

Discussion

As mangroves expand northward and shift the position of the marsh-mangrove ecotone in the southeastern U.S., once-pure salt marsh will become a mixture of vegetation types, altering habitat suitability for coastal fauna. This study compared arthropod communities associated with flowering and non-flowering range-edge *Avicennia* and neighboring salt marsh vegetation, to determine how the arthropod community is structured within this ecotone landscape. While *Avicennia* hosted fewer arthropods and less diversity than surrounding salt marsh vegetation, it did support a unique arthropod assemblage that was not simply a subset of the greater marsh community, with taxa that were entirely absent or rare in nearby marsh. Surprisingly, however, given the stark differences between mangrove and marsh arthropod assemblages, proximity to mangroves did not appear to influence community composition of the surrounding marsh. Marsh vegetation a mere 1.5m from the central trunk of *Avicennia* trees – meaning, in effect, that the mangrove canopy was often less than 1m away – hosted an arthropod community no different from marsh vegetation that was 5m distant. Consequently, the arthropod community within the

ecotone has a fine-scale spatial heterogeneity that precisely mirrors the heterogeneity of the vegetation.

Surprisingly, *Avicennia* flowers did not appear to play a large role in shaping diversity within the mangrove-marsh ecotone – at least, for the more sessile arthropods collected via vacuum sampling in this study. On mangroves themselves, Diptera were the only group to show a large response, increasing from a median of 7 individuals on *Avicennia* with no flowers to a median of 161 individuals on trees with 300 or more inflorescences (Figure 4.4). Diptera are common floral visitors in other systems, consuming nectar and pollen (Kearns 2001, Larson et al. 2001); the Diptera collected in this study were mainly small-bodied Chloropidae and Ceratopogonidae, which were likely opportunistic floral visitors but could theoretically contribute to *Avicennia* pollination (Larson et al. 2001). Hemiptera, too, tended to increase in abundance on heavily-flowering *Avicennia*, but the effect was much more modest, growing from a median of 4 individuals to 9 on trees with the most inflorescences (Figure 4.4). Importantly, this increase in Hemiptera abundance did not appear to be driven by marsh taxa migrating onto flowering mangroves; those morphospecies that were relatively common in surrounding marsh vegetation did not respond more positively to mangrove flowers than other morphospecies.

For the most part, marsh arthropods displayed no response to the quantity of nearby *Avicennia* inflorescences, although several hemipteran morphospecies in the salt marsh did statistically vary in abundance with mangrove flower number (Figure 4.5). Most of the Heteroptera observed were herbivorous mirids, a group that is known to opportunistically feed on nectar from plants they otherwise do not consume

(Wheeler and Skaftason 2010). Heteroptera were very rare on *Avicennia* (n=16), which might explain why their abundance on mangroves was unrelated to flowering. In sum, the communities in marsh and even on mangroves themselves generally were unaffected by the number of mangrove flowers present. It should be noted, however, that the sampling method used in this study selectively collects less mobile organisms (Zou et al. 2016); bees, large wasps, and other strong fliers that may visit *Avicennia* flowers within the ecotone, increasing mangrove-associated diversity, were not captured in this study.

That neighboring *Avicennia* and salt marsh host different arthropod communities is not surprising, given previous work documenting their associated fauna (Davis and Gray 1966, Pennings and Bertness 2001). Arthropod herbivores tend to be specialized on their host plants, at least to the level of family or genus (Forister et al. 2015), and *Avicennia* is a distant relative of the salt marsh plants at this study site. Furthermore, some of the most abundant salt marsh herbivores in North America – e.g. *Prokelisia marginata*, (Hemiptera: Delphacidae) and *P. dolus* – are known specialists of *Spartina alterniflora* (Denno 1977). In addition, a host plant's physical structure plays an important role in shaping the associated arthropod community (Döbel et al. 1990); the shrubby, broad-leaved *Avicennia* within the ecotone possess a very different architecture from the grasses and succulent forbs that make up the surrounding salt marsh. For example, in a salt marsh, Döbel et al. (1990) found that co-occurring *Spartina* species host distinct spider assemblages, largely due to differences in grass architecture and thatch density, which influence spider hunting strategy (e.g. active hunting vs. web-building). This study found higher spider

abundance in salt marsh than on mangroves, which could be due to the abundance of prey and/or to the fine-scale structural complexity of the vegetation. Further study of neighboring *Avicennia* and salt marsh spider assemblages at a finer taxonomic resolution would be valuable for determining species and functional turnover between the two vegetation types.

The coarse taxonomic resolution of this study undoubtedly obscured some of the distinctions between *Avicennia* and salt marsh arthropod communities. The one order which was considered at a finer taxonomic resolution illustrates this point: over 97% of all Hemiptera (n=18,310) were found in marsh samples, but within the Hemiptera, over 97% of *Telmopsylla* (n=430) were collected from mangrove samples (Figure 4.1). Consequently, this study is almost certainly conservative in its estimate of the differences between the two communities. This also means that this study may be unable to detect within-order differences in community composition that exist between marsh immediately adjacent to mangroves and marsh farther away, resulting from associational effects due to mangrove proximity or to spillover. Tellingly, and in contrast to this study, Loveless and Smees (2019) found differences in arthropod communities of Texas salt marshes that varied with the amount of *Avicennia* cover nearby. Specifically, they found that arthropod abundance and diversity were highest in marshes where *Avicennia* was rare, and that predator-to-herbivore ratios were substantially lower in marsh bordering dense stands of *Avicennia*. To better understand the causality of mangrove encroachment and changes in marsh arthropod community composition, experimental introduction of mangroves or time series data from permanent plots in marsh experiencing mangrove encroachment are needed.

This study contributes to our understanding of how mangrove encroachment into salt marsh affects arthropod diversity and community composition, by showing that the heterogeneity of the vegetation in the mangrove-marsh ecotone translates into a mosaic-like arthropod community, with sharp, sub-meter boundaries between assemblages associated with mangroves versus salt marsh. However, contrary to expectations, mangrove flowers did not meaningfully contribute to the differences between mangrove- and marsh-associated arthropod communities. This suggests that other traits, such as the plants' physical structure, are of greater importance. In sum, as *Avicennia* continues to expand northward due to climate change, settings that transition from pure salt marsh to mixed marsh-mangrove ecotone will experience an increase in regional arthropod diversity, due to increasing habitat heterogeneity (via traits other than flowers). Future work monitoring the response to mangrove encroachment in a salt marsh, over time, would help to reveal the temporal pattern of diversity change, and the pace at which the arthropod community adjusts to the shifting ecotone.

Appendices

Appendix A

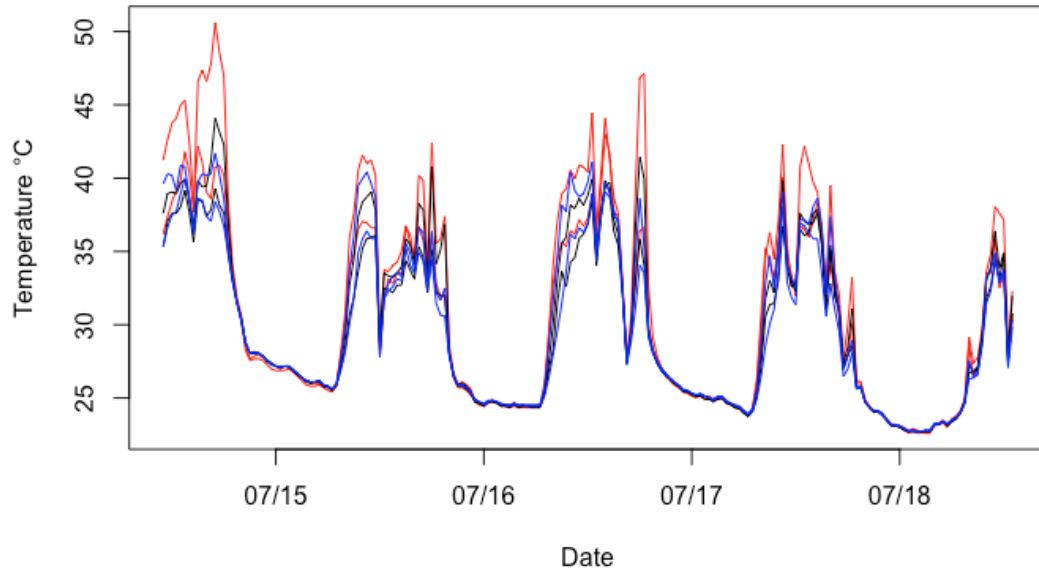


Figure A.1: Temperatures recorded inside fine-mesh pollinator exclusion bags (red lines), large-mesh pollinator bags (blue lines), and in open air on adjacent branches (black lines) over four days. Fine-mesh bags presumably blocked air flow and evaporation, leading to high temperatures during the day.

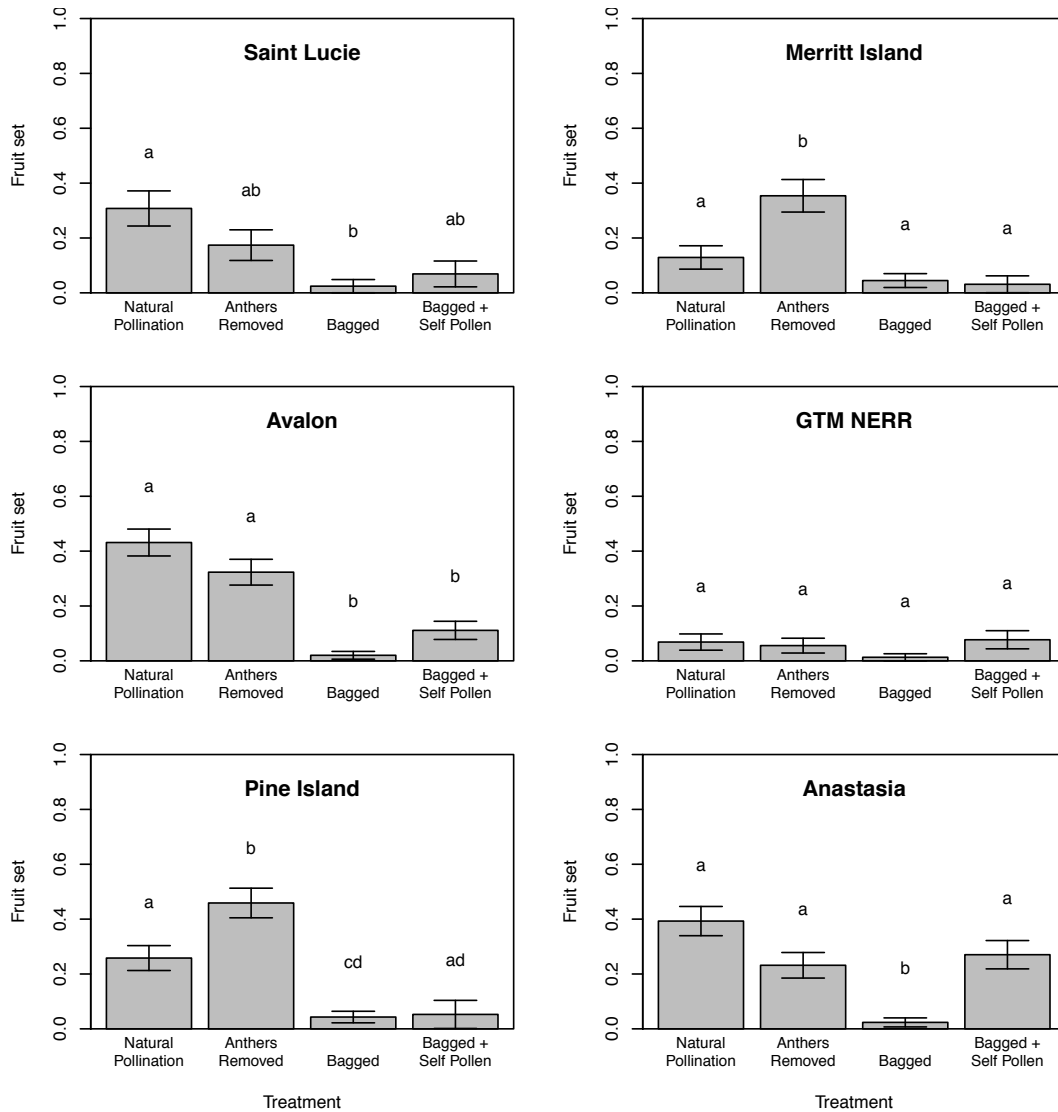


Figure A.2: Variation in treatment effects by site, in the first reproductive strategy experiment in 2014. Fruit set was defined as the number of propagules that began to develop divided by the number of flowers assigned to this treatment and site. The three southernmost sites are shown on the left; the three northernmost sites are on the right. Different letters above bars indicate significant differences, according to Tukey post-hoc comparisons ($p < 0.05$). Bars show standard errors.

Appendix B

Table B.1: Site descriptions and variables measured at each site, listed from northernmost (Anastasia State Park) to southernmost (Hobe Sound National Wildlife Refuge). See map of these sites in Figure B.1.

Site	Name	Description	Measurements
1	Anastasia S.P.	Vegetation: Mixture of mangrove (<i>A. germinans</i> only) and salt marsh (<i>S. alterniflora</i> and <i>B. maritima</i>).	Reproductive tree density and fecundity; propagule mass; floral visitation; fruit set
2	GTM-Braddocks Point	Vegetation: Mixture of mangrove (<i>A. germinans</i> only) and salt marsh (<i>S. alterniflora</i> , <i>B. maritima</i> , and <i>Salicornia</i> sp.).	Reproductive tree density and fecundity; propagule mass; floral visitation; pollen deposition
3	GTM-Matanzas	Vegetation: Mixture of mangrove (<i>A. germinans</i> only) and salt marsh (<i>S. alterniflora</i> , <i>B. maritima</i> , and <i>Salicornia</i> sp.).	Reproductive tree density and fecundity; propagule mass; floral visitation; pollen deposition; fruit set
4	GTM-Marineland	Vegetation: Mangrove (primarily <i>A. germinans</i> , scattered <i>R. mangle</i>) immediately adjacent to upland forest.	Floral visitation
5	Spruce Creek Preserve	Vegetation: Mixture of mangrove (primarily <i>A. germinans</i> and <i>R. mangle</i> , some <i>L. racemosa</i>) and salt marsh (<i>S. alterniflora</i> , <i>B. maritima</i> , and <i>Salicornia</i> sp.).	Reproductive tree density and fecundity; propagule mass; floral visitation; pollen deposition
6	Merritt Island N.W.R.	Former mosquito impoundment (natural flow restored 1997). Vegetation: Mixture of mangrove (primarily <i>A. germinans</i> and <i>L. racemosa</i> , some <i>R. mangle</i>) and salt marsh (<i>Distichlis spicata</i> , <i>B. maritima</i> , and <i>Salicornia</i> sp.).	Reproductive tree density and fecundity; propagule mass; floral visitation; pollen deposition; fruit set
7	Pine Island Conservation Area	Former dredge spoil island (restored 2006). Vegetation: Mixture of mangrove (<i>R. mangle</i> , <i>A. germinans</i> , and <i>L. racemosa</i>) and salt marsh (<i>Distichlis spicata</i> , <i>B. maritima</i> , and <i>Salicornia</i> sp.).	Reproductive tree density and fecundity; floral visitation; fruit set
8	Sebastian Inlet S.P.	Former mosquito impoundment. Vegetation: Pure mangrove (<i>R. mangle</i> , <i>A. germinans</i> , and <i>L. racemosa</i>).	Floral visitation
9	Avalon S.P.	Mosquito impoundment. Vegetation: Pure mangrove (<i>R. mangle</i> , <i>A. germinans</i> , and <i>L. racemosa</i>).	Reproductive tree density and fecundity; propagule mass; floral visitation; pollen deposition; fruit set
10	St. Lucie Inlet Preserve S.P.	Vegetation: Pure mangrove (<i>R. mangle</i> , <i>A. germinans</i> , and <i>L. racemosa</i>).	Reproductive tree density and fecundity; propagule mass; floral visitation; pollen deposition; fruit set
11	Hobe Sound N.W.R.	Vegetation: Pure mangrove (<i>R. mangle</i> , <i>A. germinans</i> , and <i>L. racemosa</i>).	Floral visitation

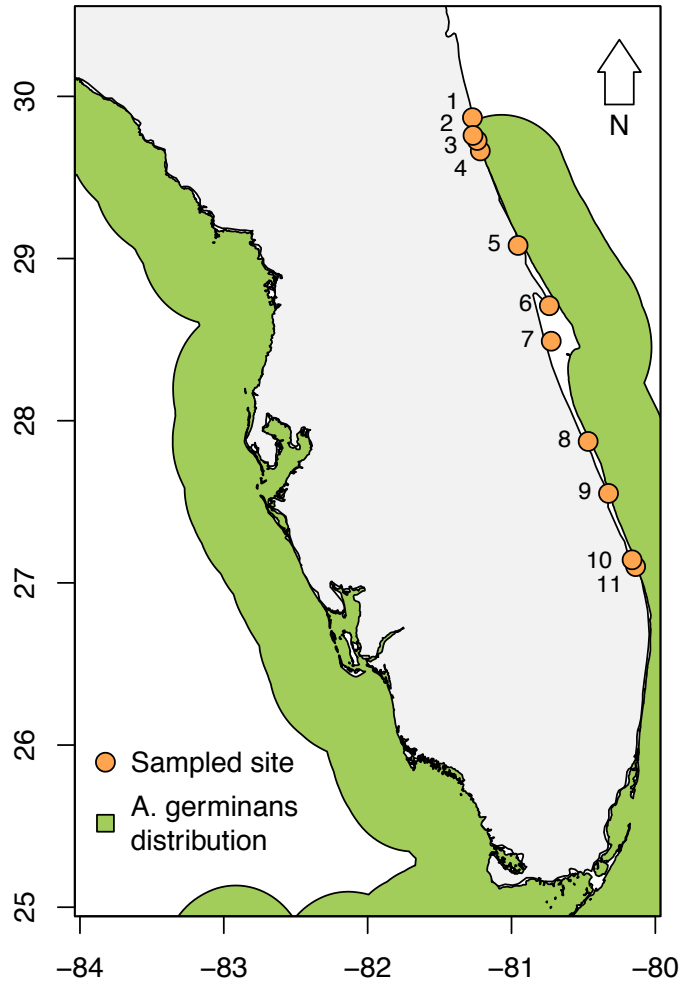


Figure B.1: Map of study sites in Florida, USA. Identities and visitation rates of pollinators were measured at all sites; pollen deposition, flowering, and propagule production were measured at a subset of these sites. See text for details; see Table B.1 for site descriptions.

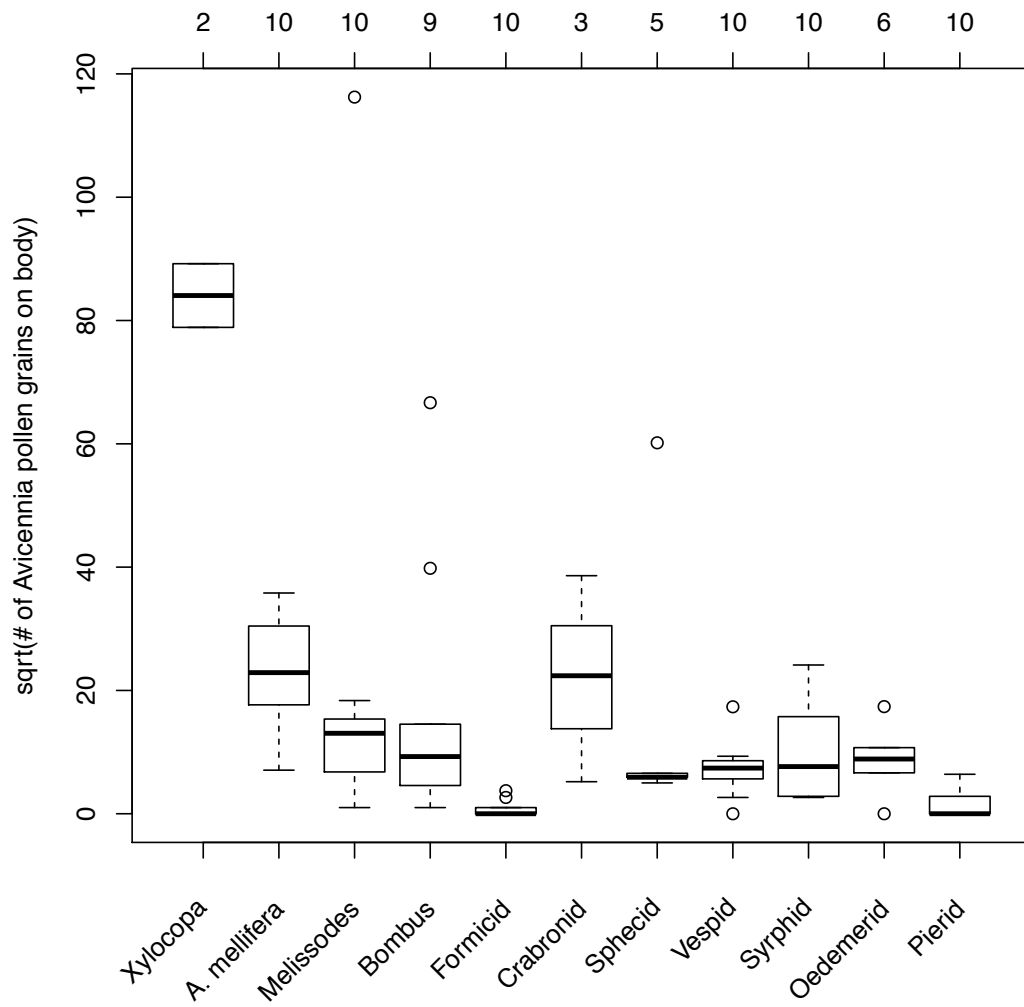
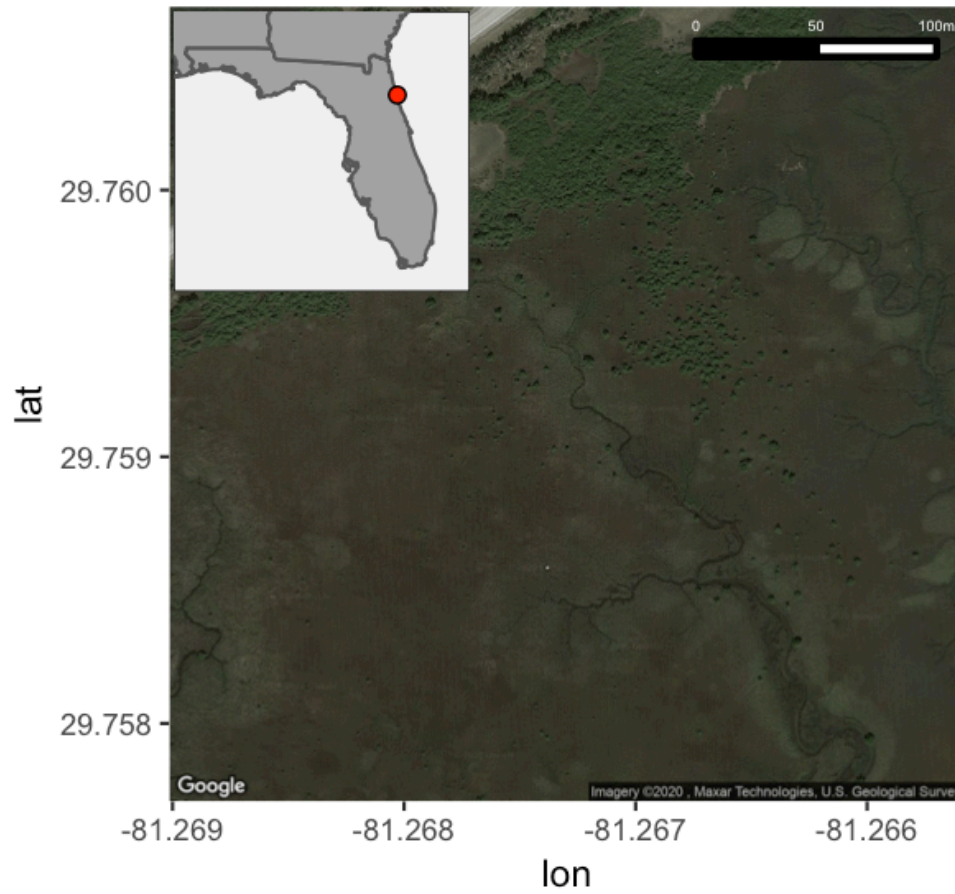


Figure B.2: Pollen load size varied significantly among insect taxa. Shown here are pollen loads collected from 11 of the 12 most frequently observed floral-visiting taxa (no pompilids were collected for pollen load measurements). Sample sizes are shown along the top of the plot.

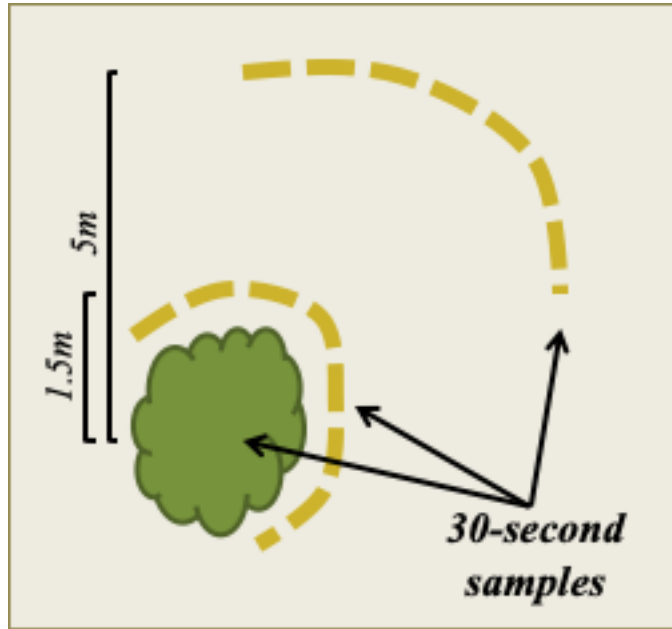
Appendix C

Table C.1: Variable loadings from a Principal Components Analysis of % cover along transects encircling each focal mangrove, at 1.5m and 5m.

Variable	PCA Axes	
	1	2
<i>Spartina</i>	0.624	
<i>Salicornia</i>	-0.508	0.162
<i>Batis</i>	-0.278	-0.672
Bare ground		0.646
<i>Avicennia</i> seedlings	-0.524	0.311
Proportion of variance explained	0.47	0.27



*Figure C.1: The study site, in northern Florida (inset), is dominated by salt marsh (brown and gray regions bordering tidal creeks). *A. germinans* is increasing in cover in parts of the marsh, and, in this satellite image, is visible as dark-green vegetation along the northern and central areas of the site.*



*Figure C.2: Birds-eye schematic showing vacuum sampling of one plot, consisting of a focal *Avicennia* mangrove and its two surrounding marsh vegetation transects (dashed lines). Each plot was sampled three times over the course of the experiment.*

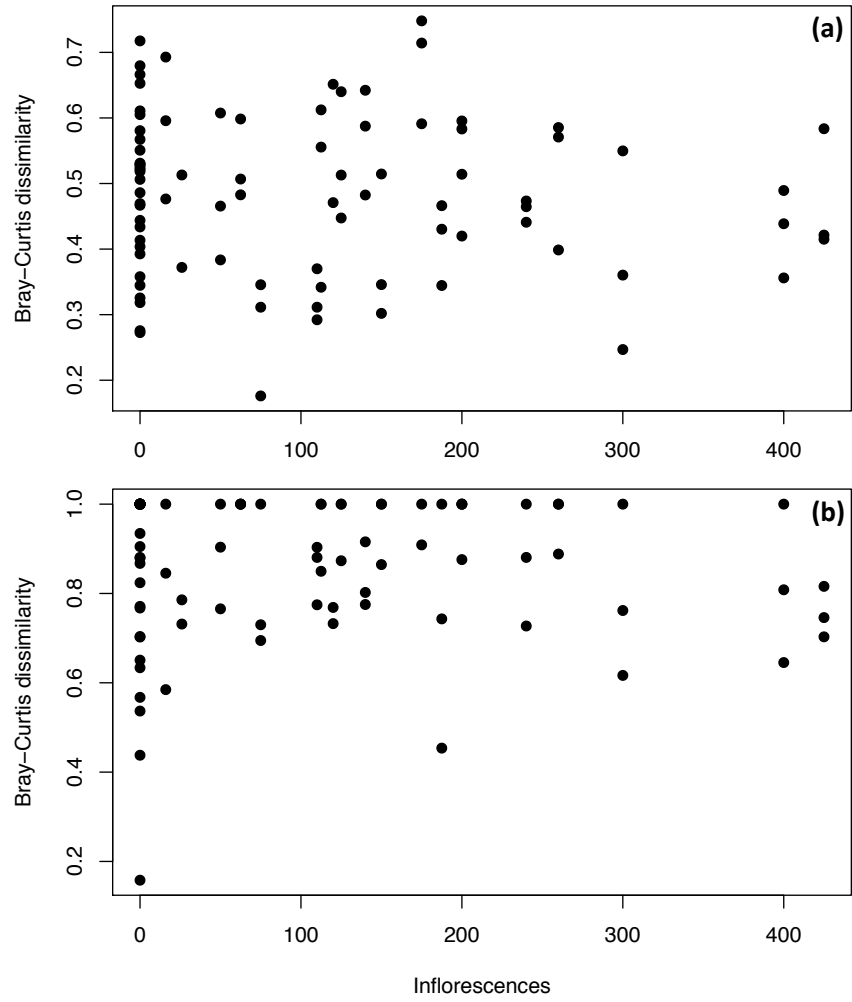


Figure C.3: Dissimilarities between mangrove and nearby marsh communities, based on (a) arthropod orders or (b) only Hemiptera morphospecies, was unrelated to the number of mangrove inflorescences present.

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