Genetic insights into climate-driven range expansion of a coastal foundation species

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Genetic insights into climate-driven range expansion of a coastal foundation species

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Abstract

In this thesis, I address gaps in our understanding of how climate-driven range expansion can shape genetic variation within a coastal foundation species and how the resulting genetic changes may have broader ecological consequences. To do this, I study the neotropical black mangrove (*Avicennia germinans*) across its distribution in the United States, and then I focus on its expanding Atlantic Florida range margin. First, in Chapter 2, I find that genetic variation within *A. germinans* declines towards three range margins in the United States, but that this reduction may not constrain adaptation within this species as range-margin *A. germinans* exhibit shifts in functional traits consistent with greater cold tolerance. Leveraging these insights and genetic data, I then document patterns in mating system and dispersal, factors that can shape intraspecific genetic variation and influence expansion success, towards the Atlantic Florida range margin. In Chapter 3, I find evidence for plastic shifts towards greater self-fertilisation at this sparsely-populated range margin, a mechanism that can facilitate colonisation of new areas. In Chapter 4, I show how extreme storm events may facilitate poleward expansion of *A. germinans* and how this form of episodic dispersal can shape genetic variation within newly-colonised populations. Finally, I evaluate potential ecological consequences of the unique genetic variation found within these Atlantic Florida *A. germinans*. In Chapter 5, at the scale of a range-margin population, I find that genetically-similar *A. germinans* harboured similar fungal communities, a relationship that may have implications for the fitness of these mangrove hosts. In Chapter 6, using a greenhouse common garden, I demonstrate a genetic basis to adaptative trait shifts within these range-margin *A. germinans* that may facilitate future range expansion of this species. These novel insights should improve our ability to predict how mangrove range margins may respond to climate change and help inform future mangrove restoration initiatives.

Declaration of alternative thesis format

This thesis is presented in an alternative format in accordance with the rules and regulations outlined in the *Guidance on Thesis Submission for Postgraduate Research Degrees* from Manchester Metropolitan University. Each data chapter differs in layout and referencing style for the following reasons. Chapters 2, 3, 4, and 5 have been published as articles in peer-reviewed journals during my PhD course. These chapters are inserted in their final typeset according to the specific format of each journal. Chapter 6 has been submitted for publication and is presented as a manuscript in the format of the target journal. Details regarding my contribution to each chapter, signed by each co-author, are presented on the respective title pages.

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CHAPTER 1

Introduction

Introduction

Background

Climate change has been linked to the expansion of diverse taxa at their cold-sensitive range margins worldwide (Parmesan and Yohe, 2003; Chen et al., 2011; Poloczanska et al., 2013). This redistribution in global biodiversity has altered ecological communities and can impact ecosystem function and human well-being (Scheffers et al., 2016; Pecl et al., 2017). As such, there is an urgent need to better predict how species will respond to continued climate change and the ecological consequences of their responses (Bellard et al., 2012; Malhi et al., 2020).

Insights into how foundation species will respond to climate change will be particularly informative. Foundation species are organisms that define the structure of ecological communities, create stable conditions necessary for the survival of associated species, and regulate ecosystem dynamics (Dayton, 1972). Trees serve as foundation species within forest ecosystems as their architecture provides physical structure and creates variation in microclimates, and their biomass and chemical composition can shape energy flow and nutrient cycling (Ellison et al., 2005). Due to their role in defining ecosystems, climate-driven expansion of tree foundation species can drastically modify landscapes and have wide-reaching ecological impacts. For instance, warming climate has been attributed to ongoing biome shifts as temperate forests replace heathlands at higher elevation in the Montseny mountains in Spain (Peñuelas and Boada, 2003) and as evergreen forests expand into tundra at higher latitude in Alaska (Beck et al., 2011). Another example, across the globe, is that of tropical mangroves expanding poleward into temperate salt marsh where their distributions overlap (Saintilan et al., 2014).

Mangroves are an ecological assemblage of woody plants that have evolved unique anatomical and physiological traits to thrive in the intertidal zone of tropical and subtropical coastlines worldwide (Tomlinson, 1986). These coastal foundation species provide vital ecosystem services of ecological and economic importance, such as habitat provision for a diverse array of associated terrestrial and marine species, coastal protection from storm events, shoreline erosion control, carbon sequestration, and water purification via nutrient retention (Nagelkerken et al., 2008; Barbier et al., 2011; Lee et al., 2014). Mangrove distributions are anchored in the tropics and radiate poleward until minimum temperature or precipitation becomes too marginal for their survival (Osland et al., 2017). At cold-sensitive range margins, mangroves are often replaced by temperate salt marsh vegetation (Spalding et al., 2010). Modern-day warming trends, however, have resulted in the proliferation of these mangroves, at the expense of salt marsh, at multiple range margins, including in Australia (Whitt et al., 2020), Brazil (Cohen et al., 2020), and the United States (Osland et al., 2021).

In this thesis, I focus on the well-documented expansion of mangroves into temperate salt marsh in the United States, and specifically on the Atlantic coast of Florida. Mangroves are the dominant coastal foundation species along Atlantic Florida until approximately 29°N, at which point salt marsh becomes dominant and mangroves mostly exist as discrete patches of isolated individuals (Spalding et al., 2010). Historically, extreme freeze events have led to periodic mangrove die-backs at the Atlantic Florida range margin (Rodriguez et al., 2016; Cavanaugh et al., 2019), but a lack of such events for more than three decades has resulted in ongoing, rapid proliferation (Cavanaugh et al., 2014; Simpson et al., 2019). Further mangrove proliferation and expansion poleward are forecast as winters continue to warm with climate change, which may result in a permanent shift from salt marsh to mangrove dominance within the region (Cavanaugh et al., 2015, 2019). This shift would lead to substantial changes in ecosystem structure and services, including increased carbon storage, greater sediment accretion in response to sea level rise, enhanced storm protection, and reduced habitat availability for certain fauna that require open vegetation (Guo et al., 2017; Kelleway et al., 2017).

Although warmer winters are fundamental for mangroves to proliferate at and expand beyond this cold-sensitive range margin, multiple additional factors will influence the establishment, survival, and growth of these mangroves, and need to also be considered to better predict how these coastal ecosystems will respond to ongoing climate change. For instance, salt marsh vegetation may facilitate mangrove establishment by trapping propagules (Peterson and Bell, 2012) and buffering against freezing temperatures (Pickens et al., 2019), but competition with salt marsh species may reduce growth (Simpson et al., 2013). Elevated hydroperiod and salinity can also reduce seedling survival and growth (Alleman and Hester, 2011) and higher salinity can reduce the ability of these plants to tolerate cold (Devaney et al., 2021). Mangrove establishment at certain sites may not even be possible because of extensive propagule predation (Langston et al., 2017) or seedling herbivory (Devaney et al., 2017). Nutrient availability can also drastically alter mangrove growth rates at their range margins (Dangremond et al., 2020; Weaver and Armitage, 2020). Another factor, that has not previously been considered, could be genetic variation within these range-margin mangroves.

Knowledge gaps

Intraspecific genetic variation is the most fundamental level of biodiversity and is increasingly recognised as an important factor that can shape community structure and ecosystem function (Raffard et al., 2019). Ecological effects of intraspecific genetic variation are often most influential in systems that are structured by dominant plant foundation species (Whitham et al., 2006). Genetic variation within these plants has been shown to impact productivity, resilience to disturbances, and the structure and diversity of associated communities within multiple natural systems (Rowntree et al., 2011).

Range-margin populations of a species may possess reduced genetic variation compared to those within more central portions of a distributional range (Eckert et al., 2008; Pironon et al., 2017) as a result of suboptimal ecological conditions that can impede population growth and survival, and increase isolation among conspecifics (Sexton et al., 2009). Limited genetic variation could constrain adaptation to the novel range-margin environment, which may prevent further expansion of a species (Bridle and Vines, 2007). Greater isolation among conspecifics and a reduced availability of pollinators can also lead to greater self-fertilisation within range-margin plants as a means of reproductive assurance (Hargreaves and Eckert, 2014). However, this shift in mating system may augment inbreeding depression that can reduce the survival and fertility of offspring over time (Charlesworth and Willis, 2009). In addition, limited gene flow to range margins can further reduce genetic variation and augment genetic drift (Bialozyt et al., 2006) that will further differentiate range-margin populations from the rest of a species' distribution (Excoffier et al., 2009). A lack of dispersal to areas beyond present-day range margins is also thought to be a principal constraint to range expansion for many plant species (Hampe, 2011). Yet, despite these natural constraints, adaptive shifts towards increased reproductive investment, greater stress tolerance, and dispersal-promoting morphological traits that could facilitate species expansion are often observed at range margins (Chuang and Peterson, 2016). These unique genetic adaptations may enable range-margin populations to persist under their extreme conditions and can dictate future responses to climate change (Rehm et al., 2015).

Although mangrove range margins often exhibit substantial reductions in intraspecific genetic variation (Pil et al., 2011; De Ryck et al., 2016; Kennedy et al., 2017; Binks et al., 2019), our understanding of the processes shaping these reductions is limited. Are range-margin mangroves genetically depauperate because of their inherent isolation and a lack of dispersal from more genetically-diverse areas? If so, will further range expansion be hindered by dispersal limitation? Does the patchiness of range-margin mangroves lead to greater self-fertilisation within these plants that, over time, can lead to even further reductions in genetic variation? In addition to these knowledge gaps, it is unknown how these reductions in genetic variation within range-margin mangroves translate into adaptive trait variation and the potential effects of this genetic variation on the wider mangrove-associated community.

Thesis outline

In this thesis, I address these knowledge gaps using the neotropical black mangrove (*Avicennia germinans*) as the study species. Mangrove range margins across the United States consist almost exclusively of *A. germinans* (Lonard et al., 2017), the most freeze tolerant mangrove within this system (Cavanaugh et al., 2015). As such, responses of this species to climate change will have the largest ecological consequences for these coastal ecosystems.

First, in Chapter 2, I document changes in *A. germinans* population genetics across the entire distribution of this species in the United States, which includes three cold-sensitive range margins. I genotype individuals using a set of nuclear microsatellite loci that I also use to genotype samples throughout this thesis. For each population, I also measure functional traits of leaves associated with cold tolerance to evaluate whether range-margin *A. germinans* exhibit phenotypic shifts consistent with adaptation to cold stress. This work determines whether genetic variation declines towards these *A. germinans* range margins and if these reductions may constrain adaptation to their marginal environments.

Leveraging the insights gained and genetic data from Chapter 2, I then focus the remainder of the thesis on the Atlantic coast of Florida to uncover processes shaping and potential ecological consequences of the genetic changes observed within *A. germinans* towards this expanding range margin. In Chapter 3, I evaluate whether self-fertilisation becomes more common as mangroves become less abundant at their range margin. I assess variation in mating system by genotyping progeny arrays from maternal trees located across the Atlantic Florida latitudinal distribution. Then, in Chapter 4, I determine the extent of *A. germinans* dispersal to its expanding range margin and to areas beyond its current distribution. I genotype both beach-stranded propagules collected after a recent hurricane and isolated trees discovered beyond this species' previously-documented range limit to determine their source(s) of origin and quantify dispersal distances.

Finally, in Chapters 5 and 6, I evaluate potential ecological consequences of genetic variation within these range-margin *A. germinans*. In Chapter 5, within a single range-margin population, I use a community-genetics approach to assess whether genetic variation within this mangrove correlates with the structure and diversity of its associated foliar endophytic fungal community. I genotype mangrove host trees and then use next-generation sequencing to characterise their associated fungal communities. In Chapter 6, I use a common garden experiment to determine whether genetic differences observed within range-margin *A. germinans* translate into adaptive trait variation better suited to thrive under their marginal environmental conditions. I monitor maternal cohorts of this species from across the Atlantic Florida distribution for their first two years of development in a greenhouse environment and measure a series of phenotypic traits.

Taken together, this body of work provides novel insights into how changes in genetic and phenotypic variation manifest within a mangrove foundation species towards an expanding range margin and how these changes may have broader ecological consequences. This knowledge should improve our ability to predict how cold-sensitive mangrove range margins may respond to ongoing climate change and help inform future mangrove restoration initiatives.

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CHAPTER 2

Is the central-marginal hypothesis a general rule? Evidence from three distributions of an expanding mangrove species, Avicennia germinans (L.) L

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Is the central-marginal hypothesis a general rule? Evidence from three distributions of an expanding mangrove species, *Avicennia germinans* (L.) L

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Abstract

The central-marginal hypothesis (CMH) posits that range margins exhibit less genetic diversity and greater inter-population genetic differentiation compared to range cores. CMH predictions are based on long-held “abundant-centre” assumptions of a decline in ecological conditions and abundances towards range margins. Although much empirical research has confirmed CMH, exceptions remain almost as common. We contend that mangroves provide a model system to test CMH that alleviates common confounding factors and may help clarify this lack of consensus. Here, we document changes in black mangrove (*Avicennia germinans*) population genetics with 12 nuclear microsatellite loci along three replicate coastlines in the United States (only two of three conform to underlying “abundant-centre” assumptions). We then test an implicit prediction of CMH (reduced genetic diversity may constrain adaptation at range margins) by measuring functional traits of leaves associated with cold tolerance, the climatic factor that controls these mangrove distributional limits. CMH predictions were confirmed only along the coastlines that conform to “abundant-centre” assumptions and, in contrast to theory, range margin *A. germinans* exhibited functional traits consistent with greater cold tolerance compared to range cores. These findings support previous accounts that CMH may not be a general rule across species and that reduced neutral genetic diversity at range margins may not be a constraint to shifts in functional trait variation along climatic gradients.

KEYWORDS

abundant-centre distribution, central-periphery hypothesis, coastal species, functional traits, genetic diversity, range limits

1 | INTRODUCTION

Understanding factors that control species distributional limits is a central objective in ecology (Gaston, 2009; Hardie & Hutchings, 2010), and necessary insight to better predict responses to climate change (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011). The basis of many theories on distributional limits are long-held “abundant-centre” assumptions, that species experience optimal conditions and highest abundances in the central distributional core and lowest abundances towards range limits, where marginal conditions impede population growth and survival (Sagarin & Gaines, 2002). While range cores are generally stable, range margins can be quite mobile as species expand or contract in response to environmental changes (Sexton, McIntyre, Angert, & Rice, 2009).

In line with these innate differences, the central-marginal hypothesis (CMH), also called the central-periphery hypothesis (Pironon et al., 2017), posits that range margins exhibit lower intrapopulation genetic diversity and higher interpopulation genetic differentiation compared to range cores because of reduced population sizes and greater isolation (Eckert, Samis, & Lougheed, 2008). Much empirical research has confirmed CMH, but exceptions remain almost as common (Eckert et al., 2008; Pironon et al., 2017). Lack of consensus could be the result of numerous factors, including interspecific life history differences (Araújo, Serrão, Sousa-Pinto, & Åberg, 2011), confounding effects of latitude (Guo, 2012), variation in past distributional fluctuations (Nadeau et al., 2015), or simply the intrinsic difficulty of defining range core and margin for many species (Sagarin, Gaines, & Gaylord, 2006). To test CMH, a common approach is to identify the range core as the geographical centre of a species range, based on the theory's underlying “abundant-centre” assumptions (i.e., decline in ecological conditions and abundances towards range margins). However, this assumed pattern occurs much less often than previously expected (Sagarin & Gaines, 2002; Santini, Pironon, Maiorano, & Thuiller, 2019). Quantitative approaches have addressed this issue with evidence-based estimates of range centre considering species biology (Schwartz, Mills, Ortega, Ruggiero, & Allendorf, 2003), climatic suitability (Lira-Noriega & Manthey, 2014; Micheletti & Storfer, 2015), and genetic differences (Griffin & Willi, 2014); and have demonstrated that disentangling the relative effects of geographic, ecological, and historical gradients is often difficult when interpreting patterns across broad spatial scales (Pironon, Villellas, Morris, Doak, & García, 2015). Another means to achieve greater insight into the generality and implications of CMH would be to identify model systems that naturally conform to “abundant-centre” assumptions and provide a test of this theory with less influence of confounding factors.

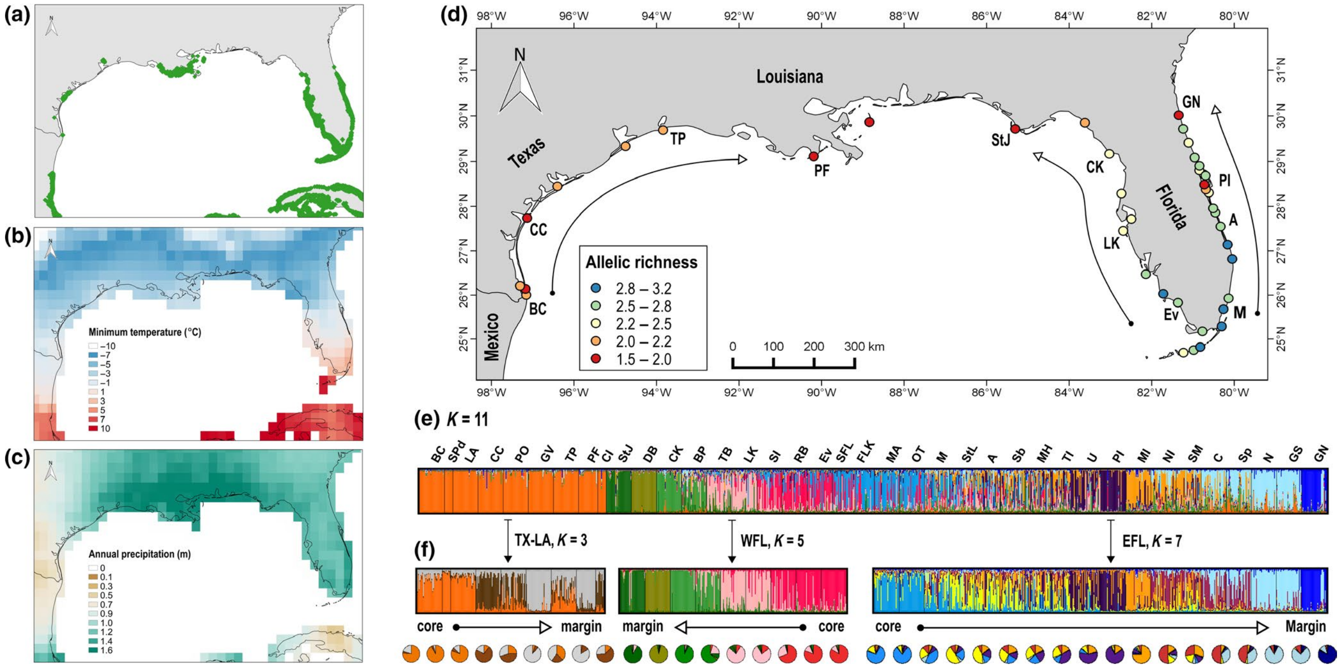

FIGURE 1 Evaluation of the central-marginal hypothesis (CMH) in *Avicennia germinans* from three distribution ranges in the United States (USA): Texas-Louisiana (TX-LA) does not conform to the underlying “abundant-centre” assumptions of this theory; West Florida (WFL) and East Florida (EFL) conform to these underlying assumptions. (a) Mangrove distribution in the USA and neighbouring countries (Giri et al., 2011). (b, c) Regional climatic factors that control mangrove abundances and distributional limits: (b) mean annual minimum temperature (°C) and (c) mean annual precipitation (m), both from 1980–2017. (d) Collection sites and neutral genetic diversity along TX-LA, WFL, and EFL (from left to right). Arrows show core to margin along each distribution range. (e) Genetic structure along the entire USA distributional range estimated in STRUCTURE 2.3 based on changes in ln Pr(X|K) (K = 11 population clusters). Collection sites are shown from west to east and strategic site codes are included in panel d to aid visualization (Note: in panel d, collection site PI corresponds to the adjacent red dot). (f) Subsequent genetic structure along each of the three distribution ranges separately from core to margin (TX-LA, K = 3; WFL, K = 5; EFL, K = 7), with averaged assignments for each collection site presented as pie charts. Refer to Table S1 for site codes [Colour figure can be viewed at wileyonlinelibrary.com]

Coastal species provide ideal systems to test many large-scale ecological theories because of their essentially one-dimensional, and often widespread, distributions (Sagarin et al., 2006). We contend that mangroves, an assortment of (sub)tropical intertidal tree and shrub species, provide an ideal model system to test CMH. Mangrove distributions are easily defined because of their restriction to narrow intertidal zones (Tomlinson, 1986), and are anchored in the tropics where these plants reach their highest abundances and experience favourable climatic conditions (Spalding, Kainuma, & Collins, 2010). Mangrove abundance and species richness decrease towards poleward range limits, as climatic variables (i.e., temperature, precipitation) become more marginal (Osland, Feher, et al., 2017). Range cores generally remain stable, unless impacted by stochastic weather events (e.g., Smith, Robblee, Wanless, & Doyle, 1994) or anthropogenic changes (Valiela, Bowen, & York, 2001); whereas range limits are highly mobile due to climatic thresholds specific to individual geographic regions (Cavanaugh et al., 2018; Osland, Feher, et al., 2017).

Black mangrove, *Avicennia germinans* (L.) L., is widespread throughout the Neotropics and the predominant mangrove species at range margins in the United States (USA) (Lonard, Judd, Summy, DeYoe, & Stalter, 2017), in part because of its greater freeze tolerance compared to co-occurring mangrove species (Cavanaugh et al., 2015). In the USA, *A. germinans* is present along the coastlines of Texas-Louisiana, West Florida, and East Florida; three natural replicates of core to margin distribution ranges (Figure 1). This system naturally controls for many common confounding factors, with a single, widespread model species and three coastlines along a similar latitudinal gradient (~26–30°N). We can also presume that these three coastlines experienced similar historical distributional fluctuations, as the present-day USA mangrove distribution is thought to be the product of complete eradication at the Last Glacial Maximum, with retraction towards the equator, and subsequent Post-Pleistocene recolonization (Sherrod & McMillan, 1985; also see descriptions in Osland et al., 2018; Rogers & Krauss, 2018; Saintilan, Wilson, Rogers, Rajkaran, & Krauss, 2014).

Although these three coastlines share many commonalities, there is a strong dichotomy between climatic factors controlling mangrove distributions in Texas compared to neighbouring Louisiana, and to Florida. Mangrove abundance and distributional limits in Florida and Louisiana are controlled by latitudinal gradients in minimum winter temperatures, whereas inverse latitudinal gradients in both minimum temperatures and precipitation control mangroves across Texas (Cavanaugh et al., 2018; Osland, Feher, et al., 2017) (Appendix S1 details this climatic information). Mangroves in the USA are most abundant in South Florida (with an assemblage of three principal species), transition into a mangrove-salt marsh ecotone along both Florida coastlines, and are eventually replaced by salt marsh as freeze events become more common (Kangas & Lugo, 1990) (Figure 1a,b). In contrast, Texas mangroves (essentially only *A. germinans*) are far less abundant, without a continuous distribution, and mostly restricted to three distributional centres with the nearest continuous mangrove forest in Mexico, approximately 300 km south of the southernmost Texas mangroves (Guo, Zhang, Lan, & Pennings, 2013; Sherrod & McMillan, 1981) (Figure 1a). Hypersaline conditions that exceed physiological thresholds are common along South and Central Texas, and limit mangrove presence and abundance (Gabler et al., 2017; Osland et al., 2016); whereas rainfall increases along North Texas, and into adjacent Louisiana where *A. germinans* reach comparatively higher abundances (Osland et al., 2016) (Figure 1a,c).

Southern range core mangroves are relatively stable in Florida (but see Ross, Ruiz, Sah, & Hanan, 2009; Zhang, Thapa, Ross, & Gann, 2016) and Texas (but see Lonard & Judd, 1991) compared to highly-mobile range margins (Cavanaugh et al., 2018). Periodic extreme freeze events lead to declines in mangrove cover at USA range margins and a cyclical pattern of expansion and contraction over time across the region (Giri & Long, 2016; Osland, Day, et al., 2017; Rodriguez, Feller, & Cavanaugh, 2016; Rogers & Krauss, 2018). An absence of extreme freezes since the late 1980s is linked to ongoing, rapid range expansion of *A. germinans* into salt marsh habitat at all USA northern limits, in Texas (Armitage, Highfield, Brody, & Louchouarn, 2015; Everitt, Yang, Judd, & Summy, 2010), Louisiana (Osland, Day, et al., 2017), West Florida (Saintilan et al., 2014; Stevens, Fox, & Montague, 2006), and East Florida (Cavanaugh et al., 2014; Rodriguez et al., 2016), with further proliferation at, and expansion past, these range margins forecast with climate change (Cavanaugh et al., 2015; Osland, Day, et al., 2017; Osland, Enwright, Day, & Doyle, 2013).

This wealth of previous research demonstrates that USA mangroves simplify tests of CMH as parallel gradients in latitude, ecological marginality (in terms of minimum temperatures), and postglacial recolonization exist along each of these three distribution ranges. West and East Florida *A. germinans* also conform to the underlying "abundant-centre" assumptions of CMH, whereas Texas-Louisiana conspecifics do not (Table 1). Instead, the entire Texas-Louisiana

TABLE 1 Results summary of central-marginal hypothesis (CMH) underlying "abundant-centre" assumptions, explicit predictions, and an implicit prediction along three *Avicennia germinans* distribution ranges in the United States: Texas-Louisiana (TX-LA), West Florida (WFL) and East Florida (EFL)

	TX-LA	WFL	EFL
CMH "abundant-centre" assumptions			
Decline in ecological conditions towards range margin	No	Yes	Yes
Decline in abundances towards range margin	No	Yes	Yes
CMH explicit predictions			
Reduced intrapopulation genetic diversity towards range margin	No	Yes	Yes
Increased interpopulation genetic differentiation towards range margin	No	Yes	Yes
CMH implicit prediction			
Constrained adaptation to environmental conditions towards range margin[a]	No	No	No

[a] To test this implicit prediction, we evaluated changes in functional traits of leaves associated with cold tolerance.

distribution resembles a range margin, with the true range core (i.e., optimal ecological conditions and highest abundances) located farther south in Mexico. Texas-Louisiana, therefore, provides a test of CMH when "abundant-centre" assumptions are not met, a proposed reason for limited consensus regarding CMH (Eckert et al., 2008; Pironon et al., 2017).

An implicit prediction of CMH is that limited adaptive genetic diversity at range margins can reduce evolutionary potential and constrain adaptation to environmental conditions at the distributional limit, which can impede further expansion (Bridle & Vines, 2007). Theoretical research predicts that range dynamics are controlled by the interactive effects of gene flow and genetic drift, and also the effect of genetic variation on trait expression (Connallon & Sgrò, 2018 and citations within). This last factor is of particular importance because genetic diversity is most often measured with neutral molecular markers, that may not reflect variation in adaptive genetic diversity of ecological significance (Gaston, 2009). Empirical research, although limited, suggests that reduced neutral genetic variation at range margins does not translate into reduced ecologically-relevant trait variation compared to range cores (Abeli, Gentili, Mondoni, Orsenigo, & Rossi, 2014; Kawecki, 2008; Pironon et al., 2017). Hence, an integration of measures of neutral genetic variation and of context-specific trait variation will improve our understanding of the potential implications of CMH.

Climatic factors shaping mangrove distributions also influence mangrove morphological traits and physiological adaptations (Clough, 1992). USA mangrove canopy heights decrease along temperature and precipitation gradients towards range margins (Feher et al., 2017). At these margins, *A. germinans* exhibit variation in xylem vessel architecture (Madrid, Armitage, & López-Portillo, 2014; Stuart, Choat, Martin, Holbrook, & Ball, 2007) and leaf traits (Cook-Patton, Lehmann, & Parker, 2015) consistent with greater freeze tolerance compared to range-core conspecifics. Here, we measured functional traits of leaves associated with cold tolerance. Functional traits are attributes that can influence establishment, survival and fitness (Pérez-Harguindeguy et al., 2013). Freeze-resistant plant species exhibit conservative leaf traits better suited to tolerate stress, including reduced leaf length and width (Jordan & Smith, 1995), reduced leaf area (Pérez-Harguindeguy et al., 2013), and reduced specific leaf area (Poorter, Niinemets, Poorter, Wright, & Villar, 2009); although similar traits are also consistent with drought resistance (Knight & Ackerly, 2003).

In this study, we measured variation in both population genetics and cold-stress associated functional traits of leaves across the entire USA distribution of *A. germinans*. We tested explicit genetic predictions of CMH and an implicit prediction along three replicate core to margin distribution ranges, two of which conform to the underlying "abundant-centre" assumptions of this theory and a third that does not (Table 1 outlines CMH assumptions and predictions). Along each of the three distribution ranges, we asked: (a) Does neutral intrapopulation genetic diversity decrease towards range margins?; (b) Does interpopulation genetic differentiation increase towards range margins?; (c) Do functional traits of leaves exhibit changes consistent with greater cold tolerance towards range margins?

2 | MATERIALS AND METHODS

2.1 | Range classification

USA *A. germinans* only represent the northern extent of this species' entire distribution, with the true range centre closer to the equator. However, radiating out from this centre, the range core remains relatively continuous until range limits are defined by abrupt ecological thresholds (Osland et al., 2016). As such, USA *A. germinans* provide three extensions of this more-equatorial range core that eventually transition into climate-sensitive northern range margins (Figure S1). We defined range core as the most southern populations and all areas progressively northward where either pure mangrove exists or mangroves are the dominant foundation species. We used published descriptions to define range margin based on latitude, abundance, and population stability (Table S1 details collection site classifications). USA mangroves are replaced by salt marsh at approximately 29°N, where isolated, low-abundance mangrove stands exist in a salt marsh-dominated landscape (Spalding et al., 2010). Range-margin sites in East Florida (29.4–30.0°N) and West Florida (29.1°N–29.8°N) are isolated from the continuous range core and are documented *A. germinans* range limits (Kangas & Lugo, 1990), with climate-driven fluctuations in abundance over time in both areas (Montague & Odum, 1997; Rodriguez et al., 2016). Range-margin sites in Texas and Louisiana (29.1°N–29.8°N) are also documented *A. germinans* range limits with evidence of fluctuations in abundance over time (Osland, Day, et al., 2017; Sherrod & McMillan, 1981), including complete mangrove die-back at Texas Point (29.6°N; code: TP) (Sherrod & McMillan, 1981) where only five trees were identified in 2010 (Guo et al., 2013).

2.2 | Sample collection: Genetic and functional trait analyses

Leaves were collected from a total of 1,083 *A. germinans* trees from 41 collection sites across this species' entire USA distributional range (Table S1; Figure 1). We collected samples along East Florida (EFL) in January 2015, along West Florida (WFL) in September–October 2015, and along Texas and Louisiana (TX-LA) in October 2015. Samples for two sites (code: TB, SFL) were obtained from preserved leaves collected in 2011. For densely-populated sites, sampled trees were located at least 20 m apart; whereas, for sparsely-populated range-margin sites, sampled trees were located as far apart as possible (generally at least 10 m) in an attempt to sample the entire site. We sampled a greater number of collection sites along EFL due to the complexity of the lagoon system along this coastline, which consists of three interconnected water bodies. This more comprehensive sampling strategy ensured that the entire system was characterized, including one site (code: PI) that has undergone substantial land modifications during conversion into a conservation area. We sampled all major distributional centres along WFL and TX-LA. For genetic analyses, we collected leaves from 30 trees per site (except for site PF, $n = 23$). Fewer samples ($n = 9–11$) were collected opportunistically at additional sites between 2015 and 2016, and were used in region-wide analyses (Figure 1d–e), but were not included in the subsequent genetic and functional trait CMH prediction tests. As such, to test CMH predictions, the EFL distribution range included 18 collection sites (25.6°N–30.0°N), WFL included nine sites (25.8°N–29.8°N), and TX-LA included seven sites (26.0°N–29.6°N), with South Florida collections from the Everglades and Florida Keys not included in prediction tests (Table S1).

We measured functional traits for all sites with $n \geq 23$ genetic samples, except for site TB where samples had been collected in 2011. We collected 10 leaves from each of ≥10 trees per site for functional trait measurements, a subset of the same trees sampled for genetic analyses. Within each site, we sampled mature (reproductive) trees that were all approximately the same height. Each of the 10 leaves per tree was from the most fully-expanded, undamaged leaf pair on an individual branch and located in direct sunlight.

2.3 | Microsatellite genotyping and data quality

Leaves were dehydrated in silica gel, and genomic DNA was isolated from 20 mg of dry tissue with the DNeasy Plant Mini Kit (Qiagen) following the standard protocol, with an extended incubation step of 45 min. Initial tests included 17 previously-published nuclear microsatellite loci (Cerón-Souza, Bermingham, McMillan, & Jones, 2012; Cerón-Souza, Rivera-Ocasio, Funk, & McMillan, 2006; Mori, Zucchi, Sampaio, & Souza, 2010; Nettel, Rafii, & Dodd, 2005) (Appendix S2). Final tree genotypes included 12 of these loci combined into two multiplex reactions (Table S2). Polymerase chain reaction (PCR) conditions followed the PCR method for a single set of cycles with 35 cycles (as outlined in Culley et al., 2013), and we used the Type-it Microsatellite PCR Kit (Qiagen). Total volume for each of two multiplex reactions was 6 µl with 2.5 µl Multiplex PCR Master Mix, 0.5 µl primer mix, 1 µl dH_2O, and 2 µl (~20 ng) of genomic DNA (Table S2 details primer combinations and concentrations [µM] in each multiplex). PCR were performed on a T100 thermal cycler (Bio-Rad). PCR products were separated on an ABI 96-capillary 3730xl DNA Analyser with ROX size standard and scored in GeneMapper 5.1 (Applied Biosystems).

We evaluated potential genotyping errors in MICRO-CHECKER 2.2.3 (van Oosterhout, Hutchinson, Wills, & Shipley, 2004) and estimated null allele frequencies with FreeNA (Chapuis & Estoup, 2007). We randomly reamplified and regenotyped 5% of DNA samples to assess genotyping accuracy and estimate a study error rate. We then tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium at each collection site after adjusting for multiple comparisons in FSTAT 2.9.3.2 (Goudet, 2002). POWSIM 4.1 (Ryman & Palm, 2006) was used to evaluate the resolving power of these microsatellite loci across all collection sites, and for each of the three distribution ranges separately.

2.4 | USA *Avicennia germinans* neutral genetic diversity & structure

For each collection site, we calculated the number of polymorphic loci and private alleles in GenAlEx 6.5 (Peakall & Smouse, 2012). We calculated observed heterozygosity, unbiased gene diversity (H_S), inbreeding coefficients, allelic richness (AR) standardized to minimum sample size ($n = 9$), and genetic differentiation (measured with F_{ST}) with corresponding p-values determined with 10^4 permutations and adjusted for multiple comparisons in FSTAT 2.9.3.2. We also calculated G''_{ST} (Meirmans & Hedrick, 2011), D (Jost, 2008), and null-allele-corrected F_{ST} in FreeNA (Chapuis & Estoup, 2007) and these metrics were highly correlated with F_{ST} (Pearson's correlation, $r = .96–1.0$, $p < .0001$; Figure S2), so we present results only in terms of F_{ST}. For all sites with $n \geq 23$ genetic samples, we also calculated a more robust estimate of AR standardized to minimum sample size ($n = 23$) in FSTAT 2.9.3.2 for statistical analyses.

To assess variation among the three distribution ranges, we tested for differences in intrasite neutral genetic diversity (H_S, AR; $n = 12$ per collection site) and intersite genetic differentiation within each distribution range (F_{ST}; $n = 6$ per collection site in TX-LA, $n = 8$ in WFL, $n = 17$ in EFL). F_{ST} sample sizes varied depending on the total number of collection sites with $n \geq 23$ genetic samples for each distribution range. We used Kruskal-Wallis tests and Dunn's tests for post hoc multiple comparisons with p-values adjusted for the false discovery rate with the Benjamini-Hochberg procedure. Unless otherwise noted, we performed statistical analyses in R 3.4.2 (R Core Team, 2013). We tested for a pattern of isolation by distance along each distribution range with Mantel tests of correlation between matrices of neutral genetic distances (intersite $F_{ST}/[1 - F_{ST}]$ [Rousset, 1997]) and geographic distances (measured along the coastline between central points within each site in Google Earth 7.1.2.2041) in the R-package ecodist (Goslee & Urban, 2007) with 10^4 permutations to determine significance.

We visualized genetic structure across the entire USA range ($n = 1{,}083$ individuals) with STRUCTURE 2.3 (Pritchard, Stephens, & Donnelly, 2000) that determines the most likely number of population clusters (K) and assigns each sampled individual to these clusters based on multi-locus genotypes. We used the admixture model with correlated allele frequencies, and did not consider geographic location. The analysis consisted of 10 replicate runs of 500,000 recorded steps after a burnin of 100,000 steps at each K value from 1 to 30. We used StrAuto (Chhatre & Emerson, 2017) in conjunction with GNU Parallel (Tange, 2011) to automate replicate runs across a 40-core standalone computer. We used CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) with default settings to align replicate runs and visualize genetic structure at each *K* value.

Determining which *K* best fits a data set remains a debated topic. One method is to identify *K* with the greatest log probability [ln Pr(*X*|*K*)] or where values reach a relative plateau (Pritchard, Wen, & Falush, 2003). An alternative, the Δ*K* method, generally identifies the highest level of genetic structure, and may require subsequent analyses on data subsets to identify additional nested structure (Evanno, Regnaut, & Goudet, 2005). Use of both methods is recommended to better interpret patterns of genetic structure, while also considering species biology and including complementary analyses (Gilbert et al., 2012; Janes et al., 2017). We determined *K* with both Δ*K* and ln Pr(*X*|*K*) in STRUCTURE HARVESTER (Earl & VonHoldt, 2012). Based on our initial results, we performed subsequent analyses on data subsets from TX-LA (*n* = 9 sites, 223 individuals) and Florida (*n* = 32 sites, 860 individuals), and then WFL (*n* = 9 sites, 270 individuals) and EFL (*n* = 18 sites, 540 individuals) separately. Run conditions were identical to the initial analysis, but we tested different ranges of *K* because of variation in collection site numbers (TX-LA: *K* = 1–9; Florida: *K* = 1–30; WFL: *K* = 1–9; EFL: *K* = 1–18). We used the LOCPRIOR model (Hubisz, Falush, Stephens, & Pritchard, 2009) to assist the clustering analysis for TX-LA only.

We performed a principal coordinates analysis (PCoA) with Nei's genetic distances in GenAlEx 6.5 as an additional line of evidence for population structure along the entire USA range. We then performed PCoA for each of the three distribution ranges separately. We plotted the first two axes with the R-package ggplot2 (Wickham, 2011).

We tested explicit predictions of CMH (Table 1) along each of the three distribution ranges with Spearman's rank correlations between neutral genetic diversity (unbiased gene diversity, H_S; allelic richness, AR) and latitude, and between genetic differentiation (F_{ST}) and latitude. For each distribution range, latitude was highly correlated with distance to range core (measured as the distance along the coast from the most southern collection site), a recommended predictor variable for these analyses (Eckert et al., 2008) (TX-LA: Pearson's correlation, $r = .85$, $p < .0001$; WFL: $r = .96$, $p < .0001$; EFL: $r = 1.0$, $p < .0001$). Use of either predictor did not qualitatively change correlation results.

Rare alleles can spread and become more frequent at expanding range margins because of strong genetic drift, a process called allele surfing (Excoffier & Ray, 2008 and citations within). We tested for this pattern of genetic drift at each of these three currently-expanding range margins, with a modification of the method outlined in Griffin and Willi (2014). We first identified the most common alleles at each microsatellite loci within each of the three range cores. Most loci exhibited 1–2 predominant allele(s) within each collection site. These alleles were present across range core sites at a frequency of 0.95 ± 0.07 (standard deviation; *SD*) in TX-LA, 0.91 ± 0.07 in WFL, and 0.93 ± 0.08 in EFL, and included at least 75% of the total allele pool per locus. We discarded these common alleles and filtered the remaining alleles based on the following criteria: (a) present in range margin site(s), (b) present in ≥ 2 range core sites, and (c) at least three copies (5% of collection site) present at range margin site(s). We included the last two criteria to avoid extremely rare or private alleles from skewing results. The resulting data set consisted of nine alleles in TX-LA (18% of TX-LA alleles), eight in WFL (11% of WFL alleles), and eight in EFL (11% of EFL alleles), with frequencies across range core sites of 0.06 ± 0.03 (*SD*), 0.09 ± 0.04, and 0.07 ± 0.04, respectively. We calculated the ratio of each allele's mean range margin frequency to its mean range core frequency, and transformed with the natural logarithm. We used a one-sided *t* test to determine whether the ratio of margin to core allele frequency for each of the three range margins was greater than zero.

2.5 | Leaf functional traits

We measured five leaf functional traits: area, length, width, ratio length:width, and specific leaf area. Area (cm^2) was measured with an area meter (Model 2100, LI-COR Inc.). Length (cm) was measured from the leaf tip to the start of the petiole and width (cm) was measured at the widest point of the leaf. Ratio length:width was also calculated as this trait proved informative to differentiate populations of *A. marina*, another member of the same genus (Saenger & Brooks, 2008). We dried leaves at 60°C for 48 hr until constant weight and measured dry weights (g). Specific leaf area (cm^2/g) was measured as leaf area divided by dry weight. We measured these traits for 10 leaves per tree and used the mean value for each tree for analyses. For each of the three distribution ranges separately, we used principal components analysis (PCA) to reduce these functional traits into a limited number of uncorrelated variables. We log-transformed trait data, centred and scaled values (mean = 0, variance = 1), and performed PCA with the R-function *prcomp*. We retained principal components (PC) with eigenvalues >1.

We tested an implicit prediction of CMH (Table 1) by evaluating whether range-margin *A. germinans* exhibit functional trait variation better suited to tolerate cold stress compared to range-core conspecifics. We performed Spearman's rank correlations between functional trait PC and latitude along each of the three distribution ranges. Microsatellite genotype and functional trait data are available at the Dryad digital repository (Kennedy, Preziosi, Rowntree, & Feller, 2020).

3 | RESULTS

3.1 | Microsatellite data quality

Across the 12 nuclear microsatellite loci, potential null alleles were identified at 15% (75 of 492) of collection site – microsatellite locus pairs, but at low frequency (0.05 ± 0.07 [*SD*]) (Tables

S3 and S4). The estimated error rate was also low at 1.39% (14 errors out of 1,007 allele comparisons), and we removed these locus-specific errors from the data set. We found no evidence of linkage disequilibrium and only 2% (12 of 492) of collection site – microsatellite locus pairs deviated from Hardy-Weinberg equilibrium. POWSIM results indicated that a true $F_{ST} \geq 0.005$ could be detected with 100% probability across all collection sites, presumably more than sufficient resolution based on observed population structure (overall $F_{ST} = 0.35$). Resolution remained high for subsets from West Florida (WFL) and East Florida (EFL) (true $F_{ST} \geq 0.005$ detected with 95.1% and 99.9% probability, respectively), but with a marked decrease for Texas-Louisiana (TX-LA) (detected with 71.1% probability).

3.2 | USA *Avicennia germinans* neutral genetic diversity & structure

We found a total of 95 alleles among 1,083 individuals. All 12 microsatellite loci were polymorphic within the most southern collection sites in Florida (except for three sites with more limited sampling, *n* = 10 per site) and had increased monomorphism towards range margins (Table S1). In contrast, multiple loci were monomorphic across TX-LA (maximum polymorphism = 8 of 12 loci per site). Twenty private alleles were identified at low frequencies (0.02 ± 0.01 [*SD*]) and were found only within range-core sites in Florida, but within both core and margin sites in TX-LA. Neutral genetic diversity was highest at lower latitudes in Florida, with maximum values in the southeast, and lowest at Florida range margins and across TX-LA (Table S1; Figure 1d). Significant inbreeding (F_{IS}) was detected within multiple sites, but at higher frequency across range margins (seven of 10 sites) compared to range cores (12 of 31 sites). We found significant genetic differentiation across all collection sites ($F_{ST} = 0.35$, $p < .0001$) with a range of intersite values from −0.02 to 0.77 (Table S5).

Among the three distribution ranges, both measures of neutral genetic diversity were significantly higher in WFL and EFL compared to TX-LA (unbiased gene diversity, H_S: Kruskal-Wallis chi-squared, H(2) = 61.0, $p < .0001$; post hoc tests, $p < .0001$, $p < .0001$) (allelic richness, AR: H(2) = 35.3, $p < .0001$; post hoc tests, $p < .0001$, $p < .0001$), but values were not significantly different between WFL and EFL (H_S: post hoc tests, $p = .07$; AR: post hoc tests, $p = .29$) (Figures S3a,b). In contrast, genetic differentiation (F_{ST}) was significantly lower in TX-LA compared to WFL and EFL (H[2] = 67.1, $p < .0001$; post hoc tests, $p < .0001$, $p < .0001$) and significantly lower in EFL compared to WFL (post hoc tests, $p = .01$) (Figure S3c). We found evidence of isolation by distance along all three distribution ranges, with the highest correlation between neutral genetic distances and geographic distances along WFL ($r_M = .85$, $p = .0003$) compared to EFL ($r_M = .33$, $p = .021$) and TX-LA ($r_M = .54$, $p = .034$).

Consistent with findings from Janes et al. (2017), Δ*K* identified *K* = 2 across all STRUCTURE analyses (except for the TX-LA subset); whereas, ln Pr(*X*|*K*) identified additional levels of genetic structure that coincided with geographic location. We interpreted these differences between methods as the highest level of genetic structure (Δ*K*) and finer-scale genetic structure [ln Pr(*X*|*K*)] for each analysis (Appendix S3, Figures S4–S10 provide detailed explanations of *K* choice, STRUCTURE results, and PCoA results). Across the USA range, Δ*K* identified a clear separation between TX-LA and Florida (Figure S5) and ln Pr(*X*|*K*) identified *K* = 11, with additional delineations between both WFL and EFL range margins and their respective cores, and admixture along multiple sections of the Florida range core (Figure 1e). Analysis of the Florida subset reached the same conclusions as the entire USA range (Figure S6). For WFL, Δ*K* identified a separation between range core and margin (Figure S7) and ln Pr(*X*|*K*) identified *K* = 5, with intersite admixture within the range core and sharp delineations at the most northern margin sites (Figure 1f). For EFL, results were analogous to WFL (Δ*K* = 2, Figure S8; ln Pr(*X*|*K*) identified *K* = 7), except for an anomalous example of within-range-core delineation (site code: PI) (Figure 1f). For TX-LA, we utilized the LOCPRIOR model and both Δ*K* and ln Pr(*X*|*K*) identified *K* = 4, with separation into southern, central, and northern clusters, plus a seemingly noninformative fourth cluster across all sites (Figure S9). *K* = 3 identified only the biologically-sensible clusters, with admixture at a recently-recolonized range-margin site (code: TP) (Figure 1f). PCoA was consistent with STRUCTURE, but indicated further separation between northern Texas and Louisiana (Figure S10).

We found that EFL and WFL distribution ranges conformed to CMH predictions, but TX-LA did not (Table 1; Figure 2). In EFL, neutral genetic diversity (unbiased gene diversity, H_S; allelic richness, AR) was negatively correlated with latitude (Spearman's rank correlation coefficient, $r_s = -.20$, $p = .004$; $r_s = -.22$, $p = .001$, respectively) and genetic differentiation (F_{ST}) was positively correlated with latitude ($r_s = .38$, $p < .0001$). In WFL, H_S was not correlated with latitude ($r_s = -.02$, $p = .83$), whereas AR was negatively correlated with latitude ($r_s = -.32$, $p = .0007$) and F_{ST} was positively correlated with latitude ($r_s = .36$, $p = .002$). In TX-LA, neither H_S or AR were correlated with latitude ($r_s = .05$, $p = .65$; $r_s = .04$, $p = .74$, respectively) and F_{ST} was not correlated with latitude ($r_s = -.08$, $p = .59$).

We found evidence of genetic drift at each of the three range margins (Table 2). Rare alleles in the range core were more frequent in the range margin in EFL (ln margin:core allele frequencies; one-sided *t* test, *t*[7] = 3.40, $p = .006$), WFL (*t*[7] = 4.08, $p = .002$), and TX-LA (*t*[8] = 3.74, $p = .003$). WFL exhibited the highest mean increase in rare allele frequencies (4.85 ± 3.12 [*SD*] times greater than range core) compared to EFL (2.66 ± 1.48) and TX-LA (2.73 ± 2.28).

3.3 | Leaf functional traits

We found the largest leaves and highest specific leaf area (SLA) in Southwest and Southeast Florida; whereas, we found lower values towards Florida range margins and across all TX-LA sites (Table S1). PCA of functional traits resulted in similar patterns for each of the three distribution ranges (Figure 3). The first two principal components (PC) had eigenvalues >1 for each distribution range, and accounted for 87% (62.1% and 24.9%, respectively) of the total

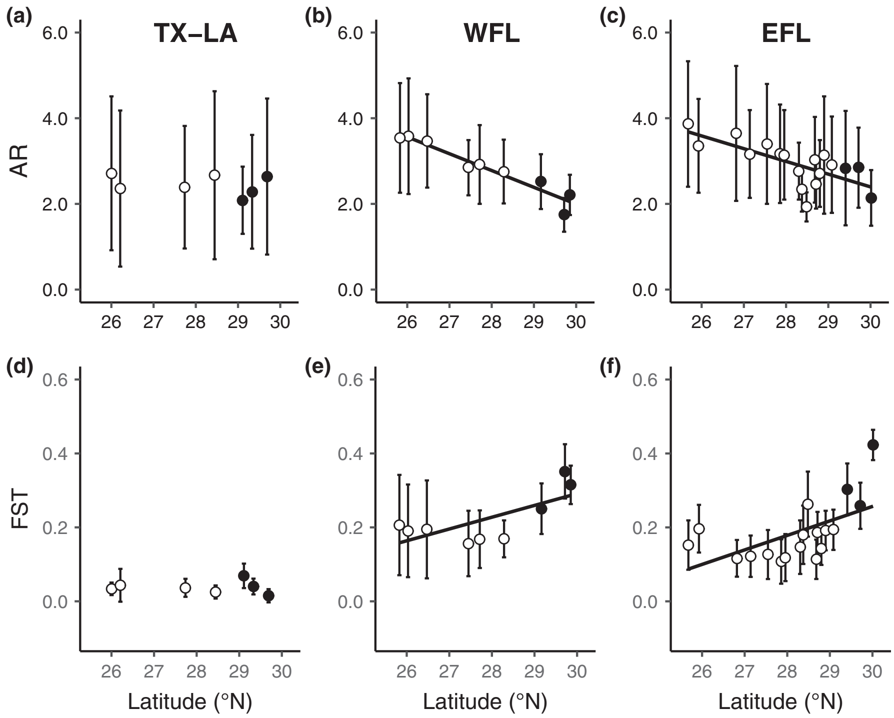

FIGURE 2 Changes in neutral genetic diversity (allelic richness; AR) and differentiation (fixation index; FST) along (a, d) Texas-Louisiana (TX-LA), (b, e) West Florida (WFL), and (c, f) East Florida (EFL). Significant ($p < .05$) correlations are depicted with a solid line. Range core sites are shown in white and margin sites in black. Error bars indicate 95% confidence intervals. AR; $n = 12$ per collection site. F_{ST}; $n = 6$ per collection site in TX-LA, $n = 8$ in WFL, $n = 17$ in EFL

variation in EFL, 89.3% (69.1% and 20.1%, respectively) in WFL, and 87.5% (55.4% and 32.1%, respectively) in TX-LA.

Leaf size (area, length and width) and life history strategy (SLA) traits had strong, positive loadings on PC1 (Figure 3). Negative values along PC1 are indicative of smaller leaves and lower SLA, both traits consistent with cold tolerance. Leaf shape (ratio length:width) had strong, positive loadings on PC2, reinforced by negative loadings for width in EFL and TX-LA and positive loadings for length in WFL (Figure 3). Positive values along PC2 are indicative of longer, narrower leaves, a trait associated with greater light capture efficiency (Takenaka, 1994), not with cold tolerance. SLA had comparatively higher positive loadings on PC2 for TX-LA compared to Florida distribution ranges, so we interpreted larger PC2 values in TX-LA as indicative of longer, narrower leaves with higher SLA.

In contrast to theory, EFL and WFL range margins exhibited functional traits consistent with greater cold tolerance, and all TX-LA collection sites exhibited similar cold-tolerant traits (Table 1). PC1 was negatively correlated with latitude along EFL ($r_s = -.69$, $p = .002$) and WFL ($r_s = -.86$, $p = .007$), indicative of functional trait variation better suited to tolerate cold stress at these range margins, but PC1 was not correlated with latitude along TX-LA ($r_s = 0$, $p = .99$) (Figure 4). PC2 was not significantly correlated with latitude along EFL ($r_s = -.24$, $p = .34$) or WFL ($r_s = .38$, $p = .35$), but was positively correlated (albeit marginally nonsignificant) with latitude along TX-LA ($r_s = .75$, $p = .052$) (Figure 4).

4 | DISCUSSION

We still lack a clear understanding of what controls distributional limits of species (Parmesan et al., 2005), in part because of limited empirical data across large spatial scales (Abeli et al., 2014; Sagarin et al., 2006). Here, we provide insights into the lack of consensus regarding the central-marginal hypothesis (CMH) and also into the implications of this theory. The model system we used naturally exhibits parallel gradients in latitude, ecological marginality, and

TABLE 2 Evidence of genetic drift at the expanding Texas-Louisiana (TX-LA), West Florida (WFL), and East Florida (EFL) range margins

Distribution	Location	Sites	Total alleles	% allele decrease	Rare alleles	Margin:core	ln (margin:core)	*t*	*df*	*p*-value
TX-LA	Core	5	45	0.16	9	2.73 ± 2.28	0.80 ± 0.64	3.74	8	.003
	Margin	3	38							
WFL	Core	6	67	0.45	8	4.85 ± 3.12	1.31 ± 0.91	4.08	7	.002
	Margin	3	37							
EFL	Core	15	71	0.44	8	2.66 ± 1.48	0.81 ± 0.67	3.40	7	.006
	Margin	3	40							

Note: Sites, number of collection sites; total alleles, number of alleles found within each location; % allele decrease, percent decrease in number of alleles from range-core to margin; rare alleles, number of identified range-core rare alleles used in analysis. Error indicates 95% confidence intervals.

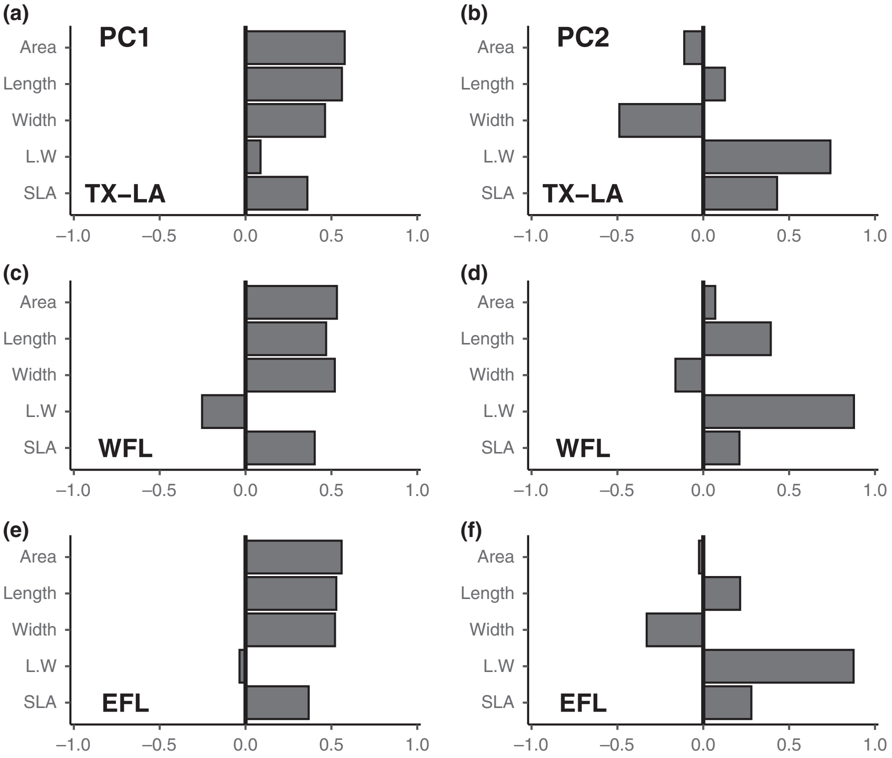

FIGURE 3 Loadings on principal components (PC) of variation in functional traits of leaves for Texas-Louisiana (TX-LA), West Florida (WFL), and East Florida (EFL). Left: PC1 for TX-LA, WFL, and EFL (a, c, e), which accounted for 55.4%, 69.1%, and 62.1% of the total variation, respectively. Right: PC2 for TX-LA, WFL, and EFL (b, d, f), which accounted for 32.1%, 20.1%, and 24.9% of the total variation, respectively. L.W, ratio length:width; SLA, specific leaf area

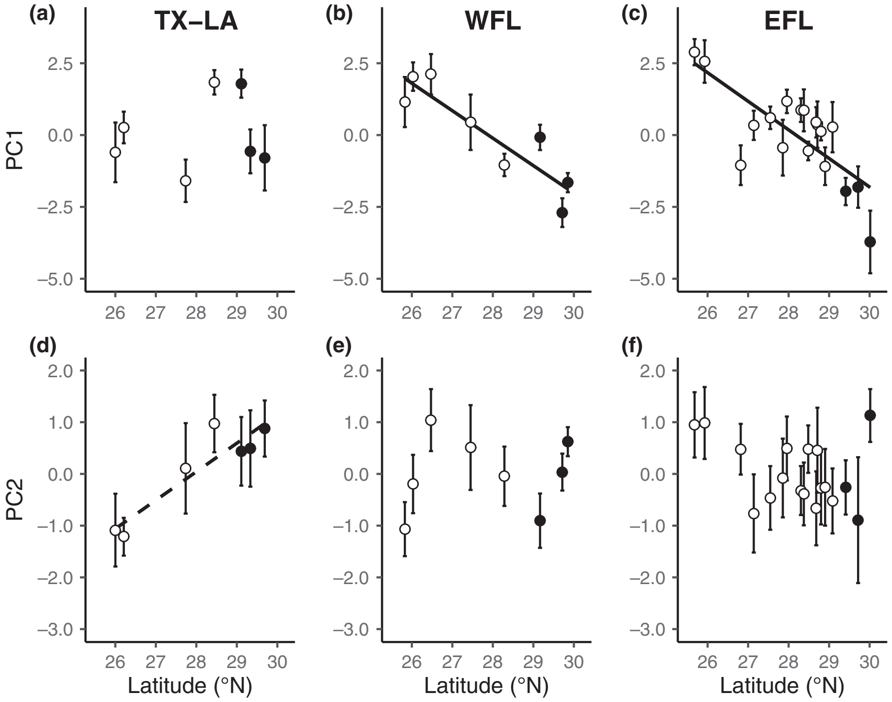

FIGURE 4 Changes in principal components (PC1, PC2) of variation in functional traits of leaves along (a, d) Texas-Louisiana (TX-LA), (b, e) West Florida (WFL), and (c, f) East Florida (EFL). Significant ($p < .05$) correlations are depicted with a solid line, and marginal nonsignificant ($p = .052$) correlations are depicted with a dashed line. Range core sites are shown in white and margin sites in black. Error bars indicate 95% confidence intervals

postglacial recolonization, which alleviates many confounding factors that may hinder research, and also provides replicate distribution ranges that either conform or do not conform to the underlying "abundant-centre" assumptions of CMH (i.e., decline in ecological conditions and abundances towards range margins). We demonstrated that CMH is validated only when "abundant-centre" assumptions are met, and that reduced neutral genetic variation at range margins does not constrain shifts in functional trait variation along climatic gradients.

4.1 | Explicit CMH predictions

A lack of consensus about the predictions of CMH is thought to be the product of multiple confounding factors and the fact that the underlying "abundant-centre" assumptions of this theory are often not met (Eckert et al., 2008; Pironon et al., 2017). Our study echoes this sentiment as we found support for CMH, but with the caveat that distribution ranges must meet these assumptions. Therefore, analogous to assumption testing in statistics, research into genetic changes across species' distributions should first confirm whether "abundant-centre" assumptions are met. For example, ecological niche modelling has proven successful in identifying patterns in ecological gradients across core to margin transects (Lira-Noriega & Manthey, 2014; Micheletti & Storfer, 2015; Pironon et al., 2015).

We contend that mangroves simplify this process with their easily-defined distributions that generally exhibit reduced abundances as conditions become more marginal towards climate-sensitive poleward range limits (Osland, Feher, et al., 2017). USA mangroves also provide three natural replicates of core to margin distribution ranges. Comparing variation across multiple distributions of the same species can provide greater insights into the processes shaping genetic change (Griffin & Willi, 2014; Kennedy et al., 2017; Leydet, Grupstra, Coma, Ribes, & Hellberg, 2018; Micheletti & Storfer, 2015). For instance, West Florida (WFL) and East Florida (EFL) exhibited a similar decline in neutral genetic diversity, with reductions in mean allelic richness of almost 50% from south to north, and greatest intersite differentiation at the northern range margins, consistent with latitudinal reductions in mangrove abundances along these coastlines (Osland, Feher, et al., 2017). However, we found a stronger effect of genetic drift at the WFL range margin, a pattern also observed in a co-occurring mangrove species, *Rhizophora mangle* (Hodel, Souza Cortez, Soltis, & Soltis, 2016; Kennedy et al., 2017). Greater genetic drift at the WFL range margin may be explained by multiple factors, including greater geographic isolation from the range core, more extreme minimum annual temperatures, limited ocean-current-driven propagule dispersal (Kennedy et al., 2017), and restricted colonization due to propagule predation (Langston, Kaplan, & Angelini, 2017).

An anomaly within the EFL range core (site code: PI) suggests another potential caveat to CMH. This collection site exhibited strong within-range-core differentiation and lowest neutral genetic diversity along this distribution range. Land modifications associated with this site's conversion into a conservation area, plus limited hydrological exchange because this portion of the EFL lagoon system experiences highest water residence times (Smith, 1993), probably explain this anomalous pattern. Identifying effects of human activity on intraspecific genetic variation is a research priority (Guo, 2012), and this example highlights that deviations from CMH predictions may also be the product of anthropogenic changes and context-specific environmental factors that may restrict recruitment to local sources.

We found a strong delineation between *A. germinans* in Florida and those in Texas-Louisiana (TX-LA), consistent with independent post-Pleistocene recolonization routes (Sherrod & McMillan, 1985) and the potential role of the Mississippi River as a biogeographic barrier (Soltis, Morris, McLachlan, Manos, & Soltis, 2006). In contrast to Florida, TX-LA did not conform to the underlying "abundant-centre" assumptions and, as such, did not support CMH predictions. TX-LA *A. germinans* are essentially a series of range margins because the entire distribution experiences marginal environmental conditions. Research from Atlantic Mexico, directly south of TX-LA and closer to this species' true range core, found highest allelic richness at lower latitudes (18°N–20°N), with values analogous to our lower-latitude Florida collection sites, and lowest allelic richness in northern Mexico (25.9°N; adjacent to our most southern site) (Ochoa-Zavala, Jaramillo-Correa, Piñero, Nettel-Hernanz, & Núñez-Farfán, 2019). Combining genetic coverage from Mexico into TX-LA highlights two important points. First, reduced neutral genetic variation seems to be a characteristic of TX-LA *A. germinans*, presumably the product of restricted population sizes, recurrent fluctuations in abundance during periods of increased aridity and/or cold, and geographical isolation among distributional centres and from more continuous forests in Mexico (Sherrod & McMillan, 1981). Second, species range margins are not always restricted to their geographical limits, and instead may exist across extensive spatial scales (~1,000 km in this case) because of widespread ecologically-marginal conditions.

As mangroves generally conform to these "abundant-centre" assumptions, we would expect additional support for CMH across their pantropical distribution. Osland, Feher, et al. (2017) evaluated mangrove distributions worldwide and determined that most range limits were controlled by either temperature or precipitation, with only four geographic regions influenced by both factors: Texas-Louisiana (TX-LA), Pacific Mexico, Western Australia, and the Middle East. CMH predictions are supported along multiple mangrove distributions controlled by either temperature or precipitation (Arnaud-Haond et al., 2006; De Ryck et al., 2016; Francisco, Mori, Alves, Tambarussi, & de Souza, 2018; Kennedy et al., 2017; Maguire, Saenger, Baverstock, & Henry, 2000; Pil et al., 2011; Sugai et al., 2016), consistent with our findings along temperature-controlled Florida. CMH is also supported in Pacific Mexico (Ochoa-Zavala et al., 2019; Sandoval-Castro et al., 2014, 2012) and Western Australia (Arnaud-Haond et al., 2006; Binks et al., 2019), where parallel declines in temperature and precipitation limit mangrove distributions. Our finding that CMH is not supported across TX-LA seems to be an exception to the general rule in mangroves, although the Middle East may also prove to be an exception as the entire region is precipitation limited (Osland, Feher, et al., 2017). Yet, CMH predictions are also not supported directly south of TX-LA where Atlantic Mexican *A. germinans* did not exhibit a systematic decline in genetic diversity due to persistence in multiple glacial refugia (Ochoa-Zavala et al., 2019), and in the wider Caribbean where post-glacial expansion seemingly occurred along separate dispersal pathways (Kennedy et al., 2016). Mangroves, and coastal species in general (Sagarin et al., 2006), seem to provide ideal models to test many large-scale ecological theories, but deviations may exist due to nonconformity to underlying "abundant-centre" assumptions (as shown here in TX-LA), and to confounding effects of variation in past distributional fluctuations (as previously shown in Atlantic Mexico and the wider Caribbean), which reiterates the need to incorporate assumption testing into future empirical research.

4.2 | Implicit CMH prediction

The underlying importance of documenting genetic changes towards range margins is that limited adaptive genetic variation could reduce evolutionary potential and constrain adaptation to novel environmental conditions, a possible mechanism defining distributional limits (Bridle & Vines, 2007). We found that reduced neutral genetic variation at three range margins was not a constraint to shifts in functional trait variation consistent with a response to cold stress. Although USA range-margin *A. germinans* are smaller than conspecifics towards the range core (Feher et al., 2017), these small-statured individuals exhibited a change in functional traits consistent with greater cold tolerance. A similar trade-off in plant size and leaf traits exists for *A. germinans* along Atlantic Mexico (Méndez-Alonzo, López-Portillo, & Rivera-Monroy, 2008), and freeze experiments have demonstrated that this transition towards cold-tolerant leaf traits in East Florida *A. germinans* correlates with greater freeze tolerance at the range margin (Cook-Patton et al., 2015). Additional systematic changes towards USA mangrove range limits include narrower xylem vessel architecture (Madrid et al., 2014), precocious reproduction and increased propagule size (Dangremond & Feller, 2016), and greater reproductive success (Goldberg & Heine, 2017). Together, these observations are consistent with evidence to date that reduced neutral genetic variation at range margins does not necessarily diminish species performance (Abeli et al., 2014; Pironon et al., 2017), and add to our growing understanding of the importance of intraspecific trait variation in explaining ecological patterns (Siefert et al., 2015).

Functional trait variation of *A. germinans* leaves towards USA range margins mirrored gradients in climatic factors (i.e., temperature, precipitation) that control these distributional limits. Both Florida distribution ranges exhibited a change in leaf traits towards those better suited to tolerate cold, consistent with gradients in minimum winter temperatures. However, while WFL exhibited a more continuous change in leaf traits towards the range margin, EFL seemed to exhibit a more abrupt change, in particular at the most northern collection site. Trait variation along environmental gradients can vary depending on rates of gene flow and the strength of genetic drift (Polechová, 2018). Differences between WFL and EFL in these two factors (i.e., WFL: stronger pattern of isolation by distance, stronger effect of genetic drift at the range margin) may explain these patterns in trait variation. In contrast, functional traits across TX-LA were comparable to those at Florida range margins, presumably the product of inverse gradients in temperature and precipitation that may blur geographic patterns, as similar leaf traits are consistent with both cold and drought tolerance (Knight & Ackerly, 2003). Our observation of a trend towards longer, narrower leaves with higher specific leaf area (SLA) at the TX-LA northern range margin is also consistent with these inverse climatic gradients. A cumulative effect of both arid conditions and periodic freeze events could explain lowest SLA in southern sites (Poorter et al., 2009), with higher SLA as rainfall increases towards the higher-latitude range margin. Less sunlight and greater abundance of co-occurring salt marsh at higher latitude could then explain changes in leaf shape as light capture becomes more critical (Takenaka, 1994).

Observations of trait variation towards range margins seldom address the relative contributions of genetic differences and environmentally-induced trait plasticity in explaining these patterns (Chuang & Peterson, 2016). Our measurements of leaf traits in situ and of putative neutral genetic variation with microsatellite loci also cannot address this question. Instead, common garden and reciprocal transplant experiments are needed to achieve a conclusive understanding of the mechanisms shaping functional trait variation at these range margins. Common garden experiments with *A. germinans* found greater chill tolerance in offspring from Texas compared to more-equatorial regions (Markley, McMillan, & Thompson, 1982), and an over-the-edge transplant experiment (i.e., individuals transplanted beyond current range limits) demonstrated greater post-freeze survival in seedlings from sources where freezes are common (Hayes et al., 2020). However, mangroves also exhibit high levels of trait plasticity in response to environmental cues (Feller et al., 2010). Additional over-the-edge transplant experiments will also further our understanding of whether these range margins are ecological niche limits or the product of dispersal limitation, important insight to better predict responses to climate change (Lee-Yaw et al., 2016).

Considering ongoing, rapid expansion at all USA range margins (Rogers & Krauss, 2018) and further expansion forecast with climate change (Cavanaugh et al., 2015; Osland, Day, et al., 2017), plus the fact that range margins are probably the primary source of recruits beyond distributional limits (Hampe, 2011), USA range-margin *A. germinans* appear well-equipped to thrive in their marginal environment, unless directly impacted by anthropogenic changes. This continued proliferation will result in wide-reaching community-level effects (Diskin & Smee, 2017; Guo et al., 2017; Kelleway et al., 2017).

In conclusion, model systems that meet underlying assumptions and alleviate the influence of common confounding factors can provide important insights into many large-scale ecological theories (Sagarin et al., 2006). We utilized a widespread mangrove species that naturally controls for common confounding factors to demonstrate that the central-marginal hypothesis (CMH) is validated, but only when underlying "abundant-centre" assumptions are met, and that reduced neutral genetic variation at range margins does not constrain shifts in functional trait variation along climatic gradients. Considering that many species do not conform to "abundant-centre" assumptions (Sagarin & Gaines, 2002; Santini et al., 2019) and that numerous confounding factors can influence genetic patterns (Eckert et al., 2008), our findings support previous accounts that CMH does not represent a general rule across species (Pironon et al., 2017), with deviations from CMH probably becoming more common with climate change and greater anthropogenic pressures that can reduce and fragment suitable habitat. Finally, we agree with the framework proposed by Pironon et al. (2017) that research needs to employ an integrated approach that not only considers geographic gradients, but also ecological and historical gradients, when interpreting patterns of genetic and trait variation across broad spatial scales.

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AUTHOR CONTRIBUTIONS

J.P.K., and I.C.F. designed the research. J.P.K. performed the research and analyzed the data. R.F.P., J.K.R., and I.C.F. supervised the research. J.P.K. wrote the manuscript with input from all coauthors.

DATA AVAILABILITY STATEMENT

Microsatellite genotype data and functional trait data are publicly available on Dryad: https://doi.org/10.5061/dryad.69p8cz8xh

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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CHAPTER 3

Mating system variation in neotropical black mangrove, Avicennia germinans, at three spatial scales towards an expanding northern distributional limit

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Mating system variation in neotropical black mangrove, *Avicennia germinans*, at three spatial scales towards an expanding northern distributional limit

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ABSTRACT

Climate-driven range expansion of ecosystem-defining foundation species can have wide-reaching ecological consequences. Expansion may also result in mating system changes in these foundation species because of the ecological characteristics of range margins, such as greater conspecific isolation and reduced pollinator availability. It is important to understand how mating systems may change during expansion due to their direct influence on intraspecific genetic and demographic dynamics. Here, we used 12 microsatellite loci to genotype progeny arrays of the neotropical black mangrove (*Avicennia germinans*) at six collection sites ($n = 23$ maternal trees; 1,612 genotyped propagules) along a latitudinal gradient towards a northern distributional limit on the Atlantic coast of Florida, USA (27.56–30.01°N), where mangroves have expanded into salt marsh over the past several decades. We assessed mating system variation at three spatial scales. First, at the species-distribution level, published outcrossing rates for tropical conspecifics were more than two times higher than those for subtropical Florida *A. germinans*, consistent with reductions in pollinator diversity and in mangrove abundance with latitude. Second, at the population level, Florida outcrossing rates did not systematically decline towards the northern range limit, but instead, a more open pollen-dispersal neighbourhood at the transition from mangrove to salt marsh dominance may elevate outcrossing until conspecific abundances become too low towards the range limit. Third, at the individual level, outcrossing increased as conspecific cover increased at the Florida range margin, consistent with density-dependent plastic shifts in mating system. These findings suggest that ecological structure influences the *A. germinans* mating system at varying spatial scales. Further research needs to evaluate the effect of *A. germinans* mating system variation on the survival and fitness of offspring and on the extent of population-level local adaptation at expanding distributional limits.

1. Introduction

Climate-driven redistributions of species are now commonplace and can lead to important changes in ecological communities, ecosystem function, and human well-being (Pecl et al., 2017). For instance, range expansion of ecosystem-defining foundation species (e.g., long-lived tree and shrub species) can result in entire biome shifts with wide-reaching ecological consequences (Beck et al., 2011; Peñuelas and Boada, 2003; Saintilan et al., 2014).

Colonisation of new habitat has long been associated with greater self-fertilisation in plants (i.e., Baker's Law; Baker, 1955) as characteristics of expanding range margins, such as greater isolation among conspecifics (Eckert et al., 2010; Ghazoul, 2005) and reductions in pollinator availability (Kalisz et al., 2004; Moeller et al., 2012; Yin et al., 2016), are known to select for increased self-fertilisation (Hargreaves and Eckert, 2014). However, adaptive shifts in mating system are not thought to be general attributes of the expansion of long-lived trees and shrubs because these species generally maintain outcrossing independent of their environment (Barrett and Harder, 2017). Instead, density-dependent plastic shifts towards greater self-fertilisation may occur during initial colonisation (Morgan et al., 2005; Peterson and Kay, 2015), with subsequent changes post-colonisation as increased conspecific density favours shifts back towards greater outcrossing (Pannell, 2015). Understanding mating system variation is important because of

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its direct influence on genetic and demographic dynamics within a species (Barrett and Harder, 2017 and cites within).

Mangroves are an assortment of intertidal tree and shrub species that are ecologically-important coastal foundation species (Tomlinson, 1986). Mangroves originate in the tropics and decline in abundance towards latitudinal range limits that correspond to ecological thresholds in temperature and/or precipitation (Osland et al., 2017). The pantropical genus *Avicennia* consists of eight species, of which three are found in the Neotropics (Duke, 1992). Of these three neotropical *Avicennia* species, *Avicennia germinans* (neotropical black mangrove) is the most widespread, with a distribution across the tropics and into the subtropics (Lonard et al., 2017). On the Atlantic coast of Florida (USA), mangroves are the dominant coastal foundation species at lower latitudes where three mangrove species (*A. germinans*, plus *Rhizophora mangle* and *Laguncularia racemosa*) form dense forests, but the higher-latitude mangrove range margin consists of discrete patches of isolated individuals (almost exclusively *A. germinans*) in a salt marsh-dominated landscape (Kangas and Lugo, 1990). Lack of extreme winter freezes over the past several decades has been linked to proliferation and expansion of *A. germinans* at this northern distributional limit (Cavanaugh et al., 2019, 2014; Rodriguez et al., 2016) and further expansion is forecast with climate change (Cavanaugh et al., 2019, 2015). This ongoing shift from salt marsh to mangrove dominance at this expanding range margin will presumably lead to significant changes in ecosystem structure and services essential to human well-being, including nutrient storage, storm protection, and habitat availability for certain fauna (Doughty et al., 2017, 2016; Kelleway et al., 2017; Osland et al., 2018; Simpson et al., 2019).

Avicennia germinans is hermaphroditic with clusters of white flowers (each with one stigma and four stamens) on axillary or terminal inflorescences (Lonard et al., 2017), and is recognised as predominantly outcrossed via insect pollination (Tomlinson, 1986). However, higher-latitude mangroves may encounter far less diverse sets of pollinators than their tropical conspecifics (Hermansen et al., 2014b). Few pollination studies exist for *A. germinans*, but those that do demonstrate greater pollinator diversity at lower latitudes (Sánchez-Núñez and Mancera-Pineda, 2012) compared to higher latitudes (Landry, 2013). Consistent with this latitudinal decline in pollinator diversity, indirect genetic evidence (via inbreeding coefficients, F_{IS}) supports predominant outcrossing in *A. germinans* populations closer to the range centre of this species (Cerón-Souza et al., 2012; Mori et al., 2015; Nettel et al., 2008; Ochoa-Zavala et al., 2019; but, see Salas-Leiva et al., 2009), with observations of elevated inbreeding towards range limits (Kennedy et al., 2020a; Mori et al., 2015; Ochoa-Zavala et al., 2019). Direct evidence via progeny arrays with *A. germinans* in Mexico also supports predominant outcrossing closer to the range centre (Nettel-Hernanz et al., 2013), but we still lack equivalent direct estimates of mating system towards *A. germinans* distributional limits.

Here, we used progeny arrays to estimate *A. germinans* outcrossing rates at six collection sites along a latitudinal gradient towards the expanding northern distributional limit of this species on the Atlantic coast of Florida. We then assessed variation in mating system (i.e., outcrossing versus self-fertilisation rates; Neal and Anderson, 2005) at three spatial scales. First, at the species-distribution level, we compared published outcrossing rates based on progeny arrays from tropical conspecifics to rates observed here for subtropical *A. germinans*. Second, at the population level, we assessed whether outcrossing rates decreased along the Florida latitudinal gradient that reaches the northern range limit of this species. Third, at the individual level, we assessed whether conspecific cover influences outcrossing rates of individual trees at the expanding range margin. We tested the following predictions: (1) outcrossing rates will decline from the tropics to subtropics; (2) population-level outcrossing rates will decline along the latitudinal gradient in Florida; (3) tree-level outcrossing rates will increase as conspecific cover increases at the Florida range margin.

2. Materials and methods

2.1. Collection sites and sampling methods

On 7–8 October 2017, we collected ~100 propagules from and recorded GPS coordinates for each of 30 maternal trees at six collection sites (n = 5 trees per site) along a latitudinal gradient (27.56–30.01°N) on the Atlantic coast of Florida. We systematically collected propagules from around the entire canopy of each maternal tree. A leaf was also collected from each maternal tree and dehydrated in silica gel to obtain maternal genotypes. Atlantic Florida mangroves are replaced by salt marsh as the dominant coastal foundation species at approximately 29 °N (Spalding et al., 2010). Our three most southern collection sites (27.56–28.37°N) are areas within the *A. germinans* continuous range core where mangroves are the dominant coastal foundation species; whereas, our three most northern collection sites (29.41–30.01°N) are areas at the *A. germinans* range margin where salt marsh species are dominant and *A. germinans* exists as discrete patches of isolated individuals (Fig. 1). Population genetic data for each of these collection sites was previously collected in 2015 (Table 1; Kennedy et al., 2020a). All propagules from an individual tree were kept together in one plastic bag during field collections. Propagules that were eventually genotyped had their pericarps removed and were stored at −20 °C until DNA extraction.

Our study design reflects two principal factors: (1) Atlantic Florida *A. germinans* exhibits a considerable reduction in genetic variation towards the northern distributional limit (Kennedy et al., 2020a) and (2) limited polymorphism can restrict our ability to detect differences using molecular markers (Arnaud-Haond et al., 2005). We were concerned that reduced genetic variation may inhibit our ability to quantify reliable outcrossing rates and, as such, we needed to focus more sampling effort on the number of offspring per tree. This concern proved valid, as we determined that a relatively large number of offspring were needed to obtain reliable tree-level outcrossing estimates (see *2.3 Descriptive analyses*). Hence, for practical reasons, we were limited in the number of sampled maternal trees that could be included in this study. We included 23 maternal trees. From south to north, we genotyped progeny arrays from n = 5, 2, and 2 maternal trees at each of the three range-core sites, and n = 4, 5, and 5 maternal trees at each of the three range-margin sites (Table 1). We focussed more effort on the range-margin sites to better address our third prediction (i.e., tree-level outcrossing rates will increase as conspecific cover increases at the range margin). Although only two maternal trees were analysed at two of the range-core sites, we attempted to capture variation across as large of an area as possible. These two forest patches (code: MH, U; Table 1) extend for approximately 2.3 and 3.9 km, respectively, along the adjacent river channel, and we selected trees that were separated by 1.3 and 1.2 km, respectively (i.e., 56% and 31% of the linear extent of these collection sites).

2.2. DNA isolation and microsatellite genotyping

For leaves from the 23 maternal trees, genomic DNA was isolated from 20 mg of dry tissue with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard protocol, with an extended incubation of 45 min. Trees were genotyped at 12 previously-developed nuclear microsatellite loci (Cerón-Souza et al., 2012, 2006; Mori et al., 2010; Nettel et al., 2005) following the protocol outlined in Kennedy et al. (2020a). We performed PCR on a Prime thermal cycler (Techne, Straffordshire, UK), analysed fragments on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA) with LIZ 500 size standard, and scored alleles in the R-package Fragman (Covarrubias-Pazaran et al., 2016). We amplified and genotyped DNA from each maternal tree twice to ensure we had the correct multi-locus genotype.

For each propagule, we removed the cotyledons and extracted DNA from portions of the hypocotyl and radicle, the eventual stem and root of

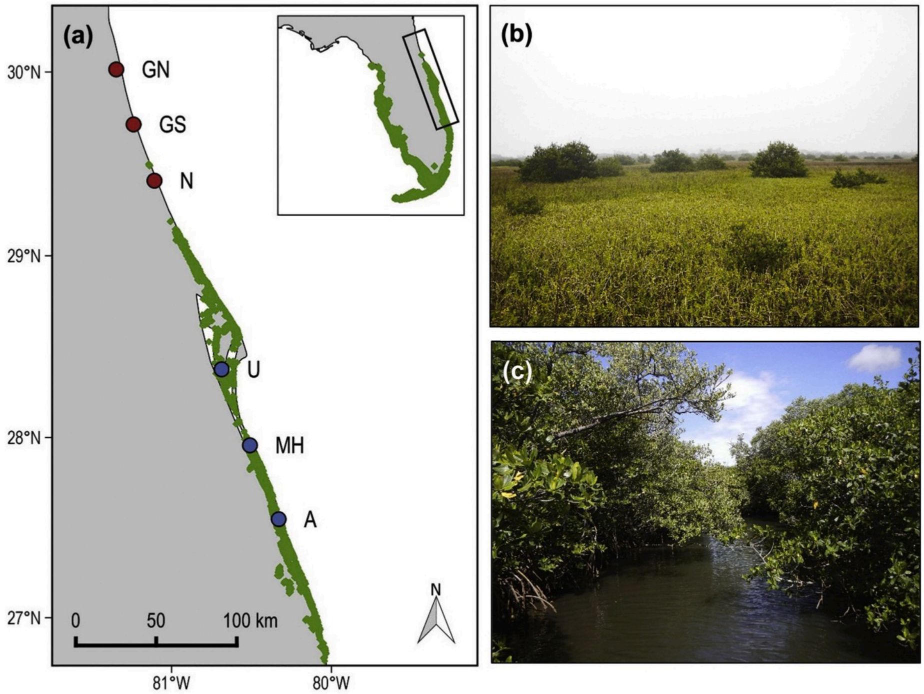

Fig. 1. Six collection sites along a latitudinal gradient (27.56–30.01°N) on the Atlantic coast of Florida (USA) for *Avicennia germinans* progeny arrays. (a) The three most southern sites (blue circles) are areas within the continuous range core of this species, where mangroves are the dominant coastal foundation species. The three most northern collection sites (red circles) are areas at the range margin of this species, where salt marsh species are dominant and *A. germinans* exists in discrete patches. Mangrove distribution is shown in green (Giri et al., 2011). (b, c) Representative picture of range-margin and range-core community structure, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Population-level genetic diversity and mating system parameters of *Avicennia germinans* from six collection sites on the Atlantic coast of Florida (USA).

Site	Code	Latitude	genetic diversity[a]				progeny arrays								
			AR		H_S		arrays	n	t_m		t_m - t_s		$r_p(m)$		$1/r_p(m)$
Avalon	A	27.5468	3.40	(0.64)	0.42	(0.05)	5	336	0.37	(0.03)	0.03	(0.01)	0.16	(0.04)	6.2
Maritime Hammock	MH	27.9566	3.14	(0.48)	0.42	(0.05)	2	169	0.22	(0.04)	0.03	(0.01)	0.17	(0.07)	5.8
Ulumay	U	28.3756	2.33	(0.24)	0.31	(0.05)	2	135	0.39	(0.05)	−0.01	(0.02)	0.08	(0.12)	13.2
North Peninsula	N	29.4096	2.83	(0.61)	0.35	(0.06)	4	277	0.49	(0.03)	0.00	(0.02)	0.05	(0.02)	20.4
GTM South	GS	29.7159	2.85	(0.42)	0.40	(0.06)	5	342	0.14	(0.02)	0.02	(0.01)	-	–	–
GTM North	GN	30.0144	2.14	(0.29)	0.21	(0.05)	5	353	0.31	(0.02)	0.06	(0.01)	0.41	(0.07)	2.5

AR, allelic richness; H_S, unbiased gene diversity; arrays, number of progeny arrays sampled; n, total number of propagules genotyped; t_m, multi-locus outcrossing rate; $t_m - t_s$, biparental inbreeding; $r_p(m)$, proportion of offspring with the same father; $1/r_p(m)$, number of effective pollen donors. Standard error in parentheses. Note: estimates of $r_p(m)$ for collection site GS could not be calculated.

[a] Data from Kennedy et al. (2020a) based on n = 30 adult trees per collection site genotyped at the same 12 nuclear microsatellite loci used here.

the germinating seedling. Genomic DNA from propagules was isolated from 50 mg of frozen hypocotyl/radicle tissue with the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) following the standard protocol. Subsequent genotyping steps were identical to those for leaves, but we modified the PCR volumes outlined in Kennedy et al. (2020a). Each of the two multiplex PCR contained a total volume of 6 μL with 2.5 μL Multiplex PCR Master Mix, 0.5 μL primer mix, and 3 μL of genomic DNA. We also randomly re-amplified and re-genotyped 5% of our propagule DNA samples to estimate a study error rate (Bonin et al., 2004).

2.3. *Descriptive analyses: maternal genotypes and sample sizes*

We performed a discriminant analysis of principal components (DAPC) (Jombart et al., 2010) in the R-package adegenet 2.1.1 (Jombart and Ahmed, 2011) as a visual assessment of genetic differences among the 23 maternal trees. We retained nine principal components, the minimum number that explained ~90% of the total variance, identified two clusters, and retained three discriminant functions. We extracted each individual's coordinates on the two principal axes of the DAPC (i.e., ind.coord) and plotted them in ggplot2 (Wickham, 2011).

We performed an initial analysis to estimate how many genotyped propagules would be needed to provide reliable tree-level outcrossing estimates. For each of two trees (code: MH1, MH3), we genotyped as close to 100 propagules as possible (n = 87, 82, respectively) and estimated tree-level detectable outcrossing rates with the direct approach outlined below (see *2.4 Outcrossing calculations*). We then reduced the number of propagules in the data set by increments of five (i.e., n = 80, 75, 70, 65, 60, 55, 50, 45) and re-calculated outcrossing rates for each of these new subsets. This approach enabled us to visualise how outcrossing estimates changed as sample sizes decreased. Estimates of these subsets remained relatively consistent with the initial estimates (MH1: 0.6–6.8% change; MH3: 2.5–9.3% change) until $n \leq 55$ when values became more variable (MH1: 7.7–13% change; MH3: 16.1–43.1% change) (Fig. 2). As such, we determined that $n \geq 60$ propagules per tree should be sufficient to estimate reliable tree-level outcrossing rates.

2.4. *Outcrossing calculations: population-level and tree-level*

To assess population-level mating system variation towards the Florida *A. germinans* range limit, we calculated multi-locus outcrossing rates (t_m) for each of the six collection sites with the maximum likelihood-based MLTR (Ritland, 2002). We also calculated levels of biparental inbreeding ($t_m - t_s$) and we used the proportion of offspring with the same father [$r_p(m)$] to calculate the number of effective pollen donors [$1/r_p(m)$] for each collection site. We used default parameters, 500 bootstraps to calculate standard errors, and resampled among

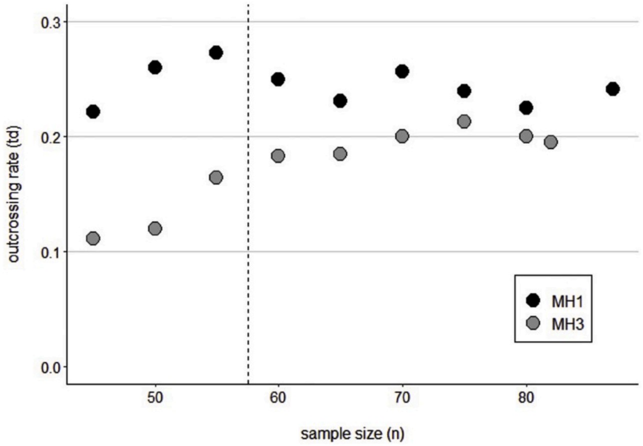

Fig. 2. Initial analysis to estimate an appropriate sample size of propagules to calculate tree-level outcrossing rates. Systematic reductions in sample size for two trees (MH1, MH3) continued to provide estimates of detectable outcrossing rate (t_d) relatively consistent with initial estimates until $n \leq 55$ when values became more variable (MH1: 7.7–13% change; MH3: 16.1–43.1% change). This threshold is shown with a vertical dashed line. We determined that $n \geq 60$ propagules should provide reliable estimates of tree-level outcrossing rates.

individuals within families.

To assess mating system variation among individual trees, we used a direct approach based on allelic differences between maternal genotypes and their sampled offspring to calculate apparent outcrossing rates (Cruzan et al., 1994). First, detectable outcrossing rates (t_d) were calculated as the number of propagules that possessed an allele not present in the maternal genotype (i.e., detectable outcross event) divided by the number of propagules genotyped. However, there may also be a percentage of offspring that appear to be the product of self-fertilisation, but are instead the result of outcrossing to individuals with similar genotypes to the maternal tree (i.e., undetectable outcross event). Hence, maternal genotypes with high-frequency alleles in a population will result in greater undetected outcrossing. Using allele frequency data from each of these six collection sites in 2015 (Kennedy et al., 2020b), we calculated the probability of an undetectable outcross event [$P(u)_j$] for each of the 23 maternal trees as the product across loci of the frequencies of each maternal allele within the corresponding collection site (Cruzan et al., 1994). For each maternal tree, we multiplied its specific $P(u)_j$ by the number of propagules that were not identified as outcrossed to obtain an estimate of the number of potential undetected outcross events. We then calculated apparent outcrossing rates (t_a) as the number of detectable outcross events plus the number of potential undetected outcross events divided by the number of propagules genotyped. We also estimated these tree-level outcrossing rates with the maximum likelihood-based MLTR, as described above for population-level estimates.

2.5. *Estimates of conspecific cover at the range margin*

It remains difficult to remotely assess mangrove cover at fragmented range margins (Bunting et al., 2018). However, mangrove patches within salt marsh can generally be manually identified with relative ease in satellite images, while also aided by knowledge of the collection site. For each of the three range-margin collection sites, we manually measured approximate estimates of area covered with *A. germinans* within a 10 m radius circle around each maternal tree (n = 14 GPS-referenced trees) with the polygon function in Google Earth Pro 7.3.2.5776. We chose this size because 10 m was the longest distance that permitted reliable estimates around maternal trees adjacent to terrestrial hammock forest patches.

2.6. *Statistical analyses*

We performed all statistical analyses in R v3.4.2 (R Core Team, 2013). To test prediction 1 (decline in outcrossing from tropics to subtropics), we compared mean multi-locus outcrossing rates (t_m) for these Florida collection sites (n = 6 sites) to published values for conspecifics in Mexico (n = 3 sites; Nettel-Hernanz et al., 2013) with a two-sample *t*-test. To test prediction 2 (decline in outcrossing along the Florida latitudinal gradient), we evaluated the relationship between t_m and latitude with a Spearman's rank correlation (n = 6 sites). To test prediction 3 (increase in outcrossing with increased conspecific cover at the range margin), we evaluated the effect of *A. germinans* cover around each range-margin maternal tree on tree-level apparent outcrossing rates (t_a) with a linear regression (n = 14 trees). We natural log-transformed *A. germinans* cover to meet the statistical assumption of normality.

3. Results

3.1. *Maternal genotypes, sample sizes and study error rate*

Each of the 23 maternal trees produced consistent multi-locus genotypes after being amplified and genotyped a second time. All 23 maternal genotypes were unique, with a range from 1 of 12 to 10 of 12 matching loci, and exhibited a clear separation between range core and margin genotypes (Fig. 3). We genotyped a total of 1,612 propagules, with a mean sample size per tree of 70.1 ± 5.3 (SD; range: 64–87). We re-amplified and re-genotyped DNA from 87 propagules (5.4% of all samples) and found an error rate of 0.01% (one error out of 1,044 locus comparisons). This locus-specific error was removed from the data set.

3.2. *Population-level mating system variation*

Population-level multi-locus outcrossing rates (t_m) in Florida ranged from 0.14 ± 0.02 (SE) to 0.49 ± 0.03 (Table 1). Florida t_m were, on average, more than two times lower than published values for tropical conspecifics in Mexico (mean t_m Florida: 0.32, Mexico: 0.71; $t_{4.8} = -4.8$, $p = 0.006$) (Fig. 4).

Population-level outcrossing (t_m) did not systematically decline along the Florida latitudinal gradient (Spearman's correlation, $r_s = -0.20$, $p = 0.70$). Instead, highest and lowest values were observed in range-margin sites (Fig. 4). All collection sites exhibited low levels of

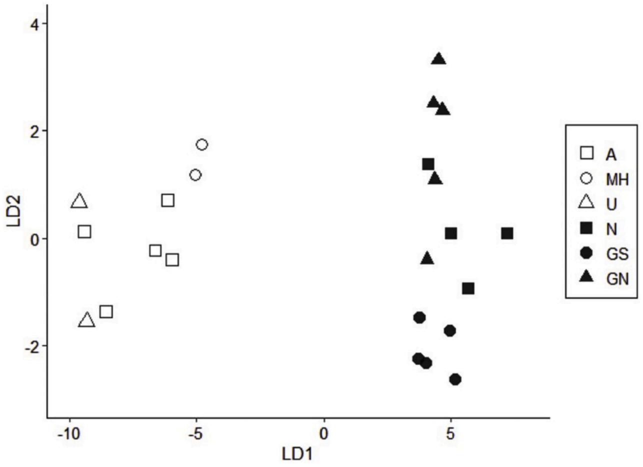

Fig. 3. Discriminant analysis of principal components (DAPC) of maternal tree multi-locus genotypes. All 23 maternal trees possessed unique multi-locus genotypes and exhibited a clear separation between range core and margin. Open shapes indicate range-core sites (A, Avalon; MH, Maritime Hammock; U, Ulumay); filled shapes indicate range-margin sites (N, North Peninsula; GS, GTM South; GN, GTM North).

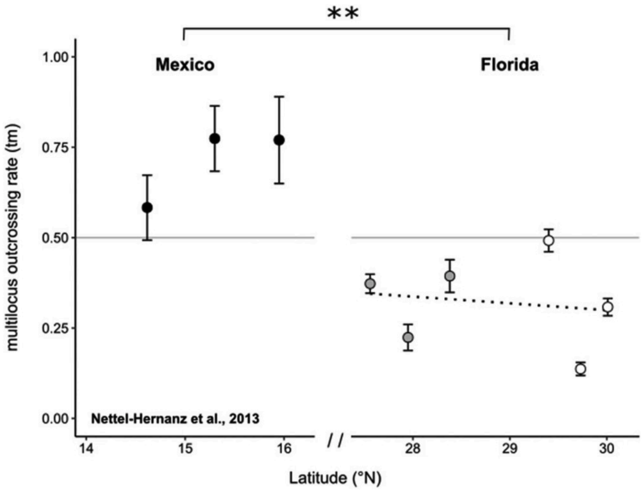

Fig. 4. Population-level multi-locus outcrossing rates (t_m) for tropical *Avicennia germinans* in Mexico (Nettel-Hernanz et al., 2013) and subtropical *A. germinans* in Florida (this study). Florida *A. germinans* mean t_m was more than two times smaller than their tropical conspecifics ($t_{4.8} = -4.8$, $p = 0.006$). Outcrossing (t_m) did not systematically decline along the Florida latitudinal gradient ($r_s = -0.20$, $p = 0.70$). Non-significant correlation is depicted with a dotted line. *A. germinans* in Mexico is shown in black, in Florida range core is shown in grey, and in Florida range margin is shown in white. Population error bars are standard error. **, $p < 0.01$.

biparental inbreeding ($t_m - t_s = -0.01 - 0.06$) (Table 1). MLTR could not calculate the proportion of offspring with the same father [$r_p(m)$] for one range-margin site (code: GS), presumably because this site exhibited the lowest outcrossing rates (Tables 1, 2). Estimates of $r_p(m)$ were calculated for all other collection sites and the number of effective pollen donors [$1/r_p(m)$] varied from 2.5 to 20.4, with the lowest number of pollen donors at the northern range limit (code: GN) and the highest number at the most southern range-margin site (code: N) (Table 1).

3.3. Tree-level mating system variation

Tree-level detectable outcrossing rates (t_d) ranged from 0.05 to 0.58 (Table 2). Probabilities of an undetectable outcross event were generally low (0–0.09) and, as such, the number of potential undetected outcross events was also generally low (0–4.7) (Table 2). However, there were two notable exceptions with probabilities of 0.17 and 0.22, and a total of 9 and 12 potential undetected outcross events (tree: GN1, GN4; Table 2). These two exceptions were trees at the northern range limit, the least genetically-diverse collection site (Table 1). After incorporating potential undetected outcross events into our calculations, tree-level apparent outcrossing rates (t_a) still ranged from 0.05 to 0.58 (Table 2) and were highly correlated with t_d (Pearson's correlation, $r = 0.97$, $p < 0.0001$). Tree-level estimates calculated with MLTR were also highly correlated with both t_d ($r = 0.99$, $p < 0.0001$) and t_a ($r = 0.97$, $p < 0.0001$).

Conspecific cover at the range margin, measured as the area covered by *A. germinans* within a 10 m radius circle around each range-margin maternal tree, varied considerably from 15.5 to 177.4 m^2 (7–57% cover), with highest values at the most southern range-margin site (tree: N2-5; Table 2). Apparent outcrossing rates (t_a) increased as conspecific cover increased ($t_a = -0.46 + 0.20 * \ln(\text{cover})$, $F_{1,12} = 21.0$, $p = 0.0006$, $r^2_{adj} = 0.61$), with a 2% increase in outcrossing with every 10% increase in *A. germinans* cover (m^2) (Fig. 5).

4. Discussion

We used progeny arrays to characterise mating system in *Avicennia germinans* at six collection sites along a latitudinal gradient towards the northern distributional limit of this species on the Atlantic coast of Florida (27.56–30.01°N). We assessed variation in mating system at three spatial scales: (1) at the species-distribution level, (2) at the population level along the Florida latitudinal gradient, and (3) at the individual level among conspecifics at the expanding Florida range margin. First, published outcrossing rates for tropical conspecifics in Mexico were, on average, more than two times higher than those for subtropical Florida *A. germinans*. Second, population-level outcrossing rates did not systematically decline with latitude towards the northern range limit in Florida. Third, tree-level outcrossing rates increased as conspecific cover

Table 2
Tree-level outcrossing rates and conspecific cover around range-margin trees. n, number of propagules genotyped; t_d, detectable outcrossing rate; $P(u)_j$, probability of an undetectable outcross event; undetected, number of potential undetected outcross events; t_a, apparent outcrossing rate; cover, *A. germinans* cover within a 10 m radius circle around each maternal tree at range-margin sites. Note: cover was not calculated for range-core collection sites.

Tree	Latitude	Longitude	n	t_d	$P(u)_j$	undetected	t_a	cover
A1	27.560644	−80.328730	72	0.33	0.000	0.0	0.33	–
A2	27.559835	−80.329391	67	0.49	0.050	1.7	0.52	–
A3	27.559641	−80.329857	66	0.42	0.002	0.1	0.43	–
A4	27.559870	−80.330614	64	0.17	0.003	0.2	0.17	–
A5	27.557966	−80.329410	67	0.42	0.014	0.6	0.43	–
MH1	27.958072	−80.515093	87	0.24	0.004	0.3	0.24	–
MH3	27.951081	−80.509048	82	0.20	0.001	0.1	0.20	–
U3	28.372275	−80.684404	70	0.23	0.088	4.7	0.30	–
U5	28.380151	−80.685456	65	0.52	0.003	0.1	0.52	–
N2	29.407971	−81.099778	65	0.40	0.006	0.2	0.40	177.4
N3	29.407679	−81.098927	70	0.40	0.035	1.5	0.42	44.5
N4	29.407872	−81.099167	70	0.53	0.026	0.9	0.54	111.1
N5	29.407941	−81.099617	72	0.58	0.002	0.1	0.58	173.2
GS1	29.729168	−81.240662	65	0.05	0.005	0.3	0.05	28.2
GS2	29.730158	−81.240638	66	0.06	0.038	2.3	0.10	21.3
GS3	29.730077	−81.241591	70	0.10	0.016	1.0	0.11	32.9
GS4	29.730190	−81.241972	71	0.14	0.013	0.8	0.15	54.1
GS5	29.729601	−81.242441	70	0.31	0.079	3.8	0.37	87.1
GN1	30.016524	−81.345922	70	0.23	0.222	12.0	0.40	45.5
GN2	30.016660	−81.345954	72	0.43	0.014	0.6	0.44	39.7
GN3	30.014781	−81.344683	72	0.49	0.081	3.0	0.53	93.2
GN4	30.014178	−81.344754	69	0.23	0.171	9.0	0.36	91.2
GN5	30.013645	−81.345022	70	0.06	0.026	1.7	0.08	15.5

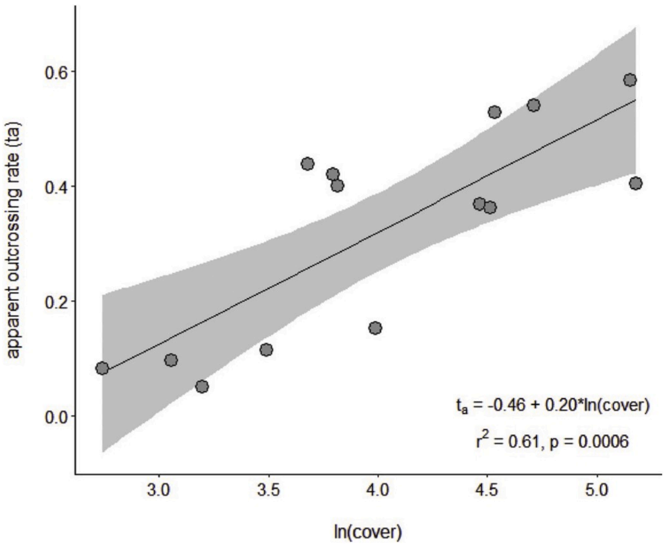

Fig. 5. Tree-level apparent outcrossing rates (t_a) increased as conspecific cover [ln(cover)] increased at the *Avicennia germinans* range margin on the Atlantic coast of Florida (USA). Conspecific cover was measured as the area covered by *A. germinans* within a 10 m radius circle around each maternal tree at the three range-margin collection sites.

increased at the Florida range margin.

4.1. Reduced outcrossing from tropics to subtropics

In contrast to the prevailing idea that *A. germinans* is predominantly outcrossed, we found that all Florida collection sites were predominantly self-fertilised. Tropical conspecifics in Mexico (14.61–15.95°N) exhibit predominant outcrossing (Nettel-Hernanz et al., 2013), with outcrossing rates more than double our observations for subtropical Florida *A. germinans*. Predominant outcrossing has also been documented for a co-occurring neotropical congener, *A. schaueriana*, at lower latitude (0.82°S) (Mori et al., 2015). Reduced outcrossing towards *A. germinans* distributional limits is consistent with a global decline in plant outcrossing rates towards higher latitude (Moeller et al., 2017). However, Moeller et al. (2017) found that this global pattern is mostly shaped by latitudinal changes in life history and growth form (i.e., lower latitude: greater frequency of perennial and tree species; higher latitude: greater frequency of annual and herbaceous species), and propose that evaluations of mating system variation in species with broad latitudinal distributions will help better understand the potential impact of plant-pollinator interactions in shaping latitudinal patterns in mating systems.

Reduced outcrossing in subtropical *A. germinans* is consistent with reductions in pollinator diversity across this species' broad distribution range. At lower latitude, four highly-effective pollinators were identified on the small, Caribbean island of San Andrés (12.54°N) (Sánchez-Núñez and Mancera-Pineda, 2012), and a diverse set of pollinators (including wasps, flies, and bees) was also identified for the neotropical congener, *A. schaueriana* (7.68°S) (Nadia et al., 2013). At higher latitude, only one principal pollinator, the exotic European honey bee (*Apis mellifera*), accounted for 87% of flower visits at a location in Southeast Florida (26.03°N) (Landry, 2013). However, we cannot disregard additional factors that could also impact the *A. germinans* mating system in Florida, such as reduced mangrove abundances towards distributional limits (Osland et al., 2017) and the highly-fragmented nature of Atlantic Florida mangroves due to anthropogenic modifications of these coastal ecosystems (Brockmeyer et al., 1996). Outcrossing often increases with conspecific density and pollinator abundance, but these two factors are not independent for animal-pollinated plants because dense groupings generally attract more pollinators (Ghazoul, 2005). Research near the southern latitudinal limits (33.94–34.63°S) of a widespread Indo-West Pacific congener, *A. marina*, highlights this point. Exotic European honey bees were also identified as the only significant pollinator for this species (Hermansen et al., 2014b) and smaller, more fragmented stands attracted fewer pollinators with altered foraging behaviour that may facilitate greater self-fertilisation (i.e., bees spent more time foraging on individual trees) (Hermansen et al., 2014a). As a result, these smaller *A. marina* stands exhibited reduced outcrossing compared to larger stands (Hermansen et al., 2015). Our observation of reduced outcrossing in subtropical Florida *A. germinans* may be the product of the interactive effects of reduced pollinator diversity and more fragmented mangrove area, that could also reduce pollinator abundances, compared to tropical mangrove forests. Further research needs to directly link assessments of mating system with plant-pollinator surveys across the broad, latitudinal distribution of *A. germinans*, as well as other *Avicennia* species, to provide definitive answers. In addition, research also needs to consider further environmental (e.g., seasonality; Chybicki and Dzialuk, 2014; Yin et al., 2016) and biological factors (e.g., fecundity, phenology; Ghazoul, 2005; Kameyama and Kudo, 2015) that can shape mating system variation.

Reductions in outcrossing are the product of a combination of biparental inbreeding and self-fertilisation. We found low biparental inbreeding at all collection sites, consistent with *A. germinans* progeny arrays in Mexico ($t_m - t_s = 0.01–0.06$; Nettel-Hernanz et al., 2013). However, progeny arrays with the neotropical congener, *A. schaueriana*, exhibited a greater effect of biparental inbreeding ($t_m - t_s = 0.15$; Mori et al., 2015), and even higher rates were found at the southern latitudinal limits of the Indo-West Pacific congener, *A. marina* ($t_m - t_s =$ 0.29–0.53; Hermansen et al., 2015). Differences among these studies may be explained by interspecific variation in reproductive biology, variation in kinship structure among collection sites (Hasan et al., 2018), or simply methodological differences (e.g., number of progeny arrays, of propagules sampled, and of loci genotyped) that can impact these parameter estimates (Ritland, 2002). Our findings suggest that reduced outcrossing in subtropical Florida *A. germinans* is predominantly via self-fertilisation, either autonomous (within the same flower) or geitonogamous (among flowers on the same plant). Although we lack evidence for *A. germinans*, multiple *Avicennia* species are self-compatible, including the neotropical congener, *A. schaueriana* (Nadia et al., 2013), and three Indo-West Pacific congeners (Aluri, 1990; Raju et al., 2012), with a much higher fruit set via geitonogamous compared to autonomous self-fertilisation for all species. Geitonogamous self-fertilisation is common in bee-pollinated plants (Harder and Barrett, 1995; Mitchell et al., 2004), is thought to aid mangrove colonisation (Primack et al., 1981), and seems to be a likely mechanism in *A. germinans* as inflorescences have multiple flowers open simultaneously.

4.2. Density-dependent mating system variation towards range limits

We did not observe a systematic decline in outcrossing rates towards the expanding *A. germinans* northern range limit in Florida, consistent with the prevailing view that adaptive shifts in mating system are not common during range expansion of long-lived trees and shrubs (Barrett and Harder, 2017). Instead, density-dependent plastic shifts in mating system may occur, with transitions between increased self-fertilisation at low density and mixed mating at higher density (Morgan et al., 2005; Peterson and Kay, 2015). We found lowest outcrossing at the range margin, but we also observed highest outcrossing at the most southern range-margin site (Fig. 4). While low density and greater spatial isolation among conspecifics at range margins can reduce outcrossing (Ghazoul, 2005), the same can also be true in areas with higher species diversity that may increase competition for pollinators (Vamosi et al., 2006). Higher-density forests can also restrict routes of pollen vectors and lead to declines in the diversity of tree-level pollen donors (González-Varo et al., 2009). Lower-latitude Florida mangroves consist of dense forests with *A. germinans* and two other mangrove species (*Rhizophora mangle*, *Laguncularia racemosa*), with evidence of competition for pollinators between *A. germinans* and *L. racemosa* (Landry, 2013). In contrast, higher-latitude range-margin Florida mangroves are patches of almost exclusively *A. germinans* individuals surrounded by lower-stature salt marsh species (Kangas and Lugo, 1990). A more open pollen-dispersal neighbourhood and limited interspecific competition at the higher-latitude range margin could facilitate inter-tree pollination among *A. germinans*, consistent with our observation of highest outcrossing and largest number of effective pollen donors at the most southern range-margin site. However, this greater potential for inter-tree pollination would be overshadowed farther north where limited numbers of conspecifics would provide very few potential pollen sources, as evidenced by the lowest number of effective pollen donors at the northern range limit.

At the range margin, tree-level outcrossing increased as conspecific cover increased. Lowest outcrossing ($t_a = 0.05$) at an *A. germinans* cover of 28.2 m^2 (7% of surrounding area covered by *A. germinans*) was more than ten times smaller than highest outcrossing ($t_a = 0.58$) at a cover of 173.2 m^2 (57% covered by *A. germinans*). We presume that continued proliferation of *A. germinans* at this range margin, forecast with climate change (Cavanaugh et al., 2019, 2015), will drive further mating system changes as increased conspecific density shifts mating systems towards greater outcrossing (Pannell, 2015). As *A. germinans* becomes more abundant, inter-individual distances will decline, presumably these denser patches will attract more pollinators, and tree-level outcrossing will increase. Hence, documented loss of genetic diversity towards *A. germinans* range limits (Kennedy et al., 2020a; Mori et al., 2015; Ochoa-Zavala et al., 2020, 2019; Sandoval-Castro et al., 2014), and for other *Avicennia* species (Arnaud-Haond et al., 2006; Binks et al., 2019; De Ryck et al., 2016; Maguire et al., 2000), may be the product of founder effects combined with density-dependent plastic shifts towards greater self-fertilisation. Areas that experience subsequent proliferation and immigration will then transition towards greater outcrossing and increased offspring genetic diversity, but will exhibit unique genetic signatures because of the disproportionate contribution of initial colonisers to the gene pool (e.g., Goldberg and Heine, 2017).

Plastic shifts towards greater self-fertilisation at range margins can be advantageous as a form of reproductive assurance (Hargreaves and Eckert, 2014) and tend to elevate seed production when pollinators or mates are unreliable (Morgan et al., 2005). However, these advantages may be offset by the genetic costs associated with inbreeding depression (e.g., reduced offspring survival and fertility) (Charlesworth and Willis, 2009). These costs are documented near the southern latitudinal limits of the Indo-West Pacific congener, *A. marina*, where smaller, less-outcrossed stands exhibited reduced reproductive success, reduced propagule size, and reduced seedling recruitment compared to larger stands (Hermansen et al., 2017). However, our finding of predominant self-fertilisation in *A. germinans* runs contrary to mangrove performance at the Atlantic Florida range margin. These mangroves have undergone proliferation and expansion for several decades (Cavanaugh et al., 2019, 2014; Rodriguez et al., 2016), with evidence from a co-occurring mangrove species, *Rhizophora mangle*, of precocious reproduction and increased propagule size (Dangremond and Feller, 2016) and greater reproductive success (Goldberg and Heine, 2017) compared to Florida conspecifics farther south. Characterisation of *A. germinans* mating system coupled with assessments of offspring performance at this range margin is needed to garner insights into the potential influence of mating system on these expanding populations.

4.3. *Considerations*

Mating system assessments are a balance between the number of progeny arrays and the number of offspring genotyped per progeny array. Here, we focussed our efforts more on genotyping larger numbers of offspring per progeny array. Our sampling design was shaped by our concern that reduced genetic variation towards this northern distributional limit could inhibit our ability to quantify outcrossing rates. This concern was valid as we found that a substantial tree-level effort ($n \geq 60$ propagules per tree) was likely needed to obtain reliable estimates. In addition, although the probability of an undetectable outcross event was low across most maternal trees, two trees at the northern range limit exhibited relatively high probabilities, with 9 and 12 potential undetected outcross events. Estimates based on smaller sample sizes per tree, comparable to research in the tropics (14–18 propagules on average per tree; Nettel-Hernanz et al., 2013), could have been overwhelmed by undetectable outcross events and potentially underestimated outcrossing at this northern range limit.

However, a greater investment of resources at the tree-level inevitably limited our ability to genotype progeny arrays from a larger number of maternal trees ($n = 2$–5 trees per collection site). Our sampling design may have provided robust tree-level estimates, but our estimates scaling up to the population level should be interpreted with caution. Sampling few maternal trees can bias these estimates towards tree-specific outcrossing rates that may not be representative of the entire collection site. Although our estimates (based on $n = 23$ maternal trees) are consistent with a substantial reduction in outcrossing compared to estimates from tropical conspecifics (based on $n = 22$ maternal trees; Nettel-Hernanz et al., 2013), and are not consistent with a systematic adaptive shift in mating system towards the northern range limit, further supported by evidence of density-dependent variation at the range margin, more intensive sampling of progeny arrays at each collection site is needed to obtain more definitive estimates of population-level mating system variation. Further research into mating systems at range margins, or areas with reduced genetic variation, should consider an investment in both more progeny arrays and large numbers of offspring per progeny array to generate reliable population-level outcrossing estimates.

5. Conclusions

This research suggests that ecological structure influences the mating system of the neotropical black mangrove, *A. germinans*, at varying spatial scales towards its expanding northern distributional limit on the Atlantic coast of Florida. First, subtropical Florida *A. germinans* exhibited significant reductions in outcrossing compared to tropical conspecifics, consistent with reductions in pollinator diversity and mangrove abundance with latitude. Second, the transition from mangrove to salt marsh dominance along Atlantic Florida may create a more open pollen-dispersal neighbourhood that is conducive to elevated *A. germinans* outcrossing, until conspecific abundances become too low towards the range limit. Third, greater inter-individual isolation at the range margin resulted in drastic reductions in tree-level outcrossing, consistent with density-dependent plastic shifts in mating system that we presume will continue to shift towards greater outcrossing as these mangroves continue to proliferate with forecast climate trends. Further research needs to evaluate the effect of *A. germinans* mating system variation on the survival and fitness of offspring and on the extent of population-level local adaptation at expanding distributional limits.

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CHAPTER 4

Hurricanes overcome migration lag and shape intraspecific genetic variation beyond a poleward mangrove range limit

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Hurricanes overcome migration lag and shape intraspecific genetic variation beyond a poleward mangrove range limit

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Abstract

Expansion of many tree species lags behind climate change projections. Extreme storms can rapidly overcome this lag, especially for coastal species, but how will storm-driven expansion shape intraspecific genetic variation? Do storms provide recruits only from the nearest sources, or from more distant sources? Answers to these questions have ecological and evolutionary implications, but empirical evidence is absent from the literature. In 2017, Hurricane Irma provided an opportunity to address this knowledge gap at the northern range limit of the neotropical black mangrove (*Avicennia germinans*) on the Atlantic coast of Florida, USA. We observed massive post-hurricane increases in beach-stranded *A. germinans* propagules at, and past, this species' present day range margin when compared to a previously surveyed nonhurricane year. Yet, propagule dispersal does not guarantee subsequent establishment and reproductive success (i.e., effective dispersal). We also evaluated prior effective dispersal along this coastline with isolated *A. germinans* trees identified beyond the most northern established population. We used 12 nuclear microsatellite loci to genotype 896 hurricane-driven drift propagules from nine sites and 10 isolated trees from four sites, determined their sources of origin, and estimated dispersal distances. Almost all drift propagules and all isolated trees came from the nearest sources. This research suggests that hurricanes are a prerequisite for poleward range expansion of a coastal tree species and that storms can shape the expanding gene pool by providing almost exclusively range-margin genotypes. These insights and empirical estimates of hurricane-driven dispersal distances should improve our ability to forecast distributional shifts of coastal species.

KEYWORDS
assignment analyses, dispersal kernels, long-distance dispersal, northernmost *Avicennia germinans*, range expansion, tropical cyclones

1 | INTRODUCTION

Species distributional shifts have become commonplace in response to anthropogenic climate change (Pecl et al., 2017; Scheffers et al., 2016). Yet, distributional responses of some species lag behind these changes (Lenoir & Svenning, 2015; Poloczanska et al., 2013). In particular, actual migration of many tree species lags behind projections based on current rates of climatic change and the consequent alterations in habitat suitability (Alexander et al., 2018; Bertrand et al., 2011; Gray & Hamann, 2013; Zhu, Woodall, & Clark, 2012).

This phenomenon, known as migration lag, is also forecast to continue or worsen in certain contexts (Gray & Hamann, 2013; Liang, Duveneck, Gustafson, Serra-Diaz, & Thompson, 2018; Prasad, Gardiner, Iverson, Matthews, & Peters, 2013), and can generate changes in forest structure, productivity, and function that have wide-reaching ecosystem-level consequences (Bonan, 2008; Solomon & Kirilenko, 1997).

Modelling efforts to project future distributional shifts are complicated by the fact that numerous factors may constrain plant migration (Corlett & Westcott, 2013; Svenning & Sandel, 2013). Dispersal limitation and niche-related constraints are the two principal factors attributed to migration lag, but temporal variation in these factors is not often considered (Renwick & Rocca, 2015). Episodic events, such as disturbance (Boisvert-Marsh, Périé, & de Blois, 2019; Lembrechts et al., 2016) or extreme climate events (Wernberg et al., 2013), can quickly overcome these migration constraints and lead to periods of rapid range shifts (Renwick & Rocca, 2015). As a result, migration rates are not constant over time, and instead, colonisation of new areas will often be limited to these transient periods of time (Zeigler & Fagan, 2014).

Extreme storm events (e.g., tropical cyclones, also known as typhoons or hurricanes) are one mechanism that can abruptly overcome migration constraints, in particular for coastal species (Lugo, 2008; Nathan et al., 2008). This is especially true for the Caribbean and Gulf of Mexico, a region frequently impacted by hurricanes (Walker, Lodge, Brokaw, & Waide, 1991) and forecast to experience more intense storms in the future (Murakami, Levin, Delworth, Gudgel, & Hsu, 2018). Numerous examples demonstrate how hurricanes are important vectors for the expansion of diverse taxa within the region, including fish (Johnston & Purkis, 2015), insects (Andraca-Gómez et al., 2015), and plants (Bhattarai & Cronin, 2014; Kendall, Battista, & Hillis-Starr, 2004). However, despite the well-recognised influence of hurricanes on distributions of species, we lack an understanding of how hurricane-driven expansion can impact variation within a species. Do hurricanes provide new recruits simply from the nearest sources? Or, do these high-energy storms provide the conditions necessary for a greater influence of long-distance dispersal? Answers to these questions have important implications for species ecology and evolution with climate change (Nadeau & Urban, 2019 and citations within). Moreover, quantitative analyses of plant dispersal driven by extreme meteorological events are absent from the literature (Nathan et al., 2008; Schurr et al., 2018), yet they would provide empirical estimates of dispersal distances that are needed to improve projections of future distributional shifts (Thuiller et al., 2008).

Hurricane Irma provided an opportunity to address this knowledge gap at the northern range limit of the neotropical black mangrove (*Avicennia germinans*) on the Atlantic coast of Florida, USA. This catastrophic storm, among the strongest and costliest Atlantic hurricanes ever recorded, devastated areas across the northern Caribbean and Florida (Cangialosi, Latto, & Berg, 2018), with massive impacts to coastal forest ecosystems (Branoff, 2020; Radabaugh et al., 2020; Ross et al., 2020). From 10–12 September, 2017, Hurricane Irma progressively weakened from a category 4 storm in the Florida Keys to a tropical storm in north Florida (Cangialosi et al., 2018). Although the storm weakened quickly over Florida, the wind field was extensive, with the strongest tropical-storm-force winds experienced on the northeast coast (Cangialosi et al., 2018; see Figure S1 for hurricane path and wind speeds).

In this study, we documented numbers of *A. germinans* propagules stranded on beaches along this northeast coast following Hurricane Irma and compared these numbers to those found previously during a nonhurricane year. We then used an extensive population-genetic data set from across the Florida *A. germinans* distribution (Kennedy, Preziosi, Rowntree, & Feller, 2020b) to determine the origin of these drift propagules and to quantify hurricane-driven dispersal distances. It is important to highlight that dispersal to these beaches (where propagules cannot establish) is not analogous to effective dispersal, which would consist of propagule transport plus successful establishment and subsequent reproductive success at the recipient location (Auffret et al., 2017). To assess effective dispersal, we also documented multiple newly-discovered *A. germinans* trees found past the most northern established population of this species. For these trees, we compared measures of their potential reproductive output to those of conspecifics at the present day range margin and used the same reference data set to determine their source of origin. We refer to these isolated *A. germinans* as “vagrant trees” throughout this publication. Vagrant trees provide evidence of prior effective dispersal along this coastline and insights into the potential filter that establishment may apply to the pool of available drift propagules.

Here, we asked: (a) Were drift-propagule densities higher following Hurricane Irma compared to a nonhurricane year?; (b) Are vagrant trees less reproductive than conspecifics at the present day range margin?; and (c) Where did drift propagules (i.e., hurricane-driven dispersal) and vagrant trees (i.e., prior effective dispersal) come from? Our findings provide novel insights into how hurricanes can overcome migration lag and shape intraspecific genetic variation in a coastal tree species and should improve our ability to forecast future distributional shifts.

2 | MATERIALS AND METHODS

2.1 | Model species

Mangroves are intertidal forests that provide ecosystem services of ecological and economic importance to coastal ecosystems worldwide (Lee et al., 2014). As coastal species, many mangrove forests are periodically impacted by hurricanes that can result in widespread tree mortality and shifts in forest structure (Krauss & Osland, 2020; Osland et al., 2020). Hurricane-driven dispersal of hydrochorous (water-dispersed) mangrove propagules is an important mechanism for forest regeneration following these episodic events and can continue for extended periods post-storm (Krauss & Osland, 2020), and may facilitate long-distance poleward expansion (Van der Stocken, Wee, et al., 2019).

The widespread neotropical black mangrove (*Avicennia germinans*) is the predominant mangrove species at northern distributional limits in the United States (Lonard, Judd, Summy, DeYoe, & Stalter, 2017). Atlantic Florida *A. germinans* inhabit protected estuaries with access to the ocean via a series of inlets. Propagules generally abscise from maternal trees in great numbers from late August through October, and some eventually exit these estuaries via inlets and become stranded on Atlantic coast beaches (I. C. Feller, personal observation). Long-distance dispersal of this species is possible as its propagules remain viable even after extensive flotation periods (Alleman & Hester, 2011b; Rabinowitz, 1978), further supported by genetic evidence for transoceanic dispersal (Cerón-Souza et al., 2015; Mori, Zucchi, Sampaio, & Souza, 2015; Nettel & Dodd, 2007). However, *A. germinans* propagules are generally retained within estuaries and most dispersal is restricted to short distances (Sousa, Kennedy, Mitchell, & Ordóñez, 2007), as evidenced by strong within-estuary spatial genetic structure (Cerón-Souza, Bermingham, McMillan, & Jones, 2012). Establishment success for *A. germinans* propagules is also inversely related to flotation time (Alleman & Hester, 2011b; Simpson, Osborne, & Feller, 2017).

Atlantic Florida mangroves decline in abundance with latitude and are eventually replaced by temperate salt-marsh vegetation at their northern range margin (Kangas & Lugo, 1990), where *A. germinans* exhibits considerable reductions in genetic variation compared to conspecifics farther south (Kennedy, Preziosi, Rowntree, & Feller, 2020a). The frequency and intensity of winter freezes has been linked to the northern extent of mangroves along this coastline (Cavanaugh et al., 2018; Osland et al., 2017), with mangrove proliferation (in particular, *A. germinans*) at this northern range margin for several decades due to a paucity of extreme freeze events (Cavanaugh et al., 2014, 2019; Osland et al., 2018). Further range expansion of *A. germinans* is forecast as winter freezes in the region become even less frequent with climate change (Cavanaugh et al., 2015, 2019).

2.2 | Beach surveys

We adapted methods used to quantify mangrove dispersal (Clarke, 1993; Sengupta, Middleton, Yan, Zuro, & Hartman, 2005) to survey Atlantic Florida *A. germinans* propagule densities on beaches adjacent to inlets. We surveyed two beaches at the established range margin of this species (29.71°N–29.91°N; Spalding, Kainuma, & Collins, 2010), three beaches past the range margin (~40–75 km to the north) where no established mangrove populations exist (30.40°N–30.70°N), and one lower-latitude beach within the mangrove-dominated continuous range core as a comparison (27.47°N; Figure 1). We performed equivalent surveys on 24–28 September, 2014 (a nonhurricane year) and 14–16 October, 2017 (five weeks after Hurricane Irma made landfall in Florida), except for the most northern beach that was only surveyed in 2017. At each survey site, we ran three to eight 100 m transects along the high tide line and counted all putatively-viable drift propagules found within 1 m of the transect line (i.e., decomposed propagules were noted, but not included in these counts). Numbers of transects varied depending on the length of the beach, and each transect line was separated from the next by 100 m. We

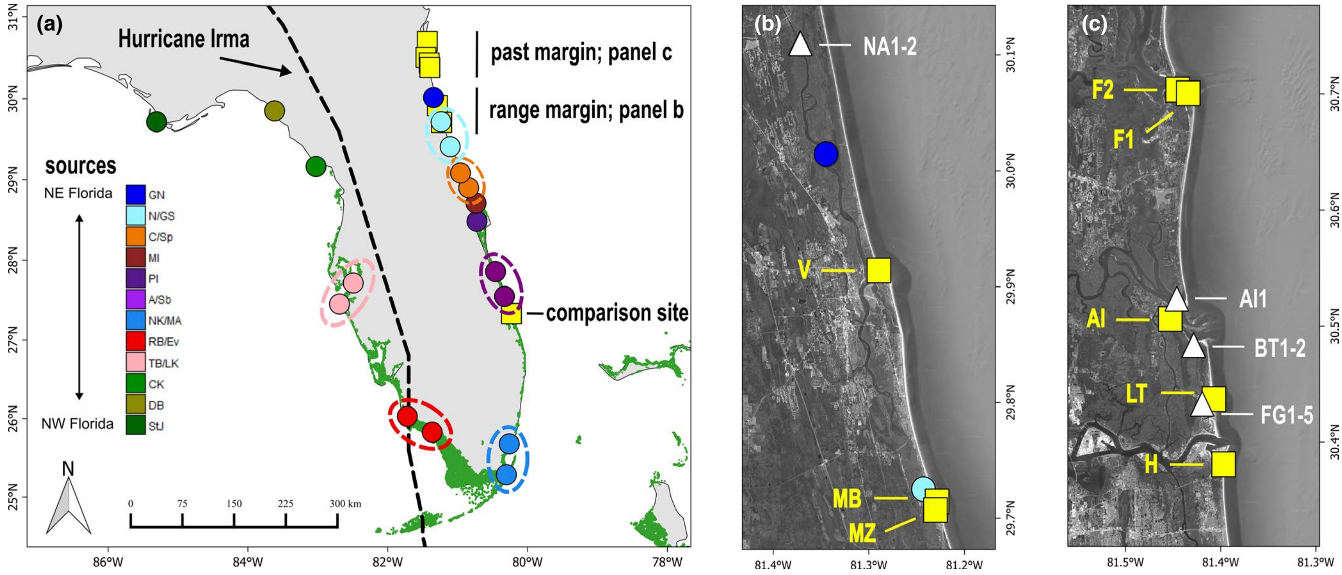

FIGURE 1 Survey sites for *Avicennia germinans* drift propagules (shown as yellow squares), vagrant *A. germinans* trees past the most northern established population of this species (shown as white triangles), and 12 potential source populations (shown as circles). (a) Path of Hurricane Irma shown with a dashed line (NOAA, 2017) and mangrove distribution shown in green (Giri et al., 2011). (b) Close-up of the location of three drift propagule survey sites, two vagrant trees, and the two northernmost Atlantic Florida source populations at the established *A. germinans* range margin (29.7°N–30.1°N). (c) Close-up of the location of five drift propagule survey sites and eight vagrant trees ~ 40–75 km past the present day *A. germinans* range margin where no established populations exist (30.4°N–30.7°N). Source population genetic data from Kennedy et al. (2020b) [Colour figure can be viewed at wileyonlinelibrary.com]

tested for differences in propagule densities between the two collection years ($n = 5$ sites per year) with a two-sample Fisher-Pitman permutation test, with 10^4 re-samplings, in the R-package coin (Hothorn, Hornik, van de Wiel, & Zeileis, 2008) in R v3.6.0 (R Core Team, 2013).

We collected all putatively-viable drift propagules during the 2017 post-Hurricane Irma beach surveys. Propagules from each survey site were stored together in plastic bags during field collections. For three of the six surveyed beaches, we subset samples into two collections that corresponded to areas within an inlet and those outside along the Atlantic Ocean (i.e., MZ and MB, F2 and F1, each respectively), or to areas separated by an inlet (i.e., H, LT; Table 1, Figure 1b, c). We haphazardly chose 100 propagules of all sizes from each of these nine collection sites for genetic analysis ($n = 900$ total propagules) and stored them at −20°C.

We assessed viability of these post-Hurricane Irma drift propagules with another subset of 100 propagules from each of the nine collection sites ($n = 900$ total propagules). We placed propagules in shallow, plastic trays with a thin layer of wet potting soil/sand until root radicles developed, and then transferred them to individual tree tubes (Ray Leach Cone-tainers, Stuewe and Sons Inc.; 2.5 cm diameter, 12.1 cm length; 49 ml volume) filled with a 2:1 mixture of commercial potting soil and sand. We placed tubes into racks of 100 and allowed propagules to grow in nonsaline, deionized water in flooded plastic tubs with the water depth maintained at 10 cm. All seedlings were grown, with no nutrient additions, in a walk-in environmental growth chamber at the Smithsonian Environmental Research Center (Maryland, USA), with chamber temperature and humidity maintained throughout this period (0:00–6:00 hours: 16°C, 6:00–12:00 hours: 21.5°C, 12:00–18:00 hours: 27°C, 18:00–0:00 hours: 21.5°C; 65% RH). We quantified the number of propagules that established and began growing true leaves (i.e., post-cotyledons).

2.3 | Vagrant tree surveys

We conducted coastal surveys by vessel over a 12-month period prior to Hurricane Irma (July 2016 to June 2017) along the intercoastal waterway between St. Augustine, Florida, and Cumberland Island, Georgia (29.9°N–31.0°N), an area past the most northern established *A. germinans* population. Surveys were conducted by trolling close to shore at low speed and visually searching for trees growing within the salt marsh. A leaf was collected from each discovered tree and dehydrated in silica gel for genetic analysis.

In August 2018, we revisited sites where we had found vagrant trees and identified four adult trees producing flowers. For these four trees, we measured height and potential reproductive output as mean inflorescence per terminal stem. We haphazardly selected a large mature branch, counted terminal stems (aiming for at least 60), and then counted how many terminal stems had inflorescence. We divided total inflorescence count by total terminal stem count to calculate inflorescence per terminal stem. We repeated this process three times for each tree and used mean values for analysis. We then selected three trees at the present day *A. germinans* range margin (29.727°N, 81.239°W) to compare with these four reproductive vagrant trees. We repeated measures of height, terminal stem counts, and inflorescence counts on these three range-margin trees. We selected these particular trees because they were larger than neighbouring trees, and presumably the most mature in the area. We tested for differences in mean inflorescence per terminal stem between the vagrant trees ($n = 4$) and range-margin trees ($n = 3$) with a two-sample Fisher-Pitman permutation test, with 10^4 resamplings, in the R-package coin (Hothorn et al., 2008).

TABLE 1 Hurricane-driven *Avicennia germinans* drift propagule survey sites and approximate over-water dispersal distances of unambiguously assigned propagules. Drift propagules were collected after Hurricane Irma at survey sites at or past the present day *A. germinans* range margin and from one lower-latitude comparison site. n_G, number of drift propagules genotyped; Assign, number of drift propagules unambiguously assigned to a source

							Dispersal distance (km)	
Site	Code	Location	Latitude	Longitude	n_G	Assign	Median	Range
Fort Clinch (inlet)	F2	Past margin	30.703	−81.445	99	53	109	74–109
Fort Clinch (beach)	F1	Past margin	30.701	−81.434	100	60	109	74–230
Amelia Island	AI	Past margin	30.506	−81.453	100	61	91	56–165
Little Talbot	LT	Past margin	30.437	−81.407	100	54	81	46–307
Hanna Beach	H	Past margin	30.381	−81.397	99	49	75	40–149
Vilano Inlet	V	Margin	29.914	−81.289	99	50	24	12–146
Matanzas Beach	MB	Margin	29.710	−81.227	99	67	2	2–124
Matanzas Inlet	MZ	Margin	29.708	−81.231	100	54	36	1–1,135[a]
Fort Pierce Inlet	FP	Comparison	27.475	−80.291	100	31	10	10–870[a]

Note: [a]Maximum Euclidean distance (the most conservative estimate of dispersal possible) differed considerably from maximum over-water distance. Maximum Euclidean distances were: MZ, 457 km; FP, 327 km.

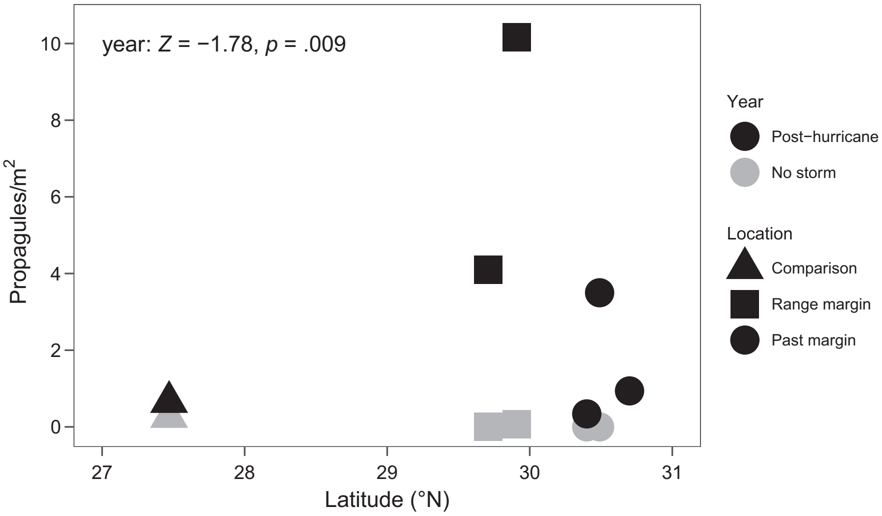

FIGURE 2 Massive increases in *Avicennia germinans* propagule dispersal to Atlantic Florida beaches following Hurricane Irma (2017) compared to a nonhurricane year (2014). Two beaches were surveyed at the established range margin (29.71°N–29.91°N), three beaches past the range margin (30.40°N–30.70°N), and one lower-latitude beach within the mangrove-dominated continuous range core as a comparison (27.47°N)

2.4 | DNA isolation and microsatellite genotyping

For drift propagules, we removed the cotyledons and isolated genomic DNA from 50 mg of frozen hypocotyl/radicle tissue with the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) following the standard protocol. For vagrant trees, we isolated genomic DNA from 20 mg of dried leaf tissue with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard protocol, with an extended incubation of 45 min. We genotyped all samples at 12 previously developed nuclear microsatellite loci (Cerón-Souza et al., 2012; Cerón-Souza, Rivera-Ocasio, Funk, & McMillan, 2006; Mori, Zucchi, Sampaio, & Souza, 2010; Nettel, Rafii, & Dodd, 2005) according to the protocol outlined in Kennedy, Sammy, Rowntree, and Preziosi (2020) for drift propagules and the protocol outlined in Kennedy et al. (2020a) for vagrant trees. We performed PCR on a Prime thermal cycler (Techne, Straffordshire, UK), analysed fragments on an Applied Biosystems 3730 DNA Analyser (Applied Biosystems, Foster City, California, USA) with LIZ 500 size standard, and scored alleles in the R-package Fragman (Covarrubias-Pazaran, Diaz-Garcia, Schlautman, Salazar, & Zalapa, 2016). We reamplified and regenotyped 5% of the drift propagule DNA samples to estimate a study error rate (Bonin et al., 2004), and did the same for all of the vagrant tree DNA samples to ensure we had the correct multilocus genotypes. Microsatellite genotype data are available at the Dryad digital repository (Kennedy, Dangremond, et al., 2020).

2.5 | Genetic assignments

For all genetic assignments, we used GENECLASS2 (Piry et al., 2004) to calculate (a) the probability that each individual (i.e., multilocus genotype) could belong to each potential source (based on the allele frequencies within each source) with the Paetkau, Slade, Burden, and Estoup (2004) Monte Carlo resampling method and 10^3 resampled individuals; and (b) source log-likelihood with the Rannala and Mountain (1997) Bayesian assignment method. For potential sources, we used a subset of an *A. germinans* reference data set with trees from 32 Florida collection sites that were genotyped at the same 12 microsatellite loci (n = 860 individuals; Kennedy et al., 2020b; Figure S2). Simulations demonstrate that the Rannala and Mountain (1997) Bayesian assignment method can achieve 100% correct assignments with ≥10 microsatellite loci, 30–50 sampled individuals from each of 10 populations, and interpopulation $F_{ST} = 0.1$, with reduced success at lower F_{ST} (Cornuet, Piry, Luikart, Estoup, & Solignac, 1999; Waples & Gaggiotti, 2006). Hence, we used intersite $F_{ST} \geq 0.1$ as a threshold to reduce the entire reference data set into 12 potential sources that encompass the entire Florida *A. germinans* distribution (Figure 1a; see Appendix S1, Table S1–S2 for detailed description).

Prior to our assignments of drift propagules and vagrant trees, we used known origin propagules to test the power of the assignment analyses and to define a priori confidence thresholds (similar to methods outlined in Sinclair et al., 2018). Known origin propagules were collected at three of the 12 potential sources (n = 50 propagules from a single tree for each site) and were genotyped at the same 12 microsatellite loci for a mating system study (Kennedy, Sammy, et al., 2020; Figure S2). As we knew the origin of these propagules, we used these assignment results to define the (a) p-value for source exclusion; and (b) acceptance threshold for unambiguous assignments based on the assignment score of the most-likely source (i.e., the relative likelihood of this source compared to all other sources; Piry et al., 2004) for subsequent genetic assignments of drift propagules and vagrant trees.

For each unambiguous assignment of a drift propagule or vagrant tree, we measured the approximate over-water dispersal distance from the assigned source in Google Earth Pro 7.3.2.5776. We measured dispersal as over-water distance because *A. germinans* propagules are hydrochorous (i.e., water is the predominant dispersal vector), but we cannot be certain how hurricane-force winds may have influenced propagule dispersal pathways. As such, we also measured Euclidean distances from assigned sources in the R-package geosphere (Hijmans, Williams, & Vennes, 2019) as the most conservative estimate possible of dispersal distance.

3 | RESULTS

3.1 | Beach surveys

In 2014, under nonstorm conditions, we found a range from 0 to 317 *A. germinans* propagules at five survey sites. We observed highest densities at the lower-latitude, within-range-core comparison site (27.47°N, 0.26 propagules/m^2), minimal propagule numbers at the two range-margin sites (29.71°N, 0.001 propagules/m^2; 29.91°N, 0.07 propagules/m^2), and no propagules at the two sites past the range margin (30.40°N, 30.49°N; Table S3; Figure 2). In 2017, five weeks after Hurricane Irma, we found a massive increase in propagule numbers, with a range from 329 to 3,048 *A. germinans* propagules at six survey sites from 27.47 to 30.70°N (Table S3). Propagules were present at higher densities post-hurricane (range: 0.34–10.16 propagules/m^2) than under nonstorm conditions (two-sample Fisher-Pitman permutation test, $Z = -1.78$, $p = .009$; Figure 2). We observed highest post-hurricane densities at the two range-margin sites (29.71°N, 4.10 propagules/m^2; 29.91°N, 10.16 propagules/m^2) where propagule numbers were orders of magnitude higher than under nonstorm conditions (29.71°N, 2014: 2 propagules, 2017: 2,462 propagules; 29.91°N, 2014: 97 propagules, 2017: 3,048 propagules; Table S3; Figure 2). Almost all post-hurricane drift propagules were viable as 99% (894 of 900) of those planted established and produced true leaves (i.e., post-cotyledons) in the environmental growth chamber.

3.2 | Vagrant tree surveys

We identified a total of 11 *A. germinans* (10 trees, one seedling) at four locations beyond the most northern established population of this species (Table 2; Figure 1b, c). From south to north, we first identified two trees on the Tolomato River (30.11°N) that are the documented northernmost *A. germinans* (Williams et al., 2014). Second, we found five trees at Fort George Inlet (30.43°N). Two larger trees were each isolated from the others by approximately 320 m and 1 km, while a third larger tree was located 40–55 m from two smaller trees. Third, we found two trees and one seedling, which was not sampled to avoid potential damage to its photosynthetic ability, on the north of Big Talbot Island (30.48°N). The larger of the two trees was located 25 m from the smaller tree. Fourth, we found one tree towards the south of Amelia Island (30.52°N).

The four vagrant trees that were reproductive (identified at three of the four locations) ranged in height from 183 to 280 cm, and the three trees sampled farther south at the range margin ranged in height from 340 to 400 cm (Figure 3a). Mean inflorescence per terminal stem was not statistically different between these vagrant trees and range-margin trees ($Z = 0.80$, $p = .57$), with a range of 0.44–1.05 inflorescence/stem and 0.41–0.67 inflorescence/stem, respectively (Figure 3b). One vagrant tree (FG3) was notably more fecund than the other measured trees (Figure 3b).

3.3 | Genotyping and genetic assignments

3.3.1 | Drift propagules

We genotyped a total of 896 drift propagules ($n = 99$–100 per survey site; Table 1). We observed a low estimated error rate of 0.97% (six errors out of 621 allele comparisons). The six individuals, that each exhibited a single locus-specific error, were reamplified a third time and we used the consensus genotype for assignment analyses.

The probability that each of the 150 known origin propagules belonged to their respective source ranged from 0.001 to 0.99 (mean = 0.44; Appendix S1). A total of 97% (146 of 150) of these propagules were correctly assigned to their source, with the highest assignment score for a misassigned propagule of 0.88 (Appendix S1; Table S4). Based on these results, we assigned the following confidence thresholds to subsequent assignment analyses of drift propagules and vagrant trees. We defined $p < .001$, the lowest probability observed, as the threshold to exclude a potential source. We also defined an assignment score ≥0.91 as the acceptance threshold for an unambiguous assignment, based on the highest score for a

TABLE 2 A total of 10 vagrant *Avicennia germinans* trees were found at four locations beyond the most northern established population of this species. Three of the 10 trees were unambiguously assigned to the most southern range-margin source (source code: N/GS) and three of the 10 trees were identified as putative offspring of adjacent larger trees. Assign, whether trees were unambiguously assigned to a source (or classified as putative offspring of adjacent trees); Source, assigned source (or putative parent); Dispersal distance, approximate over-water distance from the assigned source (Euclidean distance was identical)

Tree	Latitude	Longitude	Assign	Source	Dispersal distance (km)
AI1	30.523646	−81.446147	No		
BT1	30.483861	−81.428389	No		
BT2	30.483641	−81.428397	*offspring*	*[BT1]*	
FG1	30.421436	−81.422169	No		
FG2	30.430226	−81.421208	Yes	N/GS	80
FG3	30.432978	−81.419474	Yes	N/GS	80
FG4	30.432814	−81.419853	*offspring*	*[FG3]*	
FG5	30.433205	−81.420011	*offspring*	*[FG3]*	
NA1[a]	30.110310	−81.371722	No		
NA2[a]	30.109874	−81.371555	Yes	N/GS	45

Note: [a]Documented northernmost *A. germinans* (Williams et al., 2014).

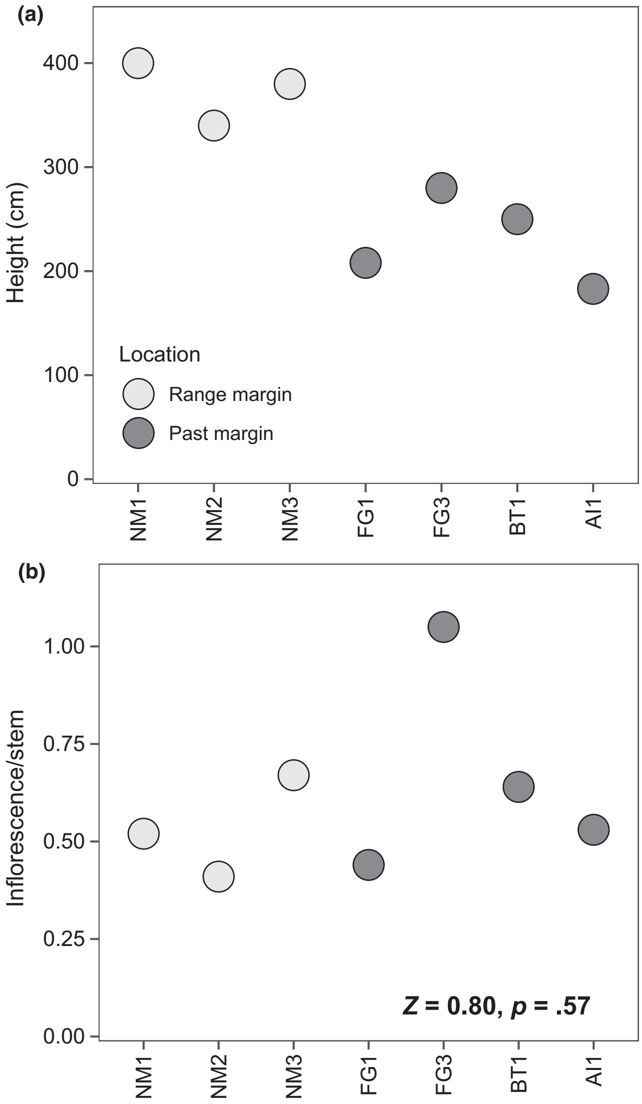

FIGURE 3 Four vagrant *Avicennia germinans* trees, discovered past the most northern established population of this species, are as potentially reproductive as three mature trees at the present day range margin of this species. (a) Heights and (b) mean inflorescence per terminal stem (i.e., potential reproductive output) of three range-margin trees (29.72°N; shown in light grey) and four vagrant trees (30.43°N–30.52°N; shown in dark grey)

misassigned, known origin propagule. This conservative acceptance threshold indicates that a multilocus genotype must be at least 10x more likely to belong to the assigned source than to any other potential source.

None of the 896 drift propagules were excluded from all 12 potential sources (i.e., $p > .001$ for at least one source; Table S5), which suggests that all the genotyped drift propagules were sourced from Florida populations. We unambiguously assigned 56% of drift propagules from the eight survey sites at or past the range margin (448 of 796), with a range within-site from 49% (site code: H) to 68% (site code: MB; Table 1, Table S6). A total of 89% (400 of 448) of these unambiguous assignments were sourced from the range margin (source code: N/GS, GN), 9% (40 of 448) were sourced from the nearest within-range-core source (source code: C/Sp; over-water distance: 75–185 km) and < 2% (8 of 448) were transported over longer distances from the Atlantic (East) and Gulf (West) coasts of Florida (over-water distance: 124–1,135 km; Table 1, Figure 4). Each of these eight survey sites exhibited similar assignment patterns (i.e., 86%–100% assigned to range-margin sources), except for the most southern range-margin site (site code: MZ; Figure 4). Almost half of the unambiguous assignments at MZ (46%; 25 of 54) were sourced to the nearest within-range-core source (39%) or via longer distances (7%; over-water distance: 225–1,135 km; Figure 4). We unambiguously assigned fewer drift propagules at the lower-latitude, within-range-core comparison site (31 of 100; Table 1; Table S6), but observed a similar pattern to the more northern survey sites. Most propagules (68%; 21 of 31) were assigned to the nearest source (source code: A/Sb), with 16% (5 of 31) from adjacent sources (source code: NK/MA, PI), and 16% (5 of 31) via longer distances from sources on the Gulf (West) coast of Florida (over-water distance: 440–870 km; Table 1, Figure 4). Euclidean distances across all unambiguous assignments (n = 479; median: 74 km, range: 1–457 km) were nearly identical to over-water dispersal distances (n = 479; median: 74 km, range: 1–1,135 km), except for the limited number (8 of 479) of dispersal events at the longest distance intervals (Table S6; Figure S3). These eight dispersal events were considerably shorter based on Euclidean distance (range: 184–457 km) compared to over-water distance (range: 434–1,135 km; Figure S3).

Applying confidence thresholds to assignment analyses (as we did here) reduces the risk of incorrect assignments, but also increases the number of unassigned individuals (Roques, Duchesne, & Bernatchez, 1999). Across all nine survey sites, 417 of 896 drift propagules (47% of all samples) were not unambiguously assigned to a source. Yet, if no acceptance threshold is used and sources are assigned simply based on the lowest log-likelihood, assignment results were equivalent to those presented here (Table S7).

3.3.2 | Vagrant trees

Each of the 10 vagrant trees exhibited consistent multilocus genotypes across two, independent PCR. In addition, multilocus genotypes were consistent with two smaller trees at Fort George Inlet (30.43°N) and one smaller tree at Big Talbot Island (30.48°N) being offspring of adjacent larger trees (Table 2; Appendix S2). As such, these putative offspring were not included in assignment analyses.

None of the seven vagrant trees included in assignment analyses were excluded from all 12 sources, with highest probabilities from the two range-margin sources and the nearest within-range-core source (Table S5). We unambiguously assigned three of the seven vagrant trees (from two of the four sampled locations), and all three trees were sourced to the most southern range-margin source (source code: N/GS; Table 2; Table S6). The first and second most-likely sources for the remaining four vagrant trees were a combination of the two range-margin and nearest within-range-core sources (Table S6). Hence, although we could not unambiguously

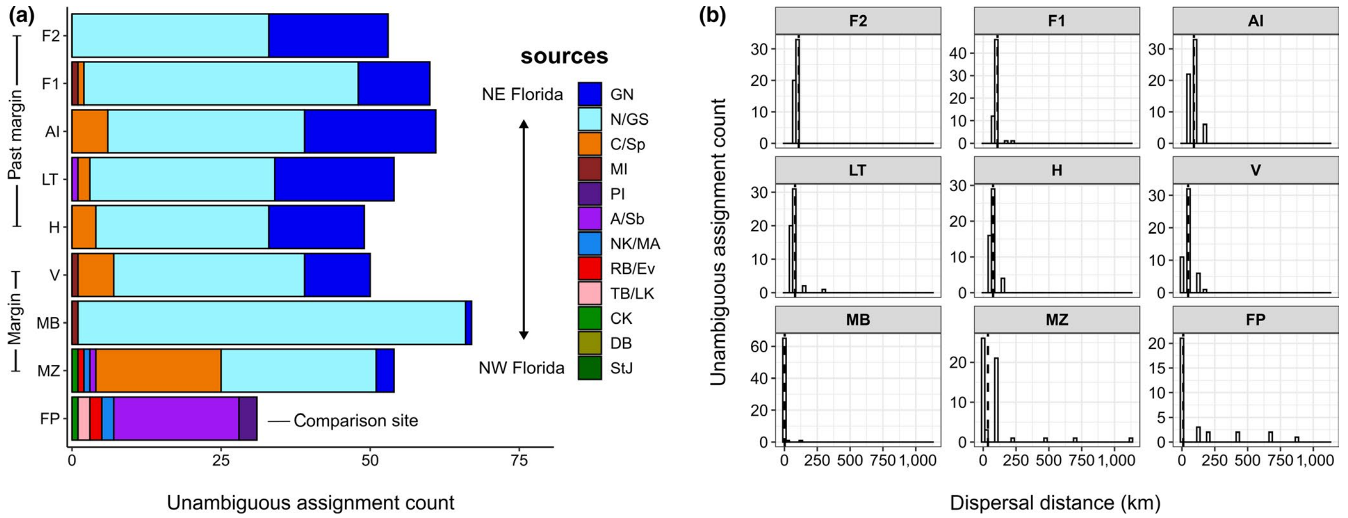

FIGURE 4 Hurricane-driven *Avicennia germinans* drift propagules were almost exclusively from the nearest sources. (a) Unambiguous assignment results for drift propagules from three survey sites at the present day *A. germinans* range margin (29.7°N–29.9°N), from five survey sites past the range margin (30.3°N–30.7°N), and from one lower-latitude comparison site within the continuous range core (27.4°N). (b) Histograms of approximate over-water dispersal distances for unambiguously assigned propagules at each of the survey sites. Median distance is shown with a dashed line and bin width is 25 km. Note: *y*-axes vary among survey sites. Refer to Figure 1 for geographic locations of the 12 potential sources from northeast Florida (Atlantic coast) to northwest Florida (Gulf coast). Refer to Table S6 and Figure S3 for Euclidean dispersal distances [Colour figure can be viewed at wileyonlinelibrary.com]

assign a source, these four trees also came from the nearest potential sources.

4 | DISCUSSION

Migration lag in trees may be the product of multiple constraints, but episodic events can quickly overcome these constraints and lead to transient periods of rapid range shifts (Renwick & Rocca, 2015). This study highlights how hurricanes create the conditions needed to drive range expansion at a northern distributional limit of the neotropical black mangrove (*Avicennia germinans*) and can shape patterns of genetic variation in expanding populations of this species. These insights, along with empirical estimates of hurricane-driven dispersal distances, should improve our ability to forecast future distributional shifts of this species, and other coastal species often impacted by extreme storm events.

4.1 | Hurricanes are a vector of range expansion

Climate models predict that, at a global scale, storm numbers may decline in the future, but that the strongest storms will become more intense and that sea level rise will exacerbate storm surge effects (Walsh et al., 2016 and citations within). In particular, a greater prevalence of major hurricanes (≥category 3) is forecast in the Caribbean and Gulf of Mexico as the tropical North Atlantic continues to warm (Murakami et al., 2018). These trends may enhance long-distance dispersal of mangrove propagules and facilitate poleward range expansion (Van der Stocken, Carroll, Menemenlis, Simard, & Koedam, 2019; Van der Stocken, Wee, et al., 2019). After Hurricane Irma, we documented large numbers of *A. germinans* drift propagules along Atlantic Florida beaches at, and past, the present day range margin of this species. Provision of propagules to these areas, where we found limited or no propagules in 2014 (a nonhurricane year), suggests that hurricanes function as episodic events that are necessary for these estuarine mangroves to expand poleward. Consistent with this conclusion, expansion patterns of invasive species within this region have been linked to hurricane frequency (Bhattarai & Cronin, 2014; Johnston & Purkis, 2015). However, we only documented one nonhurricane (2014) and one hurricane (2017) year and, due to the timing of Hurricane Irma, our beach surveys were ~2.5 weeks later in 2017. Annual and seasonal differences in propagule production and release could account for some of the variation in drift-propagule densities between our two sampling periods, although only a relatively slight increase in drift-propagule density at the lower-latitude comparison site in 2017 compared to 2014 suggests that these differences may not have had a substantial impact. Instead, *A. germinans* propagules are generally retained within estuaries (Sousa et al., 2007), as are those of a congener (*A. marina*; Clarke, 1993; Van der Stocken, Vanschoenwinkel, De Ryck, & Koedam, 2018), which would explain why we observed comparatively limited numbers of beach-stranded propagules (and no propagules past the present day range margin) under nonstorm conditions. Extreme high-water events, associated with storm surge, are also needed to disperse mangrove propagules over dense salt-marsh vegetation to enable expansion inland (Peterson & Bell, 2012; Rodriguez, Feller, & Cavanaugh, 2016). Therefore, for mangrove range expansion to occur, the pulse of energy and unusually high-water levels provided by hurricanes seem to be the prerequisite needed to flush propagules out of estuaries in large numbers and into more poleward, salt-marsh-dominated areas.

We observed highest drift-propagule densities at the lower-latitude comparison site during a nonhurricane year. As Florida mangroves decline in abundance with latitude (Osland et al., 2017), the number of propagules dispersed out of estuaries may generally be dictated by neighbouring mangrove abundance (i.e., larger forests produce greater numbers of propagules), consistent with previous mangrove dispersal studies (Sengupta et al., 2005; Van der Stocken et al., 2018). In contrast, following Hurricane Irma, much higher drift-propagule densities were observed at the range margin, where far fewer mangroves exist. This difference suggests that dispersal patterns can vary depending on the unique attributes of each storm. Hurricane Irma produced greater storm surge along northeast Florida compared to areas directly south (Cangialosi et al., 2018), which may explain why greater numbers of drift propagules were deposited on beaches at the range margin compared to the lower-latitude comparison site. Numbers of drift propagules and dispersal direction may be influenced by variation in hurricane trajectory and intensity (Krauss & Osland, 2020), as well as ocean circulation patterns (Kennedy et al., 2017) and latitudinal variation in the timing of propagule release (Van der Stocken, López-Portillo, & Koedam, 2017). Continued monitoring along expected hurricane pathways is needed to better quantify the influence of these factors and to better predict dispersal patterns associated with future storm events.

4.2 | Expanding genotypes are from the nearest sources

Where do hurricane-dispersed propagules come from? Extreme storm events have the potential to drive transoceanic dispersal (Carlton et al., 2017; Waters, King, Fraser, & Craw, 2018); however, we found that the vast majority of drift propagules collected after Hurricane Irma came from the nearest sources. Hence, hurricanes may provide an expanding gene pool that consists of a much-reduced representation of genetic variation within a species, although even limited long-distance dispersal (as observed here) can lead to substantial increases in genetic variation (Bialozyt, Ziegenhagen, & Petit, 2006). Migration models for terrestrial tree species find a similar pattern, with colonisation past present day distributions mostly influenced by the species' abundance at the range limit (Iverson, Schwartz, & Prasad, 2004). Yet, while forecast migration of these terrestrial trees for the next 100 years is mostly restricted to 10–20 km (Iverson et al., 2004; Prasad et al., 2013), we found dispersal to beaches >100 km from range-margin sources after a single storm event and vagrant trees 80 km from their assigned source. This contrast is consistent with longer transport potential for coastal species (Nathan et al., 2008) and highlights that coastal range expansions have the potential to occur rapidly over large spatial scales.

A leptokurtic dispersal kernel, where most dispersal occurs over short distances, is consistent with genetic analyses across mangrove species (Van der Stocken, Wee, et al., 2019 and citations within). Local sources are also thought to provide propagules for mangrove forest regeneration after storms (Krauss & Osland, 2020). This pattern is consistent with restricted gene flow in taxa from spatially-discrete estuarine habitats (Bilton, Paula, & Bishop, 2002). Remarkably, the proportions of unambiguous assignments from our eight survey sites at or past the *A. germinans* range margin (89% from range margin, 9% from nearest range core, 2% longer distances) were similar to the proportions of propagules from a congener (*A. marina*) collected within different zones of an East African estuary (83% adjacent to forest, 16% near estuary exit, <1% outside estuary; Van der Stocken et al., 2018). Dispersal patterns of propagules from *Avicennia* species may not change substantially whether within tidal estuaries or following extreme storm events (i.e., most propagules remain closest to their source, very few travel longer distances); however, we found that storms create a transient shift in the dispersal kernel towards massively greater spatial scales (from metres to kilometres). Further genetic research that determines the origins of drift propagules found during nonstorm periods will be needed to better quantify the effect of these storms on dispersal distances.

Although most dispersal was sourced to the nearest populations, we did find a greater proportion of dispersal from more distant sources at the most southern range-margin survey site and at the lower-latitude comparison site (maximum over-water distance: 1,135 km, 870 km, respectively). Euclidean distances were considerably shorter (maximum distance: 457 km, 327 km, respectively), but may be overly conservative as propagules would need to be dispersed overland by wind from the Gulf (West) coast to the Atlantic (East) coast of Florida. Of the nine survey sites in this study, these two sites with greater proportions of long-distance dispersal are the most geographically proximate to larger mangrove forests south of the range margin. Greater geographical isolation from these lower-latitude forests may explain the lack of dispersal over longer distances to the more northern survey sites. In addition, our observations may reflect a density-dependent process, where an overwhelming number of local propagules further dilutes the already small proportion of propagules from more distant sources (Waters, Fraser, & Hewitt, 2013). This possibility may explain why we observed numerous unambiguous assignments to the adjacent range-margin source, but almost no evidence of longer distance dispersal, at the survey site (code: MB) that borders the most southern range-margin survey site.

Almost all of the collected drift propagules were viable, but beach-stranded propagules are not analogous to effective dispersal (Auffret et al., 2017). Our documentation of vagrant *A. germinans* trees provides evidence of previous successful establishment beyond the most northern established population, and these trees were exclusively sourced to range-margin (or possibly the nearest range core) populations. Effective dispersal only from the nearest potential sources may simply be the result of the much greater local supply of propagules from these sources, or could indicate that post-dispersal establishment applies a filter to the pool of available drift propagules based on shorter flotation times for range-margin propagules (Alleman & Hester, 2011b; Simpson et al., 2017) or on local adaptation to environmental conditions (Cruz et al., 2019).

Intraspecific variation is an important consideration when formulating conservation strategies and adaptation planning with climate change (Benoliel Carvalho, Torres, Tarroso, & Velo-Antón, 2019; Chakraborty, Schueler, Lexer, & Wang, 2019). Our findings suggest that hurricanes may be a prerequisite for poleward range expansion of a coastal tree species and that these storm events can shape the expanding gene pool by providing new recruits almost exclusively from range-margin sources. Expansion of range-margin genotypes, that are presumably better adapted to climatic extremes experienced beyond the current distribution, may facilitate species range expansion with climate change (Rehm, Olivas, Stroud, & Feeley, 2015). Limited immigration from range-core sources may also expedite adaptation to these marginal environments (Kawecki, 2008). However, range margins may exhibit reduced genetic variation compared to more central portions of a distributional range (Pironon et al., 2017). Considerable reductions in genetic variation are documented in these Atlantic Florida range-margin *A. germinans* compared to conspecifics farther south (Kennedy et al., 2020a). Further reductions in genetic variation due to founder effects and minimal gene flow from more diverse sources could constrain evolutionary responses and reduce fitness in these expanding populations (Nadeau & Urban, 2019 and citations within). For instance, less genetically-diverse mangrove species were less resilient to extended flooding, analogous to forecast impacts of sea level rise (Guo et al., 2018). Yet, we found that vagrant *A. germinans* trees were not simply surviving past this species' range limit, but instead, appear to be thriving. Vagrant trees were as potentially reproductive as range-margin conspecifics and we found genetic evidence that two individuals had successfully reproduced, consistent with evidence of precocious reproduction (Dangremond & Feller, 2016) and greater reproductive success (Goldberg & Heine, 2017) in a co-occurring range-margin mangrove, *Rhizophora mangle*. Range-margin *A. germinans* also exhibit shifts towards more cold-tolerant leaf traits (Cook-Patton, Lehmann, & Parker, 2015; Kennedy et al., 2020a) and their seedlings can survive climatic conditions well past their present day range limit (Hayes et al., 2020). Further work is needed to understand how intraspecific variation at, and past, this expanding range margin may shape population-level responses to future climate change (e.g., Cruz et al., 2019, 2020).

4.3 | Insights for modelling range shifts

Plant dispersal and migration patterns are one of the most significant uncertainties for forecasting future distributional shifts with climate change (Thuiller et al., 2008 and citations within). Modelling efforts are further complicated as migration rates are not constant over time because of the transient nature of dispersal and colonisation (Zeigler & Fagan, 2014). Research that identifies mechanisms that overcome migration constraints and lead to episodic range shifts will enhance our understanding of why many species lag behind climate change projections and will advance efforts to forecast future range shifts (Renwick & Rocca, 2015).

Mangrove distributional limits are controlled by climatic thresholds in minimum temperature and/or precipitation (Cavanaugh et al., 2018; Osland et al., 2017). Forecast warming trends indicate that Atlantic Florida mangroves at their present day range margin will rarely be constrained by periodic freeze events into the future, and as a result will permanently replace neighbouring salt-marsh vegetation (Cavanaugh et al., 2015, 2019). Based on these climate projections, the distribution of *A. germinans* is forecast to expand northward ~160 km over the next 50 years (3.2 km per year; Cavanaugh et al., 2015). However, as highlighted by Cavanaugh et al. (2015), release from this climatic constraint alone does not guarantee range expansion if not accompanied by propagule dispersal and an availability of suitable habitat. Here, we found that *A. germinans* poleward expansion is probably dispersal limited under "normal" conditions, and that episodic extreme storm events are needed to move propagules past the contemporary range limit. In line with this conclusion, the present day Atlantic Florida mangrove range margin is experiencing rapid range infilling (Simpson, Stein, Osborne, & Feller, 2019), but our coastal surveys indicate very little in terms of poleward expansion. Therefore, Atlantic Florida mangrove expansion will presumably not be a progressive march poleward, and instead, this process will probably occur via a series of starts and stops driven by propagule dispersal out of estuaries and over longer distances following extreme storm events.

Incorporating biological mechanisms into predictive models should improve our ability to forecast changes in biodiversity with climate change (Urban et al., 2016). Mechanistic models can provide more realistic predictions and possibly greater transferability across geographic regions, although many uncertainties and shortcomings still remain (Yates et al., 2018). Cavanaugh et al. (2015) took the first step in this direction with their incorporation of a mechanistic predictor (i.e., freeze degree days) to forecast mangrove range expansion along Atlantic Florida, with a fully-mechanistic model of mangrove distributions as a possible next step. Our research suggests that, in addition to physiological thresholds, including hurricane projections (e.g., storm frequencies, trajectories, intensities) is essential for more realistic forecasts of Atlantic Florida mangrove expansion, as poleward dispersal will probably be restricted to these transient windows. We also provide empirical estimates of hurricane-driven dispersal distances, measured as both over-water distance and Euclidean distance (the most conservative estimate possible), that are needed to parameterise these models (Van der Stocken, Carroll, et al., 2019). Further work is necessary to understand how expansion from a restricted set of sources may shape adaptive capacity in newly-colonised populations, which can also be incorporated into future models (Bush et al., 2016). However, physiological thresholds and dispersal are not the only constraints to mangrove expansion. Smaller-scale, niche-related constraints also influence mangrove establishment, survival, and growth (Krauss et al., 2008), including hydroperiod and salinity (Alleman & Hester, 2011a; Coldren & Proffitt, 2017), salt-marsh interactions (Chen, Blaze, Smith, Peng, & Byers, 2020; Guo, Zhang, Lan, & Pennings, 2013; Pickens, Sloey,

& Hester, 2019; Simpson, Feller, & Chapman, 2013), predation or herbivory (Devaney, Lehmann, Feller, & Parker, 2017; Langston, Kaplan, & Angelini, 2017), and nutrient availability (Dangremond, Simpson, Osborne, & Feller, 2019). In closing, multiple interacting factors, at both large and small spatial scales, will influence mangrove range expansion (Rogers & Krauss, 2018) and need to be considered to better anticipate future changes in these coastal ecosystems with climate change.

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AUTHOR CONTRIBUTIONS

J.P.K., E.M.D., M.A.H., and I.C.F. designed and performed the research. J.P.K. analysed the data. R.F.P., J.K.R., and I.C.F. supervised the research. E.M.D., M.A.H., and I.C.F. drafted sections of the manuscript. J.P.K. wrote the final manuscript with input from all coauthors.

DATA AVAILABILITY STATEMENT

Microsatellite genotype data are publicly available on Dryad: https://doi.org/10.5061/dryad.2280gb5pd

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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CHAPTER 5

Evidence for the genetic similarity rule at an expanding mangrove range limit

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RESEARCH ARTICLE

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Evidence for the genetic similarity rule at an expanding mangrove range limit

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Abstract

Premise: Host-plant genetic variation can shape associated communities of organisms. These community-genetic effects include (1) genetically similar hosts harboring similar associated communities (i.e., the genetic similarity rule) and (2) host-plant heterozygosity increasing associated community diversity. Community-genetic effects are predicted to be less prominent in plant systems with limited genetic variation, such as those at distributional range limits. Yet, empirical evidence from such systems is limited.

Methods: We sampled a natural population of a mangrove foundation species (*Avicennia germinans*) at an expanding range limit in Florida, USA. We measured genetic variation within and among 40 host trees with 24 nuclear microsatellite loci and characterized their foliar endophytic fungal communities with internal transcribed spacer (ITS1) gene amplicon sequencing. We evaluated relationships among host-tree genetic variation, host-tree spatial location, and the associated fungal communities.

Results: Genetic diversity was low across all host trees (mean: 2.6 alleles per locus) and associated fungal communities were relatively homogeneous (five sequence variants represented 78% of all reads). We found (1) genetically similar host trees harbored similar fungal communities, with no detectable effect of interhost geographic distance. (2) Host-tree heterozygosity had no detectable effect, while host-tree absolute spatial location affected community alpha diversity.

Conclusions: This research supports the genetic similarity rule within a range limit population and helps broaden the current scope of community genetics theory by demonstrating that community-genetic effects can occur even at expanding distributional limits where host-plant genetic variation may be limited. Our findings also provide the first documentation of community-genetic effects in a natural mangrove system.

KEYWORDS

associated communities, *Avicennia germinans*, black mangrove, community genetics, endophytic fungi, foundation species, intraindividual heterozygosity, plant genetic variation

Intraspecific diversity can shape the ecological dynamics of communities and entire ecosystems (Raffard et al., 2019). For instance, a central principle of community genetics is that genetic variation within a host plant can influence the structure and diversity of associated communities of organisms (Whitham et al., 2003). Empirical evidence of community-genetic effects is found across diverse systems, including terrestrial forests with low (Whitham et al., 2006) and high (Zytynska et al., 2011) species diversity, agricultural landscapes (Stevenson et al., 2017), and aquatic systems (Jormalainen et al., 2017). This pattern may be most prominent in systems dominated by a limited number of plant foundation species (Whitham et al., 2006), which define ecosystems with their physical structure, and provide resources that directly influence diverse community assemblages (Ellison et al., 2005).

Community-genetic effects are measured both in terms of host-plant genetic similarity and diversity, plus spatial effects need to also be considered. First, genetically similar host plants may harbor similar associated communities, a pattern known as the genetic similarity rule (Bangert et al., 2006a, 2006b; Barbour et al., 2009; Kagiya et al., 2018). Second, increased genetic diversity at the population level may lead to concomitant increases in associated species diversity (Wimp et al., 2004; Crutsinger et al., 2006; Johnson et al., 2006). Similar patterns are also found when considering the genetic diversity of individual host plants (i.e., heterozygosity) (Tovar-Sánchez et al., 2013; Valencia-Cuevas et al., 2018). This extension of community genetics theory is in line with extensive research on the link between intraindividual heterozygosity and fitness (reviews by Hansson and Westerberg, 2002; Szulkin et al., 2010). Last, in addition to host genetic variation, the spatial context of host plants, including their relative position in relation to neighboring conspecifics and variation in environmental conditions, needs to also be considered because spatial effects can prove more influential (Tack et al., 2010; Gossner et al., 2015; Barbour et al., 2019; but see Bangert et al., 2006a; Lamit et al., 2015).

Community-genetic effects may also vary with the extent of genetic variation present in the host population. Plant systems with limited genetic variation are predicted to exhibit less prominent effects and, instead, environmental variation will exhibit a stronger effect on associated community structure (Bangert et al., 2006b). However, only one study has provided empirical evidence from such systems. Pohjanmies et al. (2015) documented that genetic variation within a tree foundation species correlates with the structure and diversity of associated herbivore communities at a distributional range limit. Range limits may exhibit limited genetic variation (Pironon et al., 2017) and are shifting for many species with anthropogenic climate change (Pecl et al., 2017). Further assessments of relationships between host-plant genetic variation and associated communities at range limits—especially those where foundation species are undergoing climate-driven range shifts—could help broaden the current scope of community genetics theory and provide insights into the ecological and evolutionary processes shaping these dynamic systems.

In this study, we evaluated relationships between genetic variation within a mangrove foundation species at its expanding distributional range limit, and the structure and diversity of associated foliar endophytic fungal communities. Mangroves are (sub)tropical, intertidal, woody plants that provide vital ecosystem services to coastal habitats worldwide (Lee et al., 2014). Mangrove forests consist of relatively few tree species (Alongi, 2009) and as such, intraspecific differences may be particularly influential in shaping ecological dynamics in these systems (Farnsworth, 1998). Numbers of mangrove species are further reduced toward climate-sensitive, poleward range limits where generally only one predominant species exists (Osland et al., 2017) and often genetic variation is limited (e.g., Pil et al., 2011; De Ryck et al., 2016; Kennedy et al., 2017; Binks et al., 2019; Ochoa-Zavala et al., 2019).

Mangrove systems harbor numerous associated communities of both terrestrial and marine origin (Nagelkerken et al., 2008), including diverse fungal communities found on or within multiple mangrove tissues (e.g., Gilbert et al., 2002; Arfi et al., 2012; de Souza Sebastianes et al., 2013; Lee et al., 2019). Fungal endophytes are ubiquitous inhabitants within plant tissues, obtain shelter and nutrition from their host plant, and may influence plant health and function (Arnold, 2007; Porras-Alfaro and Bayman, 2011). Endophytic fungi in leaves and twigs vary among host genotypes of diverse plant species (Elamo et al., 1999; Pan et al., 2008; Lamit et al., 2014; Griffiths et al., 2020); however, whether intraspecific genetic differences among mangrove host trees correlates with the structure and diversity of their associated fungal communities remains unanswered.

We sampled a natural population of neotropical black mangrove (*Avicennia germinans*) at a northern range limit on the Atlantic coast of Florida, USA. At this range limit, *A. germinans* is the predominant mangrove species (Lonard et al., 2017), exists as discrete patches within a landscape dominated by salt marsh vegetation (Kangas and Lugo, 1990), and exhibits reduced genetic variation (Kennedy et al., 2020b) and elevated levels of self-fertilization (Kennedy et al., 2021). A lack of extreme freeze events for several decades has been linked to *A. germinans* proliferation (Cavanaugh et al., 2014; Osland et al., 2018) and further expansion is forecast with climate change (Cavanaugh et al., 2015, 2019), which may have wide-reaching effects on these coastal ecosystems (Kelleway et al., 2017). We (1) genotyped *A. germinans* host trees with 24 nuclear microsatellite loci, (2) characterized communities of endophytic fungi in their leaves with ITS1 gene amplicon sequencing, and (3) accounted for potential spatial effects with host-tree global positioning system (GPS) coordinates and interhost geographic distances. We asked two questions: (1) Do interhost genetic similarity and interhost geographic distance correlate with similarity among associated endophytic fungal communities? (2) Do host-tree heterozygosity and host-tree absolute spatial location correlate with alpha diversity of the associated endophytic fungal community?

MATERIALS AND METHODS

Study design

On October 9, 2017, we sampled from and collected GPS coordinates for 40 mature *A. germinans* trees, all approximately the same height (~2 m), at a single collection site (29.7284°N, 81.2425°W) near the Atlantic Florida range limit. Mangrove area has progressively increased for several decades at this site (Rodriguez et al., 2016), which is flanked by a brackish lagoon to the west and a fringe of terrestrial hammock forest to the east. Salinity during this time of the year (September–November) increases from west to east along the site (38–67‰), then decreases adjacent to the terrestrial fringe (40‰) (Guana Tolomato Matanzas National Estuarine Research Reserve, unpublished data; Figure 1). Our sampling area covered ~0.1 km^2, which

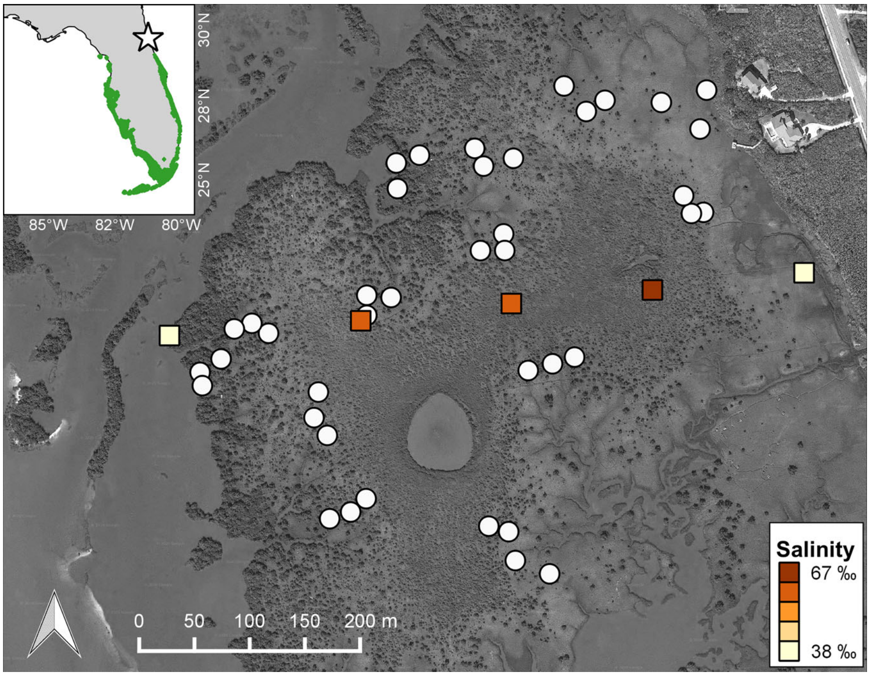

FIGURE 1 Collection site at the Atlantic Florida, USA, northern distributional limit of *Avicennia germinans* with locations of the 40 sampled *A. germinans* trees. This site is flanked by a brackish lagoon to the west and a fringe of terrestrial forest to the east. Soil salinities (‰) are mean values measured between September and November (2012–2017) (Guana Tolomato Matanzas National Estuarine Research Reserve, unpublished data). Upper panel shows the location of the collection site (with a star) and the Florida mangrove distribution in green (Giri et al., 2011)

included most of the total spatial extent of this *A. germinans* population, with a minimum intertree distance of 11 m and a maximum distance of 528 m (Figure 1). For each tree, we sampled a total of three undamaged leaves, each from the first fully mature leaf pair on branches located in direct sunlight. We collected these leaves (generally the third leaf pair) to standardize leaf age and exposure to sunlight, both of which can influence fungal community structure (Koide et al., 2017; Younginger and Ballhorn, 2017). We placed leaves from each tree into separate, labeled plastic bags and stored them in a portable cooler with an ice pack during fieldwork and subsequent transport to the laboratory.

Sample processing and DNA isolation

Leaves were kept on ice and processed within 24 hours of sampling. We rinsed individual leaves under running tap water for 30 s, then surface sterilized them with sequential immersion in 95% ethanol for 10 s, 0.5% bleach for 2 min, and 70% ethanol for 2 min under a sterile hood (U'Ren et al., 2014). We allowed the leaves to air dry and then used sterilized surgical blades to cut ~5 mm × 5 mm sections from the middle of each leaf at both sides of the midvein. We combined the cut sections from each of the three leaves per tree into a single microcentrifuge tube and isolated genomic DNA with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard protocol, with an extended incubation of 45 min. We also included two extraction blanks (negative controls) during this process. We quantified DNA extracts on a Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) and created standardized aliquots of 35 ng/μL to be used for both host-tree genotyping and fungal community sequencing. We stored DNA aliquots at –20°C until further processing.

Host-tree genotyping

We genotyped host trees at 32 nuclear microsatellite loci. Of this total, 12 loci were previously developed (Nettel et al., 2005; Cerón-Souza et al., 2006, 2012; Mori et al., 2010) and genotyped following the protocol outlined in Kennedy et al. (2020b). The remaining 20 loci were more recently developed (Craig et al., 2020a [Preprint]) and genotyped following the author's protocol. We performed polymerase chain reaction (PCR) on a Prime thermal cycler (Techne, Straffordshire, United Kingdom), analyzed fragments on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA) with LIZ 500 size standard, and scored alleles in the R package Fragman (Covarrubias-Pazaran et al., 2016). We evaluated the presence of null alleles in MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004) and randomly amplified and genotyped 10% of our DNA samples ($n = 4$) a second time to estimate a study error rate (Bonin et al., 2004). We tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium, and calculated the number of alleles and observed and expected heterozygosity per locus in FSTAT 2.9.3.2 (Goudet, 2002).

We calculated five measures of host-tree heterozygosity (i.e., proportion of heterozygous loci, observed heterozygosity, expected heterozygosity, internal relatedness, and homozygosity by loci) for each of the 40 host trees with the R-function GENHET (Coulon, 2010). We also manually calculated the number of alleles within the multilocus genotype of each host tree. All six measures were highly correlated (Pearson's correlation, $r = 0.96–1.0$, $p < 0.001$).

Hence, we present results only for homozygosity by loci (HL), an index that considers allelic variability at each locus to estimate heterozygosity and, based on simulations, correlates better than other measures with genome-wide heterozygosity (Aparicio et al., 2006). Because this index varies from 0 (all loci are heterozygous) to 1 (all loci are homozygous), we used 1 − HL for statistical analyses to provide more intuitive results (i.e., higher values represent higher heterozygosity). To evaluate genetic similarity, we calculated pairwise interindividual genetic distances (as outlined in Smouse and Peakall, 1999) and geographic distances among the 40 host trees in GenAlEx 6.5 (Peakall and Smouse, 2012).

Associated fungal community sequencing

We performed ITS gene amplicon library preparation and sequencing at the University of Salford, United Kingdom. Fungal DNA was amplified at the ITS 1F-2 gene (White et al., 1990) with modified versions of the ITS1F (5′–CTT GGT CAT TTA GAG GAA GTA A–3′) and ITS2 (5′–GCT GCG TTC TTC ATC GAT GC –3′) primer set, which included Illumina adapters, a linker, and unique barcodes (see Smith and Peay, 2014) as outlined in Griffiths et al. (2020). The PCR products for our samples and those of 80 additional fungal samples, which consisted of ITS1 gene amplicons used for an unrelated study, were then pooled to equimolar concentrations. ITS1 gene amplicon sequencing was performed using paired-end reads with an Illumina v3 (2 × 300 bp) cartridge on an Illumina MiSeq (Illumina, San Diego, California, USA). Negative (extraction blanks) and positive (synthetic mock community with 12 mock isolates; Palmer et al., 2018) controls were also included in the sequence run.

We removed adapter and primer sites from the ITS1 gene sequence data with cutadapt v2.4 (Martin, 2011), and performed all subsequent data processing and calculations in R v3.6.0 (R Core Team, 2020). A total of 275,829 raw sequences across our 40 samples were generated. We used the R package DADA2 1.12.1 (Callahan et al., 2016) with default pipelines to perform quality filtering and taxonomic assignment with the UNITE v8.0 database (UNITE Community, 2019). Here, we analyzed only forward sequence reads because lower quality and quantity of reverse reads resulted in a nearly 50% reduction in total sequence reads after quality filtering of the assembled paired-end reads (Appendix S1a). Discarding low-quality reverse reads is a common strategy that often provides better results than assembled paired-end reads (Nguyen et al., 2015; Pauvert et al., 2019). One chimera was removed. We then removed amplicon sequence variants (ASVs) with <100 reads across all samples as a conservative approach to deal with potential artifacts of high-throughput sequencing (Pauvert et al., 2019). Modal contig length was 225 bp (range: 153–251 bp). No contaminants were identified in the first negative control, and one ASV was identified in the second negative control, but was not found in other samples. All 12 expected ASVs were identified in the synthetic mock community. We did not further trim forward reads, we manually checked whether ASVs with identical taxonomic assignments were indeed unique sequences (i.e., did not simply vary at the start or end of the sequence), and all ASVs assigned as unidentified fungi were further checked with default Nucleotide Basic Local Assignment Search Tool (BLASTN) analyses on the UNITE website (Nilsson et al., 2019). We removed all ASVs that corresponded to the host-tree species (*A. germinans*), which included 64% of all sequence reads, and all additional unidentified fungi had significant alignments with public fungal ITS sequences (e-values = $1e^{-13}$ – $4e^{-88}$). The resulting data set consisted of 64,308 reads across 40 samples, with a median of 748 reads per sample (range: 104–9314).

We exported the ASV table, taxonomy table, and sample identifications to the R package phyloseq 1.28.0 (McMurdie and Holmes, 2013) for the following calculations. We calculated alpha diversity of fungal communities with Hill numbers (Hill, 1973) at the scales of $q = 0$ (species richness), $q = 1$ (exponential of Shannon index), and $q = 2$ (inverse of Simpson index), which represent the effective number of species and put more weight on abundant species as the value of q increases (Chao et al., 2014). We performed these calculations with the raw count data rarefied to a standardized number of reads equal to the sample with the lowest read count (104 reads; see Appendix S2a). Although read counts were limited for certain samples, asymptotes were reached in all rarefaction curves with few rank-order changes among samples past this lowest read count (Appendix S2a). As such, our sampling effort seems to have captured most of the diversity within these samples. Random sampling to generate rarefied counts can add noise to a data set and undermine the performance of downstream methods (McMurdie and Holmes, 2014); therefore, we also performed alpha diversity calculations and the subsequent statistical analyses with the raw count data and results were equivalent to those presented here (Appendix S1b). To evaluate community dissimilarity (beta diversity), we calculated Bray-Curtis dissimilarity with the raw count data converted to relative abundances. We also calculated Aitchison distance by centered log-ratio (clr) transforming the raw count data with the R package microbiome (Lahti et al., 2017) and then calculating pairwise Euclidean distances in phyloseq 1.28.0 (McMurdie and Holmes, 2013). Aitchison distance accounts for the compositional nature of high-throughput sequence data, which makes this measure more appropriate than many standard measures (Gloor et al., 2017; Quinn et al., 2018).

Statistical analyses

We performed all statistical analyses in R v3.6.0 (R Core Team, 2020). To address our first question, we tested for an effect of interhost genetic distance and a relative spatial effect of interhost geographic distance on dissimilarity among associated endophytic fungal communities across all samples with ranked Mantel tests of correlation. Because spatial effects may not be linear (Diniz-Filho et al., 2013; Legendre et al., 2015), we also performed multivariate Mantel correlograms to assess these patterns at five discrete distance classes. All analyses were performed in the R package ecodist (Goslee and Urban, 2007). Significance for each analysis was determined with 10^4 permutations, and p-values for correlograms were adjusted for multiple comparisons with a false discovery rate correction method using the R function p.adjust. For both Mantel tests and Mantel correlograms, we first tested for a relationship between the two predictor variables (i.e., interhost genetic distance and interhost geographic distance), then performed separate tests between fungal community dissimilarity and each of the two predictor variables, and finally performed partial analyses between fungal community dissimilarity and interhost genetic distance, while controlling for interhost geographic distance.

To address our second question, we tested for an effect of host-tree heterozygosity and an absolute spatial effect of host-tree spatial location on the alpha diversity of associated endophytic fungal communities with multiple linear regressions. We fitted three additive models, with alpha diversity of fungal communities at each Hill number ($q = 0$, 1, 2) as the response variable and heterozygosity, longitude, and latitude of each host tree as predictor variables. We also tested full models and subsets with interactions among two of the three predictor variables, but none of these interactions proved statistically significant and none of these models provided better fits based on the Bayesian Information Criterion (BIC; Schwarz, 1978). Hill numbers at $q = 1$ and $q = 2$ were natural log-transformed to meet the statistical assumption of normality, and we centered and scaled the predictor variables to standardize regression coefficients.

RESULTS

Host-tree genotyping

We discarded seven of the 32 nuclear microsatellite loci that were monomorphic across all samples, and discarded another locus that proved difficult to score. Our final host-tree genotypes included 24 loci (Appendix S1c) with no missing data, and all 40 host-tree genotypes were unique. We found no evidence for null alleles and each of the four samples that were amplified and genotyped a second time produced consistent multilocus genotypes. We found no evidence for linkage disequilibrium or deviations from Hardy-Weinberg equilibrium. Genetic variation was low across the 40 host trees, with 2.6 ± 1.4 (standard deviation [SD]) alleles per locus and expected heterozygosity of 0.37 ± 0.20 (Appendix S1c). Host-tree heterozygosity (1 – HL) ranged from 0.06 to 0.81 (mean: 0.45 ± 0.15).

Associated fungal community sequencing

A total of 49 ASVs were identified across the 40 host trees. Most ASVs were assigned to the phylum Ascomycota (35 of 49 ASVs, 87% of all reads) and 11% of all reads were assigned only to the level of kingdom Fungi (Appendix S2b). Less than half (47%) of all reads were assigned class level taxonomy, with the class Dothideomycetes as the most common (28% of all reads; Appendix S2c). The endophytic fungal community was relatively homogeneous, with one ASV (assigned taxonomy only to the level of phylum Ascomycota) representing 41% of all reads (Appendix S1d). The five most abundant ASVs represented 78% of all reads, and subsequent ASVs each represented ≤2% of all reads (Appendix S1d). Alpha diversity of fungal communities across the 40 host trees at $q = 0$ (species richness) was 4.0 ± 1.7 (SD), at $q = 1$ (exponential of Shannon index) was 2.8 ± 1.2, and at $q = 2$ (inverse of Simpson index) was 2.5 ± 1.1.

Associated fungal community structure correlates with host-tree genetics

Genetically similar host trees harbored similar associated fungal communities, with no detectable relative spatial effect of geographic distance among host trees both across all samples (Mantel tests) and at five distance classes (Mantel correlograms) (Figure 2). For Mantel tests, the predictor variables (i.e., interhost genetic distance and interhost geographic distance) exhibited no relationship (Mantel correlation, $r_M = 0.05$, $p = 0.181$; Appendix S2d). Fungal community (Bray-Curtis) dissimilarity exhibited a weak but statistically significant positive relationship with interhost genetic distance ($r_M = 0.26$, $p = 0.002$), and no relationship with interhost geographic distance ($r_M = 0.06$, $p = 0.164$) (Figure 2A, B). Accounting for interhost geographic distance did not affect the relationship with interhost genetic distance (partial $r_M = 0.26$, $p = 0.002$). Community dissimilarity measured with Aitchison distance provided equivalent results (interhost genetic distance: $r_M = 0.16$, $p = 0.041$; interhost geographic distance: $r_M = 0.05$, $p = 0.188$) (Figure 2E, F), with a weaker relationship with interhost genetic distance (partial $r_M = 0.16$, $p = 0.043$).

Mantel correlogram results were equivalent to those of the Mantel tests, with no relationships between predictor variables ($r_M = -0.07 - 0.07$, $p \geq 0.568$; Appendix S2d), and community (Bray-Curtis) dissimilarity exhibited statistically significant positive relationships with the first two genetic distance classes ($r_M = 0.16$, $p = 0.002$; $r_M = 0.14$, $p = 0.050$; respectively), a statistically significant negative relationship with the fourth genetic distance class ($r_M = -0.16$, $p = 0.008$), and no relationships with interhost geographic distance classes ($r_M = -0.06 - 0.03$, $p \geq 0.810$) (Figure 2C, D). Accounting for interhost geographic distances did not affect these relationships with interhost genetic distance classes, except for the second genetic distance class that was now statistically nonsignificant ($p = 0.090$) (Appendix S2e).

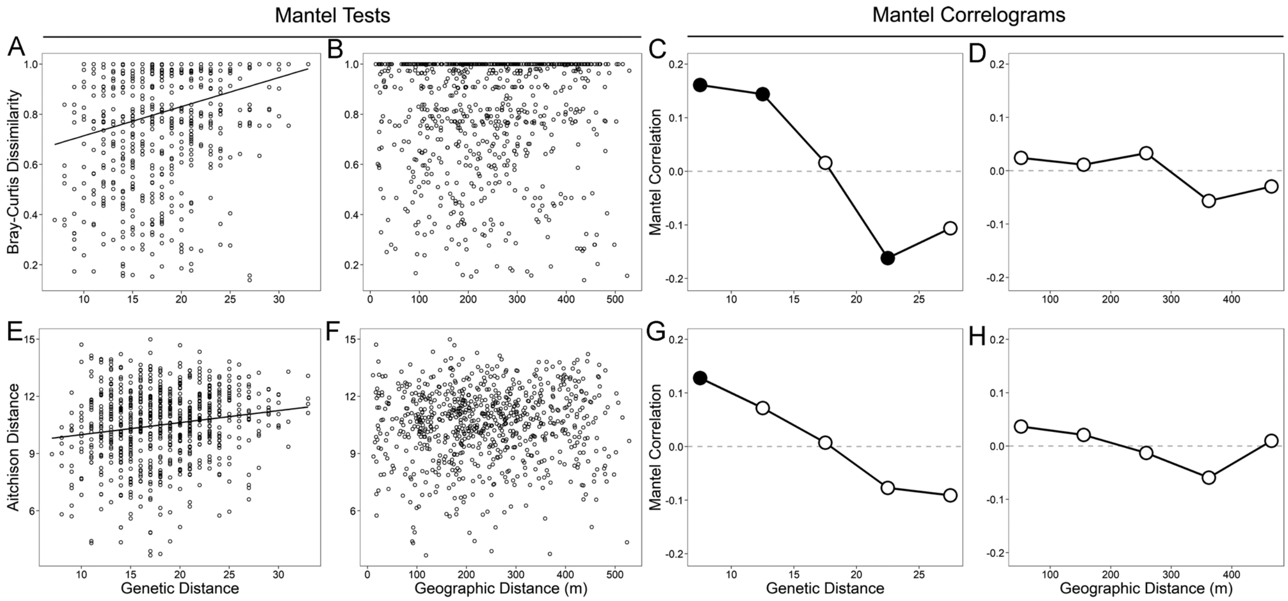

FIGURE 2 Genetically similar mangrove host trees harbored similar associated endophytic fungal communities, independent of geographic distances among these host trees. Panels show graphical representations of the relationships between fungal community dissimilarity (measured with Bray-Curtis dissimilarity and Aitchison distance) and each of the two predictor variables (interhost genetic distance and interhost geographic distance) across all mangrove host trees (Mantel tests) and at five distance classes (Mantel correlograms). Statistically significant ($p < 0.05$) correlations between fungal community dissimilarity and interhost genetic distance(s) are depicted with solid lines for Mantel tests and with black circles for Mantel correlograms

Community dissimilarity measured with Aitchison distance provided equivalent results (Figure 2G, H), with weaker relationships with interhost genetic distance classes that were statistically significant at only the first genetic distance class ($r_M = 0.13$, $p = 0.027$). Accounting for interhost geographic distances did not affect these relationships (Appendix S2e).

Associated fungal community diversity correlates with host-tree spatial location

Host-tree heterozygosity had no detectable effect on the alpha diversity of associated endophytic fungal communities. Instead, the absolute spatial location of host trees affected these associated fungal communities. Additive models explained limited variation in the alpha diversity of fungal communities at each of the three Hill numbers. The model for $q = 0$ was not statistically significant ($F_{3,36} = 1.7$, $p = 0.195$, adjusted $r^2 = 0.05$) and models for $q = 1$ ($F_{3,36} = 3.1$, $p = 0.038$, adjusted $r^2 = 0.14$) and $q = 2$ ($F_{3,36} = 3.4$, $p = 0.027$, adjusted $r^2 = 0.16$) were marginally significant. Longitude was the only predictor variable to exhibit a significant partial regression slope (for full model breakdown see Table 1). This increase in fungal community alpha diversity with increased longitude (i.e., from the brackish lagoon to the landward margin) was statistically significant at each of the three Hill numbers ($p = 0.043$, 0.009, 0.009, respectively; Table 1). Yet, instead of a systematic increase, these effects seemed to be shaped primarily by the fact that highest fungal alpha diversity was observed within trees closest to the landward margin (Figure 3).

TABLE 1 Multiple linear regressions of alpha diversity of associated endophytic fungal communities as a function of the heterozygosity and absolute spatial location of host trees. Alpha diversity of associated communities was calculated with Hill numbers at the scales of $q = 0$ (species richness), $q = 1$ (exponential of Shannon index), and $q = 2$ (inverse of Simpson index), which put more weight on abundant species as the value of q increases. Bold values indicate statistical significance ($p < 0.05$)

Response	Predictor	Estimate	SE	t	p
$q = 0$	Heterozygosity	−0.01	0.16	−0.12	0.909
	Longitude	0.37	0.18	2.10	**0.043**
	Latitude	−0.26	0.18	−1.47	0.150
$q = 1$	Heterozygosity	−0.08	0.15	−0.55	0.588
	Longitude	0.47	0.17	2.76	**0.009**
	Latitude	−0.11	0.17	−0.65	0.520
$q = 2$	Heterozygosity	−0.10	0.15	−0.66	0.515
	Longitude	0.46	0.17	2.74	**0.009**
	Latitude	−0.05	0.17	−0.29	0.772

DISCUSSION

Community-genetic effects are predicted to be less prominent in plant systems with limited genetic variation, such as those at distributional range limits. Yet, empirical evidence from such systems is limited. Here, at the scale of an expanding range limit population of a mangrove foundation species (*Avicennia germinans*), we found evidence for the genetic similarity rule whereby genetically similar host trees

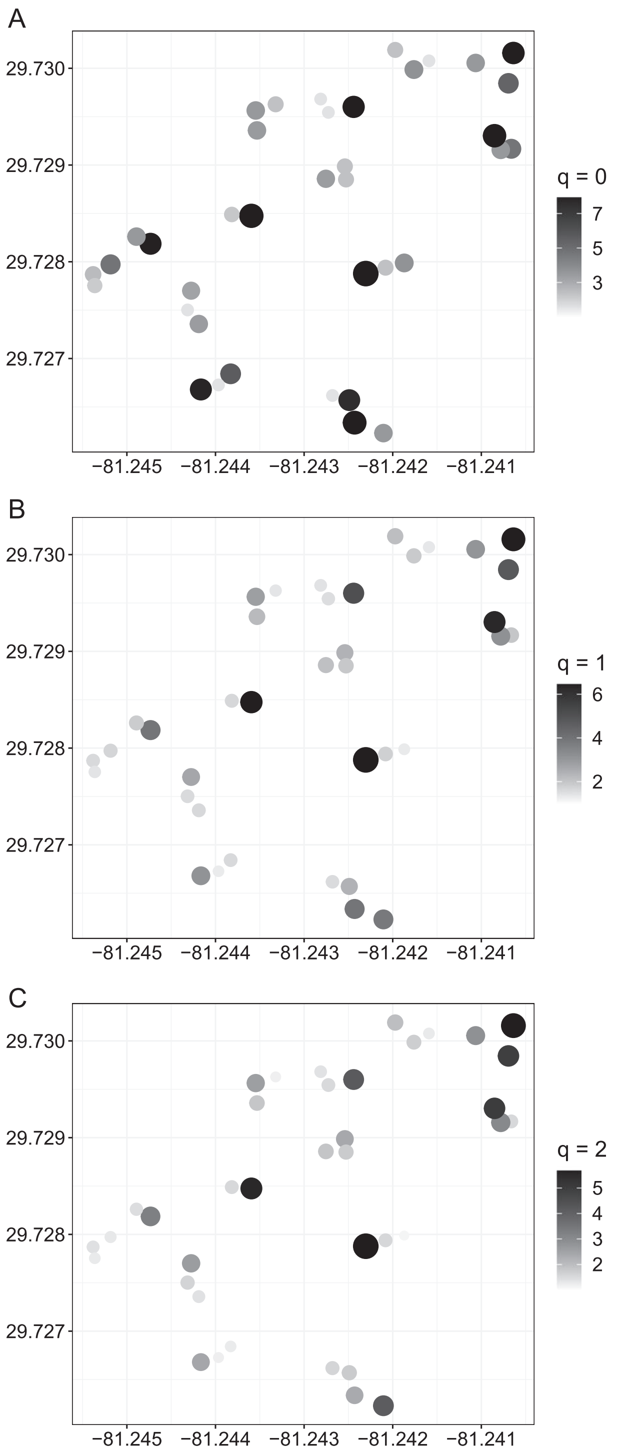

FIGURE 3 Spatial >distribution of the alpha diversity of associated endophytic fungal communities within 40 *Avicennia germinans* trees across a collection site at the northern distributional limit of this species. Alpha diversity was calculated with Hill numbers at the scales of (A) $q = 0$ (species richness), (B) $q = 1$ (exponential of Shannon index), and (C) $q = 2$ (inverse of Simpson index), which put more weight on abundant species as the value of q increases. In the figure, values of fungal alpha diversity for each tree increase with color (from white to black) and with the size of the data point

harbored similar associated endophytic fungal communities. In contrast, we found no detectable effect of host-tree heterozygosity on fungal community alpha diversity. This research demonstrates that community-genetic effects can occur even at expanding distributional limits where host-plant genetic variation may be limited and provides the first documentation of these effects in a natural mangrove system.

Genetically similar mangrove hosts harboring similar endophytic fungal communities, with no detectable relative spatial effect, may be explained by the mode of fungal transmission and/or biotic filtering dictated by the physiology and anatomy of the host plant (Ricks and Koide, 2019). Horizontal transmission via airborne fungal spores is commonly observed in woody plants (Arnold and Herre, 2003 and citations within), although vertical transmission from parent tree to seed is also possible (e.g., Vega et al., 2010). Our studied species (*A. germinans*) produces cryptoviviparous propagules (i.e., embryos emerge from the seed coat, but remain within the fruit until abscission from maternal trees), with varying degrees of vivipary across many mangrove species (Tomlinson, 1986). This form of reproduction, where developing propagules remain attached to maternal trees for extended periods, may lead to a greater contribution of fungal transfer from parent to offspring. Consistent with this hypothesis, endophytic fungi (Lee et al., 2019) and bacteria (Soldan et al., 2019) are found within surface-sterilized cryptoviviparous mangrove propagules collected directly from maternal trees. Host physiology may also dampen horizontal transfer in *A. germinans* because salt excretion through leaf glands (a mechanism to tolerate salt stress) can reduce foliar fungal colonization (Gilbert et al., 2002). Fungal communities in trees also vary with differences in phenotypic leaf traits, such as internal chemistry and surface characteristics (Valkama et al., 2005; Kembel and Mueller, 2014). Additional research that compares fungal endophytes in both *A. germinans* maternal trees and their offspring, with parallel leaf trait assessments, could evaluate the relative influence of fungal transmission mode and biotic filtering in shaping these associated communities.

We did not detect an effect of host-tree heterozygosity on fungal community alpha diversity. Instead, we found that alpha diversity varied with the absolute spatial location of host trees. Increased host-tree heterozygosity can lead to greater growth rates (Charlesworth and Willis, 2009) and greater foliar phytochemical diversity (Campbell et al., 2013), factors that may underlie increases in associated herbivore community alpha diversity observed elsewhere (Tovar-Sánchez et al., 2013; Valencia-Cuevas et al., 2018). We suggest that, within this mangrove population, the limited genetic variation present across host trees may not translate into large enough variation in host-tree phenotypic traits that would augment the alpha diversity of these associated communities. Rather, community alpha diversity increased with longitude across our collection site (i.e., from the brackish lagoon to the landward margin), an absolute spatial effect seemingly shaped by the fact that highest alpha diversity was observed within trees closest to the landward margin. Soil salinity increases with longitude across the site, but then declines at this landward margin adjacent to a fringe of terrestrial forest (Figure 1). Salinity differences can affect fungal communities associated with the *A. germinans* rhizosphere (Vanegas et al., 2019), but their effect on foliar fungal communities remains to be formally tested. Higher soil salinity closer to the center of the collection site will demand greater salt excretion through *A. germinans* leaf glands (Sobrado and Greaves, 2000; Suárez and Medina, 2008), which may further diminish foliar fungal colonization in this species (Gilbert et al., 2002). In addition, as mangrove leaves may contain fungi predominately from terrestrial sources (Lee et al., 2019, 2020), the fringe of terrestrial forest is presumably a reservoir of unique fungal diversity. Therefore, within the mangrove population studied here, trees located nearest to this landward margin may harbor slightly more diverse fungal communities than conspecifics elsewhere due to both reduced soil salinity and proximity to additional fungal sources. Whether this pattern extends to additional mangrove populations remains to be tested.

Pohjanmies et al. (2015), with their research at a distributional range limit, provided the first empirical evidence of community-genetic effects within a plant system with limited genetic variation. Our documentation of the genetic similarity rule at a mangrove range limit, where host trees possessed very limited genetic variation (on average, 2.6 alleles per locus), adds further support to these previous findings and strengthens the argument that correlations between genetic variation within foundation species and the dynamics of associated communities can occur even at distributional limits that may be genetically depauperate. These correlations, however, will ultimately depend on the strength of the community-genetic effect relative to the degree of environmental variation and how this relationship varies with spatial scale (Bangert et al., 2008). Both Pohjanmies et al. (2015) and our study assessed correlations between plant foundation species and their associated communities within single range limit populations. Environmental variation will inherently be small at this local scale compared to that across broader spatial scales where community-genetic effects may be less influential (Hughes and Stachowicz, 2009; Tack et al., 2010; Gossner et al., 2015; but see Bangert et al., 2006a; Davies et al., 2014; Lamit et al., 2015). Spatial effects on foliar endophytic fungal communities in mangroves are evident across greater geographic distances (Lee et al., 2019, 2020). As such, the relationship between mangrove host-tree genetic variation and associated fungal communities documented here may vary depending on the spatial extent under consideration and warrants additional research.

Although we sampled a relatively small spatial area, this is the scale at which species expansion occurs as small, isolated populations become colonized and begin to proliferate. This process is particularly evident at the Atlantic Florida *A. germinans* range limit where initial colonization may consist of a single individual (Kennedy et al., 2020a), and for the population studied here, which has increased from only about 10% to up to 45% mangrove cover over the past several decades (Rodriguez et al., 2016). In this context, our research demonstrates that community-genetic effects can occur across the spatial extent of an expanding range limit population, with potential implications for host fitness and population resilience because endophytic fungi can vary greatly in function within plant hosts from latent pathogens to mutualistic symbionts (Porras-Alfaro and Bayman, 2011). Symbioses with endophytic fungi can contribute to plant adaptation to high-stress environments (Rodriguez et al., 2004), with evidence that variation in soil fungal communities can influence the fitness and susceptibility of *A. germinans* to cold stress (Chen et al., 2020), although fungal infections can reduce recruitment (Devaney et al., 2017). We documented a correlation between mangrove host-tree genetics and fungal community differences, but does this relationship generate variation in stress tolerance among mangrove hosts? If so, this insight could broaden the current discussion of how a shift from salt marsh to mangrove dominance may shape these coastal communities (e.g., Kelleway et al., 2017; Johnston and Gruner, 2018; Smith et al., 2019; Armitage et al., 2020) by including mangrove intraspecific variation as a factor that could influence population resilience at these high-stress range limits.

This research also provides the first documentation of community-genetic effects in a natural mangrove system. Does the genetic similarity rule apply elsewhere across the broad distributional range of mangroves and to other mangrove-associated communities? Experimental plantings demonstrate that mangrove maternal genotypic identity can affect the composition of associated soil microbial communities (Craig et al., 2020b), which indicates that community-genetic effects can have a broader reach in mangrove systems than the more intimately associated endophytic fungal communities assessed here. Moreover, intraspecific differences in quantitative traits of mangroves, including trichome density (Piovia-Scott, 2011), plant architecture (Silva et al., 2017), and leaf chemistry (Erickson et al., 2004) can affect mangrove-associated communities. Heritable variation in these traits has been identified as a potential factor linking associated communities to host-plant genetics (Whitham et al., 2012). Assessments in additional mangrove-associated communities (of both terrestrial and marine origin) would further our understanding of how host-tree genetic variation may relate to the broader community of organisms associated with these plants, with direct implications for conservation and restoration practices.

CONCLUSIONS

We found evidence for the genetic similarity rule at an expanding mangrove range limit. This research helps broaden the current scope of community genetics theory by demonstrating that community-genetic effects can occur even at expanding distributional limits where host-plant genetic variation may be limited. Our findings also add to the growing number of diverse systems where associated communities vary with host-plant genetics. As community-level effects of host-plant genetic variation are found to be most prominent in systems dominated by few plant foundation species (Whitham et al., 2006), mangrove forests and their low tree-species diversity may prove to be a system ripe for discovery.

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AUTHOR CONTRIBUTIONS

J.P.K., R.F.P., and J.K.R. conceived and designed the research. J.P.K. performed field collections, DNA extractions, and host-tree genotyping. R.E.A. performed library preparation and sequencing, and also provided analysis tools. R.F.P. and J.K.R. supervised the research. J.P.K. conducted bioinformatics analysis and statistical analyses. J.P.K. wrote the manuscript with input from all coauthors.

DATA AVAILABILITY STATEMENT

Microsatellite genotype data are publicly available on Figshare: https://doi.org/10.6084/m9.figshare.14252660.v1. Sequence data are deposited on the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA643237/.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information tab for this article.

APPENDIX S1

Appendix S1a. Summary of ITS1 gene sequence data sets using only forward sequence reads and using assembled paired-end reads.

Appendix S1b. Multiple linear regressions of associated endophytic fungal community diversity (calculated with the raw count data) as a function of the heterozygosity and absolute spatial location of host trees.

Appendix S1c. Genetic diversity of 24 nuclear microsatellite loci used for genotyping of Avicennia germinans host trees.

Appendix S1d. Endophytic fungal diversity identified with ITS1 gene sequencing.

APPENDIX S2

Appendix S2a. Rarefaction curves of observed amplicon sequence variants (ASVs) in sampled Avicennia germinans trees.

Appendix S2b. Relative abundance across all sequence data of fungal phyla for the forwardreads data set.

Appendix S2c. Relative abundance across all sequence data of fungal class for the forward-reads data set.

Appendix S2d. Graphical representation of Mantel test and Mantel correlogram between interhost genetic distance and interhost geographic distance.

Appendix S2e. Graphical representation of partial Mantel correlograms between fungal community dissimilarity, measured with Bray-Curtis dissimilarity and Aitchison distance, and interhost genetic distance.

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Supporting information

APPENDIX S1 Supplemental Tables

Appendix S1a. Summary of ITS1 gene sequence data sets using only forward sequence reads and using assembled paired-end reads. The assembled paired-end reads data set consisted of fewer host trees because six of the host-tree samples contained less than 100 reads (range: 0 – 52 reads) after quality filtering and were removed. ASV, number of amplicon sequence variants.

		sequence reads				
Data set	trees	total	median	min	max	ASV
Forward reads	40	64,308	748	104	9,314	49
Assembled (forward + reverse)	34	38,077	557	107	8,122	38

Appendix S1b. Multiple linear regressions of alpha diversity of associated endophytic fungal communities (calculated with the raw count data) as a function of the heterozygosity and absolute spatial location of host trees. Alpha diversity of associated communities was calculated with Hill numbers at the scales of q=0 (species richness), q=1 (exponential of Shannon index), and q=2 (inverse of Simpson index). Bold values indicate statistical significance ($p < 0.05$). These results are equivalent to those obtained when alpha diversity indices were calculated with rarefied data (see Table 1 in the main text).

Response	**Predictor**	**Estimate**	**SE**	**t**	**p**
q=0	Heterozygosity	-0.04	0.16	-0.22	0.826
	Longitude	0.32	0.18	1.79	0.081
	Latitude	-0.26	0.18	-1.49	0.145
q=1	Heterozygosity	-0.08	0.16	-0.54	0.591
	Longitude	0.47	0.17	2.75	**0.009**
	Latitude	-0.12	0.17	-0.73	0.473
q=2	Heterozygosity	-0.10	0.15	-0.66	0.514
	Longitude	0.46	0.17	2.74	**0.010**
	Latitude	-0.05	0.17	-0.31	0.761

Appendix S1c. Genetic diversity of 24 nuclear microsatellite loci used for genotyping of 40 *Avicennia germinans* host trees. A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. None of these loci exhibited a significant deviation from Hardy-Weinberg equilibrium ($p > 0.05$).

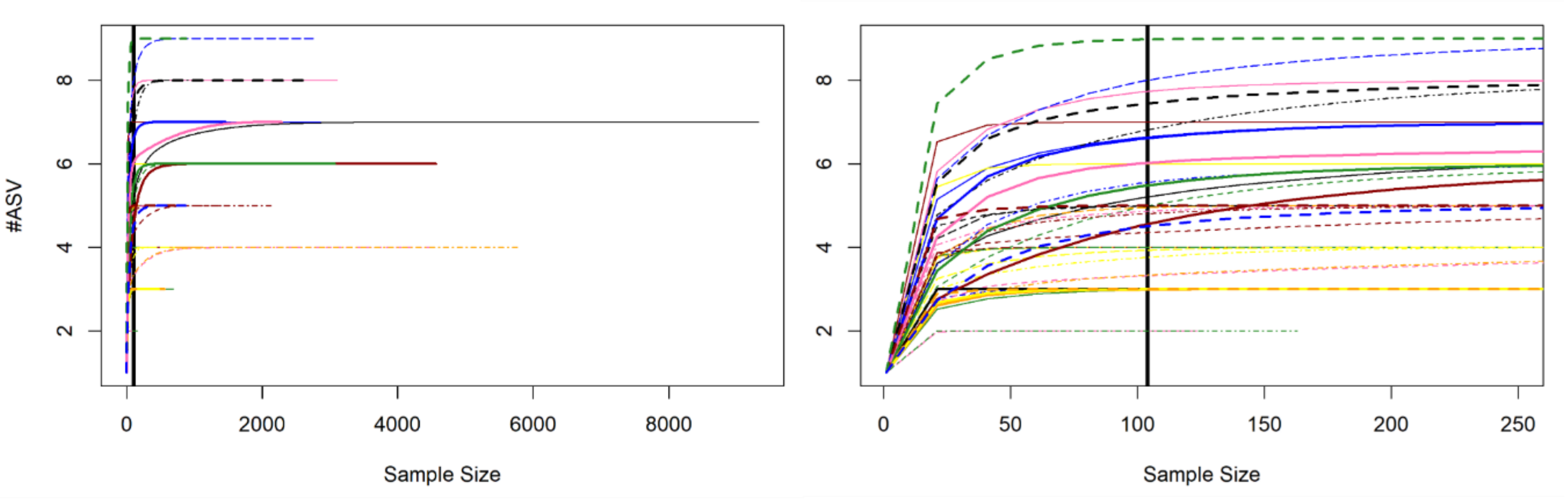
Multiplex	**Locus**	**A**	**H_O**	**H_E**	**Reference**
Multiplex 1	Agerm1-18	2	0.20	0.18	(Mori et al., 2010)
	CA_002	3	0.50	0.56	(Cerón-Souza et al., 2006)
	GT_006	2	0.38	0.44	(Cerón-Souza et al., 2012)
	CT_004	4	0.33	0.41	(Cerón-Souza et al., 2012)
	CT_003	3	0.48	0.42	(Cerón-Souza et al., 2006)
	CAT_004	2	0.50	0.51	(Cerón-Souza et al., 2012)
Multiplex 2	AgT4	2	0.38	0.51	(Nettel et al., 2005)
	AgT8	2	0.18	0.20	(Nettel et al., 2005)
	CTT_001	2	0.33	0.31	(Cerón-Souza et al., 2006)
	Agerm1-14	4	0.30	0.30	(Mori et al., 2010)
	AgD13	8	0.80	0.84	(Nettel et al., 2005)
Multiplex 3	AvGm29	2	0.18	0.16	(Craig et al., 2020)
	AvGm19	2	0.43	0.49	(Craig et al., 2020)
Multiplex 4	AvGm15	2	0.03	0.03	(Craig et al., 2020)
	AvGm18	4	0.45	0.50	(Craig et al., 2020)
	AvGm02	2	0.08	0.07	(Craig et al., 2020)
Multiplex 5	AvGm12	2	0.05	0.05	(Craig et al., 2020)
	AvGm05	3	0.53	0.49	(Craig et al., 2020)
Multiplex 6	AvGm06	2	0.13	0.16	(Craig et al., 2020)
	AvGm08	2	0.58	0.50	(Craig et al., 2020)
	AvGm26	2	0.28	0.28	(Craig et al., 2020)
	AvGm07	2	0.43	0.39	(Craig et al., 2020)
Multiplex 7	AvGm16	2	0.58	0.51	(Craig et al., 2020)
	AvGm04	2	0.43	0.50	(Craig et al., 2020)
	mean	**2.6**	**0.35**	**0.37**	

Appendix S1d. Endophytic fungal diversity identified with ITS1 gene sequencing. ASV, amplicon sequence variant; % reads, percent occurrence across all sequence reads.

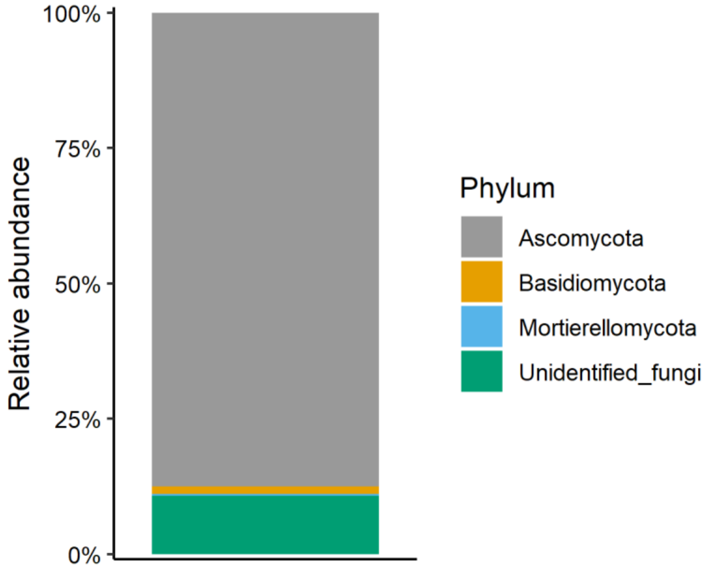
ASV	**Phylum**	**Class**	**Order**	**Family**	**Taxon**	**% reads**
SV1	Ascomycota				Ascomycota	41.2
SV2	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Phaeosphaeriaceae	11.6
SV3	Unidentified fungi				Unidentified fungi	9.0
SV4	Ascomycota	Dothideomycetes	Capnodiales	Neodevriesiaceae	*Neodevriesia* sp.	8.0
SV5	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum brasiliense*	7.9
SV6	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum brasiliense*	2.1
SV7	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Obolarina* sp.	1.6
SV8	Ascomycota	Leotiomycetes	Erysiphales	Erysiphaceae	*Erysiphe hypophylla*	1.6
SV9	Ascomycota	Dothideomycetes	Capnodiales		Capnodiales	1.2
SV10	Ascomycota	Dothideomycetes			Dothideomycetes	1.1
SV11	Ascomycota	Dothideomycetes	Capnodiales	Neodevriesiaceae	*Neodevriesia* sp.	1.1
SV12	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Phaeosphaeriaceae	1.0
SV13	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Xylariaceae	1.0
SV14	Ascomycota				Ascomycota	0.9
SV15	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Phaeosphaeriaceae	0.8
SV16	Ascomycota	Dothideomycetes	Capnodiales	Neodevriesiaceae	*Neodevriesia* sp.	0.7
SV17	Ascomycota	Sordariomycetes	Xylariales		Xylariales	0.5
SV18	Unidentified fungi				Unidentified fungi	0.5
SV19	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Paraphaeosphaeria* sp.	0.4
SV20	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum brasiliense*	0.4
SV21	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum brasiliense*	0.4
SV22	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Anthostomella* sp.	0.4
SV23	Unidentified fungi				Unidentified fungi	0.4
SV24	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinellus* sp.	0.4
SV25	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Pseudopithomyces chartarum*	0.4
SV26	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Coprinus foetidellus*	0.3
SV27	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Xylariaceae	0.3
SV28	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Anthostomella* sp.	0.3

SV29	Ascomycota	Dothideomycetes	Myriangiales	Elsinoaceae	Elsinoaceae	0.3
SV30	Basidiomycota	Agaricomycetes	Polyporales	Meripilaceae	*Rigidoporus vinctus*	0.3
SV31	Ascomycota	Dothideomycetes	Capnodiales	Neodevriesiaceae	*Neodevriesia* sp.	0.3
SV32	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Leptosphaerulina* sp.	0.3
SV33	Unidentified fungi				Unidentified fungi	0.3
SV34	Unidentified fungi				Unidentified fungi	0.3
SV35	Ascomycota	Sordariomycetes	Xylariales		Xylariales	0.2
SV36	Unidentified fungi				Unidentified fungi	0.2
SV37	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella capitata*	0.2
SV38	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Anthostomella* sp.	0.2
SV39	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Xylariaceae	0.2
SV40	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella capitata*	0.2
SV41	Ascomycota	Sordariomycetes	Xylariales		Xylariales	0.2
SV42	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Pseudopithomyces chartarum*	0.2
SV43	Unidentified fungi				Unidentified fungi	0.2
SV44	Ascomycota	Dothideomycetes	Botryosphaeriales	Phyllostictaceae	*Phyllosticta hymenocallidicola*	0.2
SV45	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinellus* sp.	0.2
SV46	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Pseudopithomyces chartarum*	0.2
SV47	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Deconica phillipsii*	0.2
SV48	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Teratosphaeriaceae	0.2
SV49	Ascomycota	Sordariomycetes	Xylariales	Xylariales	Xylariales	0.2

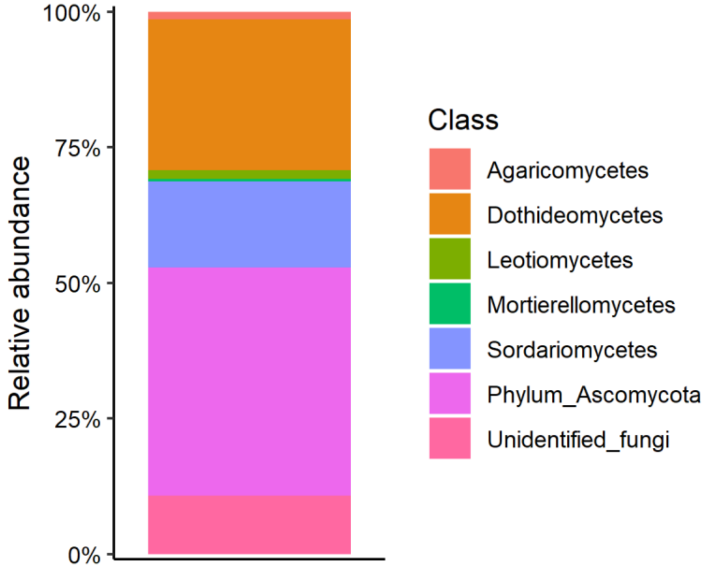
APPENDIX S2 Supplemental Figures



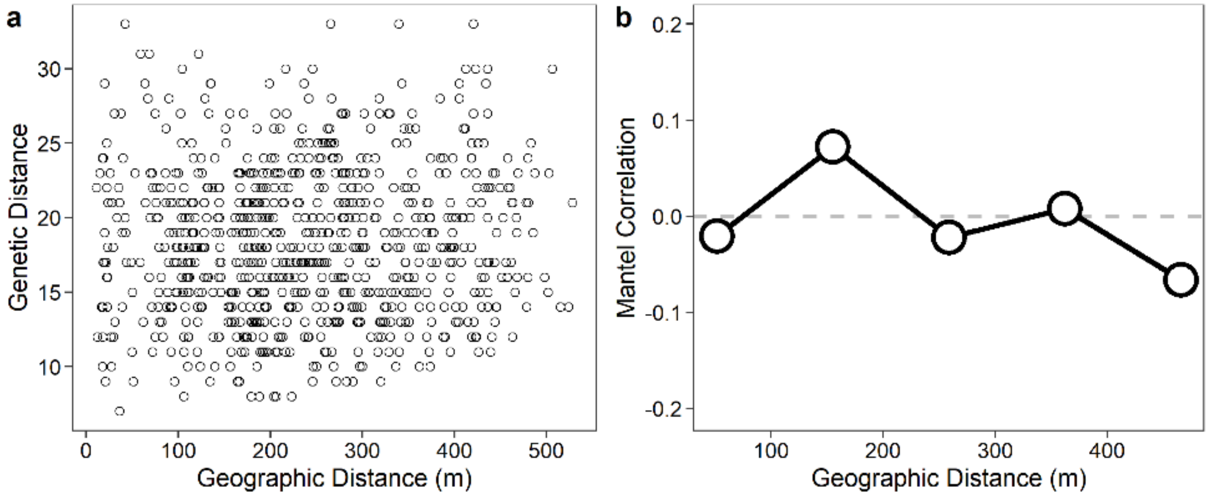
Appendix S2a. Rarefaction curves of observed amplicon sequence variants (ASVs) in sampled *Avicennia germinans* trees (n = 40) that are each shown with a unique combination of line colour, type, and width. (a) Complete sample size of reads shown (range: 104 – 9,314 reads). (b) Close-up with only the first 250 reads per sample shown. Minimum read number across all samples (104 reads) is shown with a vertical black line along the x-axis.



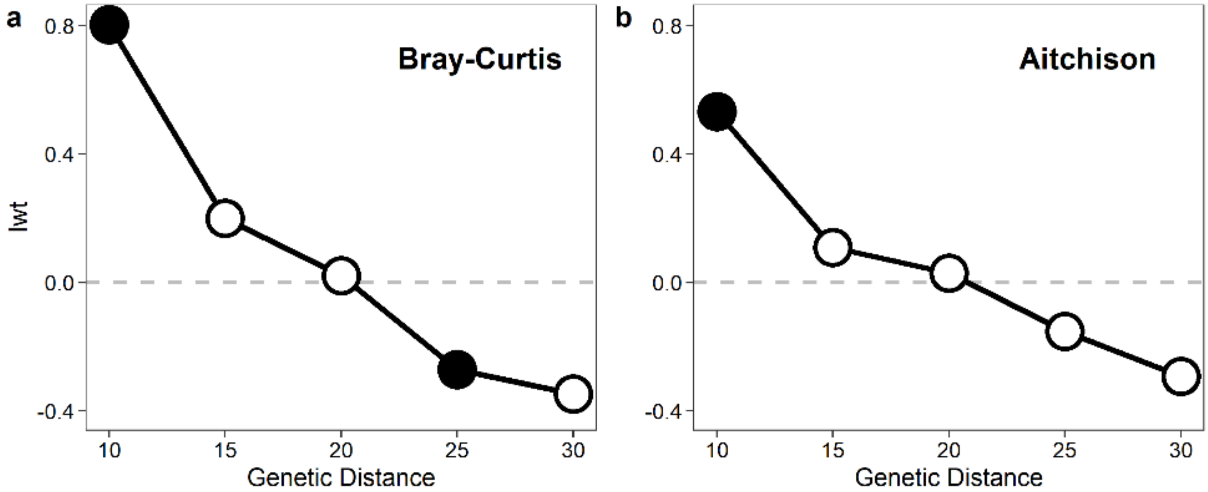
Appendix S2b. Relative abundance across all sequence data of fungal phyla for the forward-reads data set (n = 40 host trees, 49 ASV). Phylum Ascomycota represented 87% of all sequence reads and ASVs assigned only to the level of kingdom Fungi represented 11% of all reads.



Appendix S2c. Relative abundance across all sequence data of fungal class for the forward-reads data set (n = 40 trees, 49 ASV). Less than half (47%) of all reads were assigned class level taxonomy, with the class Dothideomycetes as the most common (28% of all reads).



Appendix S2d. Graphical representation of (a) Mantel test and (b) Mantel correlogram at five distance classes between inter-host genetic distance and inter-host geographic distance. No statistically significant relationship was found for either analysis (Mantel correlation, $r_M = 0.05$, $p = 0.181$; $r_M = -0.07 – 0.07$, $p \geq 0.568$; respectively).



Appendix S2e. Graphical representation of partial Mantel correlograms at five distance classes between fungal community dissimilarity, measured with (a) Bray-Curtis dissimilarity and (b) Aitchison distance, and inter-host genetic distance. Significant relationships ($p < 0.05$) shown with black circles. Iwt, statistic used for multivariate partial correlograms that converges on Moran’s autocorrelation coefficient.

CHAPTER 6

Genetically-based adaptive trait shifts at an expanding mangrove range margin

Candidate’s contribution to the publication: 95%

Candidate conceived and designed the research, performed field collections, DNA extractions, maternal tree genotyping, and experimental set-up alongside student assistants, collected data on plant survival, growth and stress, monitored greenhouse environmental conditions, harvested plants, performed chemical analyses and functional trait measurements, conducted statistical analyses, and wrote the manuscript with comments from both co-authors.

Co-authors

Jennifer Rowntree

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Genetically-based adaptive trait shifts at an expanding mangrove range margin

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ABSTRACT

1. Many species are expanding beyond their distributional range margins in response to a warming planet. Due to marginal environmental conditions and novel selection pressures, range margins may foster unique genetic adaptations that can better enable species to thrive under the extreme climatic conditions at and beyond their current distributional limits. Neotropical black mangrove (*Avicennia germinans*) is expanding poleward along Atlantic Florida, USA, with field evidence of adaptive trait shifts within range-margin populations. However, whether these adaptive shifts have a genetic basis remains to be answered.

2. We monitored twenty *A. germinans* maternal cohorts from areas in both the Atlantic Florida range core and margin in a greenhouse common garden with annual temperatures analogous to range-margin conditions. We measured variation in a series of phenotypic traits starting at initial planting of field-collected propagules and continuing until two years development.

3. Maternal cohorts from the Atlantic Florida range margin consistently outperformed those from the range core throughout the experiment. Range-margin cohorts survived in greater numbers, established more quickly, and were less stressed under winter chilling and sub-zero temperatures that are often reached at the Atlantic range margin, but not within the range core. Range-margin cohorts did not grow taller, but instead invested more into lateral growth and biomass accumulation that presumably reflects adaptation to their colder and not light-limited environment. Range-margin cohorts also exhibited leaf traits consistent with greater resource acquisition that may compensate for a shorter growing season and reduced light quality at higher latitude.

4. *Synthesis*. We confirmed that there is a genetic basis to adaptive trait shifts towards an expanding mangrove range margin. Our results suggest that genetically-based phenotypic differences better enable these range-margin mangroves to thrive within their stressful environment and may facilitate further poleward expansion in the future. In addition, our documentation of adaptive trait variation among maternal cohorts of an ecologically-important mangrove foundation species, quantitative data that is lacking for mangroves, should help inform mangrove restoration initiatives.

KEYWORDS

Atlantic Florida, *Avicennia germinans*, climate change, common garden experiment, foundation species, mangrove restoration, range expansion

INTRODUCTION

Distributional range margins are often defined by a species' inability to tolerate conditions beyond these boundaries (Brown, 1984). However, in response to a warming planet, these boundaries are expanding poleward for many species (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Osland et al., 2021; Pecl et al., 2017), with individuals that inhabit present-day range margins inherently at the forefront of this change. Due to marginal environmental conditions and novel selection pressures, individuals at range margins may exhibit strong genetic divergence and significant phenotypic differences from conspecifics within more benign portions of their range (Chuang & Peterson, 2016; Hardie & Hutchings, 2010). Understanding whether these unique range-margin genotypes are better able to thrive under the extreme climatic conditions at and beyond their current distributional limits can provide important insights into how species may respond to climate change (Nadeau & Urban, 2019; Rehm, Olivas, Stroud, & Feeley, 2015).

Evaluating genetic and phenotypic changes towards expanding range margins of plant foundation species will be particularly informative because of the direct influence of these species on ecosystem structure and function (Ellison et al., 2005). Hence, insights into how foundation species will respond to climate change will inevitably inform predictions about responses of entire ecosystems (Bernhardt & Leslie, 2013). A well-documented example of foundation species undergoing climate-driven range expansion is that of mangroves at their poleward range margins (Armitage, Highfield, Brody, & Louchouarn, 2015; Cavanaugh et al., 2014; Cohen et al., 2020; Fazlioglu, Wan, & Chen, 2020; Osland, Day, et al., 2017; Saintilan, Wilson, Rogers, Rajkaran, & Krauss, 2014; Whitt et al., 2020).

Mangroves are (sub)tropical, intertidal woody plants of significant ecological importance to coastal ecosystems (Lee et al., 2014) and a central component to a growing number of coastal rehabilitation and restoration initiatives (Friess et al., 2019; Waltham et al., 2020). Their distributional limits are defined by region-specific climatic thresholds in minimum temperatures and/or precipitation (Duke, Ball, & Ellison, 1998; Osland, Feher, et al., 2017). Along Atlantic Florida, USA, the northern extent of mangroves is controlled by a gradient in minimum winter temperatures that drives a transition from the southern range core of dense mangrove forests to the northern range margin of discrete mangrove patches within a landscape of temperate salt marsh (Cavanaugh et al., 2018; Osland, Feher, et al., 2017). Milder winters for several decades are linked to ongoing mangrove proliferation at this range margin (Cavanaugh et al., 2014; Rodriguez, Feller, & Cavanaugh, 2016) and further poleward expansion is forecast as freeze events become less common (Cavanaugh et al., 2019, 2015).

Neotropical black mangrove, *Avicennia germinans*, is the predominant mangrove species at the Atlantic Florida range margin (Lonard, Judd, Summy, DeYoe, & Stalter, 2017). Range-margin populations of *A. germinans* exhibit clear genetic differences from those directly south within the continuous range core (Kennedy, Preziosi, Rowntree, & Feller, 2020) and are the predominant source of new recruits to northern areas beyond this species' present-day distribution (Kennedy, Dangremond, et al., 2020). Field samples from these range-margin *A. germinans* also demonstrate shifts towards phenotypic traits consistent with greater cold tolerance compared to range-core conspecifics (Cook-Patton, Lehmann, & Parker, 2015; Kennedy, Preziosi, et al., 2020), with similar shifts observed at *A. germinans* range margins in the Gulf of Mexico (Madrid, Armitage, & López-Portillo, 2014; Méndez-Alonzo, López-Portillo, & Rivera-Monroy, 2008). Yet, we lack an understanding of whether these phenotypic differences in range-margin *A. germinans* have a genetic basis or are plastic responses to their marginal environmental conditions. Extensive trait plasticity in response to environmental variation is well documented in mangroves (e.g., Feller et al., 2010; Lovelock, 2008; Vovides et al., 2014), while relatively few studies provide evidence for genetically-based adaptive trait variation.

Common garden experiments are a tool to address this knowledge gap as their uniform environment allows for tests of genetic effects while controlling for trait plasticity (Hoffmann & Sgró, 2011). Furthermore, common gardens with environmental conditions analogous to those that restrict a species' distribution can provide additional insights into how genetically-based trait variation better suited to tolerate these conditions varies geographically within a species (Alberto et al., 2013; Warwell & Shaw, 2017). In this study, we monitored *A. germinans* maternal cohorts from areas in the Atlantic Florida range core and margin in a greenhouse common garden with annual temperatures that resembled those at the Atlantic Florida range margin. We assessed differences in a series of phenotypic traits starting at initial planting of field-collected propagules and continuing until two years development.

Our aim was to determine whether there is a genetic basis to previous field observations of adaptive trait shifts in *A. germinans* towards its expanding Atlantic Florida range margin (as outlined above). We predicted that, compared to range-core cohorts, (1) field-collected propagules from range-margin cohorts would survive in greater numbers and establish more quickly. (2) Range-margin cohorts would be less stressed under winter temperatures, which would result in (3) greater growth and biomass accumulation over the two-year experiment. (4) Range-margin cohorts would exhibit more conservative leaf traits (i.e., smaller, drier, reduced specific leaf area) to better tolerate marginal temperature conditions. Our documentation of adaptive trait variation among maternal cohorts of an ecologically-important mangrove species should provide not only insights into dynamics at expanding range margins, but also help inform mangrove restoration initiatives.

MATERIALS AND METHODS

Field sampling

We focused our sampling at the lowest level of genetic inheritance for our studied species (i.e., maternal cohorts). *Avicennia germinans* is a hermaphroditic, insect-pollinated tree or shrub that produces cryptoviviparous propagules (Lonard et al., 2017). Along Atlantic Florida, *A. germinans* exhibit a mixed-mating system with relatively high rates of self-fertilisation (Kennedy, Sammy, Rowntree, & Preziosi, 2021). As such, the maternal cohorts monitored in this research are a mixture of both selfed and outcrossed progeny, with outcrossed progeny being either full- or half-siblings.

On 7 – 8 October 2017, we collected mature *A. germinans* propagules systematically from around the entire canopy of maternal trees located in both the Atlantic Florida range core, where mangroves are the dominant coastal foundation species, and the range margin, where salt marsh vegetation is dominant (Figure 1A). We collected from three range-core and three range-margin sites, across similar geographic expanses (inter-site distances: 47.6 – 97.1 km for range core; 33.2 – 71.4 km for range margin), to include a broader representation of genetic variation across these areas (Figure 1A). Annual minimum temperatures decline with latitude across our sampling area, with temperatures <10°C, a threshold shown to induce chill stress in *A. germinans* seedlings (Devaney, Pullen, Feller, & Parker, 2021), common only at the range-margin sites (Figure 1B). All propagules collected from each maternal tree were stored together in one labelled plastic bag during field collections and then transported to the greenhouse facility at Manchester Metropolitan University in Manchester, UK (53.4713°N, 2.2412°W).

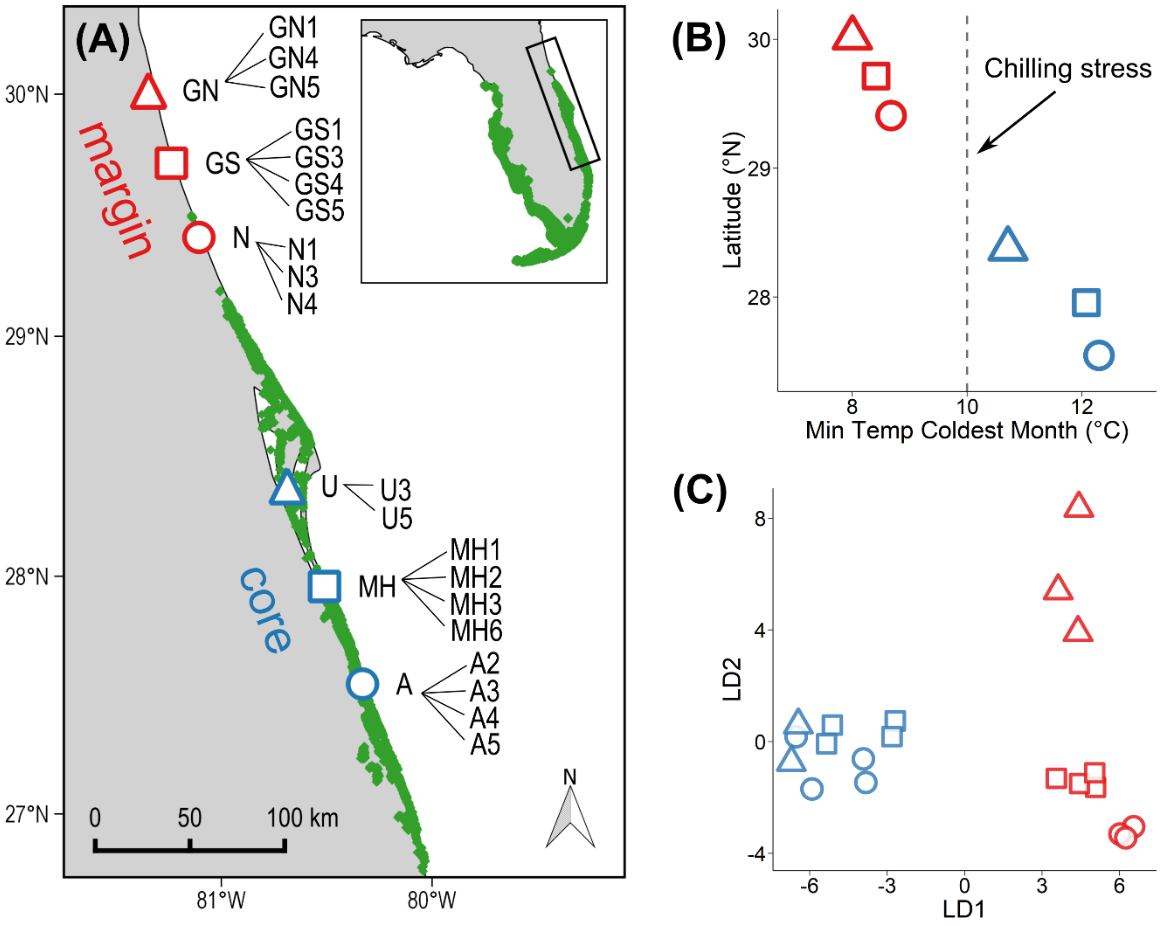

FIGURE 1. Field collections of *Avicennia germinans* propagules from Atlantic Florida, USA, for a greenhouse common garden experiment at Manchester Metropolitan University, Manchester, UK. (A) Twenty maternal cohorts from six collection sites were included in the experiment (n = 10 from range core, n = 10 from range margin). Mangrove distribution shown in green (Giri et al., 2011) (B) Latitudinal decline in annual minimum temperatures (1970–2000) across the sampled sites, with chilling stress (<10°C) common only at range-margin sites. Temperature data from WorldClim2 (Fick & Hijmans, 2017). (C) Discriminant analysis of principal components (DAPC) of the 20 maternal tree genotypes that demonstrates a clear separation between range core and margin. Throughout the figure, blue shapes depict range-core sites and cohorts, and red shapes depict range-margin sites and cohorts.

Maternal tree genotyping

During field sampling, we also collected a leaf from each maternal tree to generate their multi-locus genotypes with 12 nuclear microsatellite loci as outlined elsewhere (Kennedy, Preziosi, et al., 2020). We visualised genetic differences among maternal trees with a discriminant analysis of principal components (DAPC) (Jombart, Devillard, & Balloux, 2010) in the adegenet 2.1.1 R-package (Jombart & Ahmed, 2011). For this analysis, we retained nine principal components, which explained ~90% of the total variance, identified two clusters, and retained all five discriminant functions.

Common garden experiment

Our planting trays permitted the inclusion of 20 maternal cohorts in the common garden experiment, with range-core (n = 10) and range-margin (n = 10) cohorts equally represented (n = 2 – 4 cohorts per collection site) (Figure 1A). The experiment consisted of two components: (1) an establishment phase that monitored propagule development into seedlings until eight months post-planting (20 maternal cohorts x 30 biological replicates = 600 total propagules), and (2) a subsequent growth phase that monitored a random subset of these seedlings until two years post-planting (20 maternal cohorts x 12 biological replicates = 240 total seedlings). We used a randomised complete block design for each component, with one offspring from each of the 20 maternal cohorts present within each block (i.e., replicate planting tray) (see Figure S1 for photos of the experimental design). Greenhouse temperature and humidity were continuously monitored at 30-minute intervals with iButton data loggers (Measurement Systems Ltd, Newbury, UK). We set greenhouse temperatures to resemble those at the Atlantic Florida range margin based on mean monthly values (1981 – 2010) from St Augustine Lighthouse, Florida (29.8°N), obtained from the National Centers for Environmental Information (https://www.ncdc.noaa.gov/cdo-web/datatools). In addition, we set the duration of supplemental grow lights (54,800 lm, PLANTASTAR 400 W E40; OSRAM, Munich, Germany) each month to match mean monthly day length, also at St Augustine Lighthouse, based on data from the Earth System Research Laboratories (https://www.esrl.noaa.gov/gmd/grad/solcalc/). Due to the location of the greenhouse facility in Manchester, UK (53.4°N), summer months did experience greater photoperiods than those at the Atlantic Florida range margin (e.g., in June, greenhouse plants experienced ~3 hours more sunlight).

On 18 October 2017, 10 days after collection, we began floating field-collected propagules in a saline water solution (~15 ‰ Instant Ocean® Sea Salt) for one week, an optimal duration for seedling productivity (Simpson, Osborne, & Feller, 2017). On 25 October, we towel dried propagules and measured three size metrics to account for variation in maternal investment, specifically weight (g), length (mm), and width (mm). All three metrics exhibited strong positive correlations (Pearson's correlation, $r = .76\text{–}.88$, $p < .001$), so we decided to use propagule weight as our measure of maternal investment. Propagules were then planted in 7 × 7 × 6.5 cm square pots (LBS Horticulture, Colne, UK) filled with a 3:1 mixture of low nutrient commercial potting soil (Levington F1 Seed and Modular Compost; LBS Horticulture, Colne, UK) and sharp horticultural sand (RHS Sharp Sand; LBS Horticulture, Colne, UK), with no subsequent nutrient additions, and placed into 30 replicate trays (Gratnells shallow trays, 42.7 × 31.2 × 7.5 cm; YPO, Wakefield, UK). We added a saline water solution (~15 ‰ Instant Ocean® Sea Salt) to 3 cm depth within the trays to maintain soil saturation, and additional fresh water was added each week to return to this volume. Pots were also misted periodically with fresh water to ensure propagules remained hydrated. Every two weeks, trays were systematically rotated around the greenhouse and salinity was measured from six haphazardly-chosen trays with a handheld refractometer (VWR International, Lutterworth, UK). Complete water changes were performed at the end of each month. We determined that propagules had established as seedlings upon appearance of their first true leaves (Finney, 2011). Once the first seedling established, we began monitoring time to establishment for each propagule on a weekly basis until 35 weeks post-planting when 98.5% of surviving propagules had established. We also documented propagule mortality throughout this period.

On 10 July 2017, 8 months post-planting, we measured height (cm) and total growth as height plus length of any lateral shoots (cm) for all surviving seedlings. Then, on 18 July 2017, a random subset of 12 surviving seedlings from each of the 20 maternal cohorts was transferred to larger 11 × 11 × 12 cm square pots (LBS Horticulture, Colne, UK) filled with a fresh mix of 3:1 potting soil and sand (as detailed above), with no subsequent nutrient additions, and placed into 12 replicate trays (Garland square garden tray, 60 × 60 × 7 cm; LBS Horticulture, Colne, UK). A saline water solution (~15 ‰ Instant Ocean® Sea Salt) was added to 4 cm depth within the trays to ensure soils remained moist, additional fresh water was added each week to return to this volume, and plants were misted periodically. Every month, trays were systematically rotated around the greenhouse and salinity was measured from all trays. Complete water changes were performed every two months. We measured height (cm) and total growth (cm) for all plants at 10, 12, 14, 20, and 24 months post-planting, and documented plant mortality throughout this period.

To evaluate plant stress, we measured maximum quantum yield of photosystem II (F_v/F_m) with a FluorPen FP 100 (Photon Systems Instruments, Drásov, Czech Republic). F_v/F_m is an indicator of plant photosynthetic performance and a widely-used diagnostic to measure plant tolerance to environmental stress, with optimal values of ~0.83 for most plants (Baker, 2008; Maxwell & Johnson, 2000). Measurements were taken on a single, undamaged leaf from the youngest, fully-mature leaf pair for all surviving plants at three time points during the experiment: (1) at the start of the second winter (Dec 2018; 13 months post-planting), (2) during a series of nights with sub-zero temperatures (Feb 2019; 15 months post-planting), and (3) at the end of the second summer (Sept 2019; 22 months post-planting). Measurements were taken on three separate dates at time point 1 (4, 11, 18 Dec 2018) to establish baseline F_v/F_m values before the onset of colder winter temperatures, and a similar approach was taken at time point 2 (measurements on 1, 2, 3 Feb 2019) to assess plant responses over the course of a cold event. Values were highly correlated among measurement dates in December ($r = .83–.88$, $p < .001$) and February ($r = .89–.92$, $p < .001$), so we used mean values across these dates for statistical analyses. Measures for time point 3 were taken only on 5 September 2019 to assess plant performance under more benign conditions. All measurements were taken in the evening on plants that had been dark-adapted for at least two hours.

From 30 October to 7 November 2019, 24 months post-planting, plants were harvested and dried to obtain biomass measurements. Plants were cut at soil level, divided into roots, shoots, and leaves (roots were gently washed with fresh water to remove sediment), and then dried at 60°C for three days until constant weight (g). Prior to harvest, we collected one leaf from each surviving plant to measure functional traits. Leaves were undamaged and from the youngest, fully-mature leaf pair. We measured fresh weight (g) and used the Petiole smartphone application with calibration pad Nº 7 (Petiole LTD; https://petioleapp.com/) to measure leaf area (cm^2). We then oven dried leaves (as outlined above) and measured dry weight (g). Leaf dry-matter content (LDMC; g/g) was measured as dry weight divided by fresh weight, and specific leaf area (SLA; cm^2/g) was measured as leaf area divided by dry weight (Pérez-Harguindeguy et al., 2013). Leaves were then ground into a fine powder with a Retsch mixer mill MM 400 (Retsch, Haan, Germany) and analysed for percent carbon (%C), percent nitrogen (%N), and C:N with an Elementar vario EL cube CHNOS Elemental Analyzer (Elementar, Langenselbold, Germany), with a certified birch leaf reference standard (Elementar Microanalysis, Devon, UK). We did not obtain results from plants in the last experimental block (replicate 12) due to a technical issue during this analysis. As such, we analysed nutrient data from 214 of the 234 surviving plants (replicates 1–11).

Statistical analyses

All analyses were conducted in R v4.0.3 (R Core Team, 2020) with RStudio v1.4.1103 (RStudio Team, 2021). We tested for differences between range-core and range-margin cohorts with a series of mixed effects models using the *lmer* function in the lmerTest R-package (Kuznetsova, Brockhoff, & Christensen, 2017), with source region as a fixed effect and maternal cohort nested within region as a random effect. Although maternal cohorts were clustered by collection site nested within region, we did not include this random effect as variation attributed to environmental differences among collection sites should be accounted for with our inclusion of propagule weight as a covariate to account for maternal investment (see detailed model descriptions below). For linear models, we assessed fixed effects with the *anova* function with default Type III SS, and we assessed random effects with the *ranova* function with likelihood ratio tests. For the generalised linear model, all effects were assessed with likelihood ratio tests. Refer to Table S1 in the Supporting Information for detailed summaries of each model described below.

First, we tested whether the weight of field-collected propagules, a measure of maternal investment, varied between source regions and among maternal cohorts nested within region with a linear mixed effects model. Next, for the establishment phase of the experiment (0–8 months), we tested for effects of source region and maternal cohort nested within region on seedling survival (binary response) with a binomial generalised linear mixed effects model, and on time to establishment, height at 8 months, and total growth at 8 months with linear mixed effects models. We included propagule weight as a covariate (fixed) in these models to account for variation in maternal investment and replicate planting tray as a (random) blocking factor to account for environmental variation within the greenhouse. We included time to establishment as an additional covariate (fixed) in the height and growth models because it proved influential for both response variables, independent of propagule weight (see Table S1).

For the subsequent growth phase of the experiment (8–24 months), we tested for effects of source region and maternal cohort nested within region on height, total growth, biomass, ratios of height/growth to biomass, plant stress (F_v/F_m), and leaf traits (leaf area, LDMC, SLA, %C, %N, and C:N) with linear mixed effects models.

We log-transformed C:N for statistical analyses (Isles, 2020). We included plant size at the start of this phase of the experiment (i.e., total growth at 8 months) as a covariate (fixed) to account for variation in both propagule weight (measure of maternal investment) and time to establishment. In addition, we included replicate planting tray as a (random) blocking factor to account for environmental variation within the greenhouse. As height and total growth were measured at five time points, we first used repeated-measures models that included the effect of time (fixed) and the time × source region interaction (fixed) before analysing individual time points. We used the same approach for plant stress (F_v/F_m), which was measured at three time points.

Visual inspection of diagnostic plots for each model confirmed that linear models with a normal error distribution were suitable for all variables, except for survival that was assessed with a binomial error distribution. We did identify two large outliers for both SLA and log-transformed C:N, which were removed for analyses (see Table S1), although their inclusion did not qualitatively change the results described here. From each model, we calculated estimated marginal means for each source region in the emmeans R-package (Lenth, 2021). We also calculated marginal R^2 (variability explained by fixed effects) and conditional R^2 (variability explained by both fixed and random effects) for each model with the *r.squaredGLMM* function in the MuMIn R-package (Bartoń, 2020). Values for each model are presented in Table S1.

RESULTS

Maternal genotypes

All 20 maternal trees were genetically distinct, with a clear separation between range-core and range-margin genotypes (Figure 1C). Range-margin maternal trees exhibited greater genetic differences and greater clustering by collection site compared to those from the range core (Figure 1C).

Greenhouse conditions

Greenhouse temperatures were relatively consistent with long-term averages at the Atlantic Florida range margin (Figure S2). Chilling temperatures (≤10°C) were experienced on 29 and 82 days during the first and second year of the experiment, respectively. The number of days ≤10°C during the second year was higher than what is generally experienced at the Atlantic Florida range margin (73 ± 1.5 days; Devaney et al., 2021). Sub-zero temperatures were experienced on only two days (2 – 3 Feb 2019), both during the second year of the experiment (minimum: -1 and -3°C, respectively). Greenhouse relative humidity (%) was 55.0 ± 6.3 (SD) across the experimental period, considerably lower than annual values at the Atlantic Florida range margin (76.6 ± 7.9; data from St Augustine Airport, obtained from https://www.ncdc.noaa.gov/data-access/land-based-station-data/land-based-datasets). Salinity (‰) within replicate trays was 14.0 ± 2.7 (SD) for the initial establishment phase of the experiment and was 18.6 ± 4.3 for the subsequent growth phase.

Establishment phase (0 – 8 months)

Weight of field-collected propagules, a measure of maternal investment, varied among maternal cohorts ($\chi^2(1) = 235.7$, $p < .001$), with a mean increase of 98% from the cohort with the lightest to heaviest propagules (1.85 to 3.64 g) (Figure S3).

Propagules from range-margin cohorts were heavier than those from range-core cohorts ($F_{1, 18} = 7.7$, $p = .013$), with a 23% increase in the estimated marginal mean (2.77 and 2.26 g, respectively), although there was considerable variation among range-margin cohorts (Figure S3).

A total of 529 of 600 planted propagules (88.2%) survived to establishment. Survival ($\chi^2(1) = 31.2$, $p < .001$), time to establishment ($\chi^2(1) = 98.4$, $p < .001$), height at 8 months ($\chi^2(1) = 94.0$, $p < .001$), and total growth at 8 months ($\chi^2(1) = 122.7$, $p < .001$) all varied among maternal cohorts (Figure 2). Survival ranged from only 40% within one range-core cohort to 100% within six range-margin cohorts (Figure 2A). Mean increases from the cohort with the lowest to highest values were 14% for establishment time (26.0 to 30.2 weeks) and 90% for both height and total growth (13.6 to 25.9 cm each) (Figure 2B, C). Range-margin cohorts survived in greater numbers (96%; 289 of 300 planted propagules) compared to range-core cohorts (80%; 240 of 300 planted propagules) ($\chi^2(1) = 12.1$, $p = .005$) (Figure 2A), and established faster ($F_{1, 18.9} = 7.4$, $p = .014$) with a 4% decrease in the estimated marginal mean (27.1 and 28.3 weeks) (Figure 2B). Several range-margin cohorts grew more than their range-core conspecifics over the first eight months, but there were two notable exceptions that were among the smallest plants in the experiment (cohort: GN4, GN5; Figure 2C). As a result, estimated marginal means were nearly identical between regions for both height ($F_{1, 20.5} = 0.9$, $p = .356$) and growth ($F_{1, 20.1} = 0.3$, $p = .586$) (Figure 2C). Propagule weight did not affect survival ($\chi^2(1) = 1.2$, $p = .265$) or establishment time ($F_{1, 466.3} = 0.4$, $p = .525$), but impacted height ($F_{1, 416.3} = 52.5$, $p = .001$) and growth ($F_{1, 407.2} = 66.7$, $p = .001$). Time to establishment also impacted height ($F_{1, 512.7} = 305.5$, $p < .001$) and growth ($F_{1, 494.8} = 268.5$, $p < .001$).

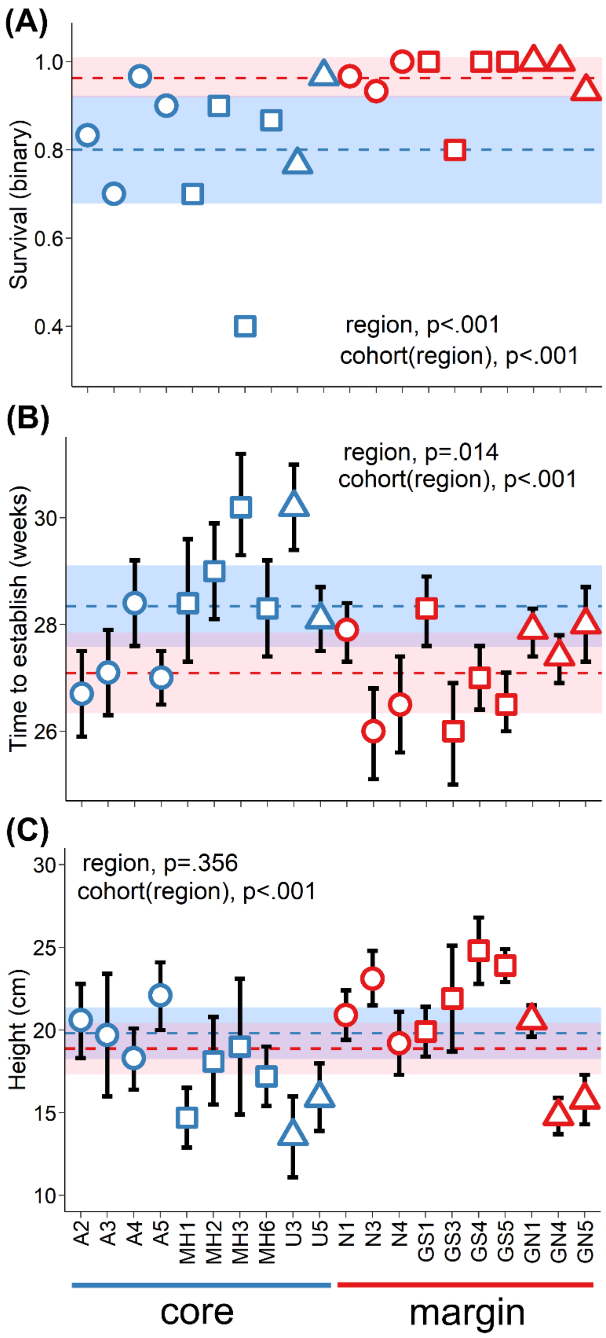

FIGURE 2. Range-margin cohorts (shown in red) (A) survived in greater numbers and (B) established faster, but (C) exhibited similar height, and total growth (*not shown*), at eight months compared to range-core cohorts (shown in blue). In the figure, different colour/shape combinations depict the six collection sites (refer to Figure 1 for geographical locations). Region-level estimated marginal means and 95% confidence intervals are shown with dashed lines and shaded areas in blue for the range core and in red for the range margin. Cohort-level means and 95% confidence intervals are calculated from the raw data.

Subsequent growth phase (8 – 24 months)

A total of 234 of 240 transferred seedlings (97.5%) survived the subsequent 16 months of the experiment. The six mortalities consisted of three range-core and three range-margin plants.

We assessed plant stress with measurements of quantum yield (F_v/F_m) in December 2018, February 2019, and September 2019. Mean temperatures were 14.3°C (min / max: 9.8 / 18.7°C), 6.6°C (-0.7 / 16.8°C), and 20.4°C (13.3 / 34.8°C) during these measurement periods, respectively. We found that the effect of source region on plant stress varied temporally (time × source region: $F_{2, 666.1} = 54.7$, $p < .001$; Table S1), with the strongest effect of source region observed during the most stressful temperature conditions (February 2019). Maternal cohorts exhibited variation in F_v/F_m at each time point (Dec 2018: $\chi^2(1) = 6.8$, $p = .009$; Feb 2019: $\chi^2(1) = 18.5$, $p < .001$; Sept 2019: $\chi^2(1) = 13.3$, $p < .001$), with mean increases from the cohort with lowest to highest F_v/F_m of 15% (0.57 to 0.66), 78% (0.26 to 0.47), and 5% (0.73 to 0.76), respectively (Figure 3**)**. Range-margin cohorts consistently had higher F_v/F_m than range-core cohorts (Dec 2018: $F_{1, 18.6} = 39.4$, $p < .001$; Feb 2019: $F_{1, 18.7} = 60.7$, $p < .001$; Sept 2019: $F_{1, 18.9} = 15.9$, $p = .001$), with increases in estimated marginal means of 9% (0.64 and 0.59), 37% (0.41 and 0.30), and 1% (0.75 and 0.74) across the three time points, respectively (Figure 3). Total growth at 8 months impacted F_v/F_m in December 2018 ($F_{1, 184.5} = 8.4$, $p = .004$) and February 2019 ($F_{1, 211.4} = 12.9$, $p < .001$), but not in September 2019 ($F_{1, 202.6} = 1.4$, $p = .246$).

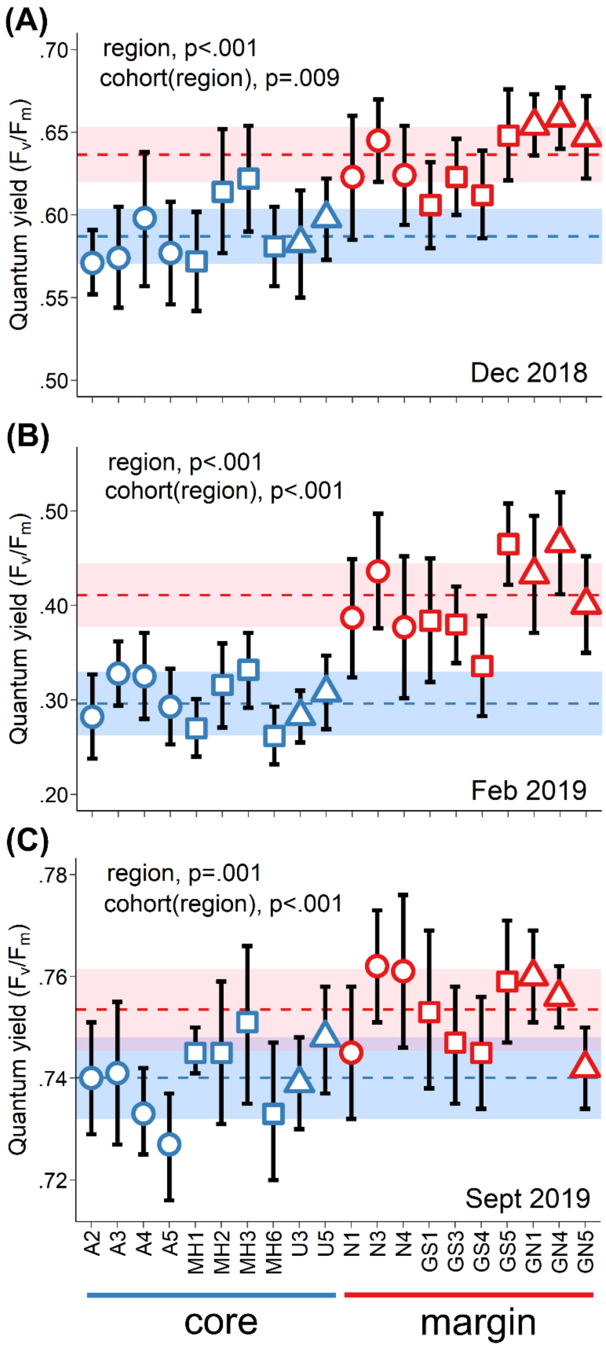

FIGURE 3. Range-margin cohorts (shown in red) were less stressed than range-core cohorts (shown in blue) in (A) December 2018, (B) February 2019 after consecutive nights of sub-zero temperatures, and (C) September 2019. Note that y-axes vary among panels. In the figure, different colour/shape combinations depict the six collection sites (refer to Figure 1 for geographical locations). Region-level estimated marginal means and 95% confidence intervals are shown with dashed lines and shaded areas in blue for the range core and in red for the range margin. Cohort-level means and 95% confidence intervals are calculated from the raw data.

We measured plant height and total growth at 10, 12, 14, 20, and 24 months post-planting. We found that the effect of source region on height varied temporally (time × source region: $F_{4, 1130.0} = 6.6$, $p < .001$); whereas, we found no temporal variation in the effect of source region on total growth (time × source region: $F_{4, 1130.0} = 0.7$, $p = .625$) (Table S1). Height varied among maternal cohorts at every time point ($p < .001$; Table S1). At 24 months, height ($\chi^2(1) = 39.0$, $p < .001$) and total growth ($\chi^2(1) = 28.0$, $p < .001$), varied among maternal cohorts, with mean increases from the cohort with lowest to highest values of 55% (36.7 to 56.9 cm) and 63% (47.2 to 77.1 cm), respectively (Figure 4A, B). As found at 8 months, the height of range-margin cohorts did not vary from those of range-core cohorts at 10 months ($F_{1, 18.7} = 0.3$, $p = .582$) or at 12 months ($F_{1, 18.5} = 2.5$, $p = .134$). However, starting at 14 months, range-margin cohorts were marginally shorter than range-core cohorts ($F_{1, 18.6} = 4.5$, $p = .047$) and this difference progressively became larger at 20 months ($F_{1, 18.4} = 5.6$, $p = .029$) and then at 24 months ($F_{1, 18.6} = 7.5$, $p = .013$), when we found a decrease in the estimated marginal mean of 8% (48.3 and 52.2 cm) (Figure 4A). In contrast, estimated marginal means for total growth at 24 months were nearly identical between regions ($F_{1, 18.6} = 0.0$, $p = .844$) (Figure 4B). As detailed for height/growth at 8 months, these patterns were partly shaped by two range-margin cohorts that were notable exceptions and among the smallest plants in the experiment (cohort: GN4, GN5; Figure 4A, B). Total growth at 8 months had a substantial impact on height at 24 months ($F_{1, 222.6} = 237.5$, $p < .001$) and total growth at 24 months ($F_{1, 226.5} = 452.3$, $p < .001$).

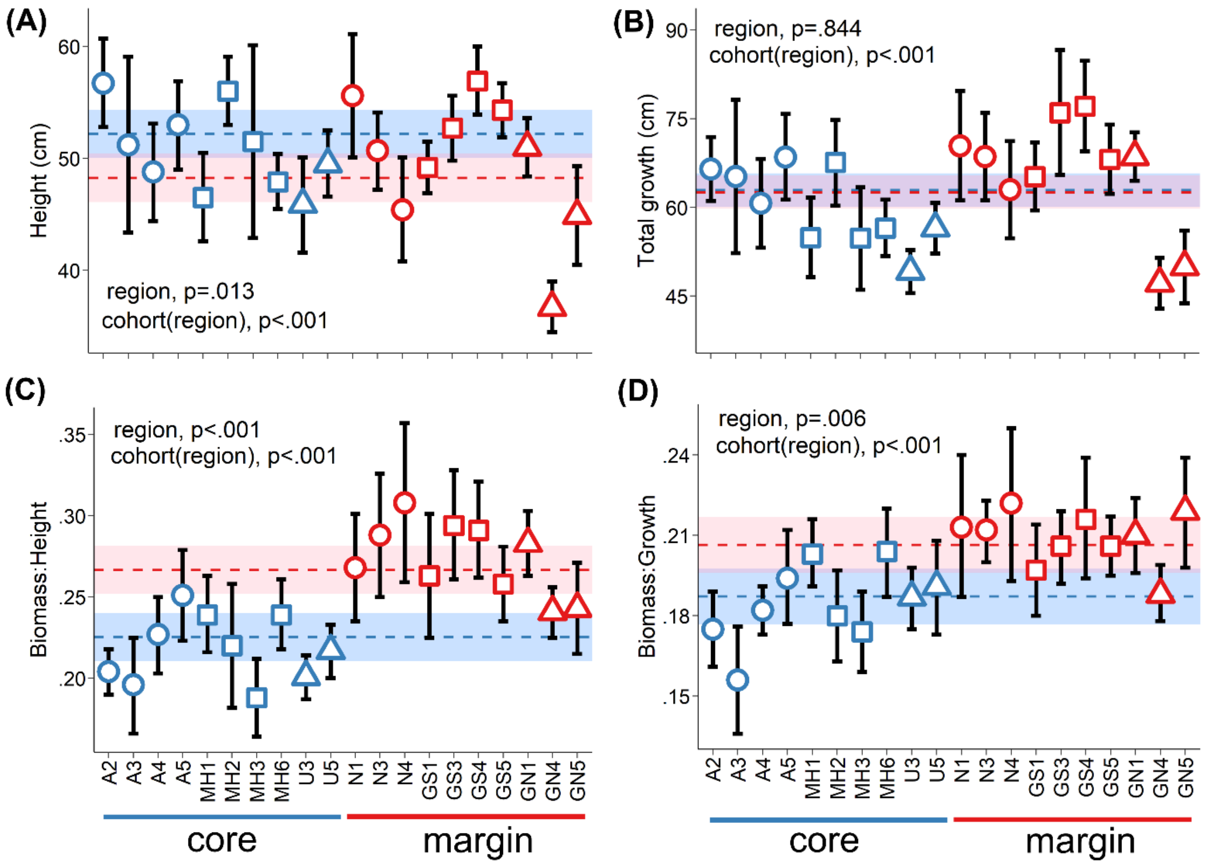

FIGURE 4. At two years development, range-margin cohorts (shown in red) were (A) shorter, but (B) exhibited similar total growth (height plus lateral growth), and accumulated a greater proportion of biomass (C) to height and (D) to growth compared to range-core cohorts (shown in blue). In the figure, different colour/shape combinations depict the six collection sites (refer to Figure 1 for geographical locations). Region-level estimated marginal means and 95% confidence intervals are shown with dashed lines and shaded areas in blue for the range core and in red for the range margin. Cohort-level means and 95% confidence intervals are calculated from the raw data.

Total biomass ($\chi^2(1) = 35.9$, $p < .001$) and the ratios of biomass to height ($\chi^2(1) = 19.3$, $p < .001$) and to growth ($\chi^2(1) = 20.5$, $p < .001$) all varied among maternal cohorts, with mean increases from the cohort with lowest to highest values of 87% (8.9 to 16.6 g), 63% (0.19 to 0.31), and 44% (0.16 to 0.23), respectively (Figure 4C, D; Figure S4**)**. Range-margin cohorts accumulated more biomass ($F_{1, 18.5} = 7.1$, $p = .015$) and exhibited greater biomass to height ($F_{1, 18.3} = 21.5$, $p < .001$) and to growth

($F_{1, 18.1} = 9.6$, $p = .006$) compared to range-core cohorts, with increases in estimated marginal means of 11% (13.1 and 11.8 g), 17% (0.27 and 0.23), and 11% (0.21 and 0.19), respectively (Figure 4C, D; Figure S4). Range-margin cohorts tended to accumulate more biomass across each measured fraction (i.e., leaves, shoots, and roots), but region-level differences were only statistically-significant for leaves ($F_{1, 18.3} = 10.8$, $p = .004$) and roots ($F_{1, 18.7} = 9.8$, $p = .006$), not shoots ($F_{1, 18.6} = 0.2$, $p = .704$) (Figure S4). Again, these patterns were partly shaped by two smaller range-margin cohorts (cohort: GN4, GN5; Figure S4). Total growth at 8 months had a substantial impact on total biomass ($F_{1, 227.3} = 422.8$, $p < .001$) and impacted the ratios of biomass to height ($F_{1, 219.3} = 97.0$, $p < .001$) and to growth ($F_{1, 220.9} = 25.9$, $p < .001$).

Leaf area ($\chi^2(1) = 39.8$, $p < .001$), leaf dry-matter content (LDMC; $\chi^2(1) = 25.8$, $p < .001$), specific leaf area (SLA; $\chi^2(1) = 14.6$, $p < .001$), and log-transformed C:N ($\chi^2(1) = 8.0$, $p = .005$) all varied among maternal cohorts, with mean increases from the cohort with lowest to highest values of 62% (6.9 to 11.2 cm^2), 15% (0.33 to 0.38 g/g), 26% (57.4 to 72.1 cm^2/g), and 8% (3.39 to 3.67), respectively (Figure 5). Leaf area ($F_{1, 18.7} = 0.0$, $p = .844$) and LDMC ($F_{1, 18.3} = 1.4$, $p = .251$) did not vary between range-margin and range-core cohorts (Figure 5A, B). Instead, range-margin cohorts exhibited greater SLA ($F_{1, 19.0} = 51.2$, $p < .001$) and lower log-transformed C:N ($F_{1, 17.0} = 12.9$, $p = .002$) compared to range-core cohorts, with an increase in the estimated marginal mean of 12% (66.5 and 59.4 cm^2/g) and a decrease of 3% (3.48 and 3.58), respectively (Figure 5C, D). Lower C:N in the leaves of range-margin cohorts was the product of greater %N ($F_{1, 16.9} = 10.8$, $p = .004$) and not changes in %C ($F_{1, 18.6} = 0.8$, $p = .370$) (Figure S5). Total growth at 8 months impacted LDMC ($F_{1, 220.3} = 8.4$, $p = .004$) and SLA ($F_{1, 210.0} = 22.0$, $p < .001$), but did not impact leaf area ($F_{1, 215.7} = 0.0$, $p = .931$) or log-transformed C:N ($F_{1, 179.1} = 0.0$, $p = .923$).

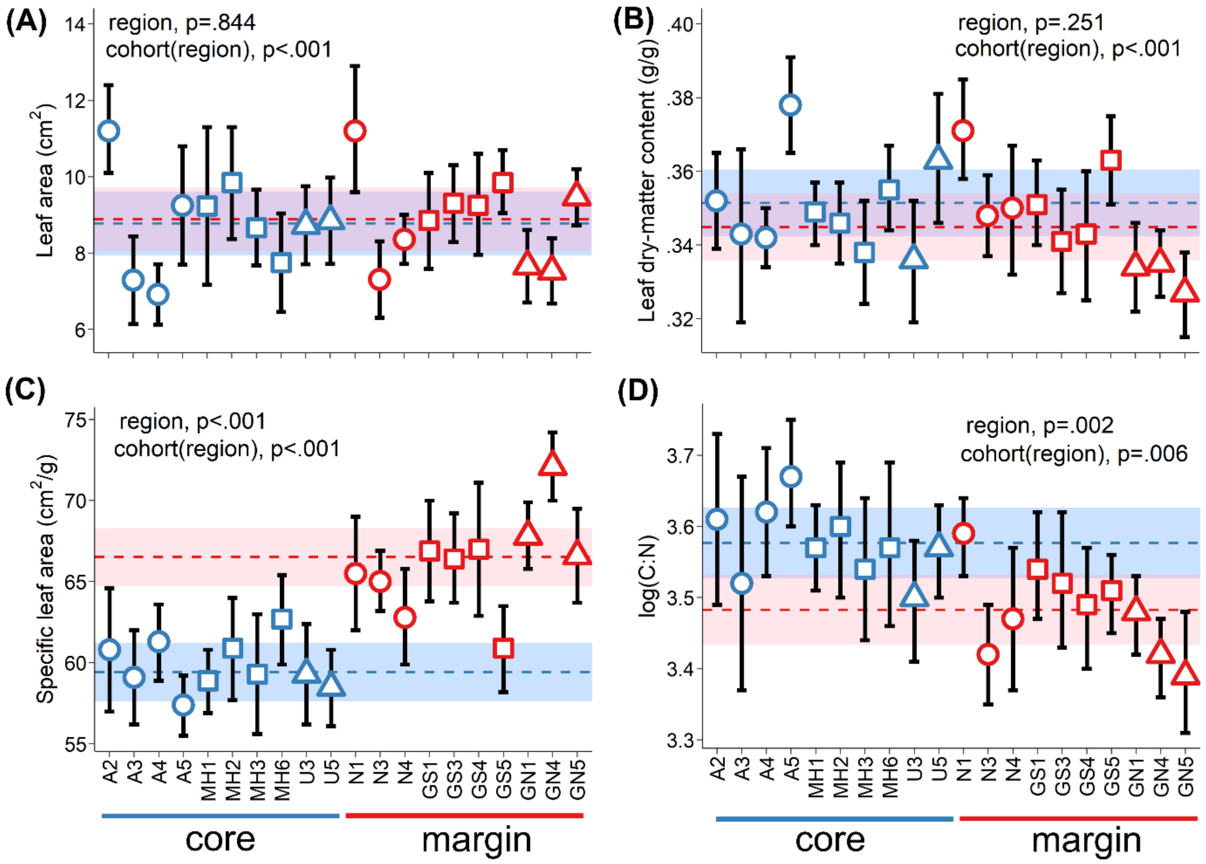

FIGURE 5. Range-margin cohorts (shown in red) produced leaves of similar (A) size and (B) leaf dry-matter content (LDMC), but with (C) greater specific leaf area (SLA) and (D) lower C:N compared to range-core cohorts (shown in blue). In the figure, different colour/shape combinations depict the six collection sites (refer to Figure 1 for geographical locations). Region-level estimated marginal means and 95% confidence intervals are shown with dashed lines and shaded areas in blue for the range core and in red for the range margin. Cohort-level means and 95% confidence intervals are calculated from the raw data.

DISCUSSION

In this study, we confirmed that there is a genetic basis to adaptive trait shifts towards an expanding range margin of a mangrove foundation species (*Avicennia germinans*). Maternal cohorts from the northern Atlantic Florida range margin consistently outperformed those from the southern range core under annual temperatures analogous to range-margin conditions in a two-year greenhouse common garden experiment. Our findings suggest that genetically-based phenotypic differences better enable these range-margin mangroves to thrive under their stressful conditions and may facilitate further range expansion with climate change. In addition, our documentation of substantial adaptive trait variation among maternal cohorts of an ecologically-important mangrove species should help inform future mangrove restoration initiatives.

Range-margin mangroves outperform range-core conspecifics

Species at their range margins are often genetically distinct from range-core conspecifics and may also exhibit adaptive shifts in morphology, reproductive strategies, and stress tolerance to facilitate establishment and survival in their marginal environment (Chuang & Peterson, 2016). The twenty *A. germinans* maternal trees sampled for this experiment exhibited a clear distinction between range-margin and range-core genotypes, consistent with population-level genetic differences along Atlantic Florida (Kennedy, Preziosi, et al., 2020). Also, we found that cohorts of field-collected propagules from range-margin trees planted in a common garden exhibited clear advantages over range-core cohorts during their critical establishment phase and under stressful winter conditions. In support of our first prediction, range-margin cohorts not only survived in greater numbers, but these survivors established, on average, more than a week earlier than range-core cohorts. We then observed that all plants exhibited signs of stress [i.e., suboptimum values of quantum yield (F_v/F_m)] under winter chilling and sub-zero temperatures, a ubiquitous plant response to winter conditions (Oliveira & Peñuelas, 2005). Yet, in support of our second prediction, range-margin cohorts exhibited higher F_v/F_m under chilling stress and the difference between range-margin and range-core cohorts was even greater when temperatures dropped below 0°C. These differences suggest that range-margin cohorts were better able to maintain photosynthetic efficiency under winter conditions, and that this ability was more pronounced under more extreme conditions often experienced at the Atlantic Florida range margin, but not within the range core. Close to optimum F_v/F_m, and minimal differences between source regions, under more benign summer temperatures suggests that all plants subsequently recovered photosynthetic efficiency, with similar values observed in *A. germinans* seedlings under optimum light and salinity conditions (Dangremond, Feller, & Sousa, 2015).

We found mixed support for our third prediction as greater stress tolerance in range-margin cohorts did translate into greater biomass accumulation, but not into greater growth. Instead, range-core cohorts gradually grew taller as the experiment progressed, while total growth (height plus length of lateral shoots) remained nearly identical between source regions throughout the experiment. In other words, over time, range-margin cohorts invested more into lateral versus vertical growth. Adult *A. germinans* at the Atlantic Florida range margin demonstrate this same pattern as they grow wider rather than taller (Chapman et al., 2021). Range-margin cohorts also accumulated a greater proportion of biomass relative to their size. This shift towards a greater investment into biomass over height may reflect adaptation to novel conditions within the harsh range-margin environment, analogous to responses across elevation gradients (Parker, Rodriguez, & Loik, 2003). At poleward range margins, shorter *A. germinans* would be less impacted by cold events due to warmer temperatures closer to the soil surface (Osland et al., 2019) and to protection offered by salt marsh vegetation (Guo, Zhang, Lan, & Pennings, 2013; Pickens, Sloey, & Hester, 2019). Also, in terms of access to sunlight, increased height would not be as beneficial for developing range-margin mangroves surrounded by low-stature salt marsh as it would be for range-core mangroves attempting to reach sunlight in closed canopy forests. It is important to note that the patterns outlined here were partly shaped by two range-margin cohorts that presented obvious exceptions in terms of growth. These cohorts, both from the most northern collection site, were consistently among the smallest plants in the experiment. However, despite their small stature, these plants were not underperforming as they exhibited clear advantages over range-core cohorts at many other measured traits, including greater stress tolerance, greater proportion of biomass to height/growth, and greater resource acquisition (see discussion in the next paragraph). A reciprocal transplant experiment with planting sites in both the range core and margin could assess whether reduced height represents local adaptation within range-margin *A. germinans*.

Our fourth prediction was not supported as range-margin cohorts did not exhibit more conservative leaf traits (i.e., smaller, drier, reduced specific leaf area), which suggests that previous field documentation of systematic shifts in these particular traits among populations of Atlantic Florida *A. germinans* (Cook-Patton et al., 2015; Kennedy, Preziosi, et al., 2020) may be the product of trait plasticity in response to environmental variation. Instead, in the common garden, we found that range-margin cohorts produced leaves of similar size and leaf dry-matter content to those of range-core cohorts, but with increased specific leaf area and lower C:N due to greater nitrogen content. These differences are consistent with a greater ability among range-margin cohorts to capture light and nutrient resources, further supported by their greater accumulation of leaf and root biomass compared to range-core cohorts. Plastic shifts towards enhanced specific leaf area and root growth are found in *A.*

germinans seedlings under limited resource availability to maximise resource acquisition (McKee, 1995). Here, we found that similar genetically-based shifts can also occur along a relatively narrow transition from mangrove range core to margin (27.5 – 30.0°N), presumably to compensate for a shorter growing season and reduced light quality at higher latitude (Spence & Tingley, 2020). An analogous genetically-based shift towards greater resource acquisition has also been documented in Brazil across greater geographic distance (0 – 28°S) between range core and margin populations of *A. schaueriana*, a closely-related congener (Cruz et al., 2019).

Genetic basis to trait variation in range-margin mangroves

Range margins may foster unique genetic adaptations that enable species to persist under extreme climatic conditions and that can dictate future responses to climate change (Rehm et al., 2015). Here, we demonstrated a genetic basis to adaptative trait shifts within a mangrove towards its expanding Atlantic Florida range margin, with evidence of greater survival during initial establishment, greater stress tolerance over winter, greater biomass accumulation, and greater resource acquisition among range-margin cohorts. Although still limited, growing evidence supports genetically-based adaptive shifts in chill tolerance (Markley, McMillan, & Thompson Jr, 1982; Short, Chen, & Wee, 2021), freeze tolerance (Hayes et al., 2020), resource acquisition (Cruz et al., 2019), and precocious reproduction (Dangremond & Feller, 2016) towards cold-sensitive mangrove distributional margins in Brazil, China, and the USA. In addition, evidence for selection along climatic gradients is found across mangrove distributions in Brazil (Cruz et al., 2020; Da Silva et al., 2021). Therefore, despite the immense trait plasticity within mangroves that enables their proliferation across highly variable environments (Feller et al., 2010), trait evolution may also be a common phenomenon in these systems, in particular towards range margins where selection pressures are inherently at their highest. Multiple interacting processes could drive this change, including selective mass mortality, genetic drift, and spatial sorting (Nadeau & Urban, 2019), as well as epigenetic changes (Robertson & Richards, 2015). A broader understanding of the processes driving these adaptive shifts could be achieved with further research that evaluates trait changes towards multiple mangrove range margins defined by distinct climatic thresholds and colonisation histories (e.g., Bardou, Parker, Feller, & Cavanaugh, 2021).

Our findings also provide insight into how an ecologically-important mangrove species (*A. germinans*) may respond to climate change at its poleward range margin. Phenotypic differences outlined above present clear advantages for range-margin over range-core genotypes in terms of proliferation within currently occupied range-margin sites and colonisation of more poleward areas. Mangrove expansion is forecast along Atlantic Florida as freeze events become less common (Cavanaugh et al., 2019, 2015), but poleward expansion of *A. germinans* along this coastline may be restricted to periods following extreme storm events that provide new recruits almost exclusively from range-margin sources (Kennedy, Dangremond, et al., 2020). Hence, an expanding gene pool with a greater representation of range-margin genotypes, that are better able to thrive under the climatic extremes beyond the current mangrove distribution, will presumably facilitate future *A. germinans* range expansion. This transition from salt marsh to mangrove dominance will inevitably have wide-reaching effects on these coastal ecosystems (Doughty et al., 2016; Kelleway et al., 2017; Osland et al., 2018; Simpson, Stein, Osborne, & Feller, 2019).

Considerations and Next steps

Offspring may exhibit phenotypic differences as a result of several factors, specifically the genetic makeup of their parents, their growing environment, and maternal effects that are shaped by both maternal genetics and maternal environment (Wolf & Wade, 2009). In this research, we monitored the development of field-collected propagules in a single greenhouse environment. Therefore, differences observed among maternal cohorts are the product of parental genetics and maternal effects. We genotyped maternal trees, but lack information on the unique genotypes of each individual plant within this experiment. As a result, some variation within maternal cohorts will be attributed to differences in pollination (i.e., proportions of progeny that are selfed, outcrossed full-siblings, and outcrossed half-siblings). Geographical variation in mating system, however, should not have systematically impacted our region-level results as there is not a systematic change in outcrossing rates along our range core to margin sampling gradient (Kennedy et al., 2021).

We found that field-collected propagules from range-margin maternal trees were, on average, heavier than those from range-core trees, consistent with previous documentation of greater propagule weight towards the Atlantic Florida range margin for *A. germinans* (Nathan, 2020) and of greater propagule size for the co-occurring mangrove, *Rhizophora mangle* (Dangremond & Feller, 2016). Propagule size is often influenced by maternal environment and is a common proxy for maternal effects. Greater maternal investment into offspring can facilitate species expansion (Estrada, Wilson, NeSmith, & Flory, 2016), although the strength of environmentally-induced maternal effects in plants generally declines as offspring age (Maruyama et al., 2016). Propagule weight, and subsequently total growth at 8 months, both proved highly influential in terms of growth and biomass accumulation across our two-year experiment. However, our observations of phenotypic differences among maternal cohorts were not merely shaped by variation in maternal investment. After accounting for this variation, we still observed significant effects of source region on height and biomass. In addition, greater propagule weight had no discernible impact on survival or establishment times and subsequent total growth at 8 months had a limited impact (compared to source region) on stress tolerance and most leaf traits.

Controlled common garden experiments can determine whether there is a genetic basis to phenotypic differences within a species, but inherently lack the reality and complexity of natural field conditions. Our greenhouse experiment demonstrates that range-margin *A. germinans* maternal cohorts may be better suited to thrive under stressful temperature conditions analogous to those at the Atlantic Florida range margin over the first two years of their development. However, in addition to temperature, multiple interacting abiotic and biotic factors will influence the establishment, survival, and growth of these range-margin mangroves (Rogers & Krauss, 2019). Longer-term *in situ* common gardens are, therefore, a logical next step to better predict how these coastal foundation species will respond to climate change. Although challenging because of long generation times, networks of common gardens have provided a wealth of knowledge regarding how forest trees have adapted to different environments and how they may respond to changing environmental conditions (Alberto et al., 2013). A series of common gardens both at and beyond mangrove range margins could further our understanding of the long-term fitness and persistence of these mangroves and of the factors that may limit or facilitate further range expansion.

Implications for mangrove rehabilitation and restoration

Initiatives to rehabilitate and restore degraded coastal ecosystems are growing in number (Waltham et al., 2020). Mangrove foundation species are a central component of many such initiatives because of the ecosystem services they provide (Friess et al., 2019). Restoration-focused experimental research demonstrates that intraspecific genetic and phenotypic variation within coastal foundation species can influence their survival and productivity, as well as ecosystem service provision (Bernik, Pardue, & Blum, 2018; Hughes, 2014; Plaisted, Novak, Weigel, Klein, & Short, 2020). Yet, only one study has documented similar quantitative data on adaptive trait variation within mangroves at the level that replanting occurs (i.e., propagules collected from maternal trees). Proffitt & Travis (2010) found that survival, growth, and age to reproduction varied among maternal cohorts of red mangrove (*Rhizophora mangle*) and that these patterns differed between two intertidal settings. Although mangrove replanting may often not be necessary (Lewis, 2005) and not a viable alternative at high-stress range margins (Macy, Osland, Cherry, & Cebrian, 2021), results from Proffitt & Travis (2010) and our documentation of substantial differences among mangrove maternal cohorts in survival, stress tolerance, growth, and biomass accumulation (key success criteria for rehabilitation and restoration projects) highlight how source selection could influence the outcome of initiatives where mangrove replanting is needed.

Clear advantages exhibited by range-margin cohorts grown under temperatures analogous to range-margin conditions could be viewed as support for using local sources, or sources with similar environmental conditions, in restoration projects (Bucharova et al., 2017). Records of propagule source and basic monitoring data on phenotypic variation within growth nurseries could help inform source selection and potentially improve replanting success. However, much more work is needed to understand how the genetic background of propagules used for replanting may influence the responses of these developing plants to the multiple interacting stressors common in mangrove systems (e.g., salinity, inundation, herbivory, irradiation) (Krauss et al., 2008). In addition, genetic variation within restoration plantings could shape the associated communities of organisms that colonise and inhabit these areas (Breed et al., 2018), with evidence that mangrove maternal genotype can influence soil microbial communities (Craig, Kennedy, Devlin, Bardgett, & Rowntree, 2020) and that genetic differences among mangrove hosts can correlate with the composition of endophytic fungal communities (Kennedy, Antwis, Preziosi, & Rowntree, *accepted*). Embedding *in situ* common garden experiments (as described in the previous section) into larger adaptive management experiments (Ellison, Felson, & Friess, 2020) could begin to uncover how intraspecific genetic variation may impact mangrove restoration and within which contexts these effects are most influential.

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AUTHOR CONTRIBUTIONS

J.P.K., R.F.P, and J.K.R. conceived the ideas and designed methodology. J.P.K. collected and analysed the data, and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

All data presented in this article will be publicly available on figshare.

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Supporting information

(a)

(b)

FIGURE S1. Photos of the common garden experiment for (a) the establishment phase (0 to 8 months; n = 600 plants) and (b) the subsequent growth phase (8 to 24 months; n = 240 plants).

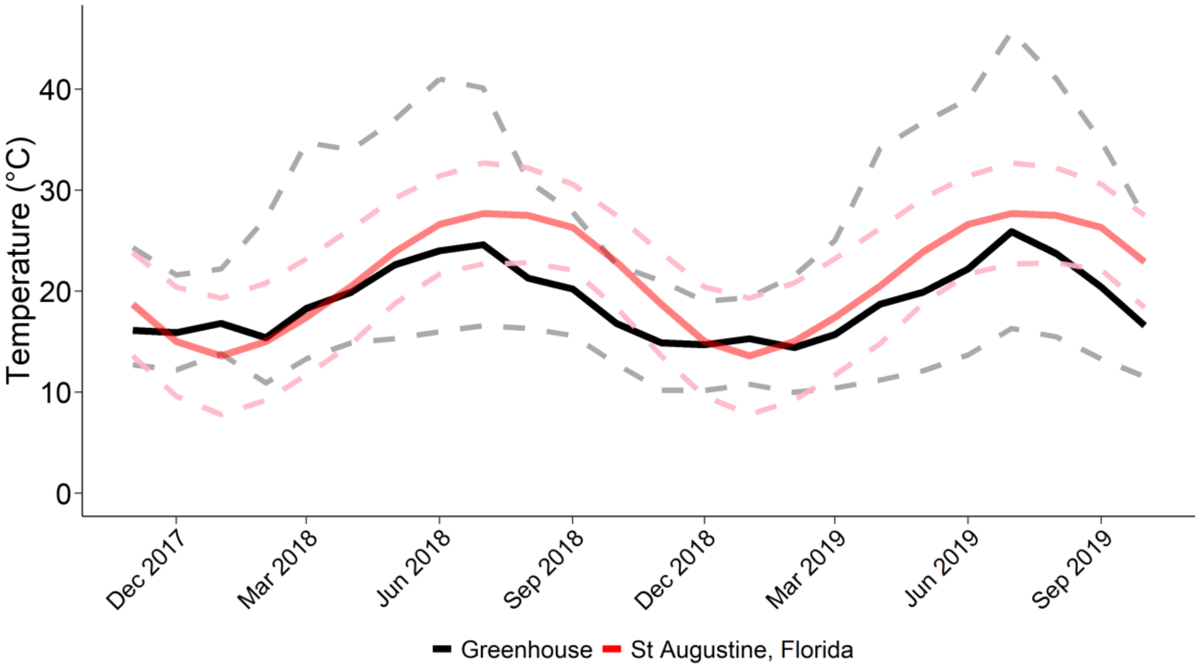

FIGURE S2. Greenhouse temperatures were relatively consistent with long-term (1981-2010) averages at the Atlantic Florida range margin (St Augustine, Florida; data from: https://www.ncdc.noaa.gov/cdo-web/datatools). Solid lines show monthly mean temperatures and dashed lines show monthly maximum and minimum temperatures.

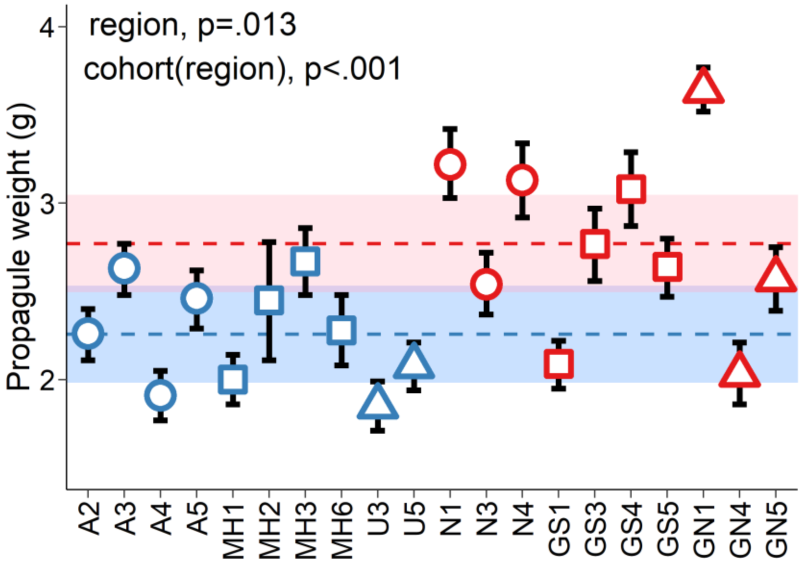

Figure S3. Field-collected propagules from range-margin cohorts (shown in red) were heavier than those from range-core cohorts (shown in blue), although considerable variation was found among range-margin cohorts. In the figure, different colour/shape combinations depict the six collection sites (refer to Figure 1 in the main text for geographical locations). Region-level estimated marginal means and 95% confidence intervals are shown with dashed lines and shaded areas in blue for the range core and in red for the range margin. Cohort-level means and 95% confidence intervals are calculated from the raw data.

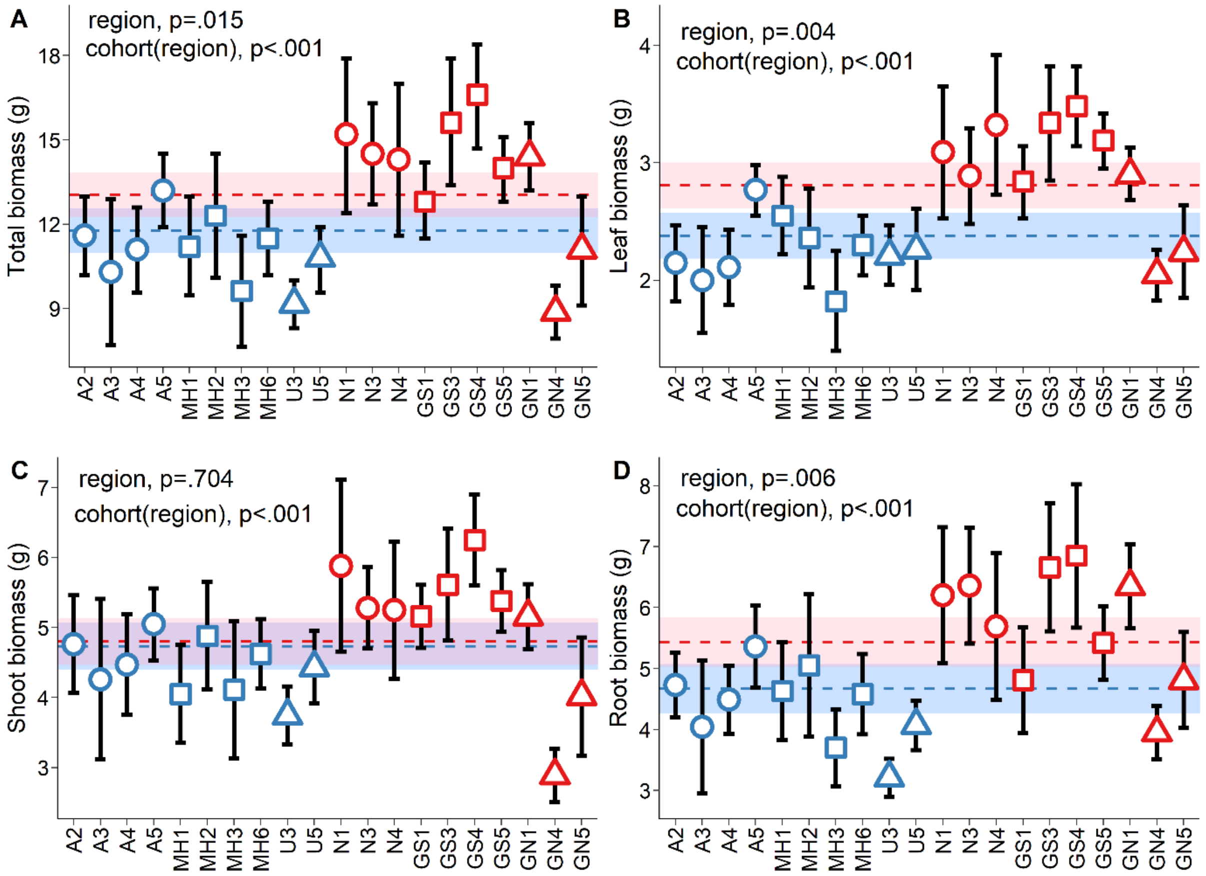

Figure S4. (A) Range-margin cohorts (shown in red) accumulated more biomass than range-core cohorts (shown in blue). Range-margin cohorts tended to accumulate more biomass across each measured fraction, (B) leaves, (C) shoots, and (D) roots, but region-level differences were only statistically-significant for leaves and roots. In the figure, different colour/shape combinations depict the six collection sites (refer to Figure 1 in the main text for geographical locations). Region-level estimated marginal means and 95% confidence intervals are shown with dashed lines and shaded areas in blue for the range core and in red for the range margin. Cohort-level means and 95% confidence intervals are calculated from the raw data.

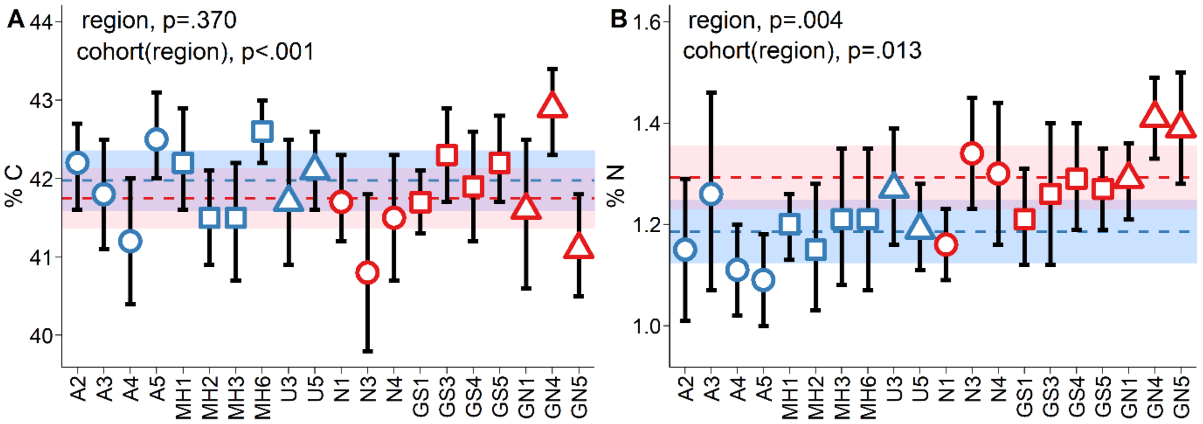

Figure S5. Range-margin cohorts (shown in red) produced leaves with (A) similar percent carbon, but (B) greater percent nitrogen compared to range-core cohorts (shown in blue). In the figure, different colour/shape combinations depict the six collection sites (refer to Figure 1 in the main text for geographical locations). Region-level estimated marginal means and 95% confidence intervals are shown with dashed lines and shaded areas in blue for the range core and in red for the range margin. Cohort-level means and 95% confidence intervals are calculated from the raw data.

Table S1. Response variables, predictor variables, and statistical results for the mixed effects models presented in the manuscript. R^2_m, variability explained by fixed effects; R^2_c, variability explained by fixed and random effects; Model, model structure used for analysis. Bold values indicate statistical significance ($p < 0.05$). [a] Two large outliers were removed to meet model assumptions. Results were equivalent when these data points were included.

Response	**Predictors**	**Results**	**p-value**	**R^2_m**	**R^2_c**	**Model**
Propagule weight	Region	$F_{1, 18} = 7.7$	**0.013**	0.14	0.49	lmer(prop_weight ~ region + (1 | cohort))
	Cohort(Region)	χ^2 (1) = 235.7	**< 0.001**			
Survival	Propagule weight	χ^2 (1) = 1.2	0.265	0.21	0.53	glmer(germ ~ prop_weight + region + (1 | cohort) + (1 | rep1), family = binomial())
	Region	χ^2 (1) = 12.1	**<0.001**			
	Cohort(Region)	χ^2 (1) = 31.2	**< 0.001**			
	Block1	χ^2 (1) = 11.0	**0.001**			
Time to establishment	Propagule weight	$F_{1, 466.3} = 0.4$	0.525	0.08	0.50	lmer(time_germ ~ prop_weight + region + (1 | cohort) + (1 | rep1))
	Region	$F_{1, 18.9} = 7.4$	**0.014**			
	Cohort(Region)	χ^2 (1) = 98.4	**< 0.001**			
	Block1	χ^2 (1) = 116.3	**< 0.001**			
Height at 8 months	Propagule weight	$F_{1, 416.3} = 52.5$	**0.001**	0.47	0.66	lmer(height1 ~ prop_weight + time_germ + region + (1|cohort) + (1 | rep1))
	Time to establishment	$F_{1, 512.7} = 305.5$	**<0.001**			
	Region	$F_{1, 20.5} = 0.9$	0.356			
	Cohort(Region)	χ^2 (1) = 94.0	**<0.001**			
	Block1	χ^2 (1) = 40.2	**<0.001**			
Total growth at 8 months	Propagule weight	$F_{1, 407.2} = 66.7$	**0.001**	0.47	0.66	lmer(grow1 ~ prop_weight + time_germ + region + (1 | cohort) + (1 | rep1))
	Time to establishment	$F_{1, 494.8} = 268.5$	**<0.001**			
	Region	$F_{1, 20.1} = 0.3$	0.586			

	Cohort(Region)	χ^2 (1) = 122.7	**<0.001**			
	Block1	χ^2 (1) = 28.5	**<0.001**			
Quantum yield (repeated measures)	Growth at 8 months	$F_{1, 488.4} = 15.8$	**<0.001**	0.91	0.92	lmer(CF ~ grow1 + time*region + (1 | cohort) + (1 | rep2))
	Time	$F_{2, 666.1} = 3861.0$	**<0.001**			
	Region	$F_{1, 18.6} = 60.8$	**<0.001**			
	Time*Region	$F_{2, 666.1} = 54.7$	**<0.001**			
	Cohort(Region)	χ^2 (1) = 22.3	**<0.001**			
	Block2	χ^2 (1) = 39.8	**<0.001**			
Quantum yield (Dec. 2018)	Growth at 8 months	$F_{1, 184.5} = 8.4$	**0.004**	0.22	0.43	lmer(DEC ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.6} = 39.4$	**<0.001**			
	Cohort(Region)	χ^2 (1) = 6.8	**0.009**			
	Block2	χ^2 (1) = 31.0	**<0.001**			
Quantum yield (Feb. 2019)	Growth at 8 months	$F_{1, 211.4} = 12.9$	**<0.001**	0.34	0.62	lmer(FEB ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.7} = 60.7$	**<0.001**			
	Cohort(Region)	χ^2 (1) = 18.5	**<0.001**			
	Block2	χ^2 (1) = 64.8	**<0.001**			
Quantum yield (Sept. 2019)	Growth at 8 months	$F_{1, 202.6} = 1.4$	0.246	0.11	0.47	lmer(CF8 ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.9} = 15.9$	**0.001**			
	Cohort(Region)	χ^2 (1) = 13.3	**<0.001**			
	Block2	χ^2 (1) = 59.9	**<0.001**			
Height (repeated measures)	Growth at 8 months	$F_{1, 1128.8} = 1505.4$	**<0.001**	0.76	0.82	lmer(height ~ grow1 + time*region + (1 | cohort) + (1 | rep2))
	Time	$F_{4, 1130.0} = 624.2$	**<0.001**			
	Region	$F_{1, 18.2} = 5.0$	**0.039**			

	Time*Region	$F_{4, 1130.0} = 6.6$	**<0.001**			
	Cohort(Region)	χ^2 (1) = 231.1	**<0.001**			
	Block2	χ^2 (1) = 41.1	**<0.001**			
Height at 10 months	Growth at 8 months	$F_{1, 215.6} = 578.9$	**<0.001**	0.74	0.80	lmer(height2 ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.7} = 0.3$	0.582			
	Cohort(Region)	χ^2 (1) = 16.2	**<0.001**			
	Block2	χ^2 (1) = 5.6	**0.018**			
Height at 12 months	Growth at 8 months	$F_{1, 210.6} = 341.1$	**<0.001**	0.62	0.69	lmer(height3 ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.5} = 2.5$	0.134			
	Cohort(Region)	χ^2 (1) = 14.0	**<0.001**			
	Block2	χ^2 (1) = 1.1	0.301			
Height at 14 months	Growth at 8 months	$F_{1, 220.2} = 421.9$	**<0.001**	0.67	0.74	lmer(height4 ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.6} = 4.5$	**0.047**			
	Cohort(Region)	χ^2 (1) = 21.4	**<0.001**			
	Block2	χ^2 (1) = 1.4	0.241			
Height at 20 months	Growth at 8 months	$F_{1, 226.4} = 307.2$	**<0.001**	0.58	0.70	lmer(height5 ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.4} = 5.6$	**0.029**			
	Cohort(Region)	χ^2 (1) = 37.5	**<0.001**			
	Block2	χ^2 (1) = 1.2	0.266			
Height at 24 months	Growth at 8 months	$F_{1, 222.6} = 237.5$	**<0.001**	0.51	0.65	lmer(height6 ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.6} = 7.5$	**0.013**			
	Cohort(Region)	χ^2 (1) = 39.0	**<0.001**			
	Block2	χ^2 (1) = 0.5	0.483			

Total growth (repeated measures)	Growth at 8 months	$F_{1, 1089.2} = 2122.4$	**<0.001**	0.83	0.87	lmer(grow ~ grow1 + time*region + (1 | cohort) + (1 | rep2))
	Time	$F_{4, 1130.0} = 910.0$	**<0.001**			
	Region	$F_{1, 18.2} = 0.2$	0.671			
	Time*Region	$F_{4, 1130.0} = 0.7$	0.625			
	Cohort(Region)	χ^2 (1) = 146.6	**<0.001**			
	Block2	χ^2 (1) = 50.6	**<0.001**			
Total growth at 24 months	Growth at 8 months	$F_{1, 226.5} = 452.3$	**<0.001**	0.69	0.77	lmer(grow6 ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.6} = 0.0$	0.844			
	Cohort(Region)	χ^2 (1) = 28.0	**<0.001**			
	Block2	χ^2 (1) = 3.8	0.051			
Biomass at 24 months	Growth at 8 months	$F_{1, 227.3} = 422.8$	**<0.001**	0.69	0.80	lmer(biomass ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.5} = 7.1$	**0.015**			
	Cohort(Region)	χ^2 (1) = 35.9	**<0.001**			
	Block2	χ^2 (1) = 19.2	**<0.001**			
Biomass:Height at 24 months	Growth at 8 months	$F_{1, 219.3} = 97.0$	**<0.001**	0.47	0.61	lmer(bio.h ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.3} = 21.5$	**<0.001**			
	Cohort(Region)	χ^2 (1) = 19.3	**<0.001**			
	Block2	χ^2 (1) = 10.2	**0.001**			
Biomass:Growth at 24 months	Growth at 8 months	$F_{1, 220.9} = 25.9$	**<0.001**	0.23	0.44	lmer(bio.gr ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.1} = 9.6$	**0.006**			
	Cohort(Region)	χ^2 (1) = 20.5	**<0.001**			
	Block2	χ^2 (1) = 12.0	**<0.001**			
Leaf area (cm^2)	Growth at 8 months	$F_{1, 215.7} = 0.0$	0.931	0.00	0.29	lmer(LA ~ grow1 + region + (1 | cohort) + (1 | rep2))

	Region	$F_{1, 18.7} = 0.0$	0.844			
	Cohort(Region)	χ^2 (1) = 39.8	**<0.001**			
	Block2	χ^2 (1) = 0.1	0.711			
Leaf dry-matter content (g/g)	Growth at 8 months	$F_{1, 220.3} = 8.4$	**0.004**	0.04	0.33	lmer(LDMC ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 18.3} = 1.4$	0.251			
	Cohort(Region)	χ^2 (1) = 25.8	**<0.001**			
	Block2	χ^2 (1) = 11.9	**<0.001**			
Specific leaf area (cm^2/g) [a]	Growth at 8 months	$F_{1, 210.0} = 22.0$	**<0.001**	0.36	0.56	lmer(SLA ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 19.0} = 51.2$	**<0.001**			
	Cohort(Region)	χ^2 (1) = 20.0	**<0.001**			
	Block2	χ^2 (1) = 22.2	**<0.001**			
log(C:N) [a]	Growth at 8 months	$F_{1, 179.1} = 0.0$	0.923	0.11	0.33	lmer(l.CNr ~ grow1 + region + (1 | cohort) + (1 | rep2))
	Region	$F_{1, 17.0} = 12.9$	**0.002**			
	Cohort(Region)	χ^2 (1) = 7.4	**0.006**			
	Block2	χ^2 (1) = 16.3	**<0.001**			

CHAPTER 7

Discussion

Discussion

Thesis summary

Within this thesis, I addressed gaps in our understanding of how climate-driven range expansion may lead to genetic changes within mangrove foundation species at their cold-sensitive range margins. I also determined whether these genetic changes may have broader ecological consequences. To do this, I studied the neotropical black mangrove (*Avicennia germinans*) across its distribution in the United States, and then I focused on its expanding Atlantic Florida range margin. I utilised a series of field surveys and a greenhouse common garden experiment that together contribute to our knowledge of how these coastal ecosystems may respond to climate change.

I started in Chapter 2 by outlining how *A. germinans* population genetics and functional traits of leaves change across this species' distribution in the United States, which consists of three cold-sensitive range margins. This research demonstrated that range-margin *A. germinans* do exhibit reduced genetic variation and increased population differentiation compared to those closer to the distributional range centre. However, the spatial extent of these range margins can vary greatly depending on ecological conditions and mangrove abundances. Both Florida range margins were restricted to their cold-sensitive poleward geographical limits; whereas, remarkably, widespread marginal ecological conditions have created a geographically-expansive (~1,000 km) range margin across the entire Texas-Louisiana distribution. Functional trait data indicated that, contrary to theoretical predictions, reduced genetic variation within *A. germinans* at each of these range margins may not constrain their adaptation to these marginal environments as range-margin *A. germinans* exhibited shifts in functional traits consistent with greater cold tolerance. These findings suggest that range-margin mangroves, although genetically depauperate, may be well-equipped to thrive under their marginal conditions and possibly under more extreme conditions farther poleward. Further experimental work, however, is needed to determine the relative importance of genetic differences versus phenotypic plasticity in shaping these patterns (as addressed in Chapter 6).

Next, in Chapters 3 and 4, I leveraged these insights and genetic data to evaluate additional factors that could shape genetic variation and influence future expansion success of *A. germinans* at one of these range margins on the Atlantic coast of Florida. In Chapter 3, I assessed mating system variation within this species along the latitudinal gradient towards the Atlantic Florida range margin. Greater isolation among conspecifics, and potentially fewer available pollinators, at range margins can result in shifts from outcrossing to self-fertilisation (Hargreaves and Eckert, 2014) with possible implications for offspring fitness over time (Charlesworth and Willis, 2009). Atlantic Florida *A. germinans* were predominately self-fertilised, in stark contrast to the commonly-held perception of this species, but outcrossing rates did not systematically decline with latitude towards the range margin. Instead, outcrossing increased as conspecific cover increased around range-margin trees. This pattern is consistent with a density-dependent plastic shift in mating system that may progressively shift towards more predominant outcrossing as these range-margin mangroves continue to increase in abundance. Plastic shifts towards greater self-fertilisation may, therefore, be a mechanism that facilitates mangrove colonisation of areas beyond their current range margins as a means of reproductive assurance. Whether this mating-system plasticity also leads to inbreeding depression in future generations remains to be evaluated.

In Chapter 4, I outlined dispersal patterns to the Atlantic Florida range margin and to areas beyond the current distribution. Surveys of beach-stranded *A. germinans* propagules during a year without a hurricane showed that dispersal to the range margin was limited, with no propagules found beyond the current distribution. However, after Hurricane Irma, propagule numbers increased massively at and beyond the range margin. Genetic assignment analyses indicated that these post-hurricane propagules were almost entirely derived from the nearest range-margin sources. Equivalent analyses for isolated *A. germinans* trees, discovered beyond the previously-documented range limit, found that these established individuals were also sourced from the range margin. These results suggest that poleward expansion of mangroves beyond the Atlantic Florida range margin may be restricted to periods following extreme storm events and, therefore, will be dispersal limited under non-storm conditions. Yet, dispersal well beyond the current distribution (>100 km) was possible after a single storm event, which indicates that poleward mangrove expansion can occur rapidly over extensive spatial scales. This work also demonstrates that storm-driven expansion can shape genetic variation within this species. Episodic dispersal from only a restricted number of sources will reduce genetic variation within newly-colonised areas, with further reductions occurring over time because of greater self-fertilisation during this initial colonisation phase (as observed in Chapter 3). These patterns of dispersal and mating system, along with periodic post-freeze declines in mangrove abundances (Cavanaugh et al., 2019), presumably shape the reduced genetic variation and increased population differentiation observed at mangrove range margins (as shown in Chapter 2). A next step will be to assess whether dispersal from range-margin sources provides genotypes that are well-adapted to the climatic extremes beyond the current distribution and, as such, may facilitate further poleward expansion (as addressed in Chapter 6).

Finally, in Chapters 5 and 6, I determined whether genetic variation within *A. germinans* at the Atlantic Florida range margin may have ecological consequences for these coastal ecosystems. In Chapter 5, I used a community-genetics approach to evaluate relationships between genetic variation within *A. germinans* host trees and the dynamics of their associated foliar endophytic fungal communities. I found that, at the scale of a range-margin population, genetically-similar host trees harboured similar fungal communities. This research helps broaden community genetics theory by demonstrating that correlations between genetic variation within plant foundation species and the structure of their associated communities can occur even at genetically-depauperate distributional range margins. In addition, this relationship may have implications for the fitness and resilience of these range-margin mangroves as endophytic fungi can contribute to stress adaptation within plants (Rodriguez et al., 2004). Further work is needed to test whether host-specific variation in endophytic fungi generates variation in the ability of these range-margin mangroves to persist within their marginal environment. This work also provides the first evidence of community-genetic effects in a natural mangrove system. Additional assessments, across the broad distributional ranges of mangroves, are now needed to determine how genetic variation within these coastal foundation species may relate to the diversity and structure of their diverse assemblage of associated organisms.

In Chapter 6, I addressed a question that repeatedly emerged throughout the course of this thesis. Does the unique genetic variation found within range-margin *A. germinans* (Chapter 2), that will likely seed further poleward expansion (Chapter 4), translate into adaptive trait variation that could better enable this species to thrive within its marginal environment? To do this, I monitored *A. germinans* maternal cohorts from areas along the Atlantic Florida distribution in a two-year greenhouse common garden experiment with annual temperatures analogous to range-margin conditions. Under these marginal conditions, range-margin cohorts consistently outperformed those from areas closer to the distributional range centre. Range-margin cohorts survived in greater numbers, established faster, exhibited greater stress tolerance over winter, accumulated greater biomass, and acquired more resources. This work demonstrates a genetic basis to adaptative trait shifts at the Atlantic Florida range margin that may facilitate the proliferation of this species within currently-occupied sites and the colonisation of more poleward areas. As a next step, longer-term *in situ* common gardens are needed to better understand the relative importance of intraspecific genetic variation compared to additional biotic and abiotic factors that can impact mangrove fitness at their present-day range margins. In addition, further evaluations of genetically-based trait changes towards multiple mangrove range margins could broaden our understanding of how variation in climatic thresholds and colonisation history, unique to each range margin, may shape these adaptive shifts.

Implications

This thesis furthers our understanding of how climate-driven range expansion can change the genetic variation within mangrove foundation species and of the broader ecological consequences of these changes. The knowledge gained from this body of work should advance efforts to forecast mangrove range expansion, as well as provide insights for a growing number of mangrove restoration initiatives.

Species distribution models are a principal tool used to predict how organisms will respond to climate change (Franklin, 2010). However, to date, most predictions are based on correlations between modern-day species occurrence and climate variables, and do not consider mechanisms that can shape biological responses to climate change (Urban, 2015). Predictive models that incorporate such mechanisms (i.e., physiology, demography, evolution, species interactions, dispersal, and environment) will be more realistic and should improve forecast projections (Urban et al., 2016). For instance, the three co-occurring mangrove species at the Atlantic Florida range margin are forecast to expand poleward at varying rates (2.2 – 3.2 km per year) based on models that incorporate differences in their experimentally-derived physiological thresholds to minimum temperature (Cavanaugh et al., 2015). Yet, as outlined in Chapter 4, there has been negligible poleward expansion along this coastline and future expansion may be dispersal limited until propagules are transported by episodic storm events. Dispersal limitation will inhibit mangroves from colonising areas that become climatically suitable in the future, which undermines these forecast results.

Incorporating projections of hurricane frequencies and trajectories into future models, and parameterising models with dispersal distances estimated in Chapter 4, should improve forecasts of mangrove expansion. Data on intraspecific trait variation, such as those presented in Chapter 6, can also be incorporated into future models, although longer-term *in situ* measurements from common gardens across a variety of potential environmental settings are needed to better predict how newly-colonised populations may perform (Chakraborty et al., 2019). In addition, continued monitoring of established range-margin populations, and the isolated *A. germinans* trees documented in Chapter 4, can provide future models with demographic parameters, such as annual rates of propagule production, seedling establishment, tree growth, and population expansion. Accumulating these mechanistic data is not a trivial task, but continued investments towards collecting this information should enable us to better anticipate how these coastal ecosystems will respond to climate change.

Although mangroves are proliferating at multiple range margins, mangrove forests have been lost or degraded across much of their global distribution as a result of modern-day, large-scale land use changes (Friess et al., 2019). In response to these losses, and to combat climate change, a growing number of global initiatives are aimed at the rehabilitation and restoration of mangrove ecosystems (Friess et al., 2020). Experimental work has shown that genetic and phenotypic variation within coastal foundation species can impact restoration success (Bernik et al., 2018; Plaisted et al., 2020), but similar insights for mangroves are limited.

Substantial differences in key phenotypic traits (e.g., survival, growth, biomass accumulation) among mangrove maternal cohorts, outlined in Chapter 6, indicate that source selection could be an important determinant shaping the outcomes of restoration initiatives. A tentative recommendation of this research would be to use local sources, or those with similar environmental conditions, because the maternal cohorts that performed best were from areas that were most climatically similar to the greenhouse growing environment. The relationship between mangrove genetic identity and the associated foliar fungal community, documented in Chapter 5, also highlights how propagule source could influence the broader community that eventually inhabits these restored areas. Evidence of this impact on soil microbial communities has been demonstrated in a restored mangrove system (Craig et al., 2020). Designing future restoration projects to test for differences among a diverse set of genetic sources could broaden our understanding of how intraspecific genetic variation may impact replanting success and subsequent ecosystem service provision.

Methodological considerations

Within this thesis, I used a panel of 12 nuclear microsatellite loci to characterise genetic differences among populations (Chapter 2) and individuals (Chapter 6) of a mangrove foundation species, as well as estimate mating system variation (Chapter 3) and quantify dispersal distances with genetic assignment analyses (Chapter 4). These genetic markers provided sufficient polymorphism to address the research objectives of this thesis, enabled data sets from earlier chapters to be integrated seamlessly into analyses for subsequent chapters, and could be easily supplemented with additional available loci to provide greater resolution when analysing differences at finer scales (Chapter 5). Future research, however, may benefit from the use of high-throughput next-generation sequencing technologies (e.g., RAD-seq, genotyping by sequencing) that can genotype hundreds to thousands of single nucleotide polymorphism (SNP) genetic markers and provide a much greater coverage of the entire genome (Davey et al., 2011). Research has begun to utilise these technologies to better understand delineations among closely-related mangroves species (Xu et al., 2017; Wang et al., 2020; Mori et al., 2021) and how genetic variation within mangroves may affect their ability to persist under climate change (Guo et al., 2018). High-throughput sequencing has also been used to characterise gene expression (i.e., transcriptomics) within mangroves (Dassanayake et al., 2009; Huang et al., 2014) and to evaluate transcriptomic variation across different environmental settings (Bajay et al., 2018; Cruz et al., 2019, 2020).

The recent availability of annotated genomes for the congener *A. marina* (Friis et al., 2021; Natarajan et al., 2021) will facilitate further research for the study species within this thesis (*A. germinans*) because sequence reads can be mapped directly to the scaffold provided by these reference genomes. Mapping sequences to a reference genome can increase the number of genetic markers recovered (Torkamaneh et al., 2016) and improve downstream inferences (Shafer et al., 2017) compared to *de novo* assembly methods without a reference genome. These genomic resources provide the tools to potentially identify adaptive loci and signatures of natural selection within mangrove populations (Cruz et al., 2019, 2020) that can complement phenotypic data collected from plants within common garden experiments (e.g., Chapter 6) to broaden our understanding of the genetic underpinnings of adaptive trait shifts towards mangrove range margins. In addition, the greater number of SNP markers provided by high-throughput sequencing can prove more effective than microsatellites for parentage analyses in species with limited genetic diversity (Tokarska et al., 2009). A greater availability of genetic markers per individual may enable further research into the *A. germinans* mating system to genotype a greater number of maternal trees with fewer offspring per tree, which improves upon the methods used here (Chapter 3) and could lead to more robust estimates of population-level mating system variation for this species.

Continuing advancements in high-throughput sequencing technologies may also improve our ability to characterise mangrove-associated microbial communities. The nuclear ribosomal internal transcribed spacer (ITS) region is recognised as the universal barcoding marker for fungal species (Schoch et al., 2012). This region consists of three subregions (ITS1, 5.8S, and ITS2) and, in Chapter 5, I characterised fungal communities within the leaves of *A. germinans* with amplicon sequencing of the ITS1 subregion. A similar environmental metabarcoding approach of ITS1 has been used to characterise endophytic fungi within multiple mangrove species (Chi et al., 2019; Lee et al., 2019, 2020). Targeting ITS1 may, however, recover less taxonomic richness compared to the ITS2 subregion because of primer bias (Tedersoo et al., 2015; Tedersoo and Lindahl, 2016), although the overall taxonomic composition recovered by either subregion may be similar (Blaalid et al., 2013). Our ability to document fungal diversity within mangroves may improve by sequencing the full ITS region (ITS1 – ITS2) as technologies continue to develop and enable the generation of longer read lengths for metabarcoding studies (Nilsson, Anslan, et al., 2019).

A major advantage of ITS is the unparalleled availability of reference sequences within curated databases compared to other fungal barcodes, which makes ITS the best alternative to broadly identify fungal species (Lücking et al., 2020). Yet, our understanding of taxonomic and functional diversity for many fungi remains limited and, as such, reference databases are incomplete (Lücking et al., 2020). For instance, 14% of fungal isolates from mangroves in Sao Paulo, Brazil, did not match any known fungal genus (de Souza Sebastianes et al., 2013) and I found that 11% of all sequence reads from a mangrove population in Florida, USA, were only assigned to the level of kingdom Fungi (Chapter 5). Ongoing efforts are focused on improving these resources (Nilsson, Larsson, et al., 2019) and beginning to uncover the functional roles of fungi within natural systems (Nilsson, Anslan, et al., 2019). Shotgun metagenomic sequencing, although not without its own challenges, provides a potential alternative to amplicon sequencing that can recover information on both microbe taxonomy and biological functions (Sharpton, 2014; Zhou et al., 2015). Efforts to incorporate this alternative approach into mangrove research may improve our understanding of not only microbial diversity within these systems, but also the functional roles of these microbes.

Conclusions

Warming temperatures have been linked to the proliferation of mangroves at multiple range margins worldwide (Cohen et al., 2020; Whitt et al., 2020; Osland et al., 2021) and forecasts predict that continued climate warming will drive further poleward expansion in the future (Osland et al., 2013; Cavanaugh et al., 2019). However, we lack an understanding of how additional, non-climatic factors may influence the establishment, survival, and growth of these expanding mangroves. This thesis provides novel contributions to our knowledge of how genetic and phenotypic variation within these coastal foundation species may change towards expanding range margins and the broader ecological consequences of these changes.

Within this thesis, I demonstrated that sparsely-populated mangrove range margins may often be characterised by reduced genetic diversity and increased genetic structure, may undergo plastic shifts towards greater self-fertilisation that can further reduce genetic diversity over time, and that dispersal beyond range boundaries may generally be limited and from a restricted number of sources. These characteristics may constrain adaptation to the range-margin environment (Bridle and Vines, 2007) and are consistent with the idea that range expansion for many plants may be dispersal limited (Hampe, 2011). Yet, despite these potential constraints, I also showed that range-margin mangroves may possess unique genetic adaptations to thrive under their marginal conditions, consistent with adaptive shifts found within diverse taxa towards distributional limits (Chuang and Peterson, 2016), and that genetic variation within these mangroves may influence their associated fungal communities, a relationship that may contribute to stress adaptation (Rodriguez et al., 2004).

The knowledge gained from this body of work improves our understanding of how mangroves at cold-sensitive range margins may respond to climate change. Mangroves will likely continue to proliferate at these range margins as temperatures continue to warm, but their poleward expansion will not simply track changes in climate. Instead, expansion will be restricted to periods following extreme storm events that disperse propagules from the nearest range-margin sources. Episodic, storm-driven dispersal has the potential to drive rapid mangrove expansion over extensive spatial scales, and the establishment of new areas will be facilitated by plastic shifts in the mating system of these mangroves. Adaptive trait variation within newly established individuals, sourced from the range margin, will presumably facilitate their survival and continued proliferation, and potentially influence the associated communities that eventually inhabit these coastal foundation species. This expanding gene pool of range-margin genotypes that are better adapted to thrive under the climatic extremes beyond the current mangrove distribution bodes well for the future resilience of these changing coastal ecosystems.

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