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Plasticity drives the trait variation of a foundation marsh species migrating into coastal forests with sea-level rise

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Keywords: climate change; marsh migration; phenotypic plasticity; *Spartina*; trait-based ecology

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Abstract

Climate-driven ecosystem shifts occur through turnover in the foundation species which structure the landscape. Therefore, to predict the fate of areas undergoing climate-driven ecosystem shifts, one approach is to characterize ecological and evolutionary responses of foundation species along dynamic environmental gradients. One such gradient is the ecotone between tidal marshes and maritime forests in coastal areas of the U.S. Mid-Atlantic region where accelerated sea-level rise and coastal storms of increased frequency and intensity are driving forest dieback and inland marsh migration. Mid-Atlantic tidal marshes are structured by marsh grasses which act as foundation species, and these grasses exhibit trait variation across their distribution from established marsh interior to their inland migration front. We conducted a reciprocal transplant experiment with *Spartina patens*, a dominant high marsh grass and foundation species, between established populations in the high marsh and range edge populations in the forest understory at three mid-Atlantic sites. We monitored environmental conditions in marsh and forest understory habitats, measured plant traits (above- and belowground biomass, specific leaf area, leaf N and C concentrations) in transplanted and reference non-transplanted individuals, and used microsatellite markers to determine the genetic identity of transplants to quantify clonality between habitats and sites. Individuals transplanted into the forest understory exhibited a plastic shift in resource allocation to aboveground structures associated with light acquisition, with shifts in transplants making them more morphologically similar to reference individuals sampled from the forest habitat. Clonal diversity and genetic distance among transplants were relatively high at two of three sites, but individuals at all sites exhibited trans-habitat plasticity regardless of clonal diversity or a lack thereof. Individuals grown in the forest understory showed lower vegetative and reproductive fitness.

Nevertheless, the trait plasticity exhibited by this species allowed individuals from the forest that were transplanted into the marsh to recoup significant biomass in only a single growing season. We predict high plasticity will facilitate the persistence of colonizing *S. patens* individuals under suboptimal forest shade conditions until forest dieback increases light availability, ultimately promoting continued inland migration of this foundation species under sea-level rise.

Introduction

Studying foundation species provides insight into the fate of ecosystems under threat of climate change, as these organisms create the structures upon which ecosystems are built (Ellison 2019, Hamann et al. 2021). Foundation species are those that meet the following criteria: 1) they are dominant species in an ecosystem, 2) they are near the base of ecological networks, and 3) their physical structures provide support for other organisms and control ecosystem functions (Ellison 2019). Due to the role their structural traits play in habitat provisioning, trait shifts in foundation species' populations can have broadscale impacts on whole ecological communities. Shifts in ecosystem functions due to changes in foundation species and their traits have been observed in alpine (Schöb et al. 2013, Zhao and An 2021), forest (Fagundes et al. 2022) and aquatic ecosystems (Irving and Bertness 2009, Narwani et al. 2019).

Coastal ecosystems are well-suited to serve as model systems for investigating the trait variation of foundation species undergoing climate-driven range shifts (Crotty et al. 2019). Coastal habitats are frequently structured by a single or few foundation species (Angelini et al. 2011). Many coastal ecosystems are experiencing rapid changes with climate change and sea-level rise (Gabler et al. 2017, Sallenger et al. 2012) and these changes can impact resource allocation and trait evolution in foundation species (Vahsen et al. 2023, Kudoh et al. 2024).

These shifts along pre-existing environmental gradients are driving changes in foundation species across the coastal landscape, in the form of transgression and marsh migration into inland areas (Donnelly and Bertness 2001, Kirwan and Gedan 2019). Studying the eco-evolutionary dynamics in transition zones can help us predict the rate of coastal change, and its outcomes for ecosystem structure and function.

The migration of tidal marshes into adjacent coastal habitats is a major phenomenon in the US Mid-Atlantic region (Schieder et al. 2018). Due to regional ocean dynamics and land subsidence, sea-level rise rates in the US Mid-Atlantic are accelerating at approximately 3 times the global average, making this area a global hot-spot of sea-level rise (Sallenger et al. 2012). Increased saltwater intrusion negatively impacts pine recruitment and survival in Mid-Atlantic maritime forests, forming a “persistence zone” characterized by a lack of forest regeneration and a thinning stand of mature pine trees (Fagherazzi et al. 2019). Over time these trees experience mortality due to a combination of chronic “press” stressors and “pulse” disturbance events (i.e. coastal storms), ultimately forming areas of ghost forest (Kirwan and Gedan 2019). The dieback of non-halophytic forest plants creates an opening for more salt-tolerant marsh species to occupy newly available niches. As a result, marsh species expand into this deteriorating forest persistence zone, resulting in a shift from loblolly pine, *Pinus taeda* L., as the primary foundation species, to halophytic shrubs and grasses, including the nonnative common reed, *Phragmites australis* (Cav.) Trin. ex Steud, the expansion of which is a major concern to land managers (Shaw et al. 2022, Smith 2013).

One dominant native species along this marsh-to-forest ecotone is *Spartina patens* (Aiton) Muhl. a clonal grass that typifies the higher elevation zones of tidal marshes in the Eastern United States (Bertness 1991, Lonard 2010). Tidal high marsh habitat undergoes less-frequent flooding than mid and low marsh zones dominated by the congener *Spartina alterniflora* Loisel. This causes periods of soil hypersalinization in the summer (Pomeroy and Wiegert 1981). *S. patens* serves as a foundation species through canopy shading that ameliorates salt accumulation and thermal stress (Gedan and Bertness 2010, Whitcraft and Levin 2007). *S. patens* provides microhabitat niches for a diverse assemblage of arthropods (Agnew et al. 2003, Denno 1977), nesting habitat for shorebirds (Greenberg et al. 2006). It is found in a wide variety of coastal habitat types, serving also as a pioneering foundation species in dune habitats (Kirschner and Zinnert 2020). *S. patens* has been shown to exhibit stark differences in traits such as resource allocation to above vs. belowground biomass, stem density, and leaf area when observed in marsh vs. dune habitats in both its native and non-native ranges (Castillo et al. 2014, Silander and Antonovics 1979). These trait differences likely alter the facilitative effects and ecosystem services provided by this species in the two habitat types and may drive differential responses to sea-level rise and coastal storm stressors.

While changes in marsh and forest extent over time have been well characterized (Field et al. 2016, Schieder et al. 2018, Smith et al. 2013), there is less known about the ecological processes that shift across a migrating marsh ecotone (Fagherazzi et al. 2019). Marsh species are present in the soil seed bank and standing vegetation of upland areas prior to noticeable changes in edaphic metrics and tree mortality (Anisfeld 2017, Kottler and Gedan 2020), and marsh plant responses to pressures in the forest are species-specific, with some species growing better at the leading edge of the ecotone and others at the trailing edge (Jobe and Gedan 2021). Soil characteristics and light availability shift dramatically across the migration front (Brinson et al. 1995), which can have direct and indirect effects on plant traits via the influence of light on evaporation, soil salinity, and moisture.

We collected ecological data at three Mid-Atlantic tidal marsh sites, using a combination of observational and experimental methods to assess intraspecific trait variation and plasticity in *S. patens* as it migrates into coastal loblolly pine forest prior to forest dieback. Through observational field work, we characterized variation in environmental conditions under open marsh and forest understory conditions within *S. patens'* expanding range extent. In a reciprocal transplant experiment, we measured trait variation, plasticity, and genetic structure in individuals moved between the marsh and forest understory habitats. We developed the following predictions: 1) open marsh and forest understory environments will exhibit different soil and light characteristics, with decreased soil salinity and light availability in the forest understory and 2) based on its demonstrable high ecological amplitude between marsh and dune habitats, *S. patens* will demonstrate strong trait variation across the marsh-to-forest gradient, with increased specific leaf area and stem height in the forest understory and 3) this variation will be plastic over the course of one growing season under altered conditions (transplantation).

Methods

Site information

During the growing season of 2018, we collected environmental data and conducted a reciprocal transplant experiment at three sites that span the natural variation of salinities and estuarine contexts (bayside, seaside mainland, and seaside back-barrier) in which *Spartina patens* naturally grows in the Mid-Atlantic region (Figure 1.A & B). Each site contains an ecotone boundary where high marsh habitat dominated by *S. patens* and *Distichlis spicata* (L.) Greene grades into a heterogeneous landscape of salt-tolerant shrubs and receding coastal maritime forest with a canopy of *Juniperus virginiana* L. and *Pinus taeda* (Figure 1.C). Moneystump marsh is a bayside, brackish tidal marsh that is part of Blackwater National

Wildlife Refuge, MD, connected to Chesapeake Bay tidal creeks that flow past Taylors Island. Sea-level rise, land subsidence, and saltwater intrusion at this site have resulted in the formation of a tidal lake and extensive ghost forests (Ganju et al. 2015, Stevenson et al. 1985). Phillips Creek Marsh is a seaside mainland salt marsh on the Delmarva Peninsula, connected to the Atlantic Ocean via Hog Island Bay. This site which is located in the Nature Conservancy's Brownsville Preserve hosts ongoing studies of marsh accretion and sedimentation dynamics (Blum et al. 2020, Kirwan and Blum 2011). Wallops Island is a barrier island that hosts a military base and NASA flight facility. The experiment was performed in a salt marsh on Wallops Island back-barrier marsh, which connects to the Atlantic Ocean through a network of tidal creeks and the Chincoteague Inlet.

Reciprocal transplant experimental design

The reciprocal transplant experiment was conducted at the three sites (Moneystump Marsh, Wallops Island, Phillips Creek Marsh) and was installed in March 2018 prior to spring growth of new aboveground biomass. We surveyed the three experimental sites and identified adjacent areas of *S. patens* in the marsh and forest habitats. In each habitat at each site, we established ten 1 m^2 plots that were at least 3 m apart from one another (10 plots per habitat, 20 plots per site, 60 plots total at all three sites). Plot location was determined within habitats based on dominance of *S. patens* as evidenced by senescent biomass from the past growing season. Two cores of *S. patens* were collected from each plot. Using random assignment, one core was transplanted into a different plot in the same habitat, and the other transplanted into a plot in the reciprocal habitat (but remaining within the same site). A 5 cm diameter half-moon corer was used to extract two 15 cm long *S. patens* plugs from each plot. We chose this depth because most *S. patens* belowground biomass occurs in the upper 10 cm of soil (Windham 2001). Each plug was wrapped in weed cloth to prevent root interference by plant competitors, and then was planted into the holes left from core removal at their destination plot. Additionally, we collected one 15 cm belowground sample per plot for destructive sampling to quantify initial belowground biomass. Plots were checked bimonthly and competitor species weeded when they emerged. Transplants that did not survive were replaced within the first month of the experiment with plugs harvested from new plots within the same habitat of origin.

Environmental data

Our environmental data collection was focused on capturing environmental variation across the marsh-to-forest gradient associated with "press" chronic stressors of sea-level rise. We monitored porewater salinity, light availability, and soil moisture throughout the growing season; these environmental measures were selected based on their identified role in driving wetland plant trait variation (Moor et al. 2017), likelihood to vary across a tidal marsh-to-forest gradient (Jobe and Gedan 2021). We also measured the elevation, redox potential, pH, and carbon content of each plot on a single occasion, as these variables were not expected to vary during the course of the short-term study. In May through August of 2018, salinity and light measurements were collected on a monthly basis at each plot. To measure salinity, two 30 ml samples of porewater were extracted with a PushPoint Sampler (MHE Products) inserted 15 cm into the soil. Salinity of porewater was determined to the nearest part per thousand (ppt) using a refractometer. For light measurement, we took readings of photosynthetic photon flux density (PPFD) in umol m^{-2} s^{-1} using a LI-250A light meter (LI-COR Biosciences, Lincoln, NE, U.S.A.) positioned ~25 cm above the plot. Six readings were averaged for each plot. Simultaneously, we collected ambient light data in the open marsh using a HOBO light pendant (Onset Computer Corporation, Bourne, MA), logging the total available light in lum ft^{-2} at 15 min intervals. Light pendant data was converted from lum ft^{-2} to umol m^{-2} s^{-1} using a calibration factor of 0.0185 (Thimijan and Heins 1983), and light availability is reported as percent available light at vegetation height relative to total ambient light in open conditions (Kottler 2022).

Soil moisture was measured gravimetrically from cores 2 x 5cm (diameter x height) collected during a low tide in a neap tide cycle (6/19/2018, 6 days after last precipitation) and low tide in a spring tide cycle (7/24/2018, 1 day after last precipitation), to capture the extremes in unsaturated soil moisture of each plot. Cores were weighed, then dried at 60°C for 48 hrs, put in a desiccator until constant weight was achieved, then weighed again to measure water loss. One set of cores was run for Loss on Ignition (LOI) to measure organic matter. For LOI, soil samples of approximately 10 g were weighed before and after being burned in a muffle furnace at 500°C for 4 hours (Morris et al. 2016). Additionally, soil pH and redox potential were measured once during the season. pH of porewater was measured in the lab using a pH electrode (IntelliCAL PHC301). Redox potential was measured with an oxygen reduction potential (ORP/RedOX) electrode (IntelliCAL MTC301) inserted into the soil to approximately 5 mm depth at low tide (as in Gedan & Bertness 2010). Finally, to assess differences in plant community composition within and between sites and habitats, we collected presence/absence data in August 2018 of all understory species within a 1 m^2 quadrat around each plot, and all shrub and tree species in the canopy directly above the plot. We chose presence/absence as opposed to other metrics of vegetation cover because our transplant manipulation moved some of the vegetation (i.e. *S. patens* tillers), which could have compromised an area/density based metric. Elevation data was collected using a Trimble R8 RTK (Trimble Incorporated, Sunnyville, CA). Where the R8 receiver was unable to obtain sufficient signal to collect data, elevation was collected using a scope and stadia rod to measure the vertical difference from a known reference point.

Plant sampling, trait measurements, and genotyping

In August 2018, when plants had reached peak biomass, we harvested reference *S. patens* plugs from natural populations at each plot (i.e. grasses not disturbed by the transplanting process) as well as the surviving reciprocal transplant individuals. All plant traits were measured in the laboratory and ~100 g of leaf tissue was collected and frozen for genotyping from each reciprocal transplant individual. The traits of above- and below-ground biomass, Specific Leaf Area (SLA), Root:Shoot ratio, leaf tissue Nitrogen per unit area (N_{area}), carbon per unit mass (C_{mass}), and flower production were measured as indicators of general plant phenotypic responses to varying abiotic conditions in wetland systems (Moor et al. 2017). Prior work with *S. patens* has observed plasticity in these traits under marsh vs. dune habitat conditions in both their native and invasive ranges (Silander and Antonovics 1979, Castillo et al. 2014). Aboveground biomass samples were sorted into live and dead stems and leaves (dead stems are plant material from either the past growing season or senesced from transplant stress, but which remains attached to the plant). Stems were counted and the five tallest stems were measured for an approximation of plant height. From each plant, three green leaves that were 3rd or 4th from the top of stems were removed and pressed for SLA measurements. Surface area of these leaves was measured on an LI-3100 leaf area meter. These were oven-dried and weighed for specific leaf area (SLA) calculations ($SLA = A / M_L$, where A is leaf area and M_L is leaf mass) and their weight was incorporated into calculations of aboveground biomass. Belowground biomass was sifted through sieves of #5 mesh (4.0 mm) to remove other debris and #60 mesh (0.2 mm) to remove soils but retain fine root matter. Biomass was then separated into root and rhizome components and oven-dried at 60°C for a minimum of 48hrs and then weighed. Leaf N_{area} and C_{mass} were quantified using a Leco CN828 elemental analyzer (Leco Corporation, St. Joseph, MI). The number of flowering stems were counted at time of harvest.

Genotyping was conducted by extracting genomic DNA from *S. patens* leaf tissue and determining a multilocus genotype based on novel polymorphic microsatellite loci (Genbank accessions OK413399-OK413402). Multilocus lineage (MLL) identity was assigned based on multilocus genotypes using a threshold genetic distance below which samples were considered members of the same clone. GenAlEx (Peakall and Smouse 2012) was used to carry out AMOVA. We used Nei's genetic distance as a metric to assess genetic differentiation between marsh and forest sites and between populations, with a bootstrap test (1000 permutations) of the Shannon index (shc) corrected for sample size for differences in clonal diversity between pairs of populations. See Appendix S1:Methods S1 for more details on the methods we used for genotyping and analysis of clonal identity data.

Data analyses

We tested for linear model assumptions of normality and homogeneity of variance, transforming data using a log or square-root transformation where needed to meet assumptions. If transformations did not resolve the issue of homoscedasticity, a non-parametric Kruskall-Wallis test was used to test the significance of predictor variables. All response variables were tested for significant interactions. All analyses were performed in R version 4.0.2 (2020-06-22).

For environmental data that was collected repeatedly over the course of the growing season, such as salinity and light availability, we ran a mixed linear effects model with site, habitat, and month as independent factors, and plot as the within-group factor. Other environmental traits were analyzed using a linear model with site and habitat as predictors. To understand the species composition of the canopy shading *S. patens* forest plots, we used non-metric multidimensional scaling (NMDS) ordination with analysis of similarity (Anosim-R) to identify significant differences in species composition by site and by habitat. The indicspecies R package (Cáceres and Legendre 2009) was used to conduct an indicator species analysis. To assess differences in *S. patens* plant traits for reference individuals naturally growing in the forest understory vs. marsh, we used a principle coordinate analysis (PCA) with traits log10 normalized, and used the resulting Principle Coordinates 1 & 2 (which explained 71.01% of variation) as response variables in ANOVA with site and habitat as predictor variables (Appendix S1: Table S1). In our analysis of reciprocal transplant plant phenotypic traits, we ran a single linear model with habitat of origin (where the plug was harvested), transplant habitat (where the plug was placed) and site as our predictor variables and all plant traits in our linear models. All plant trait response variables (with the exception of any which did not meet homoscedasticity assumption) were included together in this model to account for their nonindependence. We calculated estimated marginal means (EMMs) with contrasts for factor combinations in our linear models using the “emmeans” R package. This method allows us to determine which means for specific treatment combinations are statistically different from one another, allowing better interpretation of interactions effects. A similar model with all trait response variables was run for the common garden plant phenotypes with habitat of origin, light treatment, and plot of origin as predictor variables. We included the difference in stem density between the prior (natural growing season) and the common garden peak stem density as one of the trait response variables.

Results

Environmental data

Across all sites, forest plots had significantly lower salinity and light availability than marsh plots (Table 1). There was a slight difference in elevation between habitats, with marsh plots higher in elevation than maritime forest understory, but this is likely due to the lower resolution stadia rod-generated elevation data that had to be collected in the forest due to the fact that the forest canopy interfered with satellite signal of the RTK GPS device. Additionally, redox potential was much lower and pH slightly higher in the marsh than the forest. Most environmental factors also differed among experimental sites. Porewater salinity was lower at Moneystump Swamp (7.54 psu ± 3.15) and Phillips Creek Marsh (9.64 psu ± 3.73) than at Wallops Island (12.52 psu ± 8.4, $F_{2,185} = 4.908$ $p < 0.0001$, Appendix S1:Figure S1). Light availability in the forest plots was higher at Moneystump Swamp (40.3% ± 27.3) than at Phillips Creek Marsh (33.1% ± 32.1) and Wallops Island (29.4% ± 35.4, $F_{2,36} = 13.283$ $p < 0.0001$, Appendix S1:Figure S1). This was associated with differences in canopy composition between sites (ANOSIM, $r = 0.3257$, $p < 0.01$, Appendix S1:Figure S1), with Moneystump Swamp being typified by *Phragmites australis* versus Phillips Creek Marsh with *Juniperus virginiana* as an indicator species. Wallops Island did not have any significant indicator species. *Pinus taeda* and *Morella cerifera* were present in the forest canopy at all sites.

Reference plant traits

Spartina patens collected from the forest differed from those collected from the marsh, based on our principle components analysis (PCA) of plant traits, which explained a large proportion of variance in the model (71% explained by PC1 and 12.8% explained by PC2) (Figure 2, Table 1). Stem density and root biomass had the highest factor loadings for PC1 (Table 1). PC1 separated samples by habitat (ANOVA; $F_{1,53} = 116.296$, $p < 0.0001$), site

(ANOVA; $F_{2,53} = 6.5926$, $p < 0.01$), and their interaction (ANOVA; $F_{2,53} = 4.9682$, $p < 0.05$), indicating differences between marsh and forest habitats in stem density and root biomass. PC2 also separated samples by habitat (ANOVA; $F_{1,53} = 7.8791$, $p < 0.01$), and the interaction of site and habitat (ANOVA; $F_{2,53} = 8.4077$, $p < 0.001$), but not by site alone. Marsh *S. patens* individuals had greater total biomass than those in the forest understory (ANOVA; $F_{1,53} = 47.8084$, $p < 0.0001$). There was no effect of site on plant biomass (ANOVA; $F_{2,53} = 0.6792$, $p = 0.5114$). Plants from the forest produced no flowers, which was significantly different from flower production in the marsh (Kruskal-Wallis; $X^2_{1,53} = 6.331$, $p > 0.05$). The lack of flowering was related to the lower biomass seen in forest individuals (positive relationship between biomass and flowering: z-value = 2.488, $p = 0.0129$).

Transplant traits and genetic structure

For reciprocal transplants in the marsh and forest habitats, we observed phenotypic plasticity in several aboveground traits (Figure 3, Table 2). Planting date had an effect on total biomass, with replacement transplants that were planted later producing overall less biomass (ANOVA; $F_{12,44} = 8.733$, $p = 0.039$). This effect was accounted for in our models, and there was no measurable effect of planting date on other traits. Plants grown in the forest understory exhibited lower stem density, aboveground biomass, and leaf carbon, but greater specific leaf area (SLA) and leaf N_{area} (Figure 3). On the other hand, belowground biomass and total biomass (driven by belowground biomass) varied with habitat of origin, and there was no main effect of habitat of translocation. These findings are consistent with the fact that initial belowground biomass varied strongly by habitat, with greater belowground resources in individuals from the marsh at the start of the growing season (ANOVA; $F_{1,42} = 57.352$, s.e. = 41.0597, $p < 0.0001$).

However, there was a significant positive effect of forest origin on stem density and biomass (Figure 3). While marsh-originating plants showed little plasticity in total biomass across habitats, forest-originating plants strongly increased aboveground biomass production under open marsh conditions. Our analysis of estimated marginal means found a significant contrast between site, specifically Moneystump Swamp, and habitat of origin which impacted the overall trait model (contrast $\text{estimate}_{46} = -1.37$, s.e. = 0.486, $p < 0.01$ **).

In terms of genotype diversity, 44 multilocus lineages were identified (n = 62). Fewer multilocus genotypes were observed in the total sample and within each geographic location than expected under a model of sexual reproduction with random mating ($p >= 0.012$). AMOVA estimated 7% of genetic variation was among the three populations, 5% between marsh and forest samples within populations, and 86% was within and 2% among individuals. Wallops Island samples of *S. patens* possessed less genotype diversity than Moneystump Swamp and Phillips Creek Marsh , while clonal diversity in Moneystump Swamp and Phillips Creek Marsh sites were not different from one another (Appendix S1:Table S2). Four of 19 (21%) reciprocal transplant pairs from the forest and marsh locations had identical MLLs, and the pairs with distinct MLLs showed a wide range of genetic distances (Appendix S1:Table S3).

Discussion

This is the first field study to characterize phenotypic shifts associated with inland migration of *Spartina patens* into coastal forests of the US Mid-Atlantic. We were able to link these trait shifts to abiotic conditions in the high marsh vs. forest understory. Both reference individuals growing in the forest understory and those transplanted from the marsh to the forest in our reciprocal transplant exhibited a shift towards greater SLA and leaf N_{area}, two traits associated with light acquisition (Ryser and Eek 2000, Simioni et al. 2004). Forest-grown individuals had lower leaf C, stem density and biomass. Yet, when released from light limitation, individuals of forest origin produced greater stem density and total aboveground biomass than their marsh counterparts (Figure 3).

The three sites spanned a range of estuarine contexts and also abiotic conditions (Appendix S1:Figure S1) and still, increases in *S. patens* height and SLA under a forest canopy were consistent across sites. There was little genetic structuring by site or habitat, and we observed strong phenotypic plasticity irrespective of the level of clonal diversity within a site. High trait plasticity under variable canopy cover has also been documented in *Spartina alterniflora* in its invasive range (Xu et al. 2022) and in other wetland species (Edelkraut and Güsewell 2006, Martina and von Ende 2012). *S. patens* trait variation between habitats appears to be driven, at least in part, by plasticity to changes in light availability and other abiotic conditions, with substantial trait responses detected over a single growing season. The plant trait data from reference samples confirms that the trait plasticity we observed in reciprocal transplants mirrors the trait differentiation of natural populations found in marsh vs. forest habitats. These marsh and forest phenotypes are distinct from one another, representing discrete "ecotypes" as reflected in PCA analysis (Fig 2). The trait shifts we observed align with the predictions by Moor et al. (2017) that wetland species would exhibit high plasticity in response to light availability, and that leaf Nitrogen, SLA, and height would be some of the more plastic traits.

Site differences suggest that trait plasticity of *S. patens* across the marsh migration front will be impacted by contextual factors that vary across the Mid-Atlantic. We observed variation in light availability in forested areas between sites, which was associated with differences in the species composition of the forest canopy. Given findings of restricted marsh migration into coastal forests of southern New England that are characterized by a more closed canopy of deciduous trees, canopy composition may play an important role in the marsh migration trajectory of coastal forested areas affected by sea-level rise (Field et al. 2016) via indirect effects on understory species. A recent experiment in that region found significant trait responses to upland soil conditions but did not examine canopy cover and associated light availability as a factor in their study (Dowling et al. 2023). We did not find a difference in elevation across the marsh ecotone. Previous research at Moneystump marsh has found an extremely shallow increasing slope from the marsh to maritime forest of ~3.3 mm vertical rise per lateral meter, or 0.3 % (Kottler & Gedan 2020). However, elevation may be a relevant factor across the marsh ecotone in other regions that feature greater elevation gradients across coastal ecosystems. It is possible that additional abiotic factors not measured in this study, such as temperature (Appendix S1:Table S4), soil conditions, and leaf litter could also be involved in the observed trait changes across this ecotone. We also observed that the difference in leaf Nitrogen between forest and marsh populations was primarily driven by the Moneystump site (Appendix S1:Figure S2). Marsh habitats are generally nitrogen limited, but plant trait shifts have been observed in response to Nitrogen addition via the process of eutrophication from fertilizer runoff (Crain 2007). This was the one site connected to the Chesapeake Bay rather than the Atlantic Ocean, and, as the Chesapeake Bay has long experienced eutrophication, it is possible that this local trait variation reflects greater Nitrogen availability in bay waters (Harding et al. 2019). In the microsatellite analysis, Wallops Island *S. patens* populations exhibited lower clonal diversity than other sites (Appendix S1:Figure S3). This site has been highly impacted by anthropogenic disturbance and spread of the invasive plant *Phragmites australis* (Ailes 1993). These two factors are associated with decreased diversity of native marsh species (Silliman and Bertness

2004), and invasion has been shown to alter the genetic structure of native plant populations in other systems (Mealor and Hild 2006). Therefore, it may be that disturbance and *P. australis* invasion can decrease genetic diversity within a marsh foundation species, a hypothesis for further investigation in future research.

Individuals in the forest had lower reproductive fitness than marsh populations, confirming the effects of light-limitation on flower production in *S. patens* that has been documented in previous work in this system (Kottler and Gedan 2022). There are known to be fitness tradeoffs associated with resource allocation to sexual reproduction vs. clonal growth such that plants may shift their resource allocation towards clonal reproduction in certain environmental contexts (Mizuki et al. 2005, Tietel et al. 2015, Usui et al. 2023). In this study we found that, while habitat of origin did not have a significant impact on rhizome (clonal underground stem) production at all sites, there was greater rhizome production by forest origin plants at Phillips Creek Marsh. This could reflect differential resource availability at that site, or local adaptation within those populations leading to a shift towards clonality under unfavorable flowering conditions. Further research should be conducted to more precisely test the hypothesis that *S. patens* shifts resource allocation from sexual to asexual reproductive modes in response to light limitation, vs. the alternative hypothesis that under sufficiently resource-limited conditions individuals will be less productive in both clonal and sexual reproduction.

Genetic variation of *S. patens* populations is impacted by clonal reproduction and growth, as evidenced by sampling fewer multilocus genotypes than would be expected under strictly sexual reproduction. At the same time, clonal diversity was high, with 44 distinct multilocus lineages identified in 62 samples and a wide distribution of pairwise genetic distances. Moreover, the majority of paired tiller samples harvested from the same plot were not from the same multilocus lineage. Many studies of clonal plants assume clonal identity or independence of proximate samples without explicitly testing this assumption. Other work in related marsh grasses has found high degrees of clonal diversity at local scales (e.g. Hughes and Lotterhos 2014, Gaynor et al. 2019, Walker et al. 2021). This highlights that it is essential for studies of genotype by environment interactions in clonal species to include a confirmation of clonal identity along with estimates of the power of genetic markers to resolve clones in their methods.

A key finding of our study pertains to the beneficial role that phenotypic plasticity could play for marsh grasses as they migrate into the forest understory. Individuals from the forest had lower starting biomass, but despite this, when transplanted into the marsh, they were able to accrue significant biomass, reaching equivalent biomass levels in the marsh to transplants of marsh origin. This suggests that plasticity can serve a beneficial role for populations of *S. patens* as it colonizes the heterogeneous and shifting landscape of the marsh-to-upland ecotone. Species colonizing novel environments may experience rapid but transient increases in plasticity (Lande 2015) and this has been empirically demonstrated in various biological systems (Brancalion et al. 2018, Buckley et al. 2010). Given the acceleration of maritime forest die-off in recent decades in step with accelerating sea-level rise and more frequent and stochastic storm events, it is likely that *S. patens* in the forest understory will soon be released from light limitation as the forest retreats (Scheider et al. 2018). For this reason, the short-term and flexible strategy of phenotypic plasticity may serve as a means of tolerating suboptimal light conditions while establishing in saline conditions where non-halophytic competitors are precluded (Kottler and Gedan 2020).

The response of this foundation species to sea level rise will have implications for the entire ecological community it structures and supports. The phenotypic plasticity documented here suggests that as the forest canopy dies back, understory *S. patens* patches will be released from light limitation and exhibit greater biomass production, stem density, and flowering. Sunlight promotes evaporation and soil salinization, which will filter salt-intolerant species from the community, allowing small, sparse *S. patens* patches to expand through clonal reproduction and coalesce into marsh meadows. Recent work has found that, despite compositional differences, new marshes created by marsh migration did not meaningfully differ from established marshes in their primary production and nutrient cycling (Langston et al. 2022). We predict that these meadows will continue to provide habitat for a community of high marsh invertebrates (Denno 1977, Wimp and Murphy 2021) and birds (Greenberg 2006), providing resilience to these assemblages to persist in the face of sea level rise (Rippel et al. 2021) and a pathway to migration upslope. Ghost forests appear to support a greater diversity of marsh bird species when compared with low-lying intact forests, though not as many as older high marsh habitat (Taillie et al. 2019). Given the dire population declines and extinction predictions for many species of tidal marsh specialist birds in the Mid-Atlantic (Klingbeil et al. 2021, Wiest et al. 2019), migrating *S. patens* will be doubly important.

This study's findings contribute to the growing body of work to characterize phenotypic plasticity and shifts in intraspecific trait variation across environmental gradients as evolutionary (though not always adaptive) responses to climate change and to predict how these phenomena may in-turn impact ecosystem function. Recent experimental work with ancestral and descendant lineages of another marsh foundation species has found compelling evidence for evolutionary shifts in both trait means and the plasticity of traits which impact ecosystem function over the past century of environmental change (Vahsen et al. 2023). Researchers have also identified evolutionary shifts in trait means and in the degree of trait plasticity separate from means in other plant systems responding to climate stressors such as drought stress (Johnson et al. 2022, Blanco-

Sánchez et al. 2023). Intraspecific trait variation and plasticity are crucial to study because they can significantly impact species' abilities to undergo range shifts under climate change (Usui et al. 2023, Valladares et al. 2014) and the degree of facilitation foundation species are able to provide to other organisms – as demonstrated in alpine nurse plants along an altitudinal gradient (Schöb et al. 2013).

The results of this study suggest that intraspecific trait variation and phenotypic plasticity of marsh foundation species contribute to a migrating ecotone paradigm in Mid-Atlantic coastal ecosystems as described in Smith and Goetz (2021). More research is needed to determine the relative roles of phenotypic plasticity vs. genetic variation in trait means in the dynamics of other dynamic forest-grassland ecotones being affected by climate change, such as those in South Brazil (Müller et al. 2012). Understanding the eco-evolutionary response of foundation species can be used more generally to predict habitat and community responses to climate change in a variety of ecosystems.

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Author Contributions

E.K. and K.G. conceived and planned the experiments. E.K. carried out the experiments. E.K. and M.H. contributed to genetic data preparation and analysis. E.K. wrote the initial draft and all authors provided critical feedback and helped shape the research, analysis and manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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	High marsh mean (s.e.)	Maritime Forest mean (s.e.)	Habitat	Site	Time	Interactions
Light Availability$_{sq}$	57.3% (±3.03)	11.3% (±1.30)	M > F ***	B < C ***	Increases with time ***	Habitat x Site; Site x Time ***
Pore-water salinity$_{sq}$	13.6 ppt (±0.443)	5.5 ppt (±0.289)	M > F ***	A = B < C ***	Increases with time **	All interactions significant ***
Elevation	0.51 m (±0.052)	0. 468 m (±0.056)	NS	A < C < B ***	-	Habitat x Site ***
Soil moisture^	25.2% (±1.51)	22.5% (±1.40)	NS	NS	-	NS
Carbon Mass	0.249g (±0.049)	0.237g (±0.056)	NS	A < B < C ***	-	NS
pH^	7.58 (±0.072)	6.41 (±0.198)	M > F ***	NS	-	NS
Redox potential$_{sq}$	-288.1967 mV (±24.46)	32.427 mV (±40.02)	M > F ***	A = B < C ***	-	All interactions significant ***

Table 1: environmental differences between marsh and forest ecotone. Table displays mean and standard error values for environmental variables measured in high marsh vs. forest understory plots (first two columns) and the significance of our predictor variables on these environmental variables in linear and mixed linear effects models. M and F denote marsh plots and forest understory plots respectively, and show which habitat exhibited greater values of a given trait. Site lettering indicates: A) Moneystump Swamp, B) Phillips Creek Marsh , C) Wallops Island. Variables that were square root transformed to meet model assumptions are

denoted with the subscript “sq”, and those that could not be transformed to meet assumptions and were evaluated using a non-parametric Kruskal Wallis test are marked with a ^. Asterisks indicate significance: *p < 0.05; **p < 0.01; ***p < 0.001; †p < 0.10 (NS). "-" denotes that this factor or interaction could not be measured for these variables.

Reciprocal Transplant						
Trait	High marsh mean (s.e.)	Forest mean (s.e.)	Habitat Experienced	Habitat of Origin	Site	Interaction Effects
Stem Density$_{log}$	21 (±2.04)	10.2 (±1.14)	M > F **	M > F *	NS	Hab origin x Site (B) **
Plant Height	27 cm (±1.23)	32 cm (±1.71)	NS	NS	A < B **	Hab origin x Hab Experienced x Site (B) *
SLA$_{log}$	50 (±1.28)	58.9 (±2.12)	M < F *	NS	NS	NS
AGB$^{\wedge}$	1.71 g (±.209)	0.913 g (±.134)	M > F *	M < F *	NS	-
Root Biomass	18.4 g (±1.42)	16.6 g (±1.34)	NS	M > F ***	A = C < B **	Hab origin x Site (B) ***
Rhizome Biomass$_{sq}$	53mg (± 21)	83mg (± 26)	NS	NS	A = C < B **	Hab origin x Site (B) *
Total Biomass$_{sq}$	20.1 g (±1.50)	17.6 g (±1.34)	NS	M > F ***	A = C < B ***	Hab origin x Site (B) ***
Leaf Carbon by mass	45.4% (±0.226)	44.2% (±0.235)	M > F **	NS	NS	Hab experienced x Site (C) ***
Leaf Nitrogen by area	0.015g cm^{2} (±0.001)	0.016g cm^{2} (±0.001)	M < F *	NS	NS	Hab transplant x Site (C) **

Table 2: Reciprocal transplant trait differences. Table displays mean and standard error values plant trait response variables measured in high marsh vs. forest understory plots (first two columns) and the significance of our predictor variables on these traits in a linear model. M and

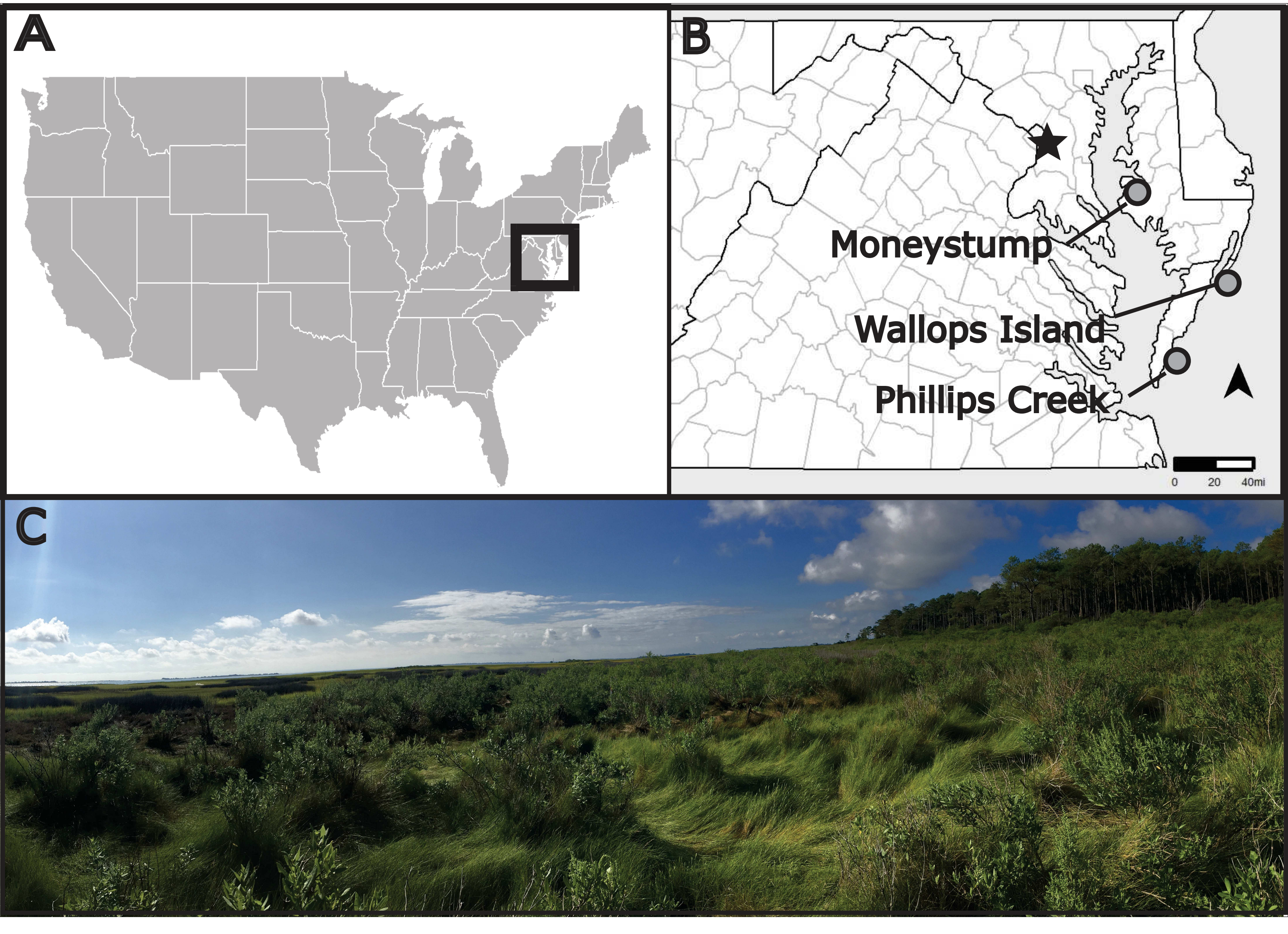
F denote marsh plots and forest understory plots respectively. Site lettering indicates: A) Moneystump Swamp, B) Phillips Creek Marsh , C) Wallops Island. Variables that were log10 transformed to meet model assumptions are denoted with the subscript log", and those that could not be transformed to meet assumptions and were evaluated using a non-parametric Kruskal Wallis test are marked with a ^. Asterisks indicate significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; †$p < 0.10$ (NS). "-" denotes that this factor or interaction could not be measured for these variables.

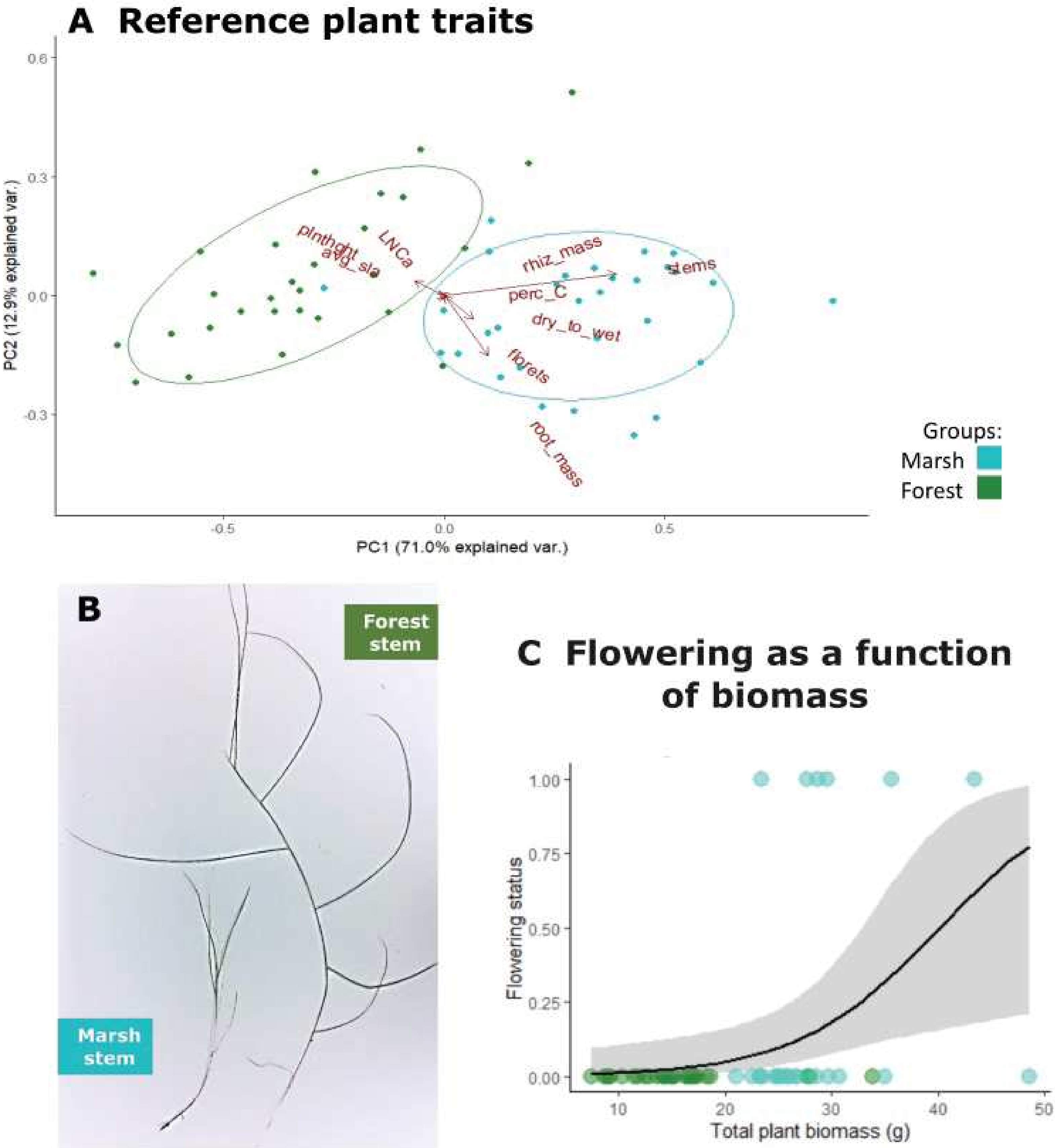
Figure 1: Site map and photographs of experiment. A) Map of the U.S. with study region outlined in black square D) Regional map; star denotes Washington, D.C., and circles denote the three reciprocal transplant experiment sites: Moneystump Marsh'(38°"5'42.7"N' 76°"3'29.7"W), Wallops Island, VA'(37°"3'02.2"N' 75°"6'18.0"W), and Phillips Creek Marsh '(37°"7'44.9"N' 75°"9'47.6"W). C) Photograph of coastal ecotone in Cape Charles, VA (Photos, CR: Kottler).

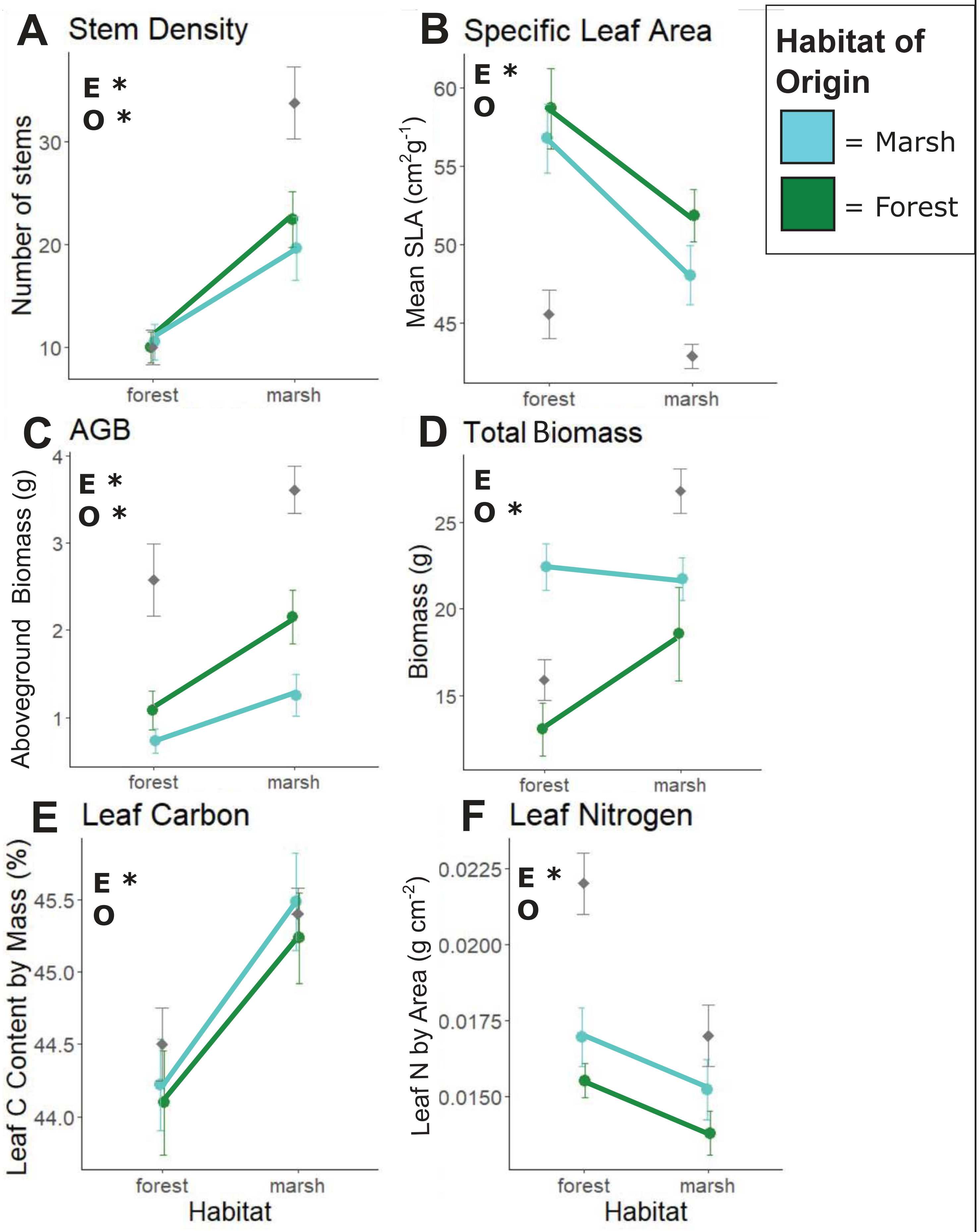
Figure 2: Ecotypic differentiation of reference individuals from high marsh and forest understory. A) Scatterplot of outputs from the Principle Components Analysis (PCA) of reference plant traits with the first principle component (PC1) on the x-axis and the second principle component (PC2) on the y-axis. Points represent principle component values for each individual plant whose traits were measured. Points are color-coded by habitat of origin, where marsh = teal, forest = green. Ellipses show 95% confidence intervals of forest and marsh groups. Arrows represent individual traits, with direction indicating correlation between each trait variable and principal components, and length indicating the contribution of said trait to principal components 1 & 2. The traits presented are as follows: plant height (plnthght), stem density

(stems), average specific leaf area (avg_sla), number of flowering stems (florets), root biomass (root_mass), rhizome biomass (rhiz_mass), plant water content (dry_to_wet), leaf carbon by unit mass (perc_C), leaf nitrogen by surface area (LNCa). B) A photograph comparing *S. patens* marsh and forest stem phenotypes from Phillips Creek Marsh, VA (CR: Kottler). C) Graph depicts total biomass of reference plants on the x-axis and flowering status (1: plant grew at least one flowering stem, 0: no flowering stems) on the y-axis. Points are color-coded by habitat of origin, where marsh = teal, forest = green. The black curve shows the fit of a binomial generalized linear model, with standard error in gray.

Figure 3: Reaction norm plots of reciprocal transplant traits. Graphs present the mean value and standard error (via error bars) for each plant trait presented on the y-axis and are color-coded according to transplant habitat of origin, where teal = marsh origin and green = forest origin. The x-axes denote the environment in which the transplants were reared, and the lines between points connect the mean values from individuals of the same habitat of origin. The slope of said lines is indicative of the degree of trait plasticity, with higher slope indicating greater cross-environment plasticity. Gray diamond points with standard error bars indicate the mean trait value of reference plants harvested from each habitat. In the upper left of each plot, there is an "E" for environment in which the transplants were reared and "O" for habitat origin, with an asterisk if this was a significant factor ($p < 0.05^*$) associated with variation observed in that trait. Graphs are shown for the following traits: A) Stem Density, B) Specific Leaf Area, C) Total Aboveground Biomass, D) Total plant biomass, E) Leaf Carbon Content by Mass, F) Leaf Nitrogen Content by Area (g/cm^2).


A
B
Moneystump
Wallops Island
Phillips Creek
0
20
40mi
C


A Reference plant traits
PC2 (12.9% explained var.)
PC1 (71.0% explained var.)
0.6
0.3
0.0
-0.3
-0.5
0.0
0.5
plnthght
avg_sla
LNCa
rhiz_mass
stems
perc_C
dry_to_wet
florets
root_mass
Groups:
Marsh
Forest
B
Forest stem
Marsh stem
C Flowering as a function of biomass
Flowering status
1.00
0.75
0.50
0.25
0.00
10
20
30
40
50
Total plant biomass (g)


A Stem Density
E *
O *
Number of stems
10
20
30
forest
marsh
B Specific Leaf Area
E *
O
Mean SLA (cm^{2}g^{-1})
45
50
55
60
forest
marsh
Habitat of Origin
= Marsh
= Forest
C AGB
E *
O *
Aboveground Biomass (g)
1
2
3
4
forest
marsh
D Total Biomass
E
O *
Biomass (g)
15
20
25
forest
marsh
E Leaf Carbon
E *
O
Leaf C Content by Mass (%)
44.0
44.5
45.0
45.5
forest
marsh
Habitat
F Leaf Nitrogen
E *
O
Leaf N by Area (g cm^{-2})
0.0150
0.0175
0.0200
0.0225
forest
marsh
Habitat