New Phytologist

Research

Complex eco-evolutionary responses of a foundational coastal marsh plant to global change

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Received: *30 November 2022*
Accepted: *6 June 2023*

New Phytologist (2023)
doi: 10.1111/nph.19117

Key words: climate change, eco-evolutionary dynamics, evolution of plasticity, *Schoenoplectus americanus*, sea-level rise, wetlands.

Summary

- Predicting the fate of coastal marshes requires understanding how plants respond to rapid environmental change. Environmental change can elicit shifts in trait variation attributable to phenotypic plasticity and act as selective agents to shift trait means, resulting in rapid evolution. Comparably, less is known about the potential for responses to reflect the evolution of trait plasticity.
- Here, we assessed the relative magnitude of eco-evolutionary responses to interacting global change factors using a multifactorial experiment. We exposed replicates of 32 *Schoenoplectus americanus* genotypes 'resurrected' from century-long, soil-stored seed banks to ambient or elevated CO_2, varying levels of inundation, and the presence of a competing marsh grass, across two sites with different salinities.
- Comparisons of responses to global change factors among age cohorts and across provenances indicated that plasticity has evolved in five of the seven traits measured. Accounting for evolutionary factors (i.e. evolution and sources of heritable variation) in statistical models explained an additional 9–31% of trait variation.
- Our findings indicate that evolutionary factors mediate ecological responses to environmental change. The magnitude of evolutionary change in plant traits over the last century suggests that evolution could play a role in pacing future ecosystem response to environmental change.

Introduction

Predicting the fate of Earth's ecosystems in the Anthropocene requires understanding how organisms respond to rapid, global environmental change. This is especially true for foundational plant species that can alter elemental cycling and ecosystem functioning (Chapin, 2003; Kell, 2011; Monroe *et al.*, 2018), where even small shifts in traits might result in meaningful outcomes (Wild *et al.*, 2011; Emery *et al.*, 2015; Mozdzer *et al.*, 2022). There has been appreciable effort to integrate trait responses into predictive ecosystem models but, thus far, most attention has focused on investigating plastic or 'ecological' responses of plants to global change factors (e.g. Maire *et al.*, 2013; Ayata *et al.*, 2014; Mastrotheodoros *et al.*, 2017). It is far less common to integrate phenotypic evolution into predictive models, despite increasing evidence that rapid evolutionary responses (i.e. evolution on 'ecologically relevant' timescales) can be an important driver of ecosystem change (e.g. Hairston *et al.*, 2005; Franks *et al.*, 2014; Geerts *et al.*, 2015; Valencia-Montoya *et al.*, 2021; Mozdzer *et al.*, 2022; Vahsen *et al.*, 2023).

Although understudied in an ecosystem-level context, evolutionary factors (i.e. evolution and sources of heritable variation) can play important roles in regulating trait-mediated ecosystem outcomes of global environmental change. For example, it has been shown that heritable trait variation in coastal marsh plants can influence the accumulation of soil organic matter (Seliskar *et al.*, 2002), soil microbial community composition (Lumibao *et al.*, 2020, 2022), and shoreline erosion (Bernik *et al.*, 2018). Similarly, Avolio *et al.* (2015) showed that genomic dissimilarity of native tallgrass prairie grass populations influence ecosystem resilience to plant invasion, and Schweitzer *et al.* (2008, 2011) have demonstrated that the influence of genetic variation in *Populus* trees on soil microbial decomposition can give rise to eco-evolutionary feedbacks. These and other findings point to the possibility that generational shifts in heritable trait variation can potentially alter ecosystem processes (Monroe *et al.*, 2018; Barton *et al.*, 2020).

Interest in the ecological importance of evolution has also been motivated by evidence that rapid environmental change can elicit evolutionary responses in plants capable of transforming ecosystems (Blum *et al.*, 2021; Mozdzer *et al.*, 2022). Avolio *et al.* (2013), for example, demonstrated that the genetic composition of tallgrass populations can rapidly shift in response to altered precipitation regimes. Similarly, Mozdzer *et al.* (2022)

demonstrated that even a brief period of exposure to elevated nitrogen and CO_2 can shift genotypic variation in populations of *Phragmites australis* marsh grasses. Several mesocosm and field-scale experiments also have demonstrated that exposure to global change pressures can elicit shifts in the variation of heritable traits that influence ecosystem processes (e.g. Grossman & Rice, 2014; García-Carreras *et al*., 2018; Blum *et al*., 2021). However, while these studies show that genotypic variation can shift rapidly in response to global change, they do not explicitly link genotypic variation to ecosystem function. Only a handful of studies have explicitly examined whether and how evolutionary responses to environmental change can alter ecosystem attributes (e.g. Barton *et al*., 2020; Vahsen *et al*., 2023).

Coastal marsh ecosystems present exceptional conditions to discover and describe ecosystem outcomes of phenotypic evolution. Plant assemblages in coastal marshes are naturally depauperate in species diversity, which increases the potential ecological significance of heritable trait variation within dominant species (Reusch & Hughes, 2006). Furthermore, many dominant marsh plants influence vital ecosystem services (Fagherazzi *et al*., 2012; Kirwan & Megonigal, 2013). For example, plants contribute to carbon accumulation in marsh soils through the production of belowground biomass. Carbon sequestration in coastal marshes – often referred to as 'blue carbon'– constitutes a globally significant sink that offsets rapidly increasing atmospheric CO_2. Plants also regulate accretion (i.e. the building of marsh surface elevation) by influencing sediment deposition and subsurface expansion, which has historically allowed marshes to keep pace with sea-level rise (Kirwan & Megonigal, 2013). Notably, decades of research on coastal marsh plant responses to global change has yielded a rich baseline of information on organismal-to-ecosystem responses to global change that can be leveraged to guide further study (e.g. Cherry *et al*., 2009; Langley *et al*., 2009, 2013; Drake, 2014; Watson *et al*., 2015; Mozdzer *et al*., 2016; Wigand *et al*., 2016; Lu *et al*., 2019; Noyce *et al*., 2019). Prior work has shown, for example, that interactions between global change factors (e.g. Deegan *et al*., 2007; Cherry *et al*., 2009; Langley *et al*., 2013; Drake, 2014; Watson *et al*., 2016) can elicit context-dependent trait change and that plant trait change can regulate marsh ecosystem functioning and persistence (Lu *et al*., 2019; Blum *et al*., 2021; Vahsen *et al*., 2023). Finally, there are mathematical models of marsh accretion and carbon accumulation that could serve as scaffolds to understand and quantify how evolutionary responses of plants to global change (e.g. sea-level rise, elevated CO_2) give rise to ecosystem-level change (e.g. Rietl *et al*., 2021; Vahsen *et al*., 2023).

Structuring predictive models to account for phenotypic evolution could deliver more accurate and precise forecasts of ecosystem functioning under possible global change scenarios. Doing so addresses simplified and possibly erroneous assumptions about how plants (and emergent ecosystem functioning) respond to global change (Vahsen *et al*., 2023). Models might be improved by accounting for selection that shifts mean trait values and exposure to global change factors that prompts the evolution of trait plasticity, constituting population-level shifts in G × E interactions (Bradshaw, 1965; Schlichting, 1986). Constructing and parameterizing representative eco-evolutionary models will require further understanding of how trait evolution proceeds, accounting for the likely possibility that responses reflect pressures imposed by multiple, interacting global change factors (Matesanz *et al*., 2010).

Here, we sought to address these knowledge gaps (Fig. 1) by conducting a mesocosm experiment with genotypes of the coastal marsh sedge *Schoenoplectus americanus* resurrected from soil cores constituting ancestral (*c*. 1895–1947) and descendant (*c*. 2003–2016) age cohorts (Summers *et al*., 2018). We exposed the genotypes to varying amounts of inundation (range = 50 cm) and either ambient (420 ppm) or elevated (700 ppm) atmospheric CO_2 at two sites that varied in salinity (4 ppt vs 6 ppt) to understand the extent to which differences in trait expression are attributable to evolutionary responses of trait means and plasticity (Fig. 1c,d). We also grew *S. americanus* in the presence and absence of a competitor, *Spartina patens*, to elucidate how community-level interactions might alter eco-evolutionary responses of *S. americanus* to environmental pressures. Considering evidence that populations of *S. americanus* can rapidly evolve over the course of a century or less (Summers *et al*., 2018; Blum *et al*., 2021; Vahsen *et al*., 2023; Fig. 1c), we hypothesized that accounting for evolutionary factors would improve statistical models describing trait-based responses of *S. americanus* to global change factors (Fig. 1c–f). This hypothesis builds from the null expectation that *S. americanus* responses to global change factors are purely a manifestation of phenotypic plasticity (i.e. ecological responses), as is currently reflected in widely used simulation models designed to forecast the state and fate of marsh ecosystems under global change scenarios (Fig. 1a,b).

To test this broad hypothesis, we purposefully exposed plants to global change factors at levels similar to previous studies investigating how environmental factors mediate plant trait variation (e.g. near doubling of atmospheric CO_2 *sensu* Drake, 2014; inundation intervals similar to other mesocosm studies *sensu* Langley *et al*., 2013). Thus, our *a priori* expectations were that the manipulated environmental variables would predictably influence trait variation in ways supported by the literature because we chose levels for those variables that were known to have a measurable effect. We introduced evolutionary factors to test whether evolution might modify these relationships in predictable (e.g. descendant genotypes consistently performed better under scenarios that reflected greater flooding or elevated atmospheric CO_2) or unpredictable ways.

Materials and Methods

Study system

Schoenoplectus americanus (Pers.) Volkart ex Schinz & R. Keller is an ecologically dominant sedge in coastal marshes in the Chesapeake Bay. Prior work has demonstrated that *S. americanus* exhibits shifts in functional traits in response to global change factors including elevated CO_2, inundation, and salinity as well as in the presence of interspecific competitors such as *S. patens* (Arp *et al*., 1993; Cherry *et al*., 2009; Langley *et al*., 2009, 2013;

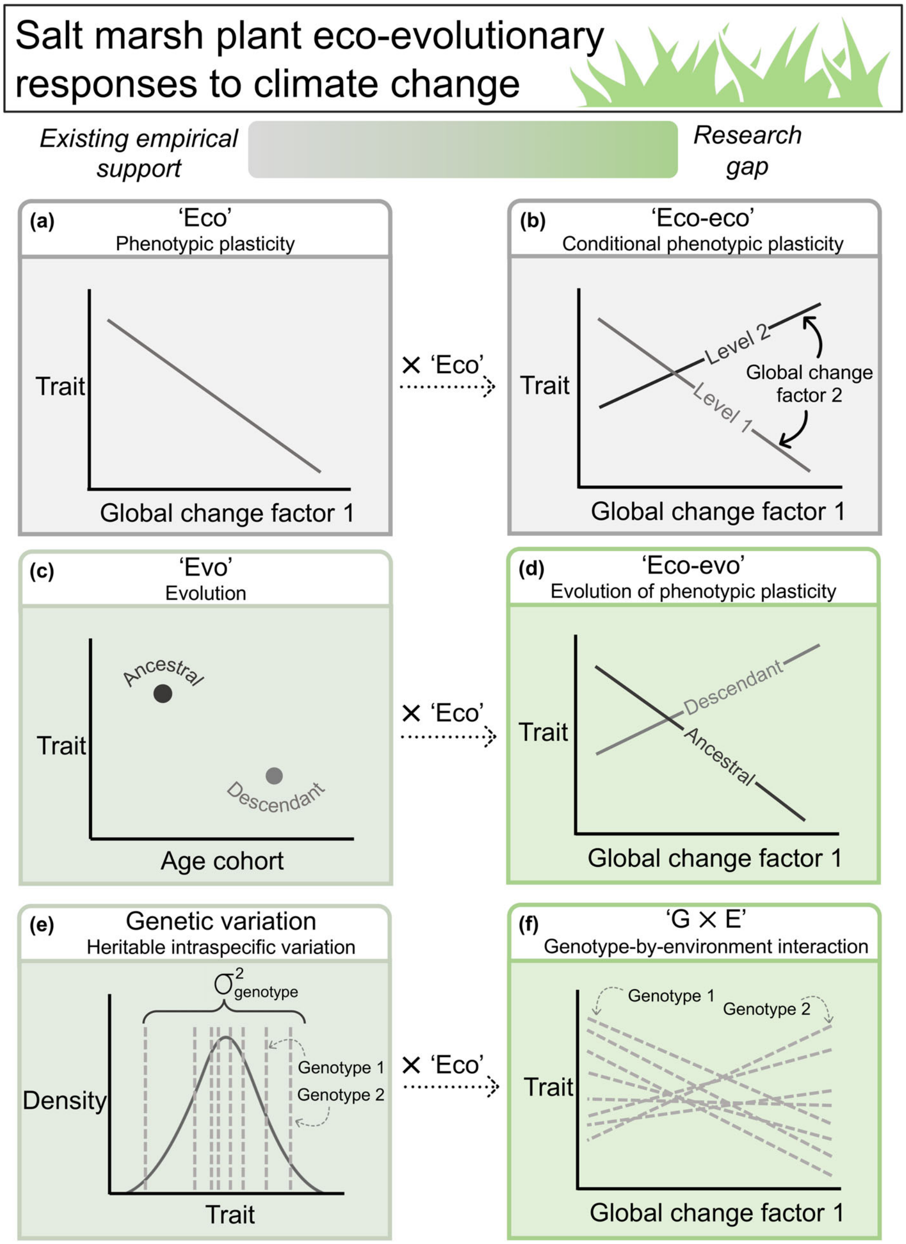


Fig. 1 Potential eco-evolutionary responses of salt marsh plants to climate change. (a) 'Eco': plant traits shift as a plastic function of exposure to one global change factor (e.g. greater inundation, elevated atmospheric CO_2). Genetic variation does not contribute to observed trait variation. (b) 'Eco-eco': an extension of 'eco' responses, where the effect of one global change factor on plant traits depends on the level of another global change factor. (c) 'Evo': plant traits have evolved, where the mean trait values are different between ancestral and descendant populations when plants are grown in a common environment. (d) 'Eco-evo': variation in response to a global change factor has evolved over time, known as 'evolution of phenotypic plasticity', where plasticity (i.e. slope of the line) is a trait capable of evolving. (e) 'Genetic variation': traits vary among plant genotypes. The Gaussian curve represents the distribution of trait values for a group of genotypes (with each genotype as a dashed line) within the same cohort. In a mixed effects statistical model of a plant trait, we can characterize $\sigma^2_{\text{genotype}}$ which is the between-genotype variance or the amount of trait variation that is due to genotype. (f) 'G × E': the effect of a global change factor on plant traits depends on the genotype, referred to as a 'genotype-by-environment' interaction.

Drake, 2014; Lu *et al.*, 2019; Noyce *et al.*, 2019). This work has increased our understanding of how plant traits have shifted over time and provided insights about how traits will shift under predicted global scenarios of increased atmospheric CO_2, as well as local increases in sea-level and accompanying shifts in salinity and inundation in the Chesapeake Bay (Hong & Shen, 2012; Rice *et al.*, 2012; Ross *et al.*, 2021). *S. americanus* reproduces asexually via vegetative tillering and sexually via seed production, the frequency of which correlates with environmental conditions (Ikegami *et al.*, 2007). *S. americanus* produces highly durable seeds that can be germinated (i.e. 'resurrected') from persistent soil-stored seed banks after over a century of burial under tidally induced sediment deposition. Genotypes that are representative of time-stratified seed depth cohorts can then be propagated into replicate clones for experiments designed to address questions about rapid evolution of ecologically relevant plant traits (Summers *et al.*, 2018; Vahsen *et al.*, 2021).

Genotype acquisition

The 32 genotypes used in this experiment were plants 'resurrected' from seeds that were sieved from soil cores collected from two locations – Kirkpatrick Marsh and Corn Island – *c.* 500 m apart on the Rhode River of the Chesapeake Bay between 2003 and 2017 (Fig. 2a; Supporting Information Table S1). Although these sites are regionally proximate, our previous analyses of genetic variation measured according to microsatellite loci (Summers *et al.*, 2018) and genomic variation measured according to SNP loci (Vahsen *et al.*, 2023) suggest that there is considerable differentiation between *S. americanus* populations at these (and

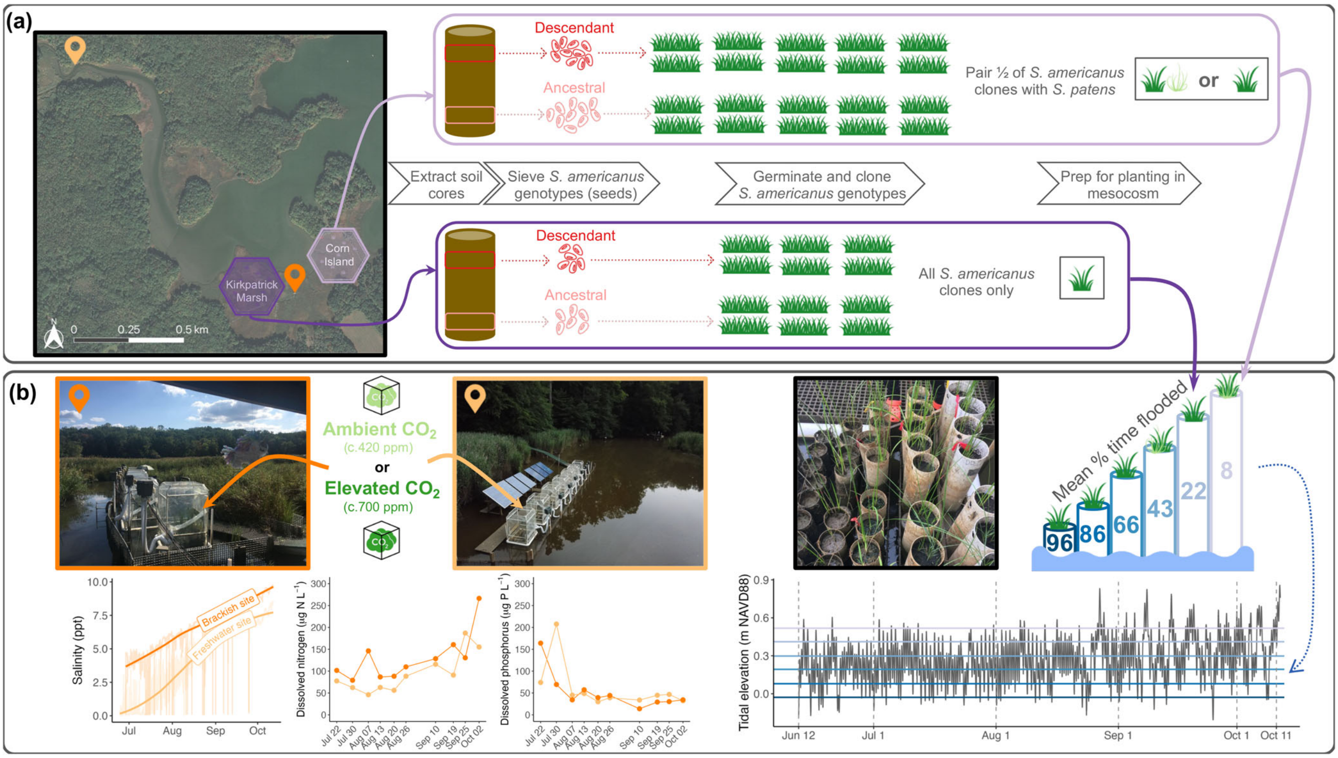


Fig. 2 Visual representation of (a) sourcing and preparing clonal propagules of *Schoenoplectus americanus* genotypes from ancestral and descendant cohorts for (b) use in the mesocosm experiment. (a) Soil cores were extracted from Kirkpatrick Marsh (dark purple) and Corn Island (light purple) marshes within the Rhode River estuary of the Chesapeake Bay; both marshes are located at the Smithsonian Environmental Research Center (Edgewater, MD, USA). Seeds were sieved from time-stratified layers of soil cores and grouped by age cohort (ancestral = pink, descendant = red), corresponding to soil depth from the marsh surface. After seeds were germinated, resulting plants were cloned to create replicates of each genotype for use in the mesocosm experiment. Half of the clones of each genotype from Corn Island were introduced into the mesocosm experiment alongside a competitor, *Spartina patens*, and half were introduced without a competitor. Due to limitations in the number of available genotypes and clones, all Kirkpatrick Marsh genotypes were introduced into the experiment without a competitor. (b) Mesocosms were constructed at two sites that differ in prevailing salinity conditions (bottom left); however, plants also experienced differing levels of dissolved nitrogen (bottom left) and dissolved phosphorus (bottom left) which may have influenced their relative growth. At each site, there were seven chambers, three with elevated atmospheric CO_2 conditions (700 ppm), and four with ambient CO_2 conditions (420 ppm). All plants were grown in peat-filled PVC pipes resting in racks within the chambers at six elevations differing by *c.* 10 cm (bottom right), varying the amount of inundation plants experienced over the course of the growing season (top right).

other nearby) sites. Seeds were wet-sieved and germinated in Conviron growth chambers (Vahsen *et al.*, 2021) and then maintained in pots containing a mixture of sand and potting soil (Fig. 2a). We estimated the age of seeds using a calibrated equation (Vahsen *et al.*, 2021) based on ^{210}Pb and ^{137}Cs dates of three soil cores collected from Kirkpatrick Marsh. We grouped seeds into two age cohorts based on estimated ages (i.e. the mean approximated year each seed was likely deposited in the sediment; Table S1): ancestral genotypes (estimated year range: 1895–1947) and descendant genotypes (estimated year range: 2003–2016). The time horizon separating the ancestral and descendant genotypes is concurrent with a period of considerable environmental change, with increased global atmospheric CO_2 as well as increases in sea-level and accompanying pressures from inundation and salinity in the Chesapeake Bay (Hilton *et al.*, 2008). The terms 'ancestral' and 'descendant' are based on genetic evidence that late 20th century *S. americanus* populations are descended from early 20th century *S. americanus* populations in Kirkpatrick Marsh (Summers *et al.*, 2018). This and other previous work (Vahsen *et al.*, 2023) report genetic variation does not decline with seed depth (i.e. genetic variation is similar in descendant and ancestral cohorts); however, further work may be warranted to fully exclude the possibility of selective pressures acting on soil-stored seed archives (Weis, 2018).

Clones of the 32 genotypes were maintained in glasshouses at the University of Notre Dame (ND) or the University of Tennessee, Knoxville (UTK) before the study, which was conducted at the Smithsonian Environmental Research Center (SERC; Edgewater, MD, USA). Before deployment, we clipped the stems to 5 cm in length and recorded the initial wet weight of all clones. We then acclimated the clones to 5 ppt salinity (Instant Ocean) for 2 wk to reduce planting stress. We included 'laboratory origin' as a categorical covariate (2 levels; ND vs UTK) in our statistical models (described below) to account for any potential maternal/carryover effects due to differences in initial rearing conditions. Finally, while we made considerable efforts to reduce possible impacts of maternal effects, our study design does not fully eliminate the possibility that observable trait variation could have been shaped by maternal effects (or other factors like epigenetic variation). We nonetheless contend that possible effects would not change the substance of our key findings or inferences.

Mesocosm experiment

We employed a now common 'marsh organ' experimental design (*sensu* Morris, 2007) that allowed us to assess plant responses to realistic tidal conditions. This involved use of PVC pots balanced on racks securely anchored in a tidal creek (Fig. 2b). Each rack in the tidal creek was composed of six elevation levels (8 pots per level × 6 levels = 48 pots per rack), thereby manipulating the amount of inundation that the plants experienced (Fig. 2b). This design allowed us to simulate past, present, and future rates of sea-level rise by varying the elevation in 10 cm increments for a total range of 50 cm difference in elevation. Past rates of relative sea-level rise in Chesapeake Bay were perhaps 1 mm yr^{-1}; present rates are *c.* 3.4 mm yr^{-1}, and rates are rapidly accelerating (Ohenhen *et al.*, 2023). Assuming an average rate of relative sea-level rise of 5 mm yr^{-1}, the 50 cm range represents *c.* 100 yr of change encompassing ancestral, descendant, and future cohorts of *S. americanus*. Because *S. americanus* plant traits can be highly sensitive to centimeter-scale fluctuations in flooding (Morris *et al.*, 2002), we used a real-time kinematic (RTK) GPS to get near-exact elevations (standard error *c.* 1 cm) of each rack to provide a more accurate estimate of inundation, measured relative to the North American Vertical Datum of 1988 (NAVD88). Thus, inundation is represented by the continuous variable of elevation in our statistical models, where low elevation values represent high inundation and high elevation values represent low inundation.

We installed a set of seven racks at two sites at SERC. One site was in a tidal creek that drains Kirkpatrick Marsh into the Rhode River estuary, and the other site was 1.6 km upstream on Muddy Creek (Fig. 2a map; Fig. 2b left). The two sites differ in salinity and were chosen to simulate near-future sea-level rise-driven effects on salinity and its interactions with other global change factors. Accordingly, we measured near-continuous salinity data from a HOBO logger installed at each site (Fig. 2b, left). The range we used for salinity was thus constrained by the characteristics of available study sites and was also subject to interannual variation in precipitation. There was a 2 ppt difference in salinity on average between the sites, which is about one-third of the interannual variation in salinity at the brackish site (Zhu *et al.*, 2022). While salinity is generally increasing in the Chesapeake Bay, it is not monotonic and often relatively slow because higher salinity from rising sea levels can be offset by higher precipitation in this estuarine system. In fact, there is no trend in mean annual growing season salinity at our study site from 1985 to 2020 (Zhu *et al.*, 2022). To characterize other site-level conditions that might contribute to trait variation, we also measured total phosphorus, dissolved phosphorus, and dissolved nitrogen (ammonium + ammonia + nitrate + nitrite) in the surface water at each site 10 times and particulate nitrogen and carbon five times over the course of the experiment (Figs 2b, S1). Although there were some differences in dissolved nitrogen between the sites, previous work suggests that in similar coastal marsh ecosystems, plant nitrogen demand is generally met via internal nitrogen cycling driven by microbial mineralization rather than external inputs of nitrogen from surface water (Neubauer *et al.*, 2005; Megonigal & Neubauer, 2019; Tobias & Neubauer, 2019).

Each rack was covered with a floating open-top chamber made of a PVC frame with the sides wrapped in translucent cellophane film (details in Langley *et al.*, 2013) that allowed us to manipulate the atmospheric CO_2 concentration (Fig. 2b, left). Specifically, we raised the CO_2 concentration to 700 ppm to simulate global concentrations that are likely to occur between 2060 and 2100 (Cheng *et al.*, 2022). At each site, three of the seven chambers maintained atmospheric CO_2 at *c.* 700 ppm via the continuous injection of CO_2 gas, which was circulated in the air using blowers within the chamber. The other four chambers maintained atmospheric CO_2 at ambient concentrations (*c.* 420 ppm), while also having air circulated using blowers within the chamber. The blowers introduced air with ambient or elevated CO_2 at the bottom of the floating chamber (sealed by the water surface), which rose slowly out of the open top past the plants (Langley *et al.*, 2013).

We planted *S. americanus* clones in a stratified random design across racks and environmental treatments, focusing on replication at the age cohort and provenance level (e.g. Corn Island ancestral vs Kirkpatrick Marsh descendant). A subset of the clones (239 out of 620 total; all from Corn Island) were grown alongside a standardized clone of *S. patens* (Fig. 2a) to determine whether the presence of a competitor mediates ecological and evolutionary responses of *S. americanus* to global change factors. We did not have sufficient clonal replication of genotypes from Kirkpatrick Marsh, so we only manipulated competition for Corn Island genotypes (Fig. 2a). Pots with both *S. americanus* and *S. patens* were randomized among and within racks and across inundation levels, and within each age and provenance cohort, genotypes were randomized across treatment combinations such that each genotype was exposed to roughly one half of the possible environmental treatment combinations.

We placed the 72 cm tall PVC pots filled with Baccto reed sedge peat into the frames before planting to allow the peat to naturally compress over multiple tidal cycles. We planted each *S. americanus* clone from 12–17 June 2019, into a PVC pot. One week after planting in the mesocosm, we replaced some propagules ($n = 84$) that failed to establish with extra propagules that were maintained in a growth chamber, keeping age cohort and provenance consistent (see Table S2 for final distribution). We planted *S. patens* propagules at the same time as the *S. americanus* propagules, following a similar schedule of replacement for those that failed to establish ($n = 12$). For analysis of *S. americanus* traits (detailed below), we included a categorical covariate (3 levels) of 'cloning group' to account for variation in traits due to differences in the date the clones were clipped for planting.

Functional traits

We measured the following traits of *S. americanus* at the end of the experiment during the week of 6–11 October 2019:

aboveground biomass, belowground biomass, root-to-shoot ratio, mean stem height, mean stem width, stem density, and root depth distribution. We chose these traits because they are linked to well-known ecosystem processes that govern plant-geomorphic feedbacks on elevation and inundation, and ultimately tidal marsh resilience to accelerated sea-level rise. Plant biomass allocation influences subsurface expansion, carbon accumulation, and sediment deposition (Kirwan & Megonigal, 2013). The distribution of belowground biomass in the soil column by depth can influence decomposition rates via 'priming' as well as nitrogen mineralization rates (Wolf *et al.*, 2007; Bernal *et al.*, 2017; Noyce *et al.*, 2022). Sediment deposition is also a function of stem morphology, as stems directly capture sediment and slow down tidal flux, which allows sediment to settle out of the water column (Mudd *et al.*, 2010; Lu *et al.*, 2019).

For each experimental unit (i.e. PVC pot), we measured the height and width of each live *S. americanus* stem and recorded the total live stem density, and then harvested live and dead aboveground biomass at the surface of the soil. We weighed total aboveground biomass by species after drying at 60°C to constant mass. We stored soil cores containing belowground biomass at −20°C to preserve roots and rhizomes until the material could be processed. We cut thawed cores into three sections (0–10 cm, 10–20 cm, and 20 cm–end) and sieved each section using a power washer over a 6.35 mm (1/4 inch) sieve frame. We considered all biomass sieved from the pots to be belowground biomass – roots, rhizomes, and bases of stems – as stem bases can contribute substantially to subsurface accretion (Cherry *et al.*, 2009).

For experimental units that contained only *S. americanus*, we calculated the root-to-shoot ratio as the ratio of total belowground biomass (roots, rhizomes, and stem bases) to total aboveground biomass (live and dead). To characterize the shape of the root depth distribution (i.e. how *S. americanus* belowground biomass is distributed proportionally across soil depth), we calculated a root depth distribution parameter, β, using the equation $y_i = 1-\beta^{d_i}$, where y_i is the cumulative proportion of biomass calculated from the top of the soil core at the marsh surface to a depth below the marsh surface, d_i (Gale & Grigal, 1987; Mozdzer *et al.*, 2016; Vahsen *et al.*, 2023). We used the function 'nls()' (R, v.4.1.2; R Core Team, 2021) to fit the nonlinear model and to extract the parameter estimate of β for each pot (for i from 1 to 224). For subsequent analyses, we considered the fitted value of β to be a trait, which excludes estimation uncertainty.

Statistical analysis

We conducted all statistical analyses in the computational language R (v.4.1.2; R Core Team, 2021). We fit (generalized) linear mixed models to *S. americanus* survival and trait data (i.e. all replicates that survived) to assess whether and to what extent ecological and evolutionary factors (and interactions thereof) explained observed trait variation. To interpret the relative effect of ecological and evolutionary factors in explaining survival and trait variation, we grouped predictor variables in the statistical models into categories that align with those depicted in Fig. 1, such that the main effect of an individual, manipulated environmental variable (i.e. inundation, CO_2 treatment, site salinity, and competition treatment) was an 'eco' effect (evidence of plasticity; Fig. 1a), the effect of an interaction between two or more of these environmental variables was an 'eco-eco' effect (evidence of conditional phenotypic plasticity; Fig. 1b), the main effect of age cohort or provenance was an 'evo effect' (evidence of temporal or spatial evolution; Fig. 1c), and the effect of an interaction between age cohort or provenance and one or more of the manipulated environmental variables was an 'eco-evo' effect (evidence of evolution of plasticity across time or space; Fig. 1d). We used random intercepts and slopes to account for genetic variation due to genotype (Fig. 1e) and genotype-by-environment interactions (Fig. 1f), respectively (detailed below). Given that we purposefully chose levels of environmental variables that should elicit effects on plant traits based on previous coastal marsh plant global change experiments, we expected that 'eco' (and potentially 'eco-eco' factors) would explain considerable variation in plant traits.

Because we applied the competition treatment for only Corn Island genotypes, competition and provenance covaried (i.e. Kirkpatrick Marsh genotypes were not subjected to competition; Fig. 2a). Thus, we fit models on two different subsets of the survival and trait data: one that included all experimental units that did not have competition (survival: $n = 430$, trait: $n = 230$) and one that only included experimental units from Corn Island (survival: $n = 482$, trait: $n = 292$). Thus, models for the 'no competition' data set included CO_2, inundation, salinity, age cohort, and provenance (and their interactions) as well as a quadratic inundation term and covariates related to propagule initial conditions (initial wet weight of propagule, laboratory origin, and cloning group) as fixed effects. We included genotype and individual chamber (as a spatial block) as random intercepts and CO_2, salinity, and inundation (and their interactions) as random slopes across genotypes. Models for the 'Corn Island only' dataset had a similar structure, except provenance was replaced with competition treatment, and competition could be included in the random slope specification.

To understand the drivers of *S. americanus* survival, we first fit a generalized linear mixed model with a binomial data distribution and logit link to binary data, where 0 = *S. americanus* did not survive to harvest and 1 = *S. americanus* was alive at harvest. Both genotype and individual chamber random effects were estimated to be near zero and were thus removed from the model. For multiple combinations of fixed effect levels, all plants either survived or died, leading to the complete separation problem (Albert & Anderson, 1984). Thus, we fit the generalized linear model with a bias-reduction method to allow for a robust approximation of standard errors (*brglm*, v.0.7.2; Firth, 1993). Using this model fitting procedure, the most complex model we fit included up to all possible three-way interactions between CO_2, inundation, salinity, age cohort, and provenance (or competition) as well as the other previously mentioned covariates.

Because of low survival of plants at the two highest levels of inundation (3% and 27% survival, respectively; Table S3; Fig. S2), we analyzed plant trait data for plants on the lowest four inundation levels only (on average 91% survival) and removed any observations for which there was zero aboveground *S. americanus* growth. We square root-transformed aboveground and belowground biomass and log-transformed root-to-shoot ratios to better meet the assumption of homogeneity of variance. We performed backward stepwise selection starting with the maximal, or most complex, model (i.e. up to five-way interactions between 'eco' and 'evo' terms) to narrow in on potentially informative interactions between ecological and evolutionary variables (LMERTEST, v.3.1-3). We assessed the significance of fixed effects in the model using a Type III ANOVA with the Satterthwaite method. We recognize that *P*-values alone are not sufficient to identify whether ecological and evolutionary factors have important effects on trait variation (i.e. statistical significance ≠ effect size; Greenland *et al*., 2016). We therefore also report effect sizes when possible (following recommendations from Greenland *et al*., 2016; Dunkler *et al*., 2020), and we have visualized model results to better understand ecological and evolutionary factors underlying trait variation. We also ran additional permutation tests *post hoc* to assess the likelihood that significant terms of interest in our model were due to chance (see Methods S1; Table S4).

Finally, we quantified the overall extent that evolution explained variation for each trait by comparing the fit of the final model to the fit of a 'null' statistical model (i.e. one that did not include the fixed effects of age cohort and provenance, the random intercept for genotype, or any random slopes across genotype; Fig. 1a,b), constructed using the stepwise approach described above. We compared the amount of variation explained by the null model to the final 'evolution' model – which included all evolutionary factors (i.e. age cohort, provenance, and genotype) – using Nakagawa's R^2 (PERFORMANCE, v.0.8.0; Nakagawa & Schielzeth, 2013). We also calculated the 'conditional' intraclass correlation coefficients (ICCs) for each of the final evolution trait models to quantify the proportion of observed trait variation explained by the random effects of genotype (and genotype-by-environment interactions) in light of fixed effects in the model (Gelman & Hill, 2006; Nakagawa *et al*., 2017). For all trait models, the spatial block random effect was dropped from the model in the stepwise procedure so the R^2 and ICC values captured variation due to genotype and G × E interactions alone.

Results

Survival of *S. americanus*

As expected, *S. americanus* survival was strongly influenced by inundation (Fig. S3a,c; Table S5). Survival was also mediated by other global change factors. For example, survival at the freshwater site was lower at higher levels of inundation than at the same levels of inundation at the brackish site (inundation × salinity, $P = 0.003$; Fig. S3a,c). Interestingly, the probability of survival was also mediated by age cohort and provenance. Ancestral genotypes from Corn Island exhibited especially lower survival in comparison with descendant genotypes from Corn Island under elevated CO_2 conditions (CO_2 × age cohort × provenance, $P = 0.026$; Fig. S3b). Furthermore, plants that originated from Kirkpatrick Marsh were more likely to survive at the freshwater site whereas plants from Corn Island were more likely to survive at the brackish site (salinity × provenance, $P = 0.018$; Fig. S3c). Competition from *S. patens* did not strongly drive or mediate the likelihood of *S. americanus* survival (Table S6), likely because most *S. americanus* mortality was due to establishment failure relatively early in the experiment.

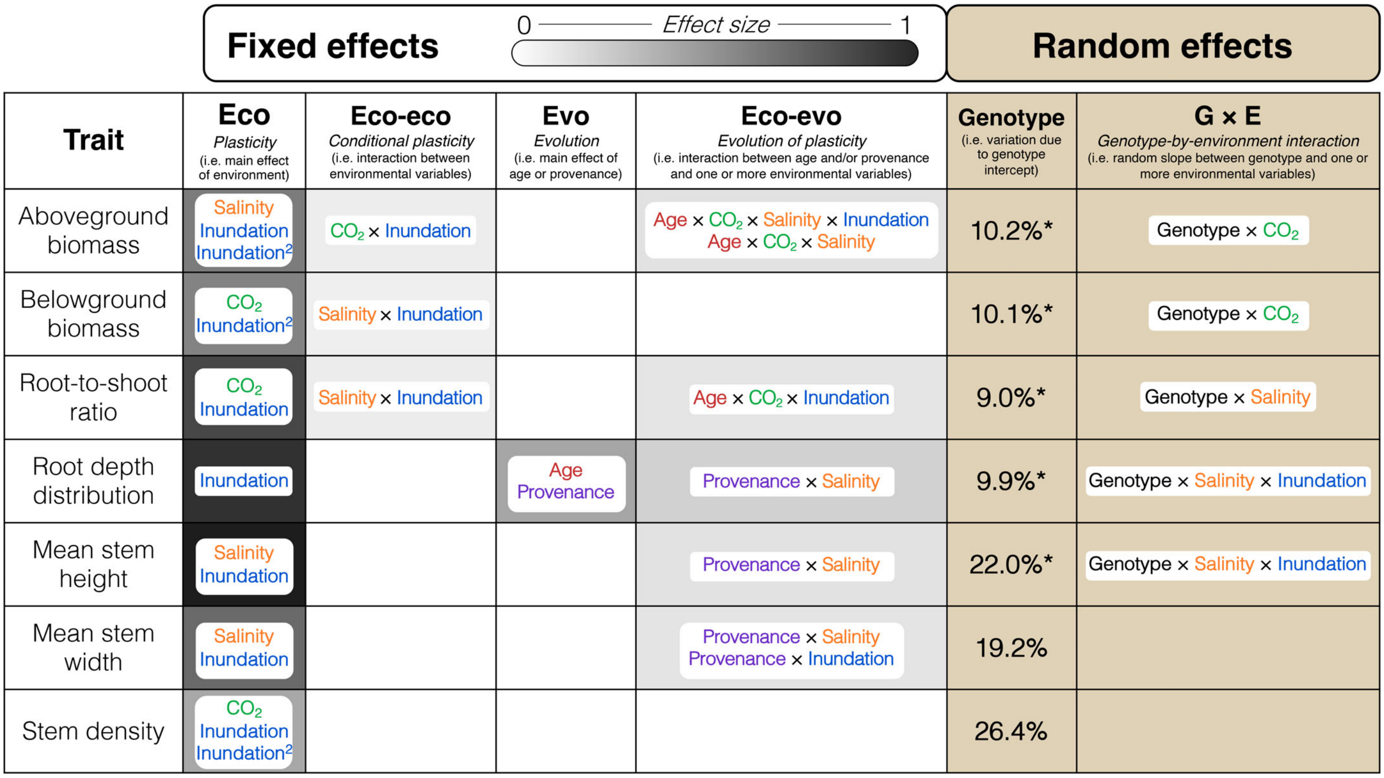
Functional traits of *S. americanus* in the absence of competition

As expected, we found evidence of trait plasticity in response to the environmental conditions that we exposed plants to in the mesocosm experiment (Fig. 3 'Eco' column; Table S7). On average (i.e. averaged across other model terms), inundation explained significant patterns in all traits measured, either with plant traits increasing linearly with inundation (mean stem height and width), decreasing linearly with inundation (root-to-shoot ratio and root depth distribution), or via a quadratic relationship with inundation (aboveground biomass, belowground biomass, and stem density) where the trait value was maximized at intermediate levels of inundation. On average, CO_2 stimulated belowground biomass production, stem density, and root-to-shoot ratios, and more saline conditions at the brackish site decreased stem height and width, resulting in decreased aboveground biomass.

We found evidence of 'eco-eco' interactions for aboveground biomass, belowground biomass, and root-to-shoot ratio (Figs 3, S4). On average, we detected a positive effect of elevated CO_2 on the aboveground biomass of plants grown at higher inundation (Fig. 4a; CO_2 × inundation: $F_{1,192.2} = 5.77$, $P = 0.017$). Root-to-shoot ratios were, on average, 23.4% higher for plants at the brackish site compared with the freshwater site, when grown at high inundation (Fig. S4a; salinity × inundation: $F_{1,183.2} = 10.69$, $P = 0.001$). Belowground biomass was 31.5% higher in plants grown at low inundation at the freshwater site compared with the brackish site (Fig. S4b; salinity × inundation: $F_{1,195.7} = 4.80$, $P = 0.030$).

Beyond expected induced shifts in plant traits due to plastic relationships with environmental variables, we found little evidence that mean trait values evolved over time or space. Only for root depth distributions were there significant differences overall between age cohorts: Root profiles were shallower for descendant genotypes compared with ancestral genotypes, indicating that plants have evolved to allocate less belowground biomass deeper in marsh soils (Fig. S5; $F_{1,26.1} = 5.79$, $P = 0.024$).

We found considerably more evidence that trait plasticity evolved across less than a century of time or across less than 1 km of space, compared with mean trait evolution (Figs 3–5). Significant 'eco-evo' interactions indicate that plasticities in aboveground biomass and root-to-shoot ratio have evolved across time in the study populations (Figs 3, 4), that is, that ancestral and descendant genotypes exhibited different responses to the same environmental pressures. For example, there was a positive effect of elevated CO_2 on aboveground biomass production at greater inundation, but only at the brackish site for ancestral genotypes



	Fixed effects (0 — Effect size — 1)				Random effects	
Trait	**Eco** *Plasticity* (i.e. main effect of environment)	**Eco-eco** *Conditional plasticity* (i.e. interaction between environmental variables)	**Evo** *Evolution* (i.e. main effect of age or provenance)	**Eco-evo** *Evolution of plasticity* (i.e. interaction between age and/or provenance and one or more environmental variables)	**Genotype** (i.e. variation due to genotype intercept)	**G × E** *Genotype-by-environment interaction* (i.e. random slope between genotype and one or more environmental variables)
Aboveground biomass	Salinity Inundation Inundation^2	CO_2 × Inundation		Age × CO_2 × Salinity × Inundation Age × CO_2 × Salinity	10.2%*	Genotype × CO_2
Belowground biomass	CO_2 Inundation^2	Salinity × Inundation			10.1%*	Genotype × CO_2
Root-to-shoot ratio	CO_2 Inundation	Salinity × Inundation		Age × CO_2 × Inundation	9.0%*	Genotype × Salinity
Root depth distribution	Inundation		Age Provenance	Provenance × Salinity	9.9%*	Genotype × Salinity × Inundation
Mean stem height	Salinity Inundation			Provenance × Salinity	22.0%*	Genotype × Salinity × Inundation
Mean stem width	Salinity Inundation			Provenance × Salinity Provenance × Inundation	19.2%	
Stem density	CO_2 Inundation Inundation^2				26.4%	

Fig. 3 Statistically significant terms in the final linear mixed model for each functional trait (on the rows), as determined by backward stepwise selection, fit to observations with extant *Schoenoplectus americanus* in the absence of competition from *Spartina patens*. Model terms in the columns 'Eco', 'Eco-eco', 'Evo', and 'Eco-evo' were statistically significant as determined by a Type III ANOVA at $\alpha = 0.05$. Cells for fixed effects are shaded by their summed effect sizes (ε^2) in each column group as calculated using the R package EFFECTSIZE. Percentages reported in column 'Genotype' represent the percent of observed variation that can be attributed to genotype in light of other (fixed effect) terms in the model (i.e. the conditional intraclass correlation coefficient). Model terms in the column 'G × E' were statistically significant as determined by a likelihood ratio test at $\alpha = 0.10$ (following default settings of the R function *lmerTest*::*step*()). *In cases where there was a significant 'G × E' random slope, the percent of observed variation attributed to genotype was calculated using an intercept-only model (i.e. without 'G × E' terms included) and should therefore be interpreted with caution.

and at the freshwater site for descendant genotypes (Fig. 4a; CO_2 × salinity × age × inundation: $F_{1,185.7} = 7.86$, $P = 0.006$). This result suggests that for aboveground biomass there was selection for plasticity in response to elevated CO_2 under certain inundation and salinity conditions. Furthermore, the stimulatory effect of elevated CO_2 due to plasticity was substantial: Aboveground biomass was 88.4% higher under elevated CO_2, on average (i.e. averaged across other explanatory variables), relative to ambient conditions for ancestral genotypes at the brackish site under high inundation and 90.2% higher for descendant genotypes at the freshwater site under high inundation. The impact of the evolution of plasticity on root-to-shoot ratios was also substantial; ancestral genotypes were stimulated by elevated CO_2 up to 18.8% at lower inundation, and conversely, descendant genotypes were stimulated up to 24.0% at higher inundation (Fig. 4b; CO_2 × age × inundation: $F_{1,198.7} = 6.87$, $P = 0.009$), suggesting that there has been selection for plasticity in response to CO_2 under different inundation conditions.

We detected further evidence of evolution of plasticity when accounting for genotype provenance. For example, comparisons of stem morphology indicate that genotypes from Corn Island exhibited greater plasticity in response to changes in inundation and salinity compared with genotypes from Kirkpatrick Marsh (Fig. 5a–c). Stems of genotypes from Corn Island were wider than those from Kirkpatrick Marsh at higher inundation, whereas the reverse was found at lower inundation (Fig. 5a; provenance × inundation: $F_{1,197.5} = 7.83$, $P = 0.006$). Stems of genotypes from Corn Island also exhibited greater plasticity in response to salinity; stems were 8.8% taller and 14.4% thicker at the freshwater site compared with the brackish site, whereas stem height and thickness did not differ substantially for Kirkpatrick Marsh genotypes (Fig. 5b,c; height model, provenance × salinity: $F_{1,74.7} = 5.95$, $P = 0.017$; width model, provenance × salinity: $F_{1,199.1} = 5.46$, $P = 0.020$). Finally, root depth distribution differed by provenance when plants were grown at the brackish site, but not at the freshwater site (Fig. 5d; provenance × salinity: $F_{1,31.1} = 5.32$, $P = 0.028$).

Across all traits, genotype was important in explaining observed trait variation (Fig. 1e,f), accounting for an additional 11–31% (mean = 20%) beyond what could be explained by global change factors and age cohort or provenance alone (Tables 1, S7, S8, marginal vs conditional R^2 for 'evolution' models; Fig. 6a). We also found that variation of five of the seven focal traits was partly attributable to genotype-by-environment interactions ('G × E'; Figs 3, 6b–f). For example, the effect of elevated atmospheric CO_2 on aboveground biomass and belowground

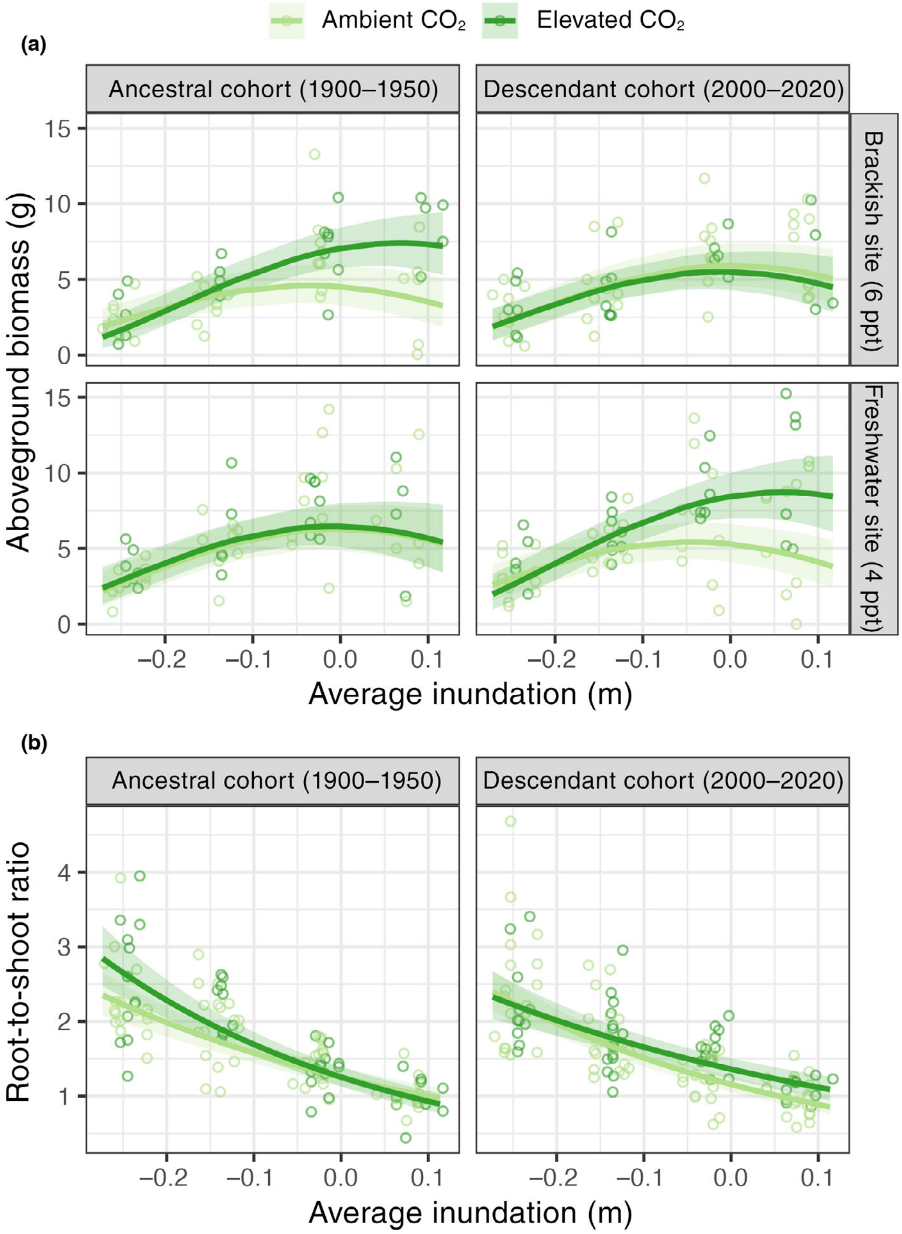


Fig. 4 Functional trait plasticity in *Schoenoplectus americanus* has evolved in less than a century. (a) Aboveground biomass of *S. americanus* was a function of an eco-evolutionary interaction between inundation, CO_2, salinity, and the age cohort of the genotype ($F_{1,185.7} = 7.86$, $P = 0.006$). For ancestral genotypes (*c.* 1900–1950; left panel), elevated CO_2 stimulated aboveground production at higher inundation at the brackish site, but not at the freshwater site, whereas for descendant genotypes (*c.* 2000–2020; right panel) elevated CO_2 stimulated aboveground production at the freshwater site, but not at the brackish site. (b) Root-to-shoot ratio was influenced by an interaction between inundation, CO_2, and age cohort ($F_{1,198.7} = 6.87$, $P = 0.009$), such that elevated CO_2 stimulated belowground production relative to aboveground production at low inundation for ancestral genotypes, and at high inundation for descendant genotypes. For both graphs, predicted means from the linear mixed models are shown as bolded lines and the shaded bands represent 95% confidence intervals. Raw data are displayed as open circles.

biomass varied by genotype (Fig. 6d,e). Similarly, the effect of salinity on root-to-shoot ratio varied by genotype (Fig. 6f). The strength of salinity × inundation interactions on root depth distribution and stem height also depended on genotype (Fig. 6b,c).

The effect of *S. patens* competition on eco-evolutionary responses of *S. americanus*

The presence of *S. patens* mediated and constrained *S. americanus* responses to global change factors (Tables 2, S9; Fig. 7). For example, *S. americanus* stems were on average thinner (Table 2; Fig. S6; $F_{1,259.1} = 14.86$, $P < 0.001$) and shorter across all treatments (Tables 2, S9; Fig. S6; $F_{1,265.4} = 3.92$, $P = 0.049$) in the presence of a *S. patens* competitor. We also found that competition had the greatest effect on decreasing *S. americanus* aboveground biomass and stem density at low inundation (Fig. 7), where *S. patens* was the most productive (Fig. S7).

Discussion

The results of this study build on previous work suggesting that evolutionary factors likely play a larger role in shaping plant traits than is currently recognized in studies of ecosystem responses to environmental change, including within wetlands (Blum *et al.*, 2021; Lumibao *et al.*, 2022; Mozdzer *et al.*, 2022; Vahsen *et al.*, 2023). Long-standing recognition that coastal marsh plants strongly regulate vital ecosystem processes (Kirwan & Megonigal, 2013) combined with mounting evidence of rapid evolution (Summers *et al.*, 2018; Blum *et al.*, 2021; Lumibao *et al.*, 2022; Mozdzer *et al.*, 2022; Vahsen *et al.*, 2023) highlights the potential

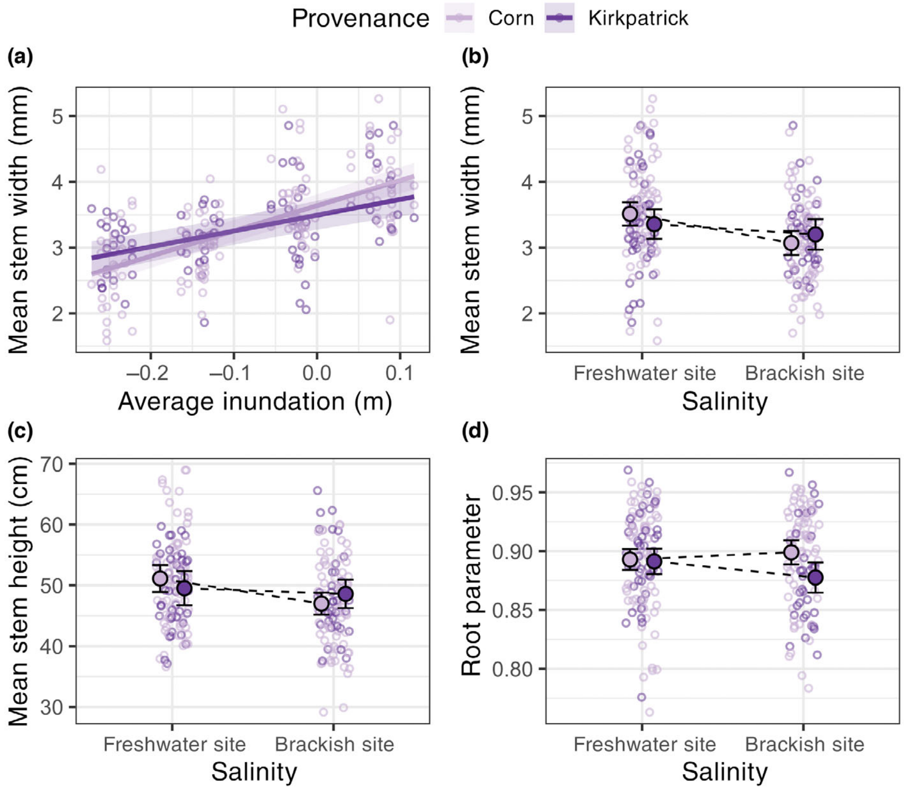


Fig. 5 Evolutionary differences in plasticity among nearby populations of *Schoenoplectus americanus*. (a) Genotypes from Corn Island (light purple) exhibited greater plasticity in mean stem width in response to changes in inundation compared with genotypes from Kirkpatrick Marsh (dark purple; provenance × inundation; $F_{1,197.5} = 7.83$, $P = 0.006$). (b) Genotypes from Corn Island (light purple) exhibited greater plasticity in mean stem width in response to changes in salinity compared with genotypes from Kirkpatrick Marsh (dark purple; provenance × salinity; $F_{1,199.1} = 5.46$, $P = 0.020$). (c) Genotypes from Corn Island (light purple) exhibited greater plasticity in mean stem height in response to changes in salinity compared with genotypes from Kirkpatrick Marsh (dark purple; provenance × salinity; $F_{1,74.7} = 5.95$, $P = 0.017$). (d) Genotypes from Kirkpatrick Marsh (dark purple) distributed belowground biomass proportionally shallower in response to increased salinity, whereas genotypes from Corn Island (light purple) were less sensitive to changes in salinity (provenance × salinity; $F_{1,31.1} = 5.32$, $P = 0.028$). For all graphs, predicted means from the linear mixed model are shown in the bolded lines (or filled points) and the shaded bands (or error bars) represent 95% confidence intervals. Raw data are displayed as the open circles and are jittered in panels b–d for ease of interpretation.

for coastal marshes to serve as a hallmark system for studying eco-evolutionary dynamics. However, our findings also highlight that traits do not necessarily evolve predictably, with evolution of plasticity being a far more common mechanism of trait change in our study than mean trait evolution, and with complex interactions between global change factors mediating the effect of evolution over space and time.

'Eco-evo': evolution of plasticity

Our results underscore that the evolution of phenotypic plasticity can be an important, though somewhat underexplored empirically, driver of trait change on decadal to centennial timescales. Appreciation for its potential importance has likely been limited in part because the evolution of plasticity can be challenging to assess, which can be overcome by leveraging 'resurrection ecology' approaches (Preston *et al*., 2022; Rauschkolb *et al*., 2022; Zhang & Jiang, 2022). We found that the stimulating effect of elevated CO_2 on aboveground biomass at high levels of inundation was conditional on an additional environmental factor (salinity) and age cohort (Fig. 4a). Though this and other 'eco-evo' responses detected in our experiment were complex, it is nonetheless possible to draw reasonable inferences and hypotheses about underlying mechanisms driving the selection of plasticity over time or space. We can postulate, for example, from measures of aboveground biomass that the additional stress of elevated salinity at the brackish site induced a novel stress on ancestral plants, which the energy subsidy afforded by higher photosynthesis at elevated CO_2 helped alleviate. This inference draws support from evidence that the Chesapeake Bay has on average become more saline since the mid-1900s (Hilton *et al*., 2008), which points to the possibility that ancestral genotypes (*c*. 1900–1950) are less capable of coping with more brackish conditions, and thus might benefit more from concurrent exposure to elevated CO_2 (Fig. 4a, left). Predicting how plant traits will shift due to the evolution of plasticity under future scenarios of accelerated sea-level rise and increased atmospheric CO_2 has direct consequences on estimates of vertical accretion and carbon sequestration rates in coastal marshes, as these processes are sensitive to traits such as aboveground biomass and

Table 1 R^2 for final linear mixed models as determined by backward stepwise selection for seven functional traits fit to observations of *Schoenoplectus americanus* in the absence of competition with *Spartina patens*.

Trait	Model	Marginal R^2 (fixed effects only)	Conditional R^2 (fixed and random effects)
Aboveground biomass (g)	Evolution	0.36	0.52
	Null	0.35	–
Belowground biomass (g)	Evolution	0.25	0.39
	Null	0.22	–
Root-to-shoot ratio	Evolution	0.59	0.70
	Null	0.61	–
Stem density	Evolution	0.18	0.44
	Null	0.19	–
Mean stem height (cm)	Evolution	0.38	0.69
	Null	0.38	–
Mean stem width (mm)	Evolution	0.44	0.63
	Null	0.42	–
Root distribution parameter	Evolution	0.51	0.77
	Null	0.48	–

The 'evolution' models represent those that might include provenance, age cohort, and genotype, whereas the 'null' model excludes those terms and assumes all trait variation can be explained by ecological factors. Marginal (fixed effects only) are calculated for both the 'evolution' and 'null' models, and conditional (fixed + random effects) R^2 values are calculated for the 'evolution' models following Nakagawa *et al*. (2017) using the R package MUMIN.

root-to-shoot ratio (Vahsen *et al.*, 2023). For example, the stimulation effect of CO_2 on plant traits in ecosystem models such as Rietl *et al.* (2021) that predict accretion and carbon accumulation may be contingent on salinity and evolutionary factors.

Evidence that responses to global change factors varied by provenance further illustrates that the evolution of phenotypic plasticity can drive trait change. For example, Kirkpatrick Marsh genotypes had lower establishment success at higher levels of inundation and salinity than Corn Island genotypes (Fig. S3). Interactions between provenance and global change factors also revealed that Corn Island genotypes exhibited greater plasticity in responses (i.e. greater sensitivity to exposure; Fig. 5), which can promote persistence of a population experiencing gradual (Chevin *et al.*, 2010) or extreme (Chevin & Hoffmann, 2017) environmental change. This finding suggests that *S. americanus* populations exhibit locally adaptive plasticity similar to that observed among populations of smooth cordgrass (*Spartina alterniflora*) from Atlantic and Gulf coast salt marshes (Bernik *et al.*, 2018). Our findings are nonetheless notable because there are so few empirical examples of locally adaptive plasticity and rapid evolution of adaptive plasticity (Reger *et al.*, 2018), despite reviews indicating that the evolution of phenotypic plasticity is a widespread phenomenon (Matesanz *et al.*, 2010; Hendry, 2016) and that plasticity can evolve on temporal scales similar to anthropogenically induced environmental change (i.e. over decades; Hendry, 2016). Additionally, our specific finding that plasticity in stem height and width in response to changes in salinity and inundation varies according to provenance (Fig. 5a–c) suggests that plant-mediated mineral sedimentation rates will likely be spatially variable under scenarios of accelerated sea-level rise. Further work is warranted to clarify the nature of adaptive plasticity in *S. americanus*. It would be worthwhile, for example, to illustrate how the spatial and temporal scales of environmental change align with genetic and phenotypic variation observed within and among populations of *S. americanus* (Summers *et al.*, 2018; Vahsen *et al.*, 2023). Conducting further empirical work using pedigreed full-sib and half-sib families generated from controlled crosses would offer a stronger basis for drawing inferences about the mechanisms underlying patterns of phenotypic variation by, for example, accounting for factors like maternal effects and epigenetic variation (*sensu* Preston *et al.*, 2022) and allowing for further exploration of responses to selection in a quantitative genetics framework.

While we found ample evidence for 'eco-evo' interactions across the traits measured, interactions between global change factors in the absence of an evolutionary component ('eco-eco') were less common (Fig. 3), despite strong plastic ('eco') responses to individual global change factors. This was surprising considering the emphasis placed on 'eco-eco' responses in prior studies characterizing exposure to global change pressures (Deegan *et al.*, 2007; Cherry *et al.*, 2009; Janousek & Mayo, 2013; Langley *et al.*, 2013; Drake, 2014; Mozdzer *et al.*, 2016; Watson *et al.*, 2016; Wigand *et al.*, 2016; Schile *et al.*, 2017). Our findings may offer a new perspective for interpreting study-specific findings as well as the (in)consistency of results across studies which could be a consequence of heritable variation (i.e. genetic variation, G × E interactions) and perhaps evolutionary processes (e.g. response to selection).

'Evo': evolution of population means

Population means for the measured traits largely did not change across time or space (i.e. 'evo'). However, on average, descendant genotypes exhibited greater survival in the experiment than did ancestral genotypes which may be a signal of adaptation to historical environmental changes. Consistent with our previous findings (Vahsen *et al.*, 2023), we also found that root depth distribution evolved to become shallower over time (Fig. S5), which could result in decreased belowground decomposition and nitrogen mineralization rates (Wolf *et al.*, 2007; Bernal *et al.*, 2017; Noyce *et al.*, 2022). The observed shift in belowground biomass distribution, proposed to be a product of nitrogen limitation being alleviated from increased anthropogenic nitrogen deposition (Mozdzer *et al.*, 2016; Vahsen *et al.*, 2023), is thus consistent across studies and across provenances within the Rhode River region of the Chesapeake Bay. This indicates that this specific finding is likely not idiosyncratic, but otherwise, limited evidence for mean trait evolution suggests that evolutionary change cannot be easily predicted.

Complexity in predicting plant trait evolution and its consequences on ecosystem processes

While the results from our study suggest that the evolution of plasticity is a more common mechanism by which plant traits

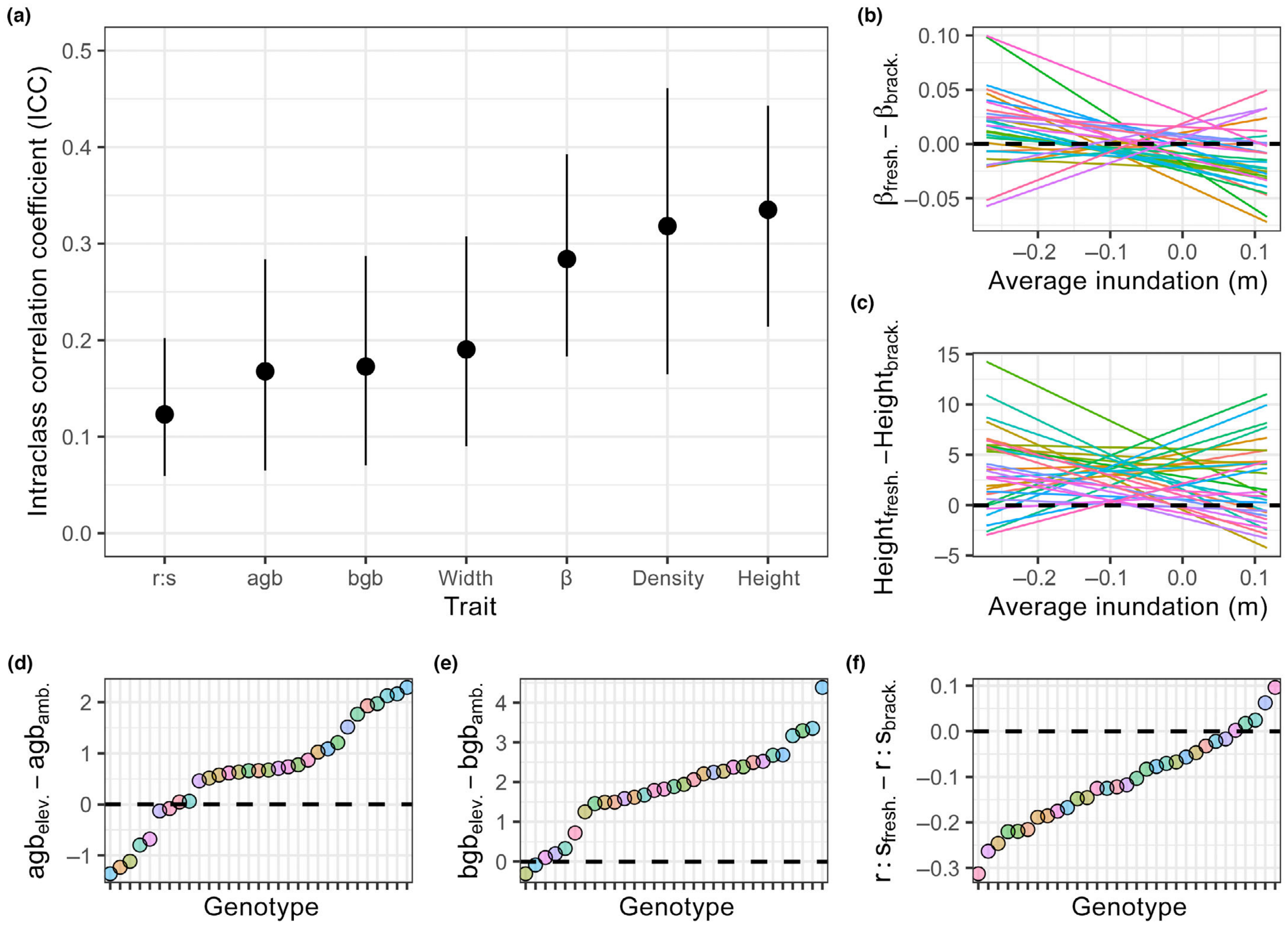


Fig. 6 Effects of genotypic variation and G × E interactions on *Schoenoplectus americanus* functional trait variation. (a) Intraclass correlation coefficients ('ICC'; the proportion of variation explained by genotype and G × E interactions (bounded between 0 and 1)) for all seven functional traits in increasing order of mean ICC: root-to-shoot ratio, aboveground biomass, belowground biomass, mean stem width, root distribution parameter (β), stem density, and mean stem height. Points represent means and error bars represent confidence intervals from bootstrapped simulations ($n = 1000$). (b–f) G × E (genotype-by-environment) interactions for (b) root depth distribution parameter (β), (c) mean stem height, (d) aboveground biomass, (e) belowground biomass, and (f) root-to-shoot ratio. The *y*-axes of panels (b–f) represents the difference between predicted trait values across either site salinity (freshwater site–brackish site) or CO_2 treatments (elevated–ambient). Each line in panels (b, c) represents the average predicted differences in response of a single genotype across an inundation gradient and each point in panels (d–f) represents the average predicted difference in response for a single genotype.

respond to rapid environmental change than has been previously understood in coastal marsh systems, our results do not necessarily suggest that accounting for complex eco-evolutionary responses will reduce uncertainty in ecosystem-level forecasting. Notably, variation among genotypes explained nearly all the additional observed variation from the inclusion of evolutionary factors in our statistical models (Table 1). This is not entirely surprising as the magnitude of genetically based differences can potentially be much larger than spatial or temporal shifts in mean trait values or trait plasticity, but it also brings into question whether accounting for the evolution of plasticity across space and time is consequential for predictive forecasting and whether accounting for genetic variation alone is important for capturing uncertainty in ecosystem processes. Explicit eco-evolutionary models that account for feedbacks between evolutionary mechanisms and ecosystem processes may be needed to reduce uncertainty in predictive forecasting.

Conclusions and future directions

Most ecosystem models of marsh accretion and carbon sequestration do not account for the influence of interacting global change factors on plant traits (but see Rietl *et al.*, 2021), and none account for heritable trait variation and changes thereof (i.e. trait evolution). Our results highlight that integrating evolutionary processes into ecosystem models may be useful but requires a complex understanding of evolution across space and time and interactions between several global change factors. Our findings illustrate that phenotypic evolution can occur on ecologically relevant timescales (i.e. fast enough to mediate ecosystem-level

Table 2 Effect of *Spartina patens* competition on *Schoenoplectus americanus* trait variation.

Trait	Additive or interactive effect	Effect of *S. patens* on *S. americanus*
Aboveground biomass (g)	Interactive	The presence of *S. patens* reduced *S. americanus* aboveground biomass at low inundation, but not at high inundation.
Stem density	Interactive	The presence of *S. patens* reduced *S. americanus* stem density at low inundation, but not at high inundation.
Mean stem height (cm)	Additive	The presence of *S. patens* reduced average *S. americanus* stem height.
Mean stem width (mm)	Additive	The presence of *S. patens* reduced average *S. americanus* stem width.

'Additive or interactive effect' signifies whether the presence of *S. patens* ('competition') as a fixed effect (0 = *S. patens* absent, 1 = *S. patens* present) is significant at the α = 0.05 level within an interaction or as a main effect.

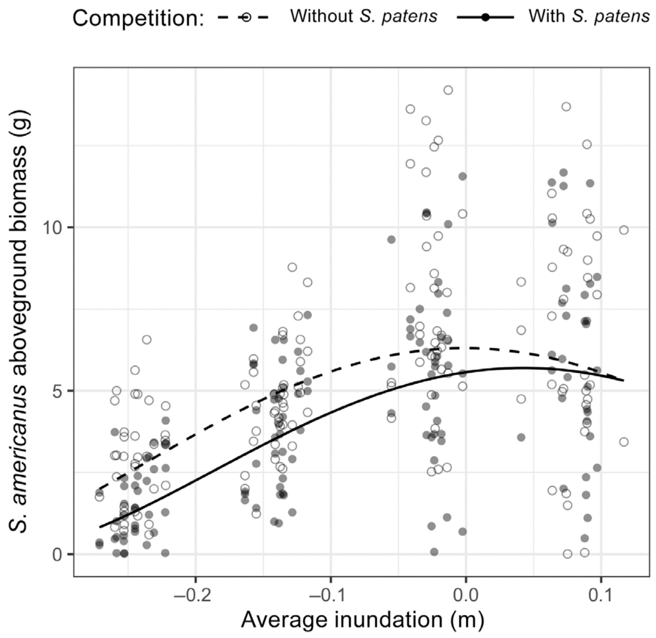


Fig. 7 Inundation mediates the effect of *Spartina patens* on *Schoenoplectus americanus* aboveground biomass production (inundation × competition, $F_{1,263.2} = 6.97$, $P = 0.009$). Raw data are displayed as the open and filled circles (without and with competition, respectively) and lines are predicted mean responses from the linear mixed model.

dynamics; Summers *et al*., 2018; Blum *et al*., 2021; Vahsen *et al*., 2023) and offer further support for the idea that global environmental change can elicit shifts in trait variation that alter vital ecosystem functions (Monroe *et al*., 2018; Mozdzer *et al*., 2022; Vahsen *et al*., 2023). We consider study sites like the Smithsonian Environmental Research Center's Global Change Research Wetland (GCReW) as highly valuable assets for pursuing research on eco-evolutionary dynamics. Long-term research sites like GCReW have generated decades-long chronosequences of data that can be revisited to investigate whether ecologically important plants like *S. americanus* have acclimated and/or evolved in response to global change. Our study can serve as a motivating resource for future retrospective and predictive analyses by serving as a framework for deconstructing the role(s) of eco-evolutionary drivers of plant trait change.

Acknowledgements

We are grateful for the support provided by technicians and volunteers at the Smithsonian Environmental Research Center. In particular, we would like to thank the following people for their assistance with experimental set-up, maintenance, and/or data processing: Adam Dunn, Johanna Hripto, Nick Iacaruso, Mikayla Manyin, Cary Pelc, Andrew Peresta, Gary Peresta, Evan Phillips, Roy Rich, Carmen Ritter, and Jenelle Whitman. We also would like to thank a large cohort of undergraduate students at Notre Dame and the University of Tennessee, Knoxville for germinating seeds and maintaining plants in the glasshouse in preparation for the mesocosm experiment. We acknowledge the following funding sources that supported this work: National Science Foundation DEB 1655781, DEB 1655702 (MJB, JPM, JSM); National Science Foundation DEB 0950080, DEB 1457100, DEB 1557009 (JPM); United States Coastal Research Program W912HZ-2020003 (MLV). Finally, we thank the editor and four reviewers for providing helpful feedback to improve the presentation and elevate the impact of our work.

Competing interests

None declared.

Author contributions

MLV, HK, MJB, JPM and JSM contributed to the conceptualization. MLV, HSK, HK, JLS, WLV and JSM contributed to the methodology. MLV, MJB, JPM and JSM contributed to the funding acquisition. MLV and JSM contributed to the writing – original draft. MLV, HSK, HK, JLS, WLV, MJB, JPM and JSM writing – review and editing.

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Data availability

All data and code for the described analyses are available via https://github.com/mlvahsen/BlueGenes.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Surface water nutrients at the Brackish and Freshwater sites over time.

Fig. S2 Trait distributions for extant experimental units for all inundation levels.

Fig. S3 Survival likelihood for *Schoenoplectus americanus* mediated by eco-evolutionary interactions.

Fig. S4 *Schoenoplectus americanus* root-to-shoot ratio and belowground biomass, as a function of inundation and site salinity.

Fig. S5 *Schoenoplectus americanus* root depth distribution as a function of age cohort and inundation.

Fig. S6 *Schoenoplectus americanus* mean stem height and width in the presence and absence *Spartina patens*.

Fig. S7 *Schoenoplectus americanus* stem density as a function of *Spartina patens* aboveground biomass.

Methods S1 Permutation tests of significant 'eco-evo' model terms.

Table S1 Summary age and provenance information for *Schoenoplectus americanus* genotypes used in the experiment.

Table S2 Distribution of genotype replicates across environmental treatments.

Table S3 List of genotypes that survived in the most flooded conditions.

Table S4 Results of permutation tests of significant 'eco-evo' model terms.

Table S5 Regression coefficients and statistics from the binomial generalized linear model assessing *Schoenoplectus americanus* survival in the absence of *Spartina patens* competition.

Table S6 Regression coefficients and statistics from the binomial generalized linear model assessing *Schoenoplectus americanus* survival for Corn Island genotypes.

Table S7 Significant model terms for all final 'evolution' models that were fit to data without *Spartina patens* competition and selected by the backward stepwise selection procedure.

Table S8 Significant model terms for all final 'null' (i.e. no evolution) models that were fit to data without *Spartina patens* competition and selected by the backward stepwise selection procedure.

Table S9 Significant model terms for all final 'competition' models that were fit to data for genotypes only from Corn Island and selected by the backward stepwise selection procedure.