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Research Article

Functional trait responses of emergent and free-floating *Alternanthera philoxeroides* to increasing salinity with sea level rise: stress tolerance, avoidance, and escape strategies

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Abstract

Sea level rise is having major impacts on estuaries due to salinity intrusion. These changes in stress profiles have ripple effects in ecosystems, including altering the invasibility of these wetlands depending on the salt tolerance of the invading species. *Alternanthera philoxeroides* Mart. (Griseb.) (alligator weed), native to South America and long recognized as one of the world's worst freshwater aquatic weeds, recently invaded tidal wetlands in California's San Francisco Bay–Delta Estuary. Generally considered a freshwater-limited glycophyte, observations suggested this invasive macrophyte may have some degree of salinity tolerance, though its degree of tolerance and capacity to spread with increased salinity intrusion were unknown. In two full-factorial greenhouse experiments, we assessed responses of emergent (soil-rooted) and free-floating growth forms of *A. philoxeroides* to four salinity concentrations (freshwater to euhaline) at the whole-plant (growth, biomass production and allocation, fitness), physiological, and biochemical levels. We also conducted a third experiment exploring the recovery potential of free-floating *A. philoxeroides* in freshwater following extended exposure to mesohaline to euhaline aqueous salinity. Although sensitivity of *A. philoxeroides* to increasing salinity was documented, the survival of both growth forms in the full range of salinity treatments was notable and unexpected. Our results indicate *A. philoxeroides* is a facultative halophyte well adapted to oligohaline–mesohaline salinity levels. Results also revealed the invasive weed's multiple strategies to survive salinity-induced physiological stress, supporting its survival even at elevated polyhaline to euhaline conditions. The macrophyte expressed functional trait responses spanning stress tolerance, avoidance, and escape strategies that may sustain its spread as estuarine salinity intrusion increases with sea level rise.

Key words: Alligator weed, Amaranthaceae, aquatic plants, glycophyte, invasion ecology, invasive plants, plant invasions, tidal wetlands

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Introduction

Accelerated climate change, concomitant sea level rise, and increasing biological invasions concurrently affect coastal species, biological diversity, and wetland ecosystem services (IPCC 2022; Hovick et al. 2023). In this context, stress gradients

in estuaries are rapidly changing due to global warming and sea level rise, resulting in spatial and temporal shifts in salinity (He and Silliman 2019; Zhu et al. 2022). In affected areas, these novel conditions can alter the distribution and spread of estuarine vegetation and other organisms, including non-native species, ultimately yielding unpredictable outcomes for native species (Rubinoff and Grosholz 2022).

The perennial aquatic herb *Alternanthera philoxeroides* Mart. (Griseb.) (alligator weed; Amaranthaceae) is endemic to southern South America (Vogt et al. 1979; Sosa et al. 2004). It is considered one of the eleven most problematic invasive aquatic plant species in freshwater habitats globally (Francis 2012), having naturalized in 32 countries across the Americas, Western Europe, Asia, and Oceania (Tanveer et al. 2018; EPPO 2024). Although generally regarded as a glycophyte (i.e., a plant species limited to freshwater or low-sodium soils; Daubenmire 1947), *A. philoxeroides* has recently been observed in more saline environments (Walden et al. 2019). Glycophytes are thought to have evolved in ecosystems with low soil sodium – yet some can develop salt-tolerant mechanisms through adaptation under natural selection pressures (Horie et al. 2012; Cheeseman 2015). Limited anecdotal reports of *A. philoxeroides* in saline habitats (Gangstad 1978; U.S. Fish and Wildlife Service 2018) suggest that at least some lineages may have developed a degree of salt tolerance.

In 2017, the first patches of *A. philoxeroides* were detected in tidal wetlands of the brackish Suisun Marsh reach of the San Francisco Bay–Delta Estuary, followed by its spread into the inland tidal freshwater Delta (Walden et al. 2019). This unexpected appearance in brackish areas raised questions about its ability to tolerate changing salinity regimes associated with seawater intrusion. Greater salt stress caused by seawater intrusion may hinder expansion or lead to die-back of freshwater plants in tidal wetlands (Craft et al. 2009; Thouvenot and Thiébaut 2018), altering native vegetation patterns and creating opportunities for invasion by alien plant species (Humphreys et al. 2021).

These plant invasions and their ecological effects arise from changing environmental conditions and corresponding shifts in plant functional trait responses (Drenovsky et al. 2012). A large meta-analysis suggests that aquatic alien plants alter their functional traits in response to global change factors more actively than native species, which accelerates their spread into new areas (Sorte et al. 2013; Wu and Ding 2019). Improved understanding of how plant functional traits support the stress response strategies of invasive species is essential for mitigating their impacts.

Plant responses to salt stress can be categorized into four main strategies: tolerance, avoidance, escape, and recovery (Munns 2002; Slama et al. 2015; Shelake et al. 2022). Tolerance may involve a slower growth rate (Voesenek and Bailey-Serres 2015; Zhang et al. 2020), production of compatible solutes to maintain osmotic balance, and generation of antioxidant enzymes (Pungin et al. 2023; Huang et al. 2024). Avoidance mechanisms reduce salt uptake through stomatal closure and leaf wax accumulation, while succulence increases leaf thickness or cell size and promotes water storage to dilute and sequester toxic ion concentrations to sublethal levels (Mann et al. 2023; Pérez-López et al. 2023). Escape mechanisms may include shortened life cycles to enable early reproduction or traits that promote dispersal away from stress (Catford and Jansson 2014), high photosynthetic capacity, remobilization of assimilates to storage organs (Tang et al. 2022), and clonal plasticity (Puijalon et al. 2008). Recovery mechanisms include cellular damage repair, stress memory formation, proline accumulation, and restoration of hydrolytic conditions to enable plants to regain fitness and growth (Gupta and Huang 2014; Shelake et al. 2022).

To date, research into the salinity responses of *A. philoxeroides* has been limited. Most previous work focused on photosynthetic metabolism and growth responses in cell-suspension cultures (Longstreth et al. 1984; Mudalige and Longstreth 2006) or on growth and gas exchange in intact young plants (Javed et al. 2019). A recent experiment evaluating limited whole-plant responses – growth rate and root versus shoot biomass – at salinity levels ranging from 0.2 to 20 parts per thousand (ppt) across 14 aquatic species in Florida found that *A. philoxeroides* survived at 20 ppt (Tootoonchi et al. 2023). Here, using three greenhouse experiments, we assessed how functional traits vary in response to increasing salinity in the invasive plant *A. philoxeroides* and how trait responses differ between its emergent and free-floating growth forms. Our specific objective was to evaluate plant trait responses to four salinity levels (freshwater to seawater) at the biochemical, physiological, and whole-plant levels in two contrasting growth forms – emergent and free-floating – in order to understand how these traits contribute to salt stress strategies ranging from escape to tolerance (sensu Negrão et al. 2017). We hypothesized that increasing salinity would lead to changes in growth, physiological, and biochemical traits that support survival and sustained invasiveness, and that these responses would vary by growth form. We predicted that belowground carbon reserves in the soil-anchored emergent form would support greater survival through tolerance or avoidance strategies, or both. In contrast, we predicted that the free-floating form would lack the capacity to survive or maintain vigor under high salinity, thereby favoring a rapid escape strategy. Our overall aim was to improve understanding of the functional traits of each growth form in relation to the potential for range expansion and persistence of *A. philoxeroides*. This work is particularly relevant in the context of rising estuarine salinity with sea level rise and has important implications for invasive species management strategies.

Materials and methods

Focal species

Alternanthera philoxeroides is a creeping emergent aquatic species (sensu Rejmánková 1992) that sprouts and emerges from belowground bud banks and grows rapidly to form long buoyant shoots that spread on the water surface and within the water column. *A. philoxeroides* typically grows as soil-rooted emergent plants in well-drained, shallow aquatic to terrestrial ecotones (Julien and Bourne 1988) and accumulates carbohydrate storage reserves in woody taproots supporting growth (Julien et al. 1992; Jia et al. 2009, 2010). In these high intertidal zones, the emergent growth form of *A. philoxeroides* grows vertically (> 2 m), often partially supported by other tall emergent macrophytes. High genetic variability supports its success as a weed in both its native and naturalized ranges (Jia et al. 2010). Although its growth rate and spread are greatest in aquatic wetland habitats, the emergent growth form of *A. philoxeroides* has also aggressively invaded adjacent terrestrial areas, including pastures, orchards, and crops (Shen et al. 2005; Xie et al. 2010; Tanveer et al. 2018).

Less common, yet ecologically significant, are free-floating plants that can form large patches of thick interwoven mats (Julien et al. 1992). Free-floating clonal ramets are also hydrochorous dispersal units, representing the primary mode of spread throughout its native and naturalized ranges (Sosa et al. 2004, 2008). The highly branching architecture of *A. philoxeroides*, leading to increased matting on the water surface, can result in more clonal propagules for dispersal, as the hollow stems of *A. philoxeroides*

are prone to breakage and dislodge from established mats in response to disturbance (Harms et al. 2023). Following fragmentation, carbohydrate storage in internodes and leaves can support the survival of free-floating shoots (Dong et al. 2010).

Experimental plant material

Alternanthera philoxeroides plants of a single haplotype and ploidy level (Ap2, hexaploid; Williams et al. 2020) invading northern California and the San Francisco Bay–Delta Estuary (SFE) were used for each experiment. Visibly undamaged apical shoot fragments (50 cm long, n = 150) from emergent floating-leaved shoots growing over the water surface were randomly collected from widely separated points along an invaded reach of the Feather River (39.456505, –121.633851), a freshwater tributary to tidelands of the SFE. Collected shoot fragments were carefully placed in coolers filled with cold river water and transported to the USDA-ARS Aquatic Weed Research Facility at the University of California, Davis. At the laboratory, shoot cuttings were cleaned, rinsed in deionized (DI) water, and floated in shallow 190-liter tanks (132 cm long × 39 cm wide × 30 cm deep; Rubbermaid®, Atlanta, Georgia, USA) filled with DI water plus a nutrient solution additive (10% Hoagland macro/20% micro/iron solution) for a 30-day recovery period prior to experimental setup.

Experimental setups

Two independent experiments (A: emergent; B: free-floating growth forms) were established due to space requirement differences for growing the contrasting life forms. Methods standardized between the experiments follow. To evaluate *A. philoxeroides* responses to salinity, mesocosms were randomly assigned to one of four aqueous salinity treatments (0.5 ppt [freshwater/low oligohaline], 12 ppt [mesohaline], 24 ppt [polyhaline], and 36 ppt [euhaline, "marine"]) using sea salts (Instant Ocean®, Aquarium Systems Inc., Mentor, Ohio, USA). Each treatment was established in a 75.7-liter plastic mesocosm (Utilitub Model 14, E. L. Mustee & Sons, Brook Park, Ohio, USA) filled with 45 liters of DI water and sea salts to achieve target salinities. Mesocosm positions were randomly assigned on greenhouse benches. Freshwater treatments were maintained at 0.5 ppt salinity. Treatments > 0.5 ppt were ramped up gradually over conditioning intervals to avoid osmotic shock, with salinity adjusted as needed. Sea salts were stirred directly into each tub, with incremental weekly increases until the target mesohaline, polyhaline, and euhaline salinities were achieved (three to four weeks for euhaline). Salinity of each treatment was monitored using a YSI Pro30 Conductivity/Salinity Meter (Yellow Springs Instruments, Inc., Yellow Springs, Ohio, USA). Specific conductance readings were taken weekly, and ocean salts were added as needed to maintain levels. Air bubblers with 5/32″ tubing (Model 9720, Pentair Aquatic Eco-systems, Cary, North Carolina, USA) were installed to circulate water, suppress algal growth, and homogenize gas concentrations across salinity treatments, ensuring valid comparisons. Controlled glasshouse conditions included 21–27 °C air temperature and approximately 1300 μmol m^{-2} s^{-1} maximum photon flux density at canopy level (LI-COR LI-250A light meter, LI-COR Inc., Lincoln, Nebraska, USA). After full treatment establishment, survivorship of plants was recorded weekly until initiation of final pre- and post-harvest measurements of plant functional traits.

Experiment A: emergent growth form

For the emergent, soil-anchored growth form treatment, single fragments of equivalent mass range, length, and number of stem nodes were selected from culture stock. Shoot fragments were trimmed to 40 cm in length. The fresh weight of the initial 20 shoot fragments was 10.5 ± 0.2 g (mean ± SE), with 5–7 stem nodes per plant. Each single fragment was transplanted into a bulb pan (12.5 cm height × 25 cm diameter) filled with a custom soil mix of moderate nutrient availability, reflecting soil bulk density and nutrient concentrations measured in 45 samples from 24 *A. philoxeroides* invasion study sites in Suisun Marsh, SFE. The soil mix was composed of 40% topsoil, 40% humus, and 20% sand, with 30 ppt C, 1.9 ppt N, and 305 ppm P. In each experimental pot, a single shoot fragment was planted with the lower 10 cm – bearing two stem nodes – buried beneath the soil surface to facilitate soil root development, and 30 cm of each fragment, with approximately four stem nodes, was left emergent above the soil. The pots were elevated on small bricks (6 cm tall) within each mesocosm (n = 5 replicates per salinity treatment), so that the soil surface remained submerged 4 cm below the water level. During the 5-day transplant adjustment period, plants were fertilized with 10% Hoagland macro/20% micro/iron nutrient solution. Emergent plants were exposed to the final salinity treatments for 42 days, and the entire experiment lasted 62 days before harvest.

Experiment B: free-floating life form

Shoot cuttings of equivalent mass range, length, and number of stem nodes were selected from culture stock, then trimmed to 45 cm, weighed, and measured. The fresh weight of the initial 72 cuttings was 11.6 ± 0.2 g (mean ± SE), with 5–9 stem nodes and 0–3 small branches per shoot. Because floating mats of alligator weed in nature typically include more than a single fragment, establishment was standardized with three floating shoot fragments in each mesocosm. Mesocosms (n = 24) were randomly assigned to one of four salinity treatments (n = 6 replicates per treatment), and cuttings were floated on the water surface in 45 L of solution per mesocosm. During the 5-day conditioning period before salinity ramp-up, plants were fertilized with 25% Hoagland macro/50% micro/iron nutrient solution. Experimental plants were then exposed to one of four aqueous salinity treatments (0.5, 12, 24, or 36 ppt) and fertilized with 50% Hoagland macro/100% micro/iron nutrient solution added to the water. Plants were exposed to the final salinity treatments for 25 days, and the entire experiment lasted 51 days before harvest.

Experiment C: free-floating salinity with freshwater recovery phase

Sixty shoot cuttings were collected two weeks following the harvest of Experiment B, from the same source population described for the initial two experiments. Shoot cuttings were trimmed to 45 cm in length, weighed, and measured. The fresh weight of the initial 36 cuttings was 18.5 ± 0.2 g (mean ± SE), with 6–7 stem nodes and 0–3 small branches per plant. Three cuttings were randomly assigned to each experimental unit (mesocosm), using the previously described salinity ramp-up methods. Given changes in day length, solar light was supplemented in the morning (6 a.m.–10 a.m.) using high-intensity discharge lights (GE Lucalox LU1000/ECO HPS 1000 W, PARsource, Petaluma, California, USA). Experimental replicates

were randomly assigned to one of three aqueous salinity treatments (12, 24, or 36 ppt; n = 4 replicates per treatment) and fertilized with 50% Hoagland macro/100% micro/iron nutrient solution. Following a four-week ramp-up period, salinity treatments were maintained for 21 days. Freshwater recovery was initiated by gradually ramping down salinity to final values of 0.5, 3, and 4.5 ppt (over a three-week ramp-down period). Plants were allowed to recover under freshwater conditions for 32 days, and the entire experiment lasted 80 days before harvest.

Pre-harvest measurements: experiments A and B

Three days before harvest, photosynthetic rate (A) and stomatal conductance (gs) were measured using an LI-COR 6400 XT portable infrared CO_2 gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) in differential mode and open circuit. Three subsamples were recorded at 10-second intervals for each plant and averaged before analysis. The LI-COR 6400 XT used a red–blue LED light source generating actinic light, and chamber conditions were set at 1000 μmol m^{-2} s^{-1} photon flux density, 400 μmol s^{-1} flow rate, and 400 μmol mol^{-1} CO_2. Intrinsic water use efficiency (iWUE) was calculated from simultaneous measurements of A and g_s. All measures were conducted on a sunny day within 2 h of solar noon. Gas exchange was measured on all plants in the emergent experiment but only on the 0.5 ppt salinity treatment in the free-floating experiment. Measurements in the 12, 24, and 36 ppt treatments of the free-floating experiment could not be conducted due to the absence of suitable live leaves above the water surface.

Post-harvest measurements: experiments A and B

Biomass production and allocation

All flowers were removed from the plants and collected for dry weight (DW) measurement. Live leaves were collected and quantified for total leaf area (m^2) using image analysis software (WinFOLIA 2009). A subset of fully expanded leaves was evaluated with WinFOLIA and used to calculate specific leaf area (SLA; m^2 kg^{-1}). Each experimental plant was harvested, separating the biomass into fractions: reproductive parts, leaves, primary stems, branch stems, soil roots, and adventitious roots. For the emergent experiment, the belowground biomass – including soil roots and the buried root crown from which the stem arises – was sieved from the soil mix, collected, and cleaned. Each biomass fraction was dried at 70 °C for 48 h, and biomass allocation and total biomass were calculated. Mass data were used to calculate relative growth rate (RGR; g day^{-1}), leaf mass ratio (LMR), shoot mass ratio (SMR), and adventitious root mass ratio (ARMR). Belowground allocation (%) was also calculated for the emergent growth form.

Stem architecture and morphology traits

Stem hollow pith cavity (SPC) diameter was measured as stem wall and pith (spongy aerenchyma cells) thickness relative to the width of the stem's hollow center. Pith cavity size is ecologically relevant as habitat and for pupal-stage survival of potential biological control insects (Li and Ye 2006; Geng et al. 2007). SPC diameters were measured on three stem sections from each plant and averaged before analysis. Each

section was taken from a different shoot and cut at the internode below the sixth node from the apical tip. For the emergent growth form, the primary stem length was measured from the soil surface. Branches were separated from the primary stem, dried separately, and used to calculate the branch intensity index (BI; ratio of branch dry weight to stem dry weight; Liu et al. 2018). Primary stem length was also used to calculate the shoot elongation rate (SER; cm day^{-1}). Primary stem length and BI could not be measured in the free-floating growth form, as the three initial shoots in each experimental unit could no longer be isolated from one another.

Plant chemistry traits

A subsample of 2–6 fresh leaves was collected and frozen at harvest. Proline concentration was determined following Bates et al. (1973). Leaf samples were extracted in 80% aqueous acetone, and supernatants from centrifuged extracts were used to determine chlorophyll (Chl) *a*, Chl *b*, and carotenoid (Car) concentrations using a spectrophotometer (Beckman DU-730, Beckman Coulter, Brea, California, USA), following Lichtenthaler (1987). Dry leaf tissue was ground to pass through a No. 40 mesh sieve prior to measurement of total nitrogen (N), sodium (Na), glycinebetaine, total phenolics, and lignin concentrations. Dry, ground stem node tissue from both growth forms and belowground roots extracted from the soil in the emergent experiment were analyzed for total N, lignin, and total non-structural carbohydrate (TNC; e.g., soluble sugars and starch) concentrations. Total N concentration was measured using a Perkin Elmer 2400 CHNS/O analyzer (Perkin Elmer, Waltham, Massachusetts, USA). Sodium concentration was determined using an Orion ROSS™ sodium ISE electrode (Thermo Scientific, Beverly, Massachusetts, USA) on dry-ashed samples dissolved in 1 M hydrochloric acid. Glycinebetaine was estimated as quaternary ammonium compounds following Grieve and Grattan (1983). Total phenolics were measured using spectrophotometric detection following reaction with Folin–Ciocalteu reagent and sodium carbonate solution (Singleton et al. 1999). Leaf and stem lignin were measured only for the emergent growth form using the acetyl bromide assay (Hatfield et al. 1999). TNC concentration was determined using a phenol–sulfuric acid assay of reducing sugars following ethanol extraction, with colorimetric determination of starch residue after enzymatic digestion (Chow and Landhäusser 2004; Quentin et al. 2015).

Post-harvest measurements: experiment C

Biomass production and allocation

Each experimental plant was harvested, separating the biomass into fractions: reproductive parts, leaves, stems, and adventitious roots. Each biomass fraction was dried at 70 °C for 48 h, and biomass allocation and total biomass were calculated. Mass data were used to calculate relative growth rate (RGR; g day^{-1}), leaf mass ratio (LMR), shoot mass ratio (SMR), and adventitious root mass ratio (ARMR).

Stem architecture and morphology traits

Total stem diameter and SPC diameter were measured on three stem sections from each plant and averaged before analysis. Stem sections were selected using the same protocol previously described.

Plant chemistry traits

Stem node tissue was ground to pass through a No. 40 mesh sieve prior to measurement of N and TNC concentrations using previously described protocols.

Statistical analysis

All analyses were conducted in R software (R-Core Team 2021). Correlation analysis of multiple independent response variables was performed to identify and eliminate highly correlated variables that could reduce the statistical power of subsequent analyses. Thirteen highly correlated variables ($r > 0.90$) – including relative growth rate, shoot elongation rate, AGB, BGB, leaf, stem, reproductive and adventitious root biomasses, leaf area, shoot mass ratio, stem and SPC diameter, and stomatal conductance – were considered redundant and removed from the statistical models for emergent plants. Plant trait responses to salinity treatments in emergent plants were categorized into three functional groups for subsequent statistical analysis: (1) biomass allocation and morphology (total plant biomass, SLA, LMR, adventitious root mass ratio, belowground mass ratio, and BI); (2) leaf gas exchange and photosynthetic pigments (*A*, iWUE, Chl *a*, Chl *b*, and Car concentrations); and (3) plant tissue chemistry (foliar N, Na, proline, glycinebetaine, total phenolic compounds, and lignin concentrations; stem lignin concentration; and stem node and soil root N and TNC concentrations). For free-floating plants, ten highly correlated traits – relative growth rate, leaf, stem, reproductive and adventitious root biomasses, leaf area, shoot mass ratio, stem diameter, Car concentration, and leaf Na concentration – were excluded. The 13 selected traits for free-floating plants were grouped as follows: (1) biomass allocation and morphology (total plant biomass, SLA, LMR, adventitious root mass ratio, and SPC diameter); and (2) plant tissue chemistry (foliar Chl *a*, Chl *b*, N, proline, glycinebetaine, and total phenolic compound concentrations; and stem node N and TNC concentrations).

For each experiment, multivariate analysis of variance (MANOVA) and Hotelling–Lawley's trace were used to compare mean values of each group of plant traits in response to salinity treatments. Multivariate analysis of variance protects against type I *error* (Scheiner 2001). Once multivariate significance was confirmed via MANOVA, main univariate differences for each plant trait were assessed using General Linear Models (GLMs), with Tukey's test used as a *post hoc* analysis. The assumptions required for parametric tests were analyzed by plotting standardized residuals against fitted values and predictor variables using the DHaRMa package (Hartig 2022). To meet the assumptions, the variable *biomass* for the free-floating plants (Experiment B) was square-root transformed.

For the experimental results of the emergent and the free-floating plants, we fitted piecewise structural equation models (SEM) in R using the *piecewise-SEM* package (Lefcheck 2016) to test for direct and indirect effects of the most important variables, using *salinity* as the only exogenous variable. Structural equation models are known to be data demanding and typically require large sample sizes for stable and generalizable estimates (Grace et al. 2010). We selected piecewise SEM over traditional SEM because it allows for the use of models with non-normal error structures – which are common in ecological data – and offers greater flexibility in model specification. Moreover, piecewise SEM is generally more tolerant of moderate sample sizes, which applies to our design ($n = 20$; five replicates across four

salinity levels) (Lefcheck 2016). This model can provide insights into the causal relationships among functional traits under salinity stress, though the moderate sample size should be considered when interpreting the strength and generality of some pathway estimates. Nevertheless, the application of piecewise SEM enables exploration of these relationships within the constraints of our experimental design (Lefcheck 2016). To construct the model, we combined 11 generalized linear models for experiment A and 18 models for experiment B, with the validity of assumptions evaluated using the *DHARMa* package (see above), and applied necessary transformations. The models, conceptually based on multi-equational analyses of dependency or correlation between variables (Grace et al. 2010), were used to explore how different functional plant traits interrelate, support plant responses to salinity stress, reveal cascading effects of increasing environmental stress on plants of the different growth forms, and identify the relative importance of causal relationships among variables. We developed our causal models *a priori* based on theoretical insights and predicted relationships (Suppl. material 1: fig. S1). For each experiment, the initial SEM was evaluated using a D-separation test to identify missing connections, and the overall model was then tested with Fisher's C statistic, where a non-significant p-value implies a well-fitting model. Direct, indirect, and overall associations were assessed as standardized partial regression coefficients with the *semEff* package (Murphy 2022). To characterize the strategies used to deal with salt stress by both *A. philoxeroides* growth forms, functional trait responses included in piecewise SEM models as direct and indirect effects related to salinity were classified into each of the main strategies: tolerance, avoidance, and escape.

Results

All plant functional trait groups revealed significant responses to salinity for both emergent and free-floating *A. philoxeroides* plants (MANOVA, Table 1; Suppl. material 1: table S1).

Table 1. Hotelling–Lawley's trace, F-statistic, degrees of freedom, and *p*-values from MANOVAs for trait response groups from experiment A: emergent plants and experiment B: free-floating plants of *Alternanthera philoxeroides* exposed to four aqueous salinity concentrations (0.5, 12, 24, 36 ppt). Significant values ($p < 0.05$) are marked in bold.

Factors	Hotelling-Lawley's Trace	F	DF	p
Experiment A: emergent plants				
Biomass and morphology	149.53	80.305	18	**<0.0001**
Gas exchange and pigments	25.556	18.173	15	**<0.0001**
Plant tissue biochemistry	136.92	19.363	33	**<0.0001**
Experiment B: free-floating plants				
Biomass and morphology	194.81	190.48	15	**<0.0001**
Plant tissue biochemistry	145.1	70.536	24	**<0.0001**

Stress tolerance trait responses to salinity

In response to salinity stress, a tolerance strategy was reflected in 12 trait responses of emergent *A. philoxeroides* plants. Total biomass of emergent plants in mesohaline salinity decreased by 78% compared to the freshwater control, whereas plants in poly-

haline and euhaline treatments experienced a severe 97.5% reduction in biomass (Fig. 1A1). The tolerance strategy to salt stress was reflected in ten trait responses by free-floating plants. Total biomass decreased exponentially by ~97% in all treatments compared to free-floating plants in freshwater (Fig. 1A2). Results of photosynthetic pigment concentrations in emergent plants indicate that Chl a concentration was approximately twice as high at 0.5–12 ppt compared to 24–36 ppt salinity (Fig. 1B1). In contrast, Chl *b* concentration was highest at 12 ppt (mesohaline level) (Fig. 1C1), whereas carotenoid concentrations decreased gradually with increasing salinity (Suppl. material 1: fig. S2A). Photosynthetic pigments Chl *a* and Chl *b* in free-floating plant ramets (Fig. 1B2, C2) both showed a gradual linear decrease with increasing salinity, showing the lowest values at 24–36 ppt. Net photosynthetic rate (*A*) of emergent plants showed a threefold decrease at elevated polyhaline and euhaline levels compared to *A* of plants in fresh and mesohaline concentrations (Fig. 1D). Foliar concentrations of proline (Fig. 1E1) and glycinebetaine in the emergent growth form (Fig. 1F1) increased linearly with salinity, while in free-floating plants these osmotic compounds increased markedly at higher salinities (Fig. 1E2, F2). Leaf N in emergent plants was elevated by ~25% in all salinity treatments compared to freshwater (Fig. 1G1), while N concentration in leaves of free-floating shoots was similar at fresh to mesohaline salinity and decreased by approximately 25% in the higher polyhaline and euhaline treatments (Fig. 1G2). Leaf Na in emergents increased by 52% in 12 ppt salinity with a three-fold increase at both higher polyhaline and euhaline salinity treatments (Suppl. material 1: fig. S2B). Responses of total phenolic concentrations with increasing salinity showed quite a different response from the emergent growth form, with a linear decline from freshwater to the maximum euhaline salinity (Fig. 1H2). This trait response mirrored the linear decline in photosynthetic pigments in free-floating plants (Fig. 1B2, C2). Emergent plants in mesohaline salinity accumulated fewer total phenolic compounds in their leaves than at either freshwater or at poly- or euhaline levels (Fig. 1H1). Total phenolic concentrations in free-floating plants showed quite a different response from the emergent growth form, with a linear decline from freshwater to the maximum euhaline salinity (Fig. 1H2). This trait response mirrored the linear decline in photosynthetic pigments in free-floating plants (Fig. 1B2, C2). All recorded plant traits of emergent *A. philoxeroides* changed significantly with increasing salinity, except for LMR, stem node TNC, and soil root TNC concentration (Suppl. material 1: table S1). Emergent plants reached the reproductive stage, producing flowers in fresh to mesohaline salinity treatments, and did not flower under polyhaline or euhaline salinity (Suppl. material 1: fig. S2D). In contrast, free-floating plants only flowered in freshwater (Suppl. material 1: fig. S3A). Stem node N concentration in free-floating plants was ca. 42% lower in freshwater than at all higher salinity levels (Suppl. material 1: fig. S3B), whereas stem node TNC concentration was generally low with a ~75% decrease with increasing salinity (Suppl. material 1: fig. S3C).

Stress avoidance trait responses to salinity

An avoidance strategy in response to salt stress was reflected in four trait responses of emergent plants (Fig. 2A–D). Leaves of emergent *A. philoxeroides* increased in thickness, as indicated by a gradual decrease in SLA with increasing salinity, reaching less than half the value at 36 ppt compared to freshwater (Fig. 2A1). The adventitious root mass ratio was ca. 80% higher at 0.5–12 ppt than at 24–36 ppt

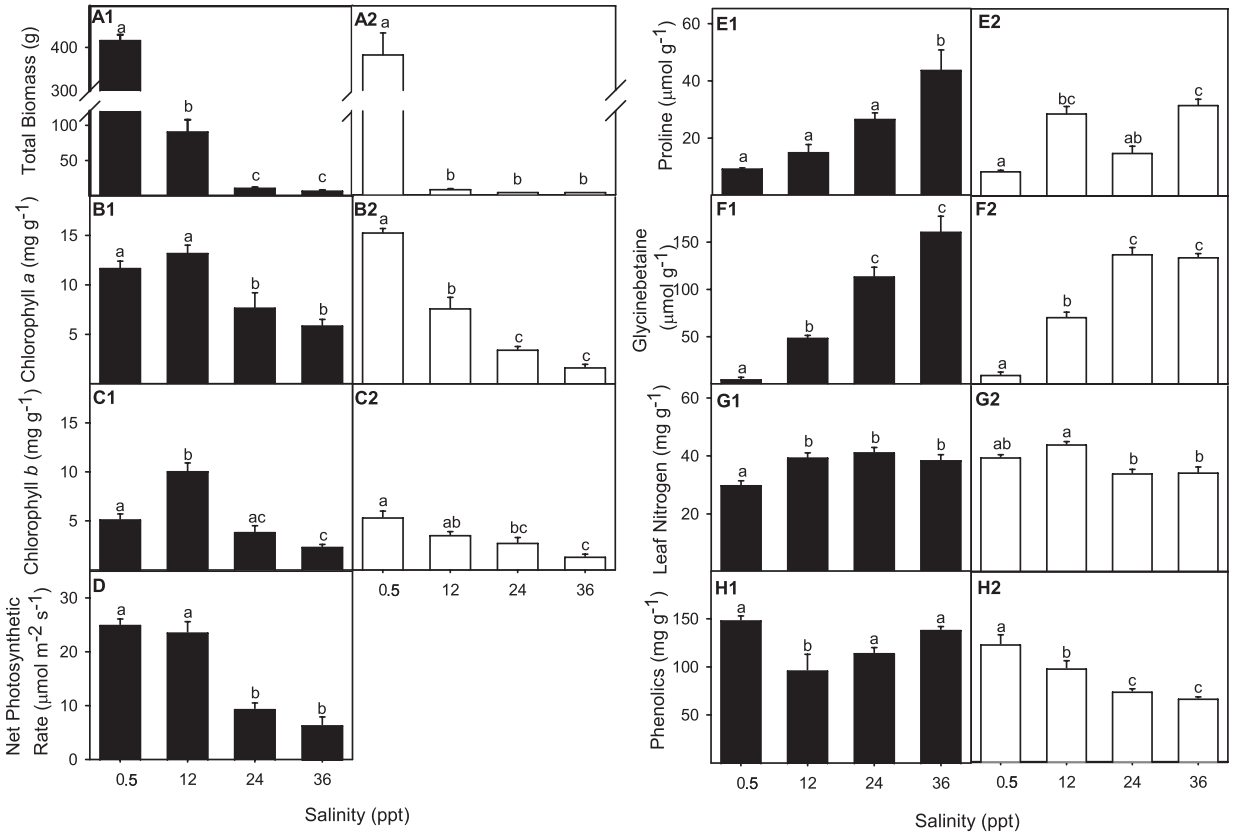


Figure 1. Tolerance responses to salinity for emergent (Experiment A; black columns) and free-floating (Experiment B; white columns) plants of *Alternanthera philoxeroides*. Means (+ SE; Exp. A, n = 5, Exp. B, n = 6) for total biomass (**A**), chlorophyll *a* (**B**), chlorophyll *b* (**C**), net photosynthetic rate (**D**), proline (**E**), glycinebetaine (**F**), leaf nitrogen (**G**), and phenolics (**H**). Letters above columns indicate significant differences between salinity treatments for the same growth form, based on post-hoc Tukey's tests. Net photosynthetic rate could not be measured for free-floating plants due to insufficient leaf mass above the water surface.

salinity (Fig. 2B1). iWUE increased at higher salinities, since the decrease in *A* was lower than that in *gs* with increasing salinity (Fig. 1C, Suppl. material 1: fig. S1E). iWUE was 73% higher at 24–36 ppt than in freshwater, with intermediate values at mesohaline salinity (Fig. 2C). Stem lignin concentration more than doubled in salinity treatments compared to that in freshwater (Fig. 2D); leaf lignin also increased with salinity exposure (Suppl. material 1: fig. S2F). An avoidance strategy of free-floating plants in response to salt stress was reflected in three trait responses: SLA, adventitious root mass ratio (Fig. 2A2, B2), and LMR (Suppl. material 1: fig. S3D), all of which decreased gradually with increasing salinity. Additionally, SPC diameter decreased gradually with increasing salinity (Suppl. material 1: fig. S3E).

Stress escape trait responses to salinity

Emergent *A. philoxeroides* expressed an escape strategy to salt stress, which was reflected in four trait responses (Fig. 3). Stem node N concentration increased linearly by 83% from freshwater to polyhaline salinity, maintaining the high N concentration at euhaline salinity (Fig. 3A). Emergent plants in the highest euhaline salinity treatment presented a 64% higher branching intensity index than plants in freshwater (Fig. 3B). The below-ground root mass ratio more than tripled at polyhaline and euhaline salinity compared to below-ground allocation in

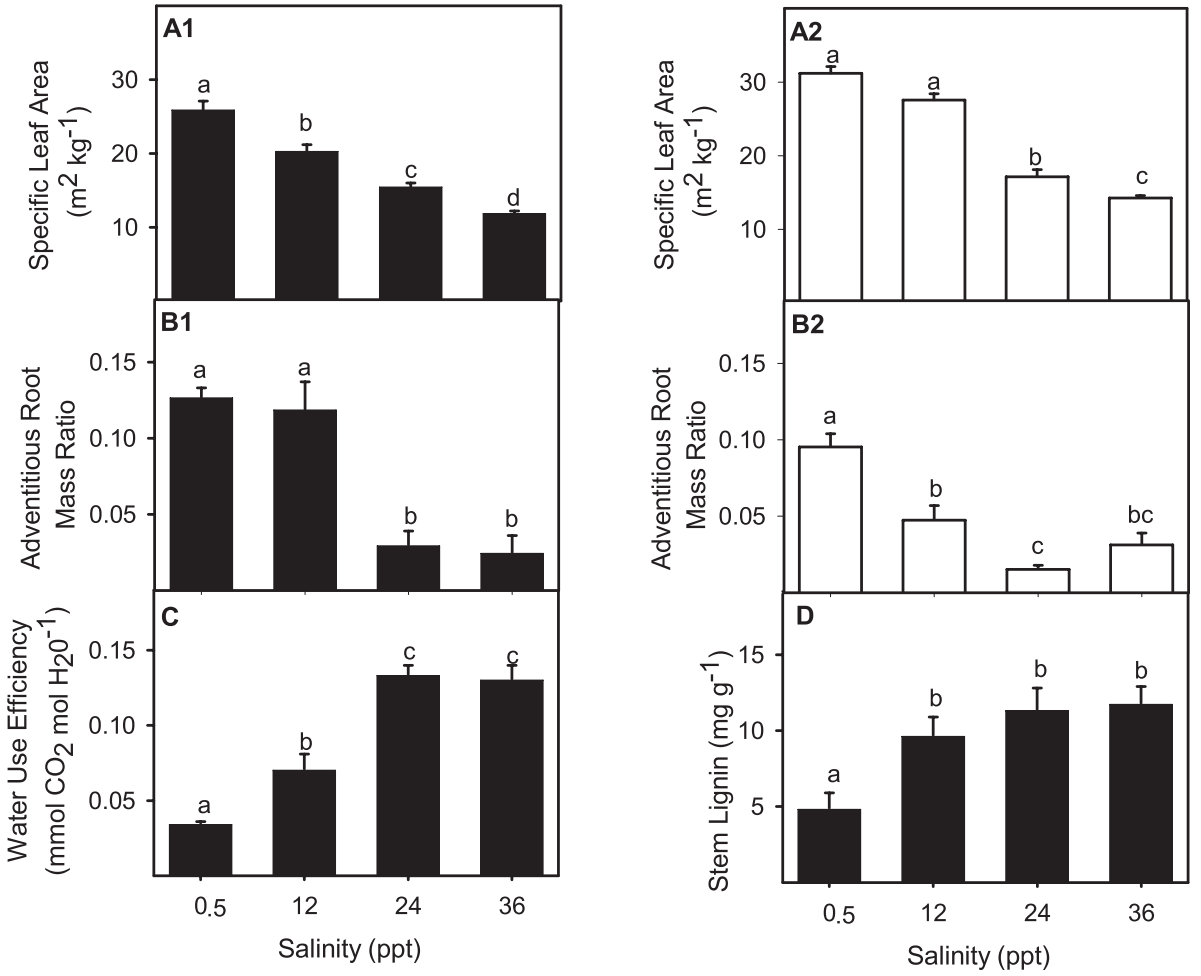


Figure 2. Avoidance responses to salinity for emergent (Experiment A; black columns) and free-floating (Experiment B; white columns) plants of *Alternanthera philoxeroides*. Means (+ SE; Exp. A, n = 5, Exp. B, n = 6) for specific leaf area (**A**), adventitious root mass ratio (**B**), water use efficiency (**C**), and stem lignin (**D**). Letters above columns indicate significant differences between salinity treatments for the same growth form, based on Tukey's post-hoc tests. Water use efficiency and stem lignin concentration were not determined for free-floating plants due to insufficient mass of plants exposed to salinity treatments.

freshwater (Fig. 3C). Stem node and soil root TNC concentrations were low (c. 1–2 mg g^{-1}) and did not change significantly between salinity treatments (Fig. 3D, Suppl. material 1: fig. S2G). The stem pith cavity was 82% greater in diameter in freshwater than at the highest two salinity concentrations (Suppl. material 1: fig. S2H). Reflecting an escape strategy, the free-floating growth form also allocated nearly 50% more of its severely reduced biomass (Fig. 1A2) to adventitious root production in all salinity treatments compared to freshwater (Fig. 2B2).

Experiment C: recovery potential of free-floating plants

At the end of the freshwater recovery phase, relative growth rate had precipitously declined following salinity exposure, and mortality of one to two shoots in each 3-shoot treatment occurred in the highest brackish and marine salinity treatments under which surviving biomass was quite low (Suppl. material 1: fig. S4A, B). Following potential recovery in freshwater, total biomass of free-floating *A. philoxeroides* was less than shoot biomass prior to initiation of salinity treatments, and the magnitude

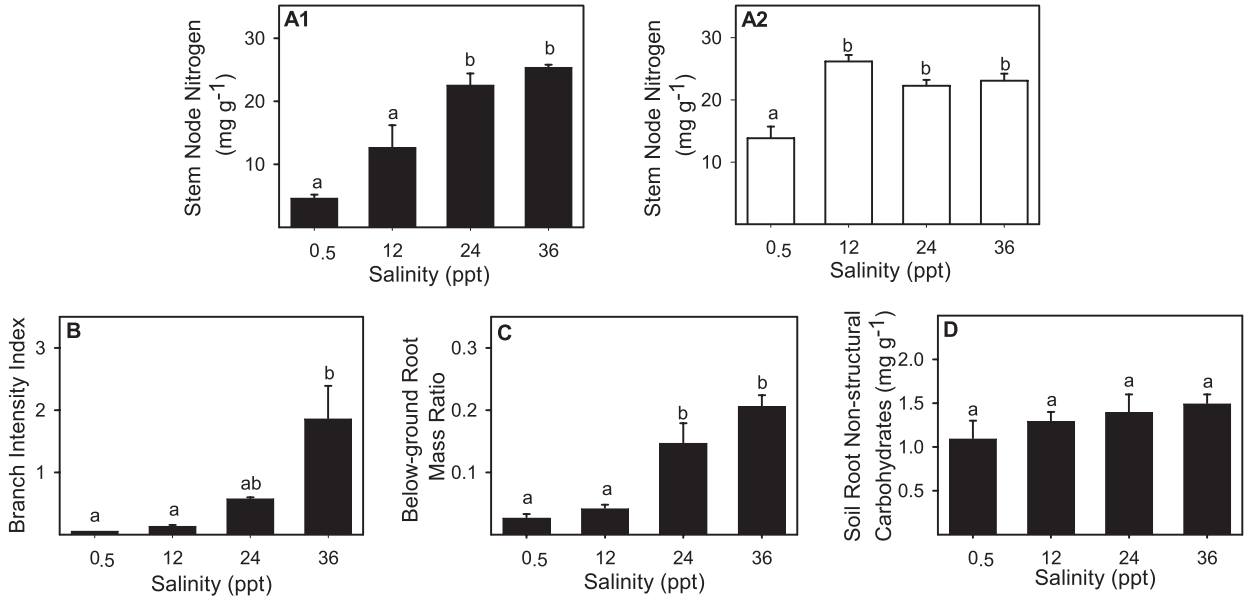


Figure 3. Escape responses to salinity for emergent (Experiment A; black columns) and free-floating (Experiment B; white columns) plants of *Alternanthera philoxeroides*. Means (+ SE; Exp. A, n = 5, Exp. B, n = 6) for stem node nitrogen (**A**), branch intensity index (**B**), below-ground root mass ratio (**C**), and soil root non-structural carbohydrates (**D**). Letters above columns indicate significant differences between salinity treatments for the same growth form, based on Tukey's post-hoc tests. It was not possible to evaluate the branch intensity index for the free-floating plants, as the initial three-shoot treatments were intertwined at harvest and could not be separated.

of biomass reduction corresponded to that of increased salinity (Suppl. material 1: fig. S4B). One of the shoots in euhaline salinity did not survive the salinity exposure period (8% treatment mortality), and during the freshwater recovery phase, an additional six shoots, three shoots from both the polyhaline and euhaline treatments, did not survive (25% mortality from each treatment). Plants exposed to freshwater after free-floating in mesohaline salinity on average produced 20% less total dry biomass at harvest than the initial dry mass of subsamples evaluated for initial conditions, yet there was high variance in the response at this brackish level, with significant recovery noted in some plants (Suppl. material 1: fig. S4B). Plants potentially recovering from 21 days in polyhaline and euhaline aqueous salinity had biomass reductions of 83% and 93%, with final biomass < 1 g. The only plants that flowered while in potential freshwater recovery were from the mesohaline salinity treatment. Pre-exposure to higher salinity was reflected in lower adventitious root biomass, stem diameters, SPC diameters, and LMR after the potential recovery period. However, plants recovering from mesohaline salinity had 44–50% larger stem diameters and 177–213% larger stem pith cavity diameters than plants recovering from polyhaline and euhaline salinity, respectively (Suppl. material 1: fig. S4C–D). Adventitious root biomass declined steeply with increasing salinity; some plants in the highest two salinity treatment levels did not regrow adventitious roots during recovery, and the mass of roots in these treatments was measurable but quite low (Suppl. material 1: fig. S4E). TNC concentration in stem nodes was generally low in all treatments following the freshwater recovery phase, but plants subjected to euhaline salinity slightly exceeded allocation of resources to carbohydrate storage compared to those exposed to lower salinity (Suppl. material 1: fig. S4F), whereas total N concentration in stem nodes was approximately doubled in the higher two salinity treatments compared to N in nodes following mesohaline exposure (Suppl. material 1: fig. S4).

Response strategies to salt stress: structural equation model results

The piecewise SEM on the drivers of salinity responses of the emergent growth form of *A. philoxeroides* showed that the most negative direct effects of salinity were on biomass, SLA, photosynthesis rate, carotenoids, and Chl *a* (Fig. 4). In contrast, salinity stimulated the amount of glycinebetaine, BI, BGMR, root N, stem lignin, proline, leaf N, and root TNC (Fig. 4).

However, the primary indirect effects of salinity involved an increase in proline and a reduction in phenolics, Car, and Chl *a*, mediated by changes in leaf N. Salinity also indirectly reduced *A* via Chl *a*, while improving iWUE through its influence on Chl *a* and *A*. The main endogenous relationships included an increase in Chl *a* driven by LMR and Car concentration. At the same time, Chl *a* concentration enhanced *A*, which in turn lowered iWUE that was increased by stem lignin. Leaf N concentration directly increased resource allocation to proline production, while it reduced phenolic and Car concentrations. Sixty percent of the trait responses to salinity, accounting for 55% of the direct and indirect effects, were related to a tolerance strategy. Only 20% of emergent plant trait responses to salinity were related to a stress avoidance strategy, and 20% of traits support an escape strategy, accounting for 19 and 25 of the effects, respectively.

The piecewise SEM indicated that the most negative effects of salinity on the free-floating growth form were on phenolics, ARMR, leaf N, Car, Chl *b*, and SLA, whereas salinity induced higher glycinebetaine and proline concentrations in leaf tissue (Fig. 5). Additionally, salinity indirectly and negatively affected biomass through allocation to glycinebetaine and ARMR, affected Chl *a* through proline and ARMR, and affected stem node TNC through ARMR. Leaf N also directly increased SLA, while Chl *a* increased with Car and Chl *b* concentrations. The majority (73%) of the trait responses to salinity were related to a tolerance strategy, accounting for 70% of direct and indirect effects. Only 27% of the trait responses to salinity, accounting for 30% of the effects, corresponded to a stress avoidance strategy. The model results indicate none of the trait responses of free-floating *A. philoxeroides* were related to an escape strategy. While our experimental findings provide insights into causal relationships among the functional trait responses, the moderate sample sizes used (n = 20) should be considered when interpreting the strength and generality of some pathway estimates.

Discussion

Our study suggests invasive *A. philoxeroides*, a widely presumed glycophyte, is a facultative halophyte (*sensu* Flowers 1972). Although optimal growth was in freshwater, all experimental emergent plants survived in fresh-oligohaline water to euhaline salinity conditions for more than 30 days. The emergent and the floating growth forms responded to salinity mainly using a stress tolerance strategy mediated by a complex array of functional trait responses. However, avoidance and escape strategies also played key roles to counter salt stress in emergent plants.

Stress tolerance traits

The high sensitivity of *A. philoxeroides* to salinity was reflected in changes to several integrated plant functional traits providing stress tolerance. The dramatic decreases in net photosynthesis rate and biomass production with increasing salinity by

Indirect effects

Response	*Predictor*	*Standarized estimate*	*Mediator*	*Standarized estimate*
Proline	Salinity	0.11	Leaf N	0.11
Phenolics	Salinity	-0.36	Leaf N	-0.36
Carotenoids	Salinity	-0.17	Leaf N	-0.17
Chl *a*	Salinity	-0.06	Leaf N	-0.06
A	Salinity	-0.07	Chl *a*	0.10
	Leaf N	-0.02		
WUE	Salinity	0.15	Chl *a*	-0.03
	Chl *a*	-0.05	A	0.21

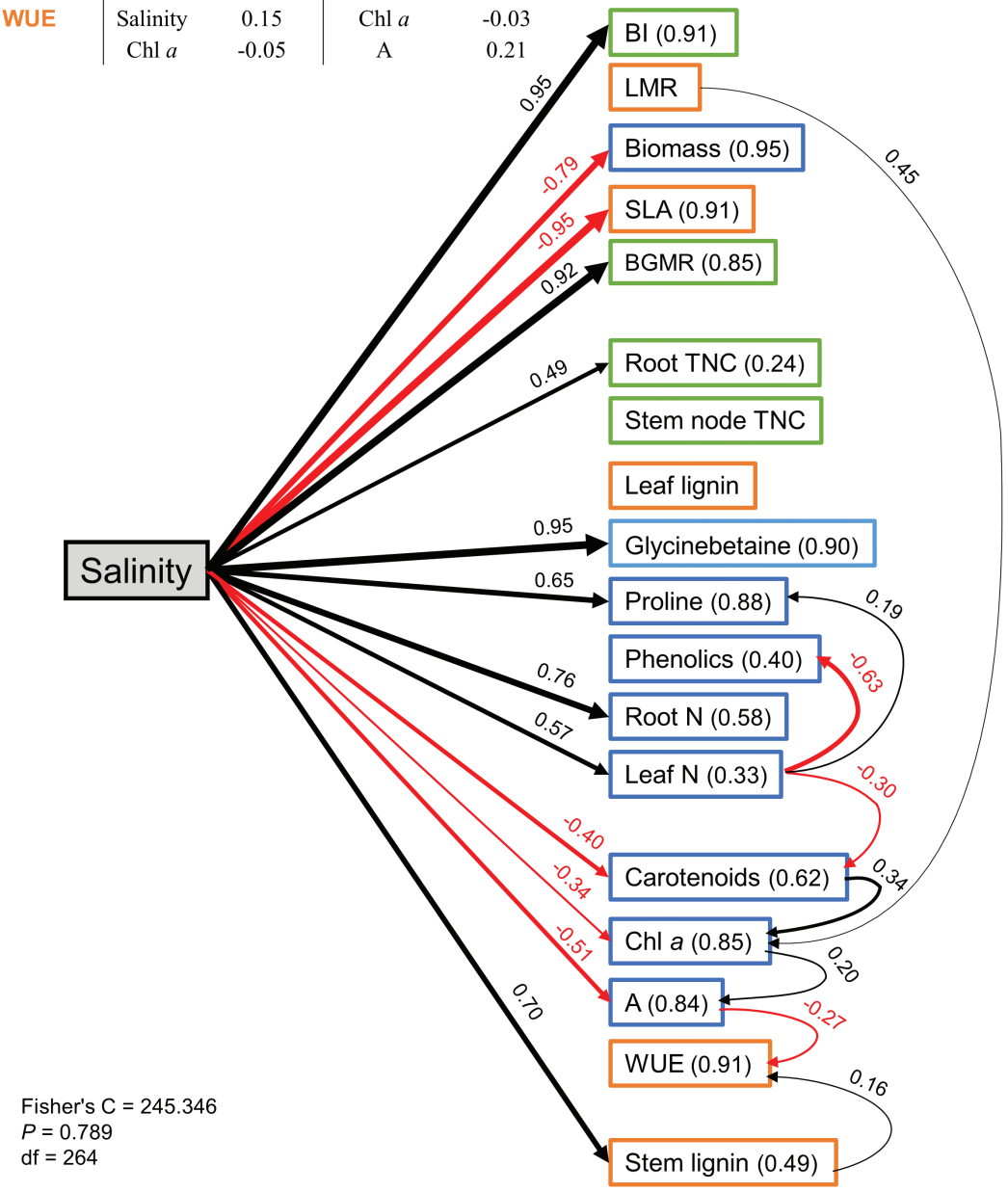


Figure 4. Results from the structural equation model (SEM) showing the main relationships between metrics of functional trait responses and salinity for the emergent growth form of *Alternanthera philoxeroides* (Experiment A; n = 20, five replicates across four salinity levels). The variable in grey represents the main explanatory variable (salinity), and the response variables are enclosed in hollow boxes. Black arrows indicate positive causal links, and red arrows indicate negative causal links. Double-headed arrows represent correlations. Standardized parameter estimates (β) are shown next to each arrow and are proportional to the thickness of the lines. The coefficient of determination (R^2) is shown in parentheses for each response variable. Statistical power is indicated by the Fisher's C statistic, p-value (*P*), and degrees of freedom (df). The table shows the indirect effects of predictors on response variables through mediators, along with their corresponding standardized estimates. Salt stress response strategies included: tolerance (blue), avoidance (orange), and escape (green).

Indirect effects

Response	*Predictor*	*Standarized estimate*	*Mediator*	*Standarized estimate*
Biomass	Salinity	-0.15	Glycinebetaine	-0.12
			ARMR	-0.02
SLA	Salinity	-0.1	Leaf N	-0.09
Chl *a*	Salinity	-0.05	Proline	0.05
			ARMR	-0.04
Stem Node TNC	Salinity	-0.16	ARMR	-0.15

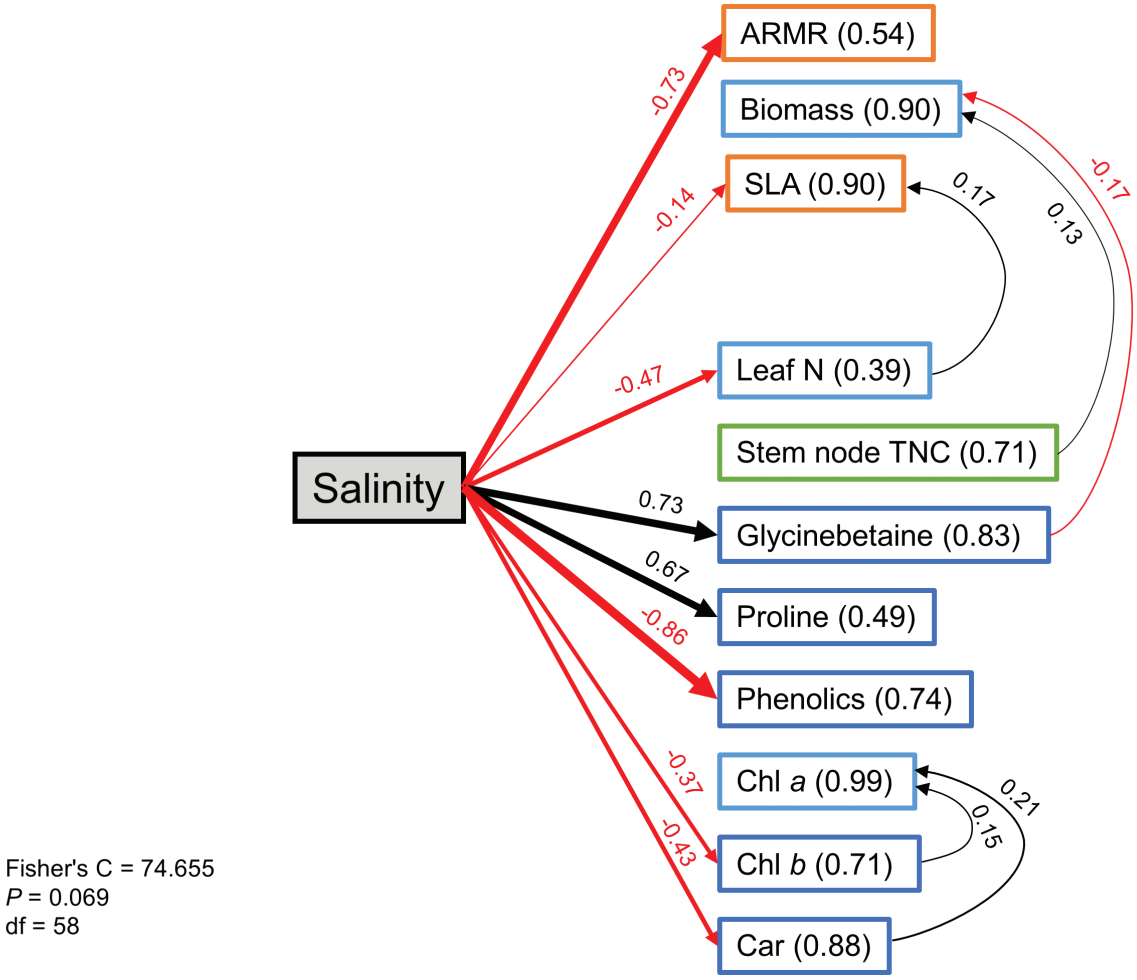


Figure 5. Results from the structural equation model (SEM) showing the main relationships between metrics of functional trait responses and salinity for the free-floating growth form of *Alternanthera philoxeroides* (Experiment B; n = 20, five replicates across four salinity levels). The variable in grey represents the main explanatory variable (salinity), and the response variables are enclosed in hollow boxes. Black arrows indicate positive causal links, and red arrows indicate negative causal links. Double-headed arrows represent correlations. Standardized parameter estimates (β) are shown next to each arrow and are proportional to the thickness of the lines. The coefficient of determination (R^2) is shown in parentheses for each response variable. Statistical power is indicated by the Fisher's C statistic, p-value (*P*), and degrees of freedom (df). The table shows the indirect effects of predictors on response variables through mediators, along with their corresponding standardized estimates. Salt stress response strategies included: tolerance (blue), avoidance (orange), and escape (green).

the emergent growth form reflect a classic stress tolerance strategy (Shelake et al. 2022) that was mediated in our study by decreasing levels of photosynthetic pigments (Ashraf and Harris 2013). Diminishing photosynthetic protein levels and increasing dark respiration rates also have been documented as functional trait

responses related to reduced net photosynthetic rates under salinity stress in *A. philoxeroides* (Longstreth et al. 1984; Mudalige and Longstreth 2006). A salt-tolerant strategy also was reflected in increasing accumulation of organic osmolytes by both growth forms. Proline and glycinebetaine accumulation are well-known mechanisms plants use to maintain osmotic balance for alleviation of salinity stress (Gupta and Huang 2014), as previously reported for *A. philoxeroides* (Longstreth et al. 2004; Islam et al. 2019). Stem node TNC in emergent plants was not affected by salinity, but it decreased with increasing salinity in free-floating plants, suggesting mobilization of storage reserves to survive in harsh conditions.

Stress avoidance traits

Emergent and free-floating growth forms of *A. philoxeroides* expressed comparable avoidance responses to salt stress. They produced thicker, succulent leaves to dilute salt concentrations and allocated less biomass to adventitious roots with increasing salinity, potentially reducing salt uptake (Karlova et al. 2021). Moreover, we recorded increasing stem lignin accumulation for emergent plants in response to increasing salt stress, yielding thicker, hardened stems with reduced pith cavities more typically observed in *A. philoxeroides* in terrestrial habitats (Vogt 1973). Although increased stem lignin would promote tissue impermeability and thereby reduce Na uptake and water loss (Du et al. 2024), it may reduce success of biological control, as plants with thin, hollow stems are needed for successful pupation by the flea beetle, *A. hydrophila* (Sosa et al. 2008).

Stress escape traits

Functional trait responses to salinity supported alternative stress escape strategies for emergent *A. philoxeroides*, whereas the free-floating growth form had a much more limited capacity to counter salt stress. Root investment may support accumulation of non-structural carbohydrate storage reserves in response to salt stress (Gupta and Huang 2014). In our study, rooted plants increased their subterranean TNC reserves in response to salt stress, a response not available for free-floating plants. Reallocation of these stored assimilates from belowground storage organs provides energy for stress escape and supports resprouting during recovery from salinity exposure. TNC reserves may also be stored in stem node tissues of floating stoloniferous shoots that can influence establishment success of floating ramet fragments (Dong et al. 2011; Suzuki and Stuefer 1999). In our results, stem node TNC in emergent plants was not affected by salinity, though it decreased with increasing salinity in free-floating plants, reflecting that they were mobilizing these storage reserves to escape and survive in harsh conditions. Carbohydrate storage patterns can vary greatly among seasons, growth forms, and life history stages in coastal aquatic macrophytes (Lammerant et al. 2024), highlighting the importance of considering differences in stress responses among growth forms.

Trait responses to salinity differ between growth forms

Like *A. philoxeroides*, other mat-forming, soil-rooted, emergent macrophytes (e.g., *Myriophyllum aquaticum* Vell. (Verdc.); parrot's-feather; *Ludwigia peploides* (Kunth) P.K. Raven and other water primroses), or free-floating species (e.g., *Eichhornia crassipes*

(Martius) Solms; water hyacinth; *Pistia stratiotes* L.; water lettuce) exhibit both emergent and free-floating strategies. To date, experiments evaluating salinity effects on such macrophytes have studied single growth forms within a species, potentially misrepresenting stress tolerance of species that shift between alternate growth forms (Moreira et al. 2023). Overall, our results indicate free-floating *A. philoxeroides* experienced greater deleterious effects with increasing salinity than the emergent growth form. The higher capacity of emergent *A. philoxeroides* to sustain salinity stress appeared to be optimal in mesohaline (12 ppt) conditions. Emergent plants reached the flowering life stage and produced higher biomass than free-floating plants in polyhaline to euhaline conditions. In our recovery experiment, most of the free-floating *A. philoxeroides* plants were able to survive even in euhaline conditions for 21 days, and they always retained some adventitious roots. However, their biomass was very low at 24–36 ppt (< 1 g), suggesting very limited capacity, if any, to recover in freshwater conditions after high salinity exposure. Nevertheless, free-floating plants were able to flower in freshwater conditions after being exposed to 12 ppt, a signal of recovery from salt stress in brackish conditions. In light of these recovery results, the escape strategy from salt stress by fragmentation of free-floating ramets detached from emergent plants could be successful if dispersal of floating stems to low salinity water occurs within a few weeks following exposure to detrimentally brackish salinity conditions.

Implications for biotic stress (herbivory) tolerance

Given the high genetic diversity of non-native *A. philoxeroides* across the U.S. range (Williams et al. 2020) and an incomplete understanding of salt tolerance thresholds and mechanisms underlying responses of contrasting growth forms of invasive *A. philoxeroides* to increasing salinity stress, a comprehensive research approach was needed to understand the risk of range expansions and support climate adaptive management plans in the context of sea level rise. Biological control of *A. philoxeroides* by the *Agasicles hygrophila* Selman & Vogt beetle has been highly successful in freshwater wetlands throughout the southeastern USA. Although *A. hygrophila* has been important for management of *A. philoxeroides*, we know little about how salinity might change plant traits important to the survival of the beetle (e.g., hollow stems, Maddox et al. 1971; branching architecture, Harms et al. 2023).

On the whole, in response to increasing salinity, more traits would seem to support herbivory by the biocontrol agent. Leaf N concentration and stem branching increased with increasing salt stress in emergent plants, potentially increasing palatability to herbivores (Reich 2014; Harms and Cronin 2019) and mat habitat for *A. hygrophila*, respectively. Increased stem branching may provide greater opportunities for hydrochorous dispersal of ramet hosts with insect herbivores from higher to lower salinity zones (or vice versa) following disturbance (Harms et al. 2023), with potentially contrasting outcomes for herbivory. Although phenolic compounds protect plants from multiple abiotic and biotic stressors, including salinity (Kumar et al. 2020; Chauhan et al. 2023; Ahlawat et al. 2024), emergent plants in mesohaline conditions and free-floating plants in mesohaline to euhaline salinity accumulated fewer total phenolic compounds than under freshwater conditions, suggesting plant defense against herbivory will decline with increasing salinity. However, low allocation to phenolics in freshwater conditions suggests the plants may be better defended against herbivory in freshwater (Singh et al. 2021). Also, as salinity increased, less hollow, more rigid stems were produced, potentially

reducing suitability for flea beetle pupation and dispersal by hydrochory. Given the contrasting implications of trait responses by *A. philoxeroides*, the potential efficacy of insect biological control agents across salinity gradients merits further study.

Implications for invasiveness of *Alternanthera philoxeroides* with sea level rise

Coastal flooding by extreme tide levels impacting low-lying wetlands around the San Francisco Bay–Delta Estuary is projected to increase in magnitude and frequency concurrent with continued temperature warming and sea level rise (Wang et al. 2025). In this system, salinity influences vegetation patterns as it decreases with distance from the Pacific Ocean and as it increases with intertidal elevation within brackish wetlands. The Mediterranean climate with hot, dry summers results in elevated interstitial soil salinity in brackish and salt marshes (Callaway et al. 2007; Schile et al. 2011). The recent invasion of *A. philoxeroides* in our study area has now spread along a longitudinal salinity gradient ranging primarily from freshwater inland Delta to oligohaline–high mesohaline (12–18 ppt) Suisun Marsh reaches and down-estuary into seasonal polyhaline conditions (Authors, unpublished data). This occupied area includes the elevational salinity stress gradient from waterways upslope to the maximum tidal elevation along natural banks and artificial levees and into high marsh plains and terrestrial ecotones.

Native plant species locally dominant to subdominant in the lower intertidal zones in freshwater and oligohaline–mesohaline reaches at the onset of the invasion of *A. philoxeroides* (Authors, unpublished data) are now experiencing sea level rise and impacts of the invasion. These include tall clonal stands of flood-tolerant sedges, cattails, and reeds (e.g., *Schoenoplectus*, *Typha*, and *Phragmites* species). Saltgrass (*Distichlis spicata* (L.) Greene), pickleweed (*Sarcocornia pacifica* (Standley) A.J. Scott; per Ball 2003), and other succulent halophytes prevail at higher intertidal elevations. Importantly, the tidal wetland vegetation invaded by *A. philoxeroides* that is vulnerable to changing salinity stress also supports endemic plant species and the highest productivity and biological diversity in the estuary (Parker et al. 2011). In general, mesocosm and field observations suggest dominant taxa differ in their sensitivity to increasing salinity, with some more sensitive than what we observed in our study with *A. philoxeroides*. For example, in a mesocosm experiment, increasing salinity reduced the growth of the native succulent *S. pacifica* from San Francisco Estuary, although it tolerated 30 ppt levels (Woo and Takekawa 2012). Native *Typha domingensis* Persoon and *T. latifolia* L. are reported to be moderately tolerant of brackish salinity but at lower thresholds (5–8 ppt) than observed for *A. philoxeroides* in our experiment (Macek and Rejmánková 2007; Hadad et al. 2018). However, field observations suggest *T. domingensis* maintains growth into somewhat higher brackish conditions in our study estuary, and sympatric, non-native *T. angustifolia* L. tolerates higher mesohaline salinity than its congeners (Bansal et al. 2019; Moreira et al. 2023). Likewise, salt tolerance is reported as "low" for native *Phragmites australis* subsp. *americanus* Saltonstall, P.M. Peterson & Soreng compared to invasive non-native European and African *Phragmites australis* genotypes (Vasquez et al. 2005), which can tolerate salinity more concentrated than seawater (Achenbach et al. 2013; Achenbach and Brix 2014).

The relative success of an invasive plant species is dependent on the level of environmental stress in the habitat (Alpert et al. 2000) and on native species' abilities

to respond to the concomitant competitive and environmental pressures. In California's estuarine wetlands, Callaway et al. (2007) predicted that increases in tidewater salinity will impart more immediate stresses on tidal wetland vegetation than greater flood-induced stress. Increased physiological stresses imposed by sea level rise can weaken the competitive abilities of vulnerable native species already near their stress tolerance thresholds, opening the disturbed habitat to establishment by opportunistic invaders (Gonzalez et al. 2024). In this context, greater salt tolerance of invasive plant species may support their superior competitive ability over native species and their invasiveness in saline environments (Liu et al. 2017; Liu et al. 2019; Guo et al. 2023). Paradoxically, plant communities in harsh habitats are often less invaded by non-native species than those in more benign habitats (Uyeda et al. 2013; Zefferman et al. 2015; Zhao et al. 2025). Increased salinity stress can significantly reduce biomass production of both native and invasive species, though negative stress effects have not resulted in the elimination of many invasive aquatic plant species. The invasion outcomes of native vs. non-native interspecific interactions appear to depend on the number and intensity of environmental stress factors in effect and on functional plant traits related to performance, stress tolerance/avoidance strategies, and propagule pressure (van Kleunen et al. 2010; Zefferman et al. 2015; Zhao et al. 2025). Outcomes from interacting effects of multiple stresses imposed by salinity, other environmental change factors, and invasion of tidal wetlands by *A. philoxeroides* are therefore complex and uncertain.

Although stress tolerance strategies are likely at play throughout the invasion continuum, the competitive ability of alien invaders compared to native plants is key to their invasiveness (Guo et al. 2022). In aquatic systems, strong competitive effects of *A. philoxeroides* have been attributed to its mat-forming growth form, comprised of interwoven shoots that spread and blanket over native wetland plants (see review, Tanveer et al. 2018). In complementary field research in progress, we have been evaluating the rapid spread of *A. philoxeroides* in our study estuary, where its emergent mats are displacing tall clonal macrophytes, including native *Schoenoplectus acutus* (Muhl. ex Bigelow) Á. Löve & D. Löve var. *occidentalis* (Watson) S.G. Sm., *S. californicus* (C.A. Meyer) Soják, *Typha domingensis*, *T. latifolia*, *Phragmites australis* subsp. *americanus*, and other species in fringing wetlands along tidal sloughs and island shores in the brackish Suisun Marsh (Suppl. material 1: fig. S5).

Conclusion

Overall, our results indicate that *A. philoxeroides* is a facultative halophyte well adapted to oligohaline to mesohaline salinity levels. Remarkably, plants survived across the full range of freshwater to euhaline experimental conditions, with traits revealing the invasive weed's stress tolerance, avoidance, and escape strategies for enduring salinity-induced physiological stress. These diverse strategies may enable *A. philoxeroides* to withstand temporal and spatial increases in salinity in tidal wetlands until more favorable environmental conditions are reached. Having multiple strategies suggests the invader will be resilient to a range of conditions that might accompany sea-level rise (e.g., altered herbivory or disease, temperature extremes). Collectively, the growth, physiological, and biochemical traits underlying these strategies can contribute to maintaining the competitive ability of *A. philoxeroides*, supporting its continued invasiveness as estuarine salinity intrusion increases with

sea-level rise. Improved understanding of the mechanistic traits underlying the capacity of *A. philoxeroides* to survive salinity stress can also be used to improve predictive models of invasive plant growth (Javed et al. 2022) relative to climate change effects on biological invasions, thereby improving invasive species risk assessments and management prioritization.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

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Author contributions

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Data availability

Data supporting this study are available from John Carroll University, Graselli Library's "Carroll Collected" repository: https://collected.jcu.edu/alligator_weed_salinity_study/1/.

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Supplementary material 1

Supplemental tables and figures supporting functional trait responses of *Alternanthera philoxeroides* to increasing salinity in tidal wetlands

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