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Interactive effects of salinity and inundation on native Spartina foliosa, invasive S. densiflora and their hybrid from San Francisco Estuary, California

Blanca Gallego-Trevar

Brenda J. Grewell

Caryn J. Futrell

Rebecca E. Drenovsky

Jesus M. Castillo

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• **Background and Aims** Sea level rise (SLR) associated with climate change is intensifying permanent submersion and salinity in salt marshes. In this scenario, hybridization between native and invasive species may result in hybrids having greater tolerance of abiotic stress factors than their parents. Thus, understanding the responses of native and invasive halophytes and their hybrids to interacting physiological stresses imposed by SLR is key to native species conservation. We analysed how salinity, inundation depth and their interaction impact the functional traits of native and invasive cordgrass species and their hybrid (genus *Spartina*; Poaceae).
• **Methods** In a mesocosm experiment, we evaluated interactive stress effects of three inundation depths (4.5, 35.5 and 55 cm) and four aqueous salinities (0.5, 10, 20 and 40 ppt) on 27 functional traits of native *Spartina foliosa*, invasive *S. densiflora* and their hybrid *S. densiflora* × *S. foliosa* from San Francisco Estuary.
• **Key Results** The combined effect of salinity and inundation led to synergistic effects on leaf biochemical stress indicators. *Spartina foliosa* behaved as a stress-tolerant species, with high leaf sodium exudation rate and glycine betaine concentrations that also increased with stress. *Spartina foliosa* was less sensitive to salinity than *S. densiflora* and the hybrid but was highly growth-limited in response to increased inundation and salinity. *Spartina densiflora* was fast-growing in low-stress conditions and tolerated moderate interactive stresses. The hybrid produced more biomass, rhizome reserves and tillers than its parents, even under the most stressful conditions. Transgressivity improved the hybrid's capacity to deal with flooding stress more so than its response to increasing salinity.
• **Conclusions** Based on our observations, we predict that established populations of both native and invasive cordgrasses will experience reduced vegetative and sexual fitness in response to SLR. In particular, the combined effects of high salinity and deep inundation may decrease floret production in *S. densiflora*, a key trait for the spread of its invasive populations. In contrast, the hybrid likely will be able to sustain its invasiveness under SLR based on its ability to maintain growth and biomass production under stressful conditions.

Key words: Abiotic stress, climate change, estuarine ecosystems, evolutionary biology, functional traits, halophytes, hybridization, invasiveness, plant invasion, salt marsh, sea level rise.

INTRODUCTION

Salinity and inundation are among the most important abiotic drivers of plant species distribution and abundance within tidal salt marshes (Engels and Jensen, 2010). Variation in the salinity of tidewater and the depth, duration and frequency of inundation reflect differences in distance from oceanic tidal sources, fluctuations in tidal ranges, local hydrogeomorphology, precipitation and evapotranspiration rates, and freshwater river inflow. These physical drivers are coupled with complex biotic factors such as interspecific interactions among halophytes (Gedan and Bertness, 2010). Together, these processes lead to variation within and among intertidal habitats along estuarine hydrological gradients of coastal marshes and influence the response of wetland vegetation to sea level rise (SLR) (Morris *et al.*, 2002).

Accelerating rates of SLR associated with climate change are modifying the patterns of salinity and inundation in salt marshes (IPCC, 2015). Vertical accretion in salt marshes driven by organic matter and sediment deposition may compensate for accelerated SLR (Craft *et al.*, 2009). However, salinity and permanent inundation depth are increasing in salt marshes, in which sediment accretion will not compensate for SLR, intensifying abiotic stress levels on halophytes (Stralberg *et al.*, 2011) and threatening intertidal habitats with extreme submergence by the end of the century (Thorne *et al.*, 2018). Hence, studies on the response of plant species to varying levels of salinity and inundation stress imposed by SLR are essential for the preservation of wetlands (Tabot and Adams, 2012). In tidal wetlands, it is especially important to understand how SLR will limit or extend the role of invasive species known to function as ecosystem engineers that can alter habitat, with cascading ecological effects on resident biota (Crooks *et al.*, 2002).

Halophytes by definition have developed morphological, anatomical and physiological adaptations that support stress

tolerance or avoidance mechanisms to complete their life cycles in saline environments (Mishra and Tanna, 2017). Sea level rise progressively imposes greater salinity and flooding stress with constant to more frequent and deeper inundation by saline tidewater across tidal elevations. Responses to these stresses by wetland plant species can negatively impact their photosynthetic capacity, modify biomass allocation patterns and accumulation, and increase maintenance costs (Pezeshki, 2001; Munns and Tester, 2008). Osmotic and flooding stresses, independently, induce common and differentiated responses in wetland halophytes. Synergistic effects of these physiological stresses can increase negative effects on marsh vegetation (Spalding and Hester, 2007; Barrett-Lennard and Shabala, 2013; Janousek and Mayo, 2013), yet the direct and indirect interactions of osmotic and flooding stresses on halophytes are still poorly understood (Flowers and Colmer, 2015). In fact, recent work recommended additional research into plant physiological responses to combinations of climate change impacts (Parker and Boyer, 2017).

Biological invasions of plant species well beyond their native ranges are a significant anthropogenically derived component of global environmental change (Vitousek *et al.*, 1997). In natural ecosystems such as salt marshes, the interactive effects of changing environmental conditions derived from SLR on native versus invasive plant species remain unclear (Hellmann *et al.*, 2008; Parker *et al.*, 2011). Some studies suggest that, in general, invasive species can benefit from the effects of climate change (Loebl *et al.*, 2006; Vilà *et al.*, 2007). Other authors emphasize that the effects may be species-specific, given the complex interaction between the new environmental conditions and the invasive species (Hellmann *et al.*, 2008; Rahel and Olden, 2008). In order to elucidate how invasive plants will respond to environmental changes, it is fundamental to study their responses through a functional trait framework (Drenovsky *et al.*, 2012). In general, invasive species are able to colonize environments significantly different from their native range by exhibiting traits that enable them to tolerate spatial heterogeneity of abiotic conditions (Dukes and Mooney, 1999), frequently developing high phenotypic plasticity (Castillo *et al.*, 2014; Grewell *et al.*, 2016).

In this context of global environmental change, plant species with a greater tolerance of modifications in environmental factors and broader ecological niches will influence the future configuration of ecosystems (Thuiller *et al.*, 2005). This is the case with invasive plant hybrids, since interspecific hybridization with an invasive parent species frequently results in increased invasiveness (Hovick and Whitney, 2014), with hybrids having greater tolerance of abiotic factors and competitive ability than their parents. This heterosis or 'hybrid vigour'(Rieseberg *et al.*, 1999) is associated with a suite of superior traits that lead to high fitness (Lippman and Zamir, 2007). As a result, hybrids provide an opportunity to document adaptive changes in response to climate change from an evolutionary perspective (Taylor *et al.*, 2015). Some studies have assessed the effect of increasing salinity (Favre and Karrenberg, 2011; Lee *et al.*, 2016) or of waterlogging (Waldren *et al.*, 1988; Boers and Zedler, 2008) on plant hybrids, finding patterns of enhanced tolerance. However, little is known of the combined effects of physiological stress from increasing salinity and inundation stress on the ecological tolerances of invasive plants and their hybrids in tidal wetlands facing SLR. To address this knowledge gap, we performed an experiment with plants from the genus of polyploid halophyte cordgrasses *Spartina*, in which interspecific hybridization and invasiveness are common processes (summarized in Strong and Ayres, 2013).

In this study, we evaluated how salinity, inundation depth and their interaction impact the functional traits of native and invasive halophyte congeners and their hybrid in the context of SLR. With this aim, our study focused on two cordgrass species and their hybrid (genus *Spartina;* Poaceae) that colonize different habitats along the intertidal gradient in Californian salt marshes: (1) *Spartina densiflora*, native to the south-west coast of South America (Bortolus, 2006); (2) *Spartina foliosa* (syn. *Sporobolus foliosus*), a native cordgrass endemic to California; and (3) their hybrid *S. densiflora* × *S. foliosa*, which formed in the San Francisco Estuary (Ayres *et al.*, 2008). *Spartina densiflora* shows low genetic diversity and high phenotypic plasticity in its invaded range along the Pacific coast of North America (Castillo *et al.*, 2014, 2016, 2018; Grewell *et al.*, 2016), where it has become highly invasive (Strong and Ayres, 2013). In the San Francisco Estuary, *S. densiflora* may co-occur with *S. foliosa* (Ayres *et al.*, 2003), where it colonizes low salt marshes subjected to long flooding periods and anoxic sediments (Mahall and Park, 1976). Reciprocal hybridization processes have been described between the two species, with both *S. foliosa* and *S. densiflora* acting as seed parents (Ayres *et al.*, 2008).

To evaluate the performance of native *S. foliosa*, invasive *S. densiflora* and their hybrid *S. densiflora* × *S. foliosa* along increasing salinity and inundation gradients, we conducted a randomized, full factorial experiment using aquatic mesocosms in a greenhouse to study responses of the *Spartina* taxa under controlled environmental conditions. We hypothesized: (1) *Spartina foliosa* would be more tolerant than *S. densiflora* to flooding stress given its adaptation to low marsh habitats; (2) both cordgrass species would present high tolerance to salinity; (3) the hybrid *S. densiflora* × *S. foliosa* would show higher tolerance of abiotic stresses due to hybrid vigour; and (4) the interaction between salinity and flooding stress would induce high stress levels in each taxon.

MATERIALS AND METHODS

Experimental design

Plants of *Spartina foliosa* ($2n$ = 62 chromosomes) were collected from a poorly drained middle elevation near the bayshore of a tidal wetland in the Carquinez Straits, where the species also occurs in fringing wetlands at other, lower elevations below mean high water (Southampton Marsh, Benicia, 38°3′57″ N, 122°11′36″ W). *Spartina densiflora* ($2n$ = 70) and the hybrid *S. densiflora* × *S. foliosa* were also collected in July to November 2016 from well-drained middle elevations of a tidal wetland at Corte Madera Creek (Creekside Park, Greenbrae, 37°56′27″ N, 122°31′2″ W) in the north and central reaches of San Francisco Estuary, California. Diploid and triploid hybrids of *S. densiflora* × *S. foliosa* have been detected in San Francisco Estuary (Ayres *et al.*, 2008). Somatic cell counts for collected hybrids ($2n$ = 66) indicated our experimental plants

were all diploid, indicating their seed parent was most likely *S. densiflora*.

Experimental plants were cleaned, potted and grown in culture at the USDA-ARS Aquatic Weed Research Facility at the University of California (Davis, USA). In February 2017, rhizomes were separated from aerial tillers and roots and cleaned to obtain similar-sized experimental individuals according to the growth form of each taxon [initial rhizome mass (fresh weight): *S. foliosa*, 103 ± 8 g; *S. densiflora*, 200 ± 11 g; hybrid, 34 ± 1 g). On 3 March 2017, rhizomes were transplanted to pots (15 cm diameter × 17.5 cm height] with bottom drainage holes, using sterile sand as substrate, and grown until the beginning of the experiment. The pots were sub-irrigated to 5 cm depth, using freshwater, and nutrients were added as 10 mL of 40 % Hoagland's nutrient solution pipetted onto the sediment surface of each pot once per week. Plants of each taxon were randomly assigned to experimental treatments. To avoid osmotic shock in the plants, the higher salinity treatments were obtained by increasing the irrigation water by 10 ppt salinity per week until the target experimental salinity level was reached.

After salinity conditioning, on 8 May 2017, plants were arranged in a randomized complete block design within treatments nested within sixteen 500-L (1.3 m × 0.8 m × 0.6 m) structurally reinforced polyethylene mesocosms (Rubbermaid, Atlanta, GA). This approach allowed precise control of flood depth and salinity treatments that cannot be obtained under field conditions. It also allowed us to accurately harvest and quantify above- and below-ground biomass of experimental plants for analyses. A split-plot, full factorial experiment was designed in which salinity was assigned as the main plot in the different mesocosms in a randomized complete block design, inundation was assigned to the subplots, and the three taxa, including the parental species and the hybrid *S. densiflora* × *S. foliosa* (n = 4 plants per treatment), nested within each subplot (Supplementary Data Fig. S1). The salinity treatments ranged from freshwater to hypersalinity (0.5, 10, 20 and 40 ppt) and were created using Instant Ocean® sea salt (Aquarium Systems, Mentor, OH) plus 20 % Hoagland's nutrient solution and Eco Pond Clear biological product (Grow More, Gardena, CA) for reducing algal growth. Three permanent inundation treatments [shallow inundation (4.4 cm deep from the base of the pots); intermediate inundation (35.5 cm deep); and deep inundation (55.0 cm deep)] were established by placing the plants on top of concrete block platforms at different heights inside each mesocosm (Supplementary Data Fig. S2). Our experimental design did not mimic the temporal flooding to which salt marsh vegetation may also be exposed during high tides, but halophytes are also subjected to permanent flooding at lower elevation ranges due to SLR. We imposed static inundation as a treatment to evaluate stress responses to a water depth gradient from constant flooding to aerated condition during our experiment. Our experimental design allowed us to control the salinities and level of anoxia imposed by fixed inundation levels, providing relevant data regarding the combined effects of salinity and waterlogging. Glasshouse conditions were maintained with controlled air temperature (21–25 ºC) and a 12 h daily photoperiod set with high-intensity discharge lights (GE Lucalox LU1000/ECO HPS 1000 W, PARsource, Petaluma, CA). The photon flux density at the canopy level measured by a LI-COR LI-250A light meter (LI-COR, Lincoln, NE) varied between 500 and 1500 µmol m^{-2} s^{-1} during the photoperiod. The experiment was carried out for 44 d prior to biomass harvest (see initial and final display of the greenhouse in Supplementary Data Fig. S3).

Pre-harvest measurements

Phenology. The number of flowering tillers of each experimental plant was counted weekly to monitor differences in phenology and reproductive potential of the three *Spartina* taxa under the different flooding and salinity treatments.

Leaf gas exchange. Prior to harvest, measurements of net photosynthesis rate (A) and stomatal conductance (g_s) were carried out using a LI-COR 6400 portable infrared CO_2 analyser (LI-COR Biosciences, Lincoln, NE) in differential mode and in an open circuit. The CO_2 concentration inside the chamber was fixed at 400 µmol mol^{-1}, photon flux density was 1000 µmol m^{-2} s^{-1} (LED light source, 90 % red and 10 % blue, generating actinic light) and flow rate 400 µmol s^{-1}. Before taking each measurement, one leaf per plant was wiped with a tissue moistened with distilled water to remove surface salts and left to dry. Each individual measurement was the mean of three subreplicates separated by a 10-s interval, beginning when conditions stabilized inside the chamber. All measurements were conducted on sunny days between 36 and 40 d after the start of the experiment (13–17 June 2017) within the 2 h around solar noon.

Root and rhizome porosity. Aerenchyma presence, or porosity, of roots and rhizomes was determined following the method described in Kercher and Zedler (2004). Fragments of live roots and rhizomes of 5 cm length were gently dried with a tissue and weighed to the nearest 0.001 g. The air volume in the plant fragments was then immediately extracted and replaced with water using a vacuum pump connected to a 250-mL flask containing 200 mL of distilled water running for 5 min. Finally, the piece of root or rhizome was blotted dry with a tissue and weighed again. Porosity (%) was calculated as the difference between initial and final weight in relation to the initial weight.

Plant tissue chemistry. Fresh leaf tissue [0.5–2.0 g per plant from the middle part of randomly chosen flag leaves (first unfolded adult leaf from the apical leaf)] was collected prior to harvest and immediately frozen for later analyses of photosynthetic pigments and proline.

Post-harvest evaluations

Biomass and growth. At the end of the experiment, aboveground biomass (AGB) and below-ground biomass (BGB) of each tussock were separated, and BGB was divided into roots and rhizomes. Dry weight (DW; g) was obtained after oven-drying each fraction of biomass at 70 ºC for 48 h, and total biomass was calculated (Castillo *et al.*, 2008). Root mass ratio (RMR) was also calculated as the proportion of roots (DW) in relation to the total plant tussock biomass (Martin and Hine, 2008). The number of live tillers was counted for each tussock at the beginning and at the end of the experiment. Tiller relative

growth rate (TGR, tillers tillers^{-1} year^{-1}) was calculated as the difference between the number of final and initial tillers divided by the number of experiment days (Castillo *et al.*, 2010). Tiller length was measured for five adult tillers of each tussock at the end of the experiment. The final number of flowering tillers per plant was counted at harvest. As a measure of reproductive fitness (Kulheim *et al.*, 2002), flowers were separated and the number of florets per plant was determined from the average number of florets per inflorescence for each taxa at each treatment (n = 1 inflorescence per plant) and the number of inflorescences per individual (n = 4 plants per treatment).

Leaf morphology. All leaf measurements were conducted on flag leaves to avoid effects due to leaf ontogeny. Foliar width was recorded twice on the central zone of each leaf: prior to manipulation and after unrolling it. Leaf adaxial rolling was calculated as the percentage reduction in leaf width after rolling (Premachandra *et al.*, 1993). Specific leaf area (SLA, m^{-2} g^{-1}) was determined for three leaves per plant as the ratio between leaf area and its DW (Garnier *et al.*, 2001). Leaf area was calculated using WinFOLIA (Regent Instruments, Saint-Foy, Quebec, Canada) and DW was obtained after oven-drying the leaves at 70 ºC for 48 h.

Leaf chemical analysis. Plant tissue samples (from frozen samples) were extracted in 80 % aqueous acetone. The extracts were centrifuged and supernatants were used for the determination of the concentrations of chlorophylls ([Chl *a*+*b*]) and carotenoids ([Car]) from their absorbance at 664, 647 and 470 nm (Lichtenthaler, 1987) using a spectrophotometer (Beckman DU-64, Beckman Coulter, Brea, CA). Additionally, dry leaf tissue was ground to pass through a No. 40 mesh screen for nitrogen (N), carbon (C), sodium (Na), total non-structural carbohydrates (TNC) and glycine betaine analyses. Total leaf C and N concentrations were determined using a Perkin Elmer 2400 CHNS/O analyser (Perkin Elmer, Waltham, MA, USA). The C:N ratio was calculated. Free proline concentration in leaves was determined following the procedure in Bates *et al.* (1973). Foliar glycine betaine concentration in leaves was estimated as quaternary ammonium compounds following the protocol in Grieve and Grattan (1983). Leaf Na concentration was measured using a sodium electrode on dry-ashed samples that were dissolved in 1 M HCl (Grewell *et al.*, 2016). The leaf Na exudation rate was measured following the protocol in Christman *et al.* (2009). Randomly selected flag leaves were rinsed with distilled water and marked. The marked area was harvested after 48 h and measured to calculate leaf area. Each harvested leaf section was submerged in 2 mL of distilled water and shaken, and salinity (measured as electrical conductivity) was recorded. The leaf Na exudation rate was reported as salt excreted per unit area per time, in which the time interval represented the time elapsed between leaf rinsing and harvest.

Subterranean resource storage. Total C and N concentrations in rhizomes were analysed as reported above for leaves, and we calculated the C:N ratio. Rhizomes were analysed for TNC concentration using a modified enzymatic digestion procedure as detailed by Swank *et al.* (1982), followed by spectrophotometric assay for reducing sugars (Nelson, 1944).

Statistical analyses

All statistical analyses were performed using IBM SPSS V. 20 for Windows, applying a significance level (α) of 0.05. Plant traits were classified into four functional groups: (1) growth and biomass response (total biomass, percentage of root and rhizome in BGB, tiller length, RMR, TGR and floret number); (2) morphological and anatomical stress responses (leaf rolling, SLA, and rhizome and root porosity); (3) biochemical stress responses (leaf proline, leaf glycine betaine, N and Na concentrations, leaf Na exclusion, leaf N:C ratio, rhizome N, rhizome N:C ratio, rhizome TNC and change in rhizome TNC); and (4) photosynthetic responses ([Chl *a*+*b*], [Car], Chl *a*:Chl *b* ratio, Chl *a*:Car ratio, A and g_s). Data series were tested for homoscedasticity using Levene's test and for normality using the Shapiro–Wilk test. The trait of leaf exudation rate was transformed using the functions $\sqrt{x}$ and $1{:}x$, respectively, to meet the assumption of homogeneity of variances for parametric tests. To protect analyses from type I error, we used the protected analysis of variance (ANOVA) protocol (Scheiner, 2001). The means of the dependent variables of each trait group were compared using multivariate analysis of variance (MANOVA) and Pillai's trace to evaluate the significance of the factors taxon (*S. foliosa*, *S. densiflora* and *S. densiflora* × *S. foliosa*), salinity (0.5, 10, 20 and 40 ppt) and inundation depth (shallow, intermediate and deep) avoiding type I error (Scheiner, 2001). Redundant, highly correlated variables ($r > 0.95$) were identified prior to MANOVA analysis. Highly correlated variables were omitted from the statistical models. Once multivariate significance was confirmed via MANOVA, the main univariate differences were evaluated for each functional plant trait with general linear models (LMs) and the Bonferroni–Dunn test as a *post hoc* analysis. When homogeneity of variance was not achieved after data transformation, univariate differences were analysed using the γ generalized linear model (GLM) with Wald's χ^2 (Ng and Cribbie, 2017).

Ordination analysis was used to explore patterns in our data without constraining the analysis based on treatment (ter Braak and Šmilauer, 2012). As is typical with functional trait data, our data responses were linear, not unimodal, suggesting that principal components analysis (PCA) was a better approach than correspondence analysis. Therefore, PCA was conducted for the three studied taxa independently to investigate linear relationships between traits, in order to express covariation in numerous variables in a smaller number of composite factors. The PCA was carried out analysing the correlation matrix with 25 maximum iterations for convergence without rotation to extract independent PCA factors with eigenvalues >1. The PCA factors obtained for the response traits of each taxon were correlated with salinity (ppt) and inundation (cm deep) treatments. Linear regression analysis was applied to analyse the relationships between changes in AGB (as percentage of its maximum) and increments in salinity and inundation depth.

RESULTS

Salinity, inundation depth and taxon each had a significant effect on growth and biomass production and allocation, leaf morphology and rhizome anatomy, leaf biochemistry and photosynthetic rates (MANOVA, $P < 0.05$) (Supplementary Data Table S1). Results of all the LMs for the 27 measured plant traits are summarized in Supplementary Data Table S2. Herein we report the most significant results for each trait group.

Growth and biomass responses

These traits were affected by salinity, inundation depth, taxon and their interactions (MANOVA, $P < 0.005$), with the exception of interactions between salinity and inundation depth [Supplementary Data Table S1]. In general, the hybrid accumulated 1.5-fold and 3.5-fold more total biomass than *S. densiflora* and *S. foliosa*, respectively (taxon: $F_{2,96} = 169.5$, $P < 0.0001$). The hybrid reduced its biomass ~20 % more than its parents with increasing stress. *Spartina foliosa* was able to maintain its biomass with increasing salinity (taxon × inundation depth: $F_{4,96} = 11.3$, $P < 0.0001$; taxon × salinity: $F_{6,96} = 4.0$, $P < 0.001$) (Fig. 1A). Root mass ratio was ~ 45 % lower for the hybrid than its parents (taxon: $F_{2,96} = 85.9$, $P < 0.0001$), increasing by ~30 % under increasing inundation depth, particularly, for both parents (taxon × inundation depth: $F_{4,96} = 2.3$, $P < 0.07$) (Fig. 1B). The percentage of root biomass in BGB was higher for *S. foliosa* and the hybrid (~47 %) than for *S. densiflora* (41 ± 1 %) (taxon: $F_{2,96} = 11.448$, $P < 0.0001$). In contrast, production of rhizome mass was higher in *S. densiflora* BGB than in *S. foliosa* and the hybrid (taxon: $F_{2,96} = 11.5$, $P < 0.0001$). The hybrid increased its root and decreased its rhizome accumulation at deeper inundations, whereas these below-ground growth responses of the parents remained constant (taxon × inundation depth: root percentage, $F_{4,96} = 3.3$, $P < 0.05$; rhizome percentage, $F_{4,96} = 3.3$, $P < 0.05$) (Supplementary Data Fig. S4A–C).

In general, tiller length was highest for the hybrid (129.4 ± 2.3 cm), followed by *S. densiflora* (80.2 ± 2.3 cm) and *S. foliosa* (49.1 ± 2.3 cm) (taxon: $F_{2,96} = 306.5$, $P < 0.0001$). Tiller length was reduced for each taxon by inundation depth (inundation depth: $F_{2,96} = 28.5$, $P < 0.0001$) and salinity (salinity: $F_{3,12} = 56.1$, $P < 0.0001$); the reduction at higher salinities tended to be lower for the hybrid (taxon × salinity: $F_{6,96} = 2.0$, $P = 0.08$) (Fig. 1C). In general, TGR was maximal for the hybrid (0.72 ± 0.02 tillers tillers^{-1} year^{-1}), followed by *S. foliosa* (0.27 ± 0.01 tillers tillers^{-1} year^{-1}) and *S. densiflora* (0.13 ± 0.01 tillers tillers^{-1} year^{-1}) (taxon: $\chi^2 = 211.0$, $P < 0.0001$,

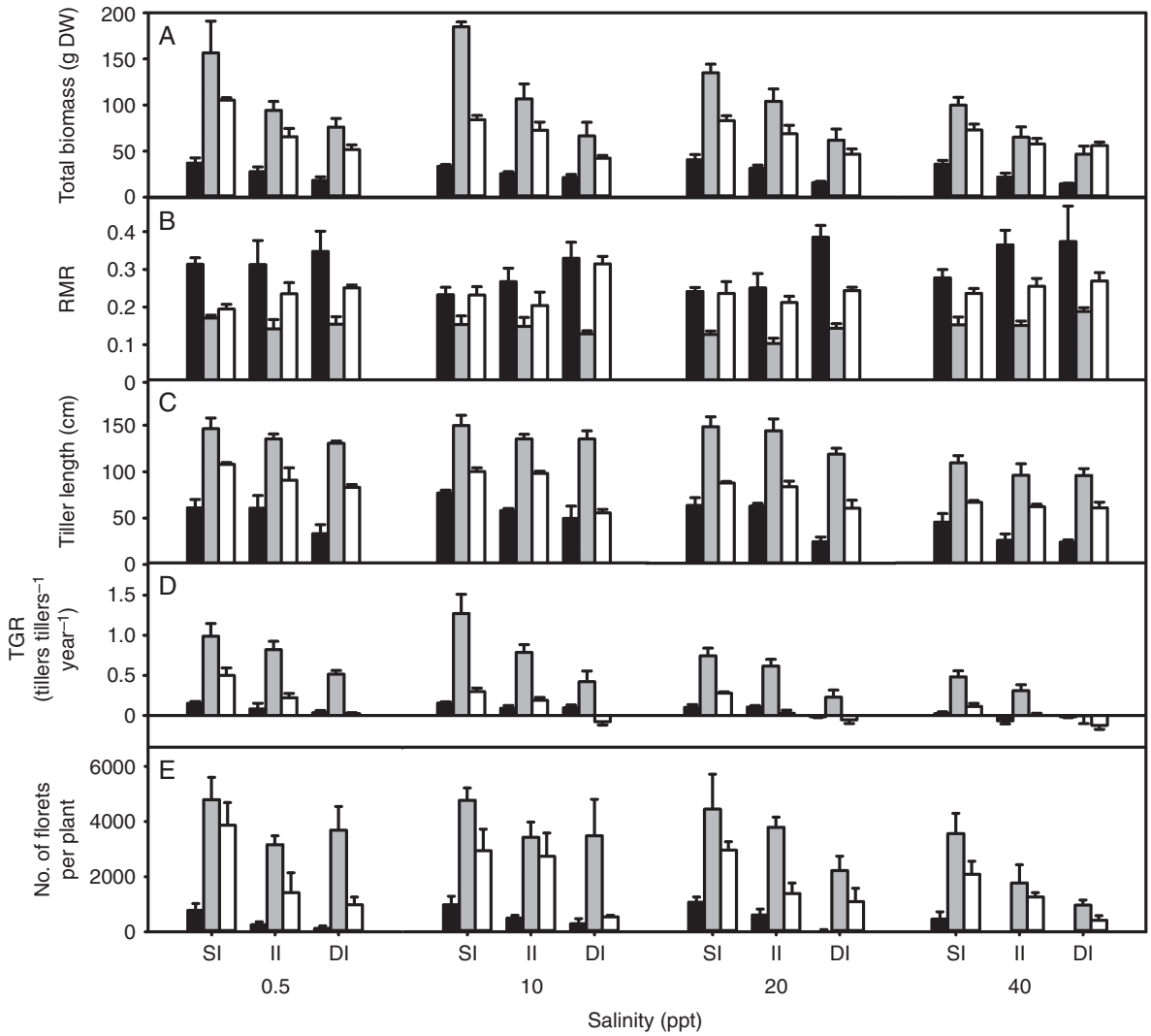

FIG. 1. Growth and biomass responses. (A) Total biomass, (B) root mass ratio (RMR), (C) tiller length, (D) tiller growth rate (TGR) and (E) number of florets per plant for *Spartina foliosa* (black bars), the hybrid *S. densiflora* × *S. foliosa* (grey bars) and *S. densiflora* (white bars) under different aqueous salinity levels (0.5, 10, 20 and 40 ppt) and inundation depths (SI, shallow inundation, 4.4 cm deep; II, intermediate inundation, 35.5 cm deep; DI, deep inundation, 55.0 cm deep). Values are mean ± s.e. ($n = 4$).

d.f. = 2). The TGR was greatest at 10 ppt salinity and lowest at hypersalinity for all taxa (salinity: $\chi^2 = 70.4$, $P < 0.0001$, d.f. = 3). The TGR was also negatively affected by inundation depth for all taxa (inundation depth: $\chi^2 = 78.2$, $P < 0.0001$, d.f. = 2), with *S. foliosa* having highest values at intermediate inundation and *S. densiflora* and the hybrid at shallow inundation (taxon × inundation depth: $\chi^2 = 18.5$, $P < 0.001$, d.f. = 4) (Fig. 1D). The hybrid produced the greatest number of florets, followed by *S. densiflora* and *S. foliosa* (taxon: $F_{2,93} = 87.0$, $P < 0.0001$).

The number of florets per plant decreased by ~10–15 % at hypersalinity for all taxa (salinity: $F_{3,12} = 8.8$, $P < 0.005$). The decrease in the number of florets per plant was 86 % for *S. foliosa*, 76 % for *S. densiflora* and 41 % for the hybrid (taxon × inundation depth: $F_{4,93} = 2.2$, $P = 0.070$) (Fig. 1E).

Morphological and anatomical responses

This trait group was affected by salinity, inundation depth, taxon and the interaction between salinity and taxon (MANOVA, $P < 0.005$) (Supplementary Data Table S1). Leaf rolling was higher for *S. densiflora* (25 ± 1 %) than the hybrid (20 ± 1 %) and *S. foliosa* (14 ± 1 %) (taxon: $F_{2,93} = 22.1$, $P < 0.0001$). Leaf rolling to reduce evaporative water loss increased from freshwater (10 ± 1 %) to hypersalinity (35 ± 1 %) for all taxa (salinity: $F_{2,12} = 83.9$, $P < 0.0001$), with the hybrid being the only taxon showing higher leaf rolling at 20 ppt than at freshwater (taxon × salinity: $F_{6,93} = 3.6$, $P < 0.005$). In contrast, leaf rolling decreased at deeper inundations, from 26 ± 1 % at shallow inundation and 14 ± 1 % at deep inundation for all taxa (inundation depth: $F_{2,93} = 26.5$, $P < 0.0001$) (Fig. 2A).

Specific leaf area decreased from freshwater to hypersalinity (salinity: $F_{3,13} = 3.6$, $P < 0.05$). It was lowest with shallow inundation (~100 $cm^2\ g^{-1}$) and increased for leaves of all taxa subjected to greater inundation stress (~120 $cm^2\ g^{-1}$) (inundation depth: $F_{2,90} = 5.1$, $P < 0.01$) (Fig. 2B).

Rhizome porosity was higher for *S. foliosa* (34 ± 3 %) than for *S. densiflora* (6 ± 1 %), with the hybrid showing intermediate values (taxon: $F_{2,95} = 62.0$, $P < 0.001$) (Supplementary Data Fig. S5A). Root porosity increased with inundation depth for *S. densiflora* (from 42 to 55 %), with *S. foliosa* and the hybrid showing constant values of ~50 % (taxon × inundation depth: $F_{4,95} = 3.0$, $P < 0.05$) (Supplementary Data Fig. S5B).

Nutrient and biochemical stress responses

This trait group was affected by salinity, inundation depth, taxon and all interactions (MANOVA, $P < 0.0001$) (Supplementary Data Table S1). In general, leaf N concentration was higher for *S. foliosa* (23.2 ± 0.3 mg N g^{-1}) and the hybrid (23.5 ± 0.3 mg N g^{-1}) than for *S. densiflora* (20.1 ± 0.3 mg N g^{-1}) (taxon: $F_{2,95} = 34.0$, $P < 0.0001$). Leaf N increased and C:N ratio decreased at higher salinities for both parents, being independent of salinity for the hybrid (taxon × salinity: LM, $P < 0.05$). In addition, leaf N was higher and C:N ratio was lower at deeper inundations for all taxa (inundation depth: LM, $P < 0.0001$) (Supplementary Data Fig. S6A,B).

Spartina densiflora showed lower rhizome N concentration and a 2-fold higher C:N ratio than *S. foliosa* and the hybrid (taxon: LM, $P < 0.0001$). Rhizome N concentration decreased at higher inundation for *S. densiflora* and the hybrid but not for *S. foliosa* (taxon × inundation depth: $F_{4,96} = 4.1$, $P < 0.005$) (Supplementary Data Fig. S6C, D). On the other hand, the hybrid accumulated ~ 50 % more rhizome TNC than both parents (taxon: $F_{2,96} = 121.8$, $P < 0.0001$) (Supplementary Data Fig. S4D). Moreover, the hybrid increased its rhizome TNC by ~20 % when exposed to stress, whereas the value was reduced by ~50 % in its parents (taxon: $F_{2,96} = 410.7$, $P < 0.0001$) (Supplementary Data Fig. S4E). The hybrid and *S. densiflora* maintained higher rhizome TNC (110.9 and 52.6 mg g^{-1}, respectively) than *S. foliosa* (28.5 mg g^{-1}) under deep inundation (taxon × inundation depth: $F_{4,96} = 4.5$, $P < 0.005$) (Supplementary Data Fig. S4D).

A five-fold increase in leaf Na response occurred from freshwater to hypersalinity levels for *S. foliosa*, while leaf Na concentration increased ~2.5-fold for *S. densiflora* and the hybrid (taxon × salinity: $F_{6,94} = 10.4$, $P < 0.0001$) (Fig. 2C). Although leaf Na exudation rate increased for every taxon in response to increasing salinity and inundation depth, *S. foliosa* showed a 50–70 % higher exudation rate than *S. densiflora* at 20–40 ppt, with the hybrid showing intermediate values (taxon × salinity: $F_{6,90} = 5.2$, $P < 0.0001$) (Supplementary Data Fig. S5C).

Leaf glycine betaine concentration was ~25 % higher for *S. foliosa* and the hybrid than for *S. densiflora* (taxon: $\chi^2 = 181.7$, $P < 0.0001$, d.f. = 2), increasing with salinity and inundation depth for all taxa (LM, $P < 0.0001$, d.f. = 2–3) (Fig. 2D). Foliar proline concentration increased with salinity for all taxa (taxon × salinity: $F_{3,12} = 103.5$, $P < 0.0001$), but only increased under deeper flooding for *S. foliosa* (taxon × inundation depth: $F_{2,60} = 5.7$, $P < 0.0001$). Thus, proline concentration was greater for *S. foliosa* than for *S. densiflora* and the hybrid under deep inundation at all salinity levels (taxon × salinity × inundation depth: $F_{12,96} = 2.0$, $P < 0.05$) (Fig. 2E).

Photosynthetic responses

This trait group was affected by salinity, inundation depth, taxon and all interactions (MANOVA, $P < 0.05$) except for those between salinity and taxon (Supplementary Data Table S1). The highest [Chl *a*+*b*] was in leaves of the hybrid, with lower concentrations in leaves of *S. foliosa* and *S. densiflora* (taxon: $F_{2,96} = 30.0$, $P < 0.0001$) (Fig. 3A). The hybrid and *S. foliosa* had ~20 % higher [Car] than *S. densiflora* (taxon: $F_{2,96} = 21.9$, $P < 0.0001$) (Fig. 3B). Increasing salinity caused a gradual decrease of ~33 % in [Chl *a*+*b*] and ~20 % in [Car] for all taxa (salinity: LM, $P < 0.0001$). Only the hybrid had ~14 % higher [Chl *a*+*b*] and ~10 % higher [Car] under depth than under shallow inundation (taxon × inundation depth: LM, $P < 0.05$). At extreme interacting stress levels, the hybrid produced higher leaf pigment concentrations than either parental species (Fig. 3A, B). The Chl *a*:Car and Chl *a*:Chl *b* ratios were highest for the hybrid, followed by *S. densiflora* and *S. foliosa* (taxon: LM, $P < 0.0001$). The Chl *a*:Car ratio decreased by ~10 % and Chl *a*:Chl *b* ratio increased by ~25 % at higher salinities for all taxa (salinity: LM, $P < 0.001$). *Spartina foliosa*

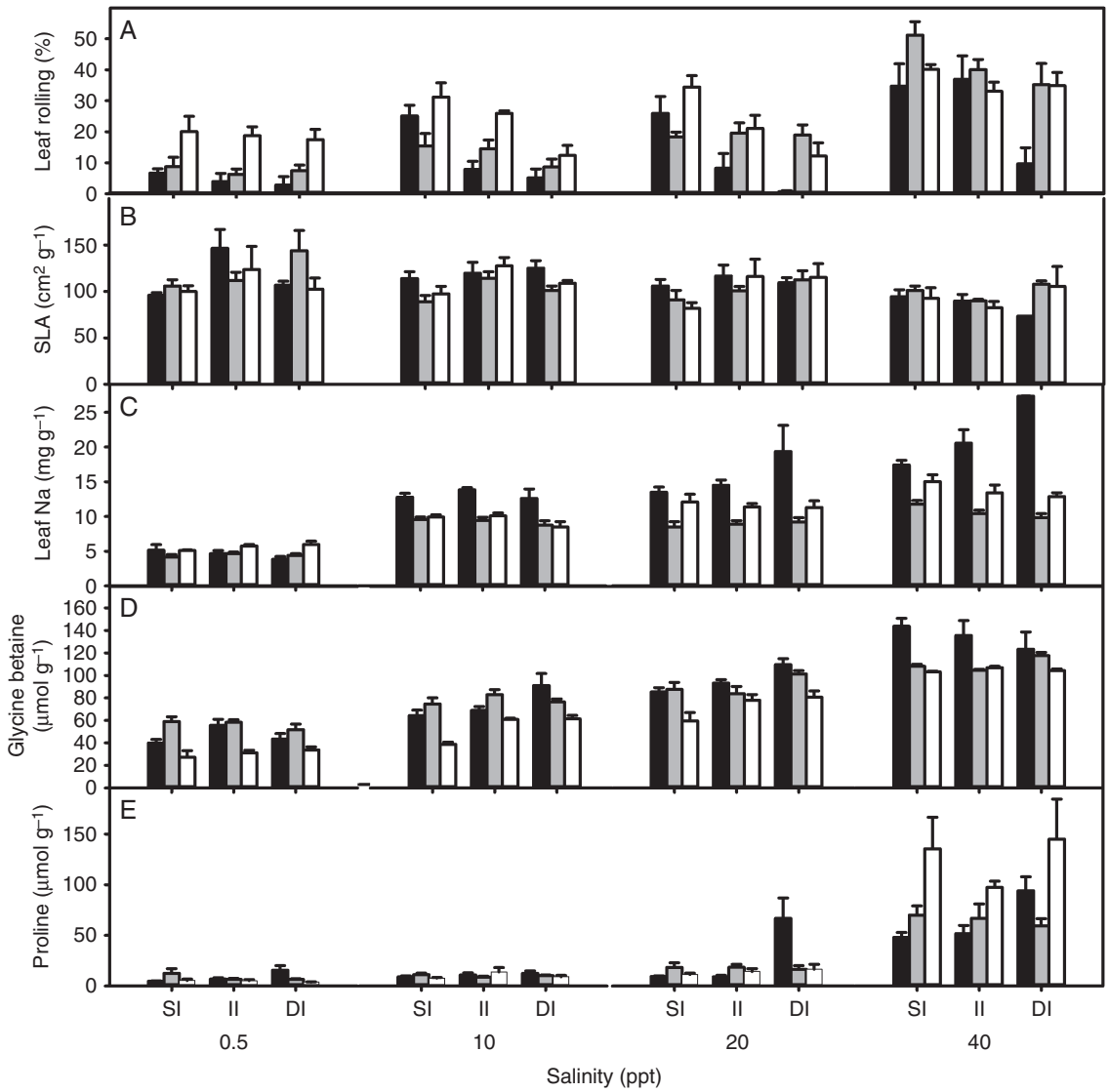

FIG. 2. Leaf morphological and biochemical responses. (A) Leaf rolling, (B) specific leaf area (SLA), (C) foliar sodium concentration, (D) glycine betaine concentration and (G) proline concentration for *Spartina foliosa* (black bars), the hybrid *S. densiflora* × *S. foliosa* (grey bars) and *S. densiflora* (white bars) under different aqueous salinity levels (0.5, 10, 20 and 40 ppt) and inundation depths (SI, shallow inundation, 4.4 cm deep; II, intermediate inundation, 35.5 cm deep; DI, deep inundation, 55.0 cm deep). Values are mean ± s.e. ($n = 4$).

decreased its Chl *a*:Chl *b* ratio at deeper inundations (taxon × inundation depth: $\chi^2 = 12.1$, $P < 0.05$, d.f. = 4) (Supplementary Data Fig. S5D, E).

Net photosynthesis rate (A) decreased at higher salinities for all taxa (salinity: $F_{3,13} = 7.0$, $P < 0.01$), being higher for *S. foliosa* and the hybrid (~8.5 µmol CO_2 m^{-2} s^{-1}) than for *S. densiflora* (6.4 ± 0.4 µmol CO_2 m^{-2} s^{-1}) (taxon: $F_{2,89} = 7.7$, $P < 0.001$) (Fig. 3C). In general, *S. foliosa* and the hybrid also had stomatal conductance rates that were 50 % higher than *S. densiflora* (taxon: $F_{2,93} = 11.4$, $P < 0.0001$) (Fig. 3D). The reduction in A coincided with lower g_s with increasing salinity (salinity: $F_{3,13} = 3.4$, $P = 0.05$) for all taxa (*S. densiflora*: $r = +0.538$, $P < 0.0001$; *S. foliosa*: $r = +0.635$, $P < 0.0001$; *S. densiflora* × *S. foliosa*: $r = +0.485$, $P < 0.0005$). Moreover, A was positively correlated with Chl *a*+*b* and Car concentrations for both parental species (Chl *a*+*b*: *S. densiflora*: $r = +0.302$, $P < 0.05$; *S. foliosa*: $r = +0.429$, $P < 0.01$; Car: *S. densiflora*: $r = +0.311$, $P < 0.05$; *S. foliosa*: $r = +0.421$, $P < 0.01$).

Relationships between plant traits and abiotic factors.

The PCA for *S. foliosa* (*Sf*) traits yielded eight factors, revealing the most interesting and strongest covariation among variables and explaining 84 % of the variance in trait responses relative to abiotic variables (Fig. 4A). PC1-*Sf* explained 27 % of the variance and was positively correlated with biomass accumulation, tiller height, number of florets and subterranean resource storage traits. PC1-*Sf* was negatively correlated with foliar biochemical responses. PC1-*Sf* was negatively correlated with salinity ($r = -0.726$, $P < 0.0001$, $n = 48$) and inundation depth ($r = -0.483$, $P < 0.001$, $n = 48$). The second factor (PC2-*Sf*,

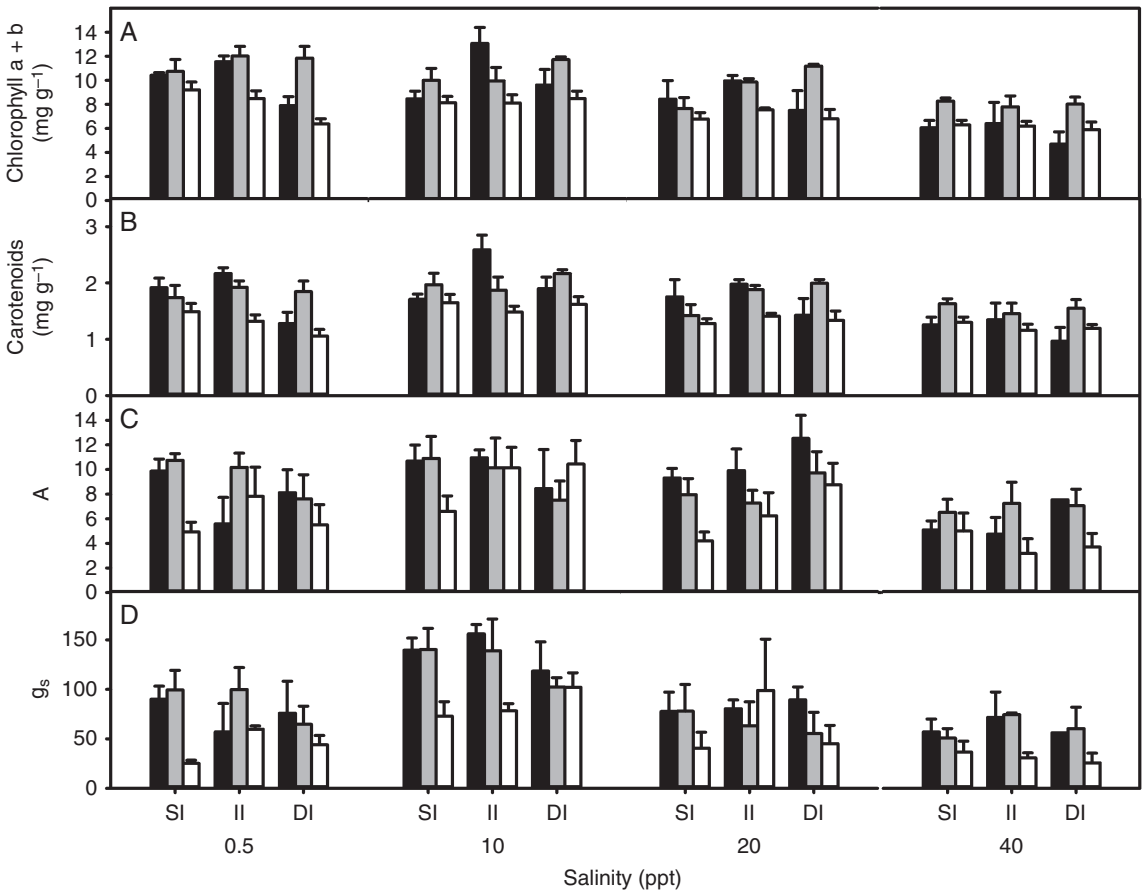

FIG. 3. Photosynthetic responses. (A) Chlorophyll *a*+*b* concentration, (B) carotenoid concentration, (C) net photosynthesis rate (*A*) and (D) stomatal conductance (g_s) for *Spartina foliosa* (black bars), the hybrid *S. densiflora* × *S. foliosa* (grey bars) and *S. densiflora* (white bars) at different aqueous salinity levels (0.5, 10, 20 and 40 ppt) and inundation depths (SI, shallow inundation, 4.4 cm deep; II, intermediate inundation, 35.5 cm deep; DI, deep inundation, 55.0 cm deep). Values are mean ± s.e. ($n = 4$).

explaining 15 % of the variance) was positively correlated with rhizome biomass and negatively with root biomass allocation; PC2-*Sf* increased with salinity ($r = +0.512$, $P < 0.0001$, $n = 48$) and decreased with inundation depth ($r = -0.488$, $P < 0.0001$, $n = 48$) (Fig. 4A) (Supplementary Data Table S3).

Eight factors revealed the highest covariation among variables in the PCA for *S. densiflora* (*Sd*) traits, explaining 81 % of the total variance (Fig. 4B). PC1-*Sd* (explaining 26 % of the variance) was positively correlated with total biomass accumulation, tiller length, number of florets, TGR, leaf C:N ratio and [Chl *a*+*b*], and negatively correlated with leaf N and foliar biochemical stress responses. PC1-*Sd* decreased with increasing salinity ($r = -0.829$, $P < 0.0001$, $n = 48$) and inundation depth ($r = -0.464$, $P < 0.001$, $n = 48$). PC2-*Sd* explained 13 % of the variance, being positively correlated with biomass accumulation and leaf rolling, increasing with salinity ($r = +0.414$, $P < 0.005$, $n = 48$) and decreasing with inundation depth ($r = -0.671$, $P < 0.0001$, $n = 48$). PC3-*Sd* also explained 13 % of the variance, being positively correlated with rhizome reserves, and it decreased with inundation depth ($r = -0.367$, $P < 0.01$, $n = 48$) (Fig. 4B) (Supplementary Data Table S4).

Seven factors were recorded for the responses of the hybrid (H) to salinity and inundation in the PCA, explaining 79 % of the variance (Fig. 4C). The first factor (PC1-H, explaining 26 % of the variance) was positively correlated with foliar biochemical stress responses and negatively with Chl concentration. PC1-H decreased with increasing salinity ($r = -0.965$, $P < 0.0001$, $n = 48$). The second factor (PC2-H, explaining 17 % of the variance) was positively correlated with total biomass and rhizome accumulation, tiller length, number of florets and leaf C:N ratio, and negatively with root accumulation and leaf N concentration. PC2-H was related negatively with inundation depth ($r = -0.740$, $P < 0.0001$, $n = 48$) (Fig. 4C) (Supplementary Data Table S5). The AGB of *S. densiflora* and the hybrid were more sensitive to increased salinity than *S. foliosa* (Fig. 5A, C, E). The hybrid's AGB was more robust than its parental species to changes in inundation (Fig. 5B, D, F).

DISCUSSION

Our results supported our first and second hypotheses: (1) *S. foliosa* was more tolerant than *S. densiflora* to flooding stress, and (2) both species tolerated high salinity levels. However, these tolerances were compromised when salinity was combined with deep inundation, which supported our fourth hypothesis: the interaction between the environmental factors would induce high stress levels in each taxon. In addition, the

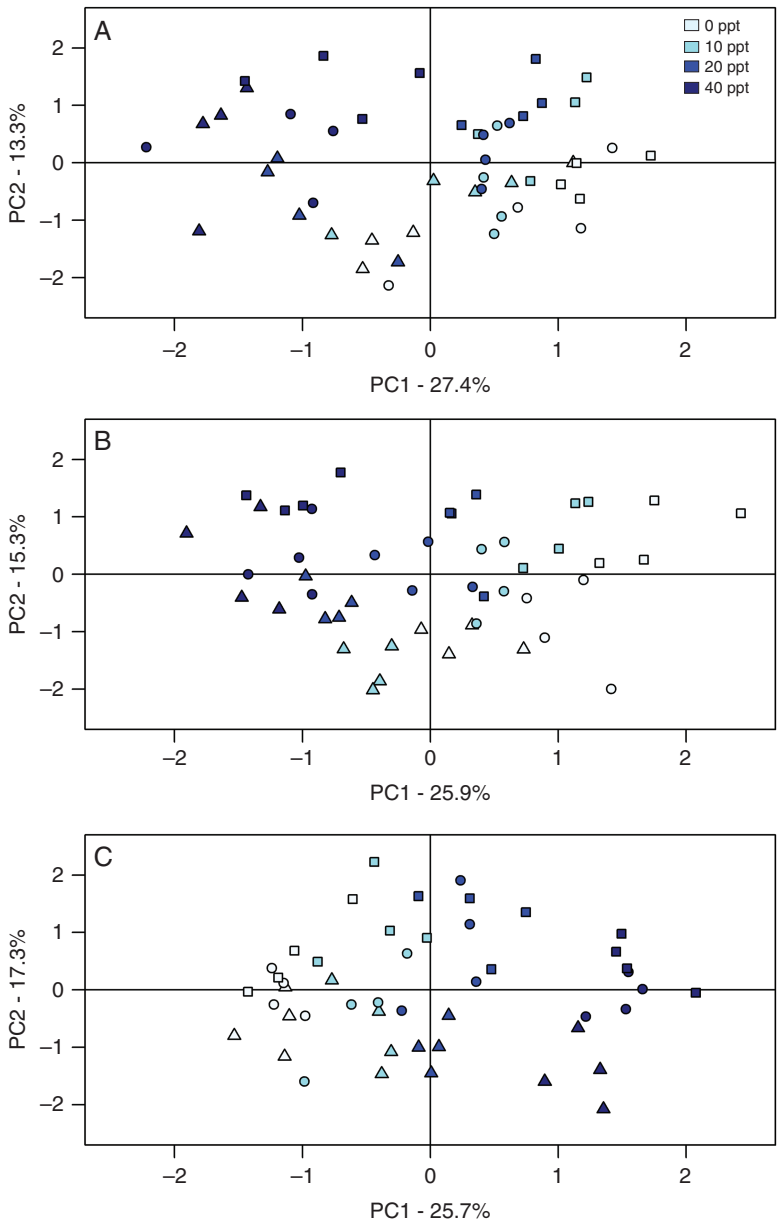

FIG. 4. PCA plots showing first and second axes (composite variables) for responses (n = 27 traits) of (A) *Spartina foliosa*, (B) *S. densiflora* and (C) their hybrid *S. densiflora* × *S. foliosa* under different aqueous salinity levels (indicated by blue shading) and inundation depths (shallow, 4.4 cm deep, triangles; intermediate, 35.5 cm deep, circles; deep, 55.0 cm deep, squares).

hybrid *S. densiflora* × *S. foliosa* had higher tolerance than its parental species to both abiotic stressors, contributing to hybrid vigour as predicted in our third hypothesis. Among all taxa, tiller length and TGR, proxies of vegetative fitness (Castillo *et al.*, 2016; Lee *et al.*, 2016), were reduced by the imposed salinity and flooding stresses.

The most dramatic responses to combined salinity and flooding stresses were synergistic changes in leaf biochemical stress indicators in our focal taxa. Similar to our study, N-containing compounds, such as proline and glycine betaine, accumulated in leaves of salt- and flood-stressed plants with functions related to osmoregulation, N storage, detoxification and enzyme protection (Parida and Das, 2005; Chen *et al.*, 2010). However, it is remarkable that we did not observe any variation in leaf N concentration or C:N ratio with increasing inundation depth at hypersalinity, or with increasing salinity at deep inundation, since the extreme stress of one of these abiotic factors could potentially mask some biochemical responses (Harley and Helmuth, 2003).

Spartina foliosa *stress responses*

Overall, the native *S. foliosa* expressed the traits of a slow-growing and stress-tolerant species (Grime, 1977). *Spartina foliosa* was less sensitive to increasing salinity than

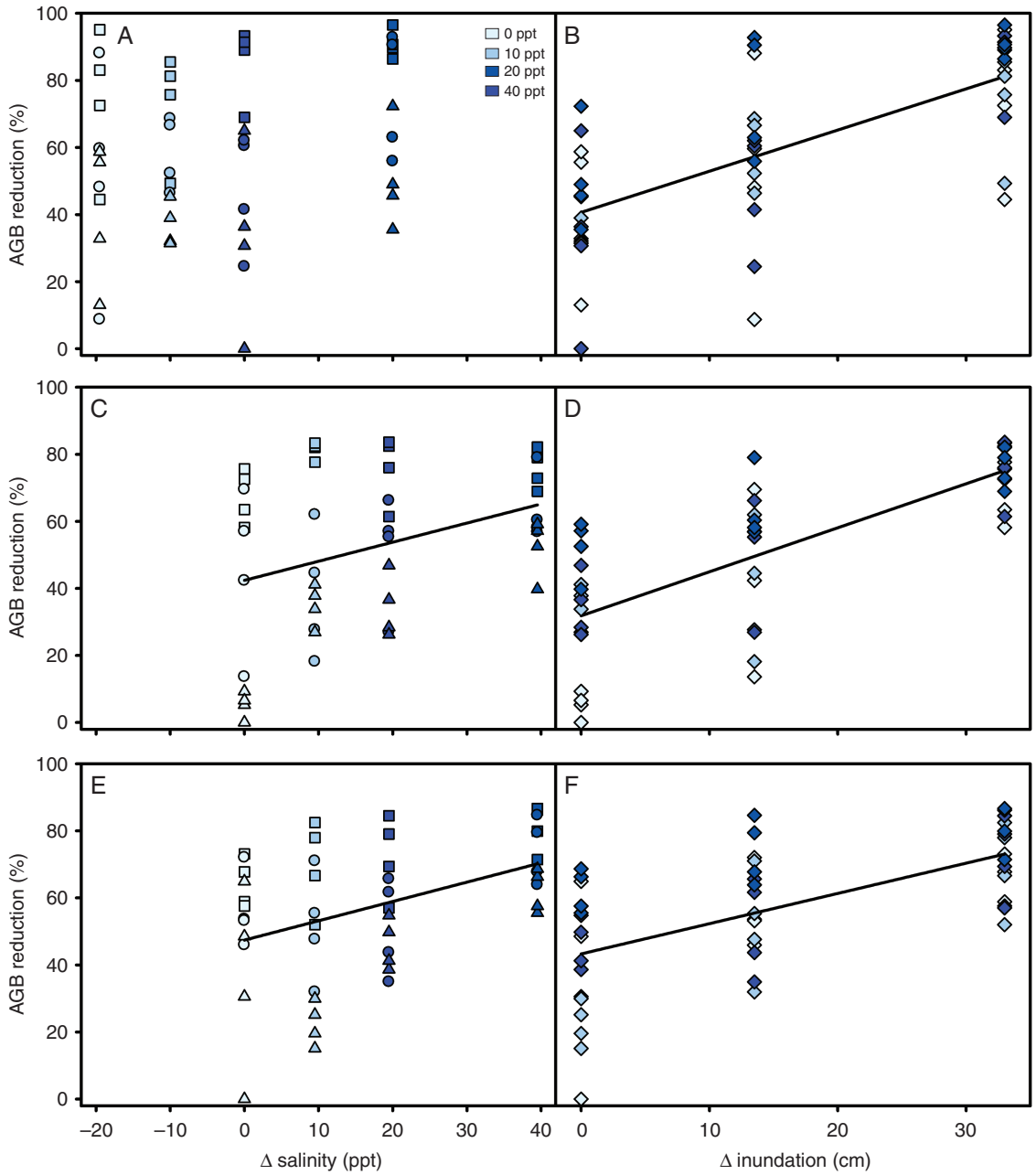

FIG. 5. Relationships between the percentage of above-ground biomass (AGB) reduction in relation to maximum individual AGB for any treatment and changes (Δ) in salinity (indicated by blue shading) and inundation depth (shallow, 4.4 cm deep, triangles; intermediate,35.5 cm deep, circles; deep, 55.0 cm deep, squares) for (A, B) native *Spartina foliosa*, (C, D) invasive *S. densiflora* and (E, F) their hybrid *S. densiflora* × *S. foliosa*. Regression equations: (B) $y = 1.23x + 40.67$ ($R = 0.672$, $P < 0.001$, $n = 48$); (C) $y = 0.57x + 42.44$ ($R = 0.352$, $P < 0.05$, $n = 48$); (D) $y = 1.31x + 31.81$ ($R = 0.753$, $P < 0.001$, $n = 48$); (E) $y = 0.58x + 47.42$ ($R = 0.426$, $P < 0.005$); (F) $y = 0.90x + 43.30$ ($R = 0.618$, $P < 0.001$).

S. densiflora or the hybrid. Along broad gradients of salinity and inundation depth, *S. foliosa* generated relatively low total biomass, with a large proportion of biomass allocated below ground. *Spartina foliosa* also prioritized asexual over sexual reproduction, with few florets produced per plant across treatments. *Spartina foliosa* maintained high photosynthetic and stomatal conductance rates (except at hypersalinity), supported by high leaf photosynthetic pigments, N concentrations and accessory pigments (low Chl:Car and Chl *a*:Chl *b* ratios), favouring photoprotection (Demmig-Adams and Adams, 1996). Nevertheless, salinity was the only factor affecting its net photosynthesis rate due to lowered stomatal conductance rates and photosynthetic pigment concentrations, which are frequently reduced under salt stress (Parida and Das, 2005; Grewell *et al.*, 2016). *Spartina foliosa* also showed high leaf Na concentration that increased with salinity, even with high Na exudation rates. This was likely due to its high stomatal conductance. The Na stress was balanced by increasing levels of glycine betaine

in leaves, which acts as an organic osmolyte maintaining cell turgor and enzyme and membrane integrity (Ashraf and Foolad, 2007). *Spartina foliosa* also expressed traits that enabled it to withstand inundation. Below ground, investment in root and rhizome biomass, increased root porosity with inundation depth and investment in subterranean reserves supported its growth under inundation. *Spartina foliosa* is known to establish and expand in anoxic sediments exposed to long submersion periods and short photoperiods (Mahall and Park, 1976). Overall, the combination of plant traits we measured, along with the ability of *S. foliosa* to maintain highest TGR at intermediate inundation depth, indicates mechanisms enabling this native species to act as a primary colonizer. While *S. foliosa* exhibited stress tolerance, it approached its physiological tolerance limit under the level of high salinity and deep inundation imposed in this experiment, as evidenced by decreases in rhizome reserves and floret set inhibition.

Spartina densiflora *stress responses*

In contrast with the native species, the invasive *S. densiflora* may be typified as a fast-growing species able to take advantage of low-stress conditions while tolerating moderate stress levels. Under non-stressful conditions, this species maintained high sexual reproduction and biomass accumulation with tall tillers. However, extreme conditions (hypersalinity and/or deep inundation) induced high leaf-rolling, increased proline concentrations and marked decreases in net photosynthesis rate and floret production. *Spartina densiflora* rhizomes had low porosity across all treatments, but produced extensive aerenchyma tissue in roots, measured as root porosity, and decreased below-ground C storage reserves in response to flooding. Leaf-rolling helped *S. densiflora* minimize light exposure, and thus photoinhibition, and reduced transpiration rates (in conjunction with decreased g_s), while proline potentially detoxified free radicals, and root porosity promoted oxygen delivery below ground (Yeo *et al.*, 1991; Kadioglu and Terzi, 2007; Yordanova and Popova, 2007; Ahmad *et al.*, 2013). Thus, invasive *S. densiflora* tolerated some degree of stress related to flooding. The ability of *S. densiflora* to tolerate moderate stress levels and to show elevated growth rates under low-stress conditions may be related to its ancestral hybrid origin (Fortune *et al.*, 2008). Hybridization is a frequent process in plants, which may lead to speciation and relevant ecological consequences (Arnold, 1992), whereas the high invasiveness of *S. densiflora* can be explained by its flexibility as an opportunistic species under low-stress episodes and as a stress-tolerant species with high phenotypic plasticity under moderately stressful conditions (Castillo *et al.*, 2018).

Spartina densiflora × S. foliosa *stress responses*

The hybrid *S. densiflora* × *S. foliosa* produced more biomass, tall tillers and rhizome reserves than the parental species. The hybrid also achieved high vegetative and reproductive fitness, even under the most stressful conditions imposed in our experiment. High tiller production would drive rapid lateral expansion rates to colonize surrounding sediments and high floret production would increase the colonization capacity of the hybrid to medium and long distances if this taxon were not sterile (Ayres *et al.*, 2008). These high hybrid fitness responses can be related to transgressive traits such as higher pigment concentrations, taller tillers and higher rhizome reserves than its parents. Tall tillers would enable the hybrid to keep a higher proportion of aerial tissues out of the water, facilitating oxygen transport below ground (Naidoo and Mundree, 1993), light collection (Burdick *et al.*, 2001) and CO_2 fixation (Castillo *et al.*, 2005). A high rhizome reserve, which increased even during exposure to stress, would enable it to obtain energy by anaerobic fermentation in situations of hypoxia (Sharma *et al.*, 2008). These reserves would also maintain cell metabolism and energy generation under adverse conditions (Martínez-Vilalta *et al.*, 2016), favouring its recovery after stress release (Chen *et al.*, 2005). In contrast with both parents, which were affected by salinity and inundation depth to similar degrees, the response of the hybrid was characterized overall by a much higher influence of salinity than inundation depth. This was reflected in salinity being related to 26 % of the recorded variance in plant traits (whereas inundation depth was related to just 17 %) and in a greater number of plant traits being affected by salinity (20) than by inundation (16).

Conclusions

Based on our greenhouse results and the predicted increases in salinity and inundation with SLR (Parker *et al.*, 2011; Stralberg *et al.*, 2011; IPCC, 2015), we predict that established populations of both native and invasive cordgrass species will experience reduced vegetative and sexual fitness. In fact, these observations may be conservative estimates of plant responses in the field, where plants also experience the additional stressors of sediment oxygen deprivation and sulphur toxicity, as well as potential light limitation due to inundation with turbid waters (Castillo *et al.*, 2000). The native *S. foliosa* may be threatened as tidewater salinity and inundation in extant habitat exceeds the salinity and flooding tolerance of the species, given its extreme sensitivity to the combined effects of high salinity and deep inundation. Conservation measures to protect potential migration pathways are needed in San Francisco Estuary, where migration is severely constrained by urban development or topography (Parker and Boyer, 2017). The invasive *S. densiflora* accumulated less biomass and produced fewer florets under high salinity and deep inundation, which are key to the spread of its invasive populations. From this perspective, SLR would reduce *S. densiflora* invasiveness (Kittelson and Milton, 1997; Nieva *et al.*, 2001; Castillo *et al.*, 2010). Finally, the sterility of the hybrid *S. densiflora* × *S. foliosa* currently limits its invasiveness, but, once established, it has a high ability to maintain growth and biomass production with increasing levels of salinity and inundation.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: *F*-statistics and Pillai's trace from MANOVAs for seven trait response groups for the factors taxon, salinity and inundation depth and their

interactions. Table S2: general linear models with taxon, salinity (S) (0.5. 10. 20 and 40 ppt) and inundation depth treatments as fixed factors and their corresponding interactions for biochemical, physiological anatomical and morphological plant traits. Table S3: factor loadings of the individual variables obtained by PCA on traits of native *S. foliosa* exposed to different salinities and inundation depths. Table S4: factor loadings of the individual variables obtained by PCA on traits of native *S. densiflora* exposed to different salinities and inundation depths. Table S5: factor loadings of the individual variables obtained by PCA on traits of native *S. densiflora* × *S. foliosa* exposed to different salinities and inundation depths. Figure S1: a split-plot, full factorial experiment design in which salinity was assigned as the main plot in the different mesocosms in a randomized complete block design, inundation was assigned to the subplots, and the three taxa, including the parental species and the hybrid *S. densiflora* × *S. foliosa*, were nested within each subplot. Figure S2: design of the 16 tanks with the arrangement of the different inundation levels for *S. foliosa*, *S. densiflora* and *S. densiflora* × *S. foliosa* individuals. Figure S3: display of the experiment in the greenhouse on the first day after setting up the experimental conditions of salinity and inundation and 31 d later, when plant harvest began. Figure S4: below-ground responses. Figure S5: stress responses. Fig. S6: plant tissue nitrogen responses.

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