Nitrogen further promotes a dominant salt marsh plant in an increasingly saline environment

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Abstract

Aims
Human alterations of the environment are combining in unprecedented ways, making predictions of alterations to natural communities a difficult and pressing challenge. Estuarine systems have been subject to a high degree of modification, including increased nitrogen (N) inputs and altered salinity, factors important in shaping estuarine plant communities. As human populations increase and the climate changes, both N and salinity levels are likely to increase in these coastal marshes. Our objective was to evaluate the interactive effects of N and salinity on US West Coast salt marsh species; in particular, the performance of the dominant species *Sarcocornia pacifica* (pickleweed) alone and in mixed species assemblages. We expected increased salinity to favor *S. pacifica* but that N enrichment could help maintain greater species richness through use of N in salinity tolerance mechanisms.

Methods
We crossed treatments of N (added or not) and salinity (salt added or not) in a field experiment at a salt marsh in the San Francisco Estuary, California, USA, in each of three habitats: (i) monotypic pickleweed on the marsh plain, (ii) monotypic pickleweed along channels and (iii) mixed assemblages along channels. In a greenhouse experiment, we crossed treatments of N (added or not) and salinity (at three levels to simulate brackish to saline conditions) in (i) pots of pickleweed only and (ii) the same species mix as in the field.

Important Findings
N addition doubled *S. pacifica* biomass and branching in both channel and marsh plain habitats regardless of salinity and greatly increased its dominance over *Distichlis spicata* and *Jaumea carnosa* in mixed assemblages along channels. In the greenhouse, *S. pacifica* biomass increased 6- to 10-fold with N addition over the range of salinities, while *D. spicata* and *J. carnosa* biomass increased with N addition only at lower salinity levels. Thus, while localized management could influence outcomes, expected overall increases in both N and salinity with human population growth and climate change are likely to enhance the production of *S. pacifica* in US West Coast marshes while reducing the diversity of mixed species assemblages. This decline in diversity may have implications for the resilience of marshes already subject to multiple stressors as the climate changes.

Keywords:
Distichlis • eutrophication • *Jaumea* • salinity • *Sarcocornia*

Received: 2 February 2011 Revised: 15 November 2011 Accepted: 5 January 2012

INTRODUCTION

Human manipulation of the environment has increased in intensity over the last century (Chapin *et al.* 2000; Meyer and Turner 1992; Vitousek *et al.* 1997b). Changes in temperature and precipitation are proceeding concurrent with sea level rise, increased ocean acidity, pollution, increased drought and fire and nitrogen deposition (Aber *et al.* 2001; Crain *et al.* 2009; Fox 2007; Zavaleta *et al.* 2003), making predictions of alterations to natural communities a difficult and pressing challenge.

The amount of biologically available nitrogen (N) has more than doubled over the last hundred years, principally due to growing use of chemical fertilizers and the burning of fossil fuels (Vitousek *et al.* 1997a). Elevated N has been linked to a suite of changes in plant communities, including an increase in the vulnerability of forest systems to drought and disease (Takemoto *et al.* 2000), an increase in the susceptibility of serpentine grasslands to invasion (Weiss 1999) and a general loss of species diversity (Suding *et al.* 2005). Even very small amounts of additional N, equivalent to worldwide averages

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of atmospheric deposition, can cause long-term changes to plant communities (Clark and Tilman 2008).

Estuaries receive a disproportionate share of the additional N load. In highly developed estuaries such as Chesapeake Bay in the USA (Cloern 2001) and the Seine, Somme and Scheldt estuaries in Europe (Billen *et al.* 2009), up to 80% of N comes from anthropogenic sources. Increases in waterborne N may have significant implications for the plant communities that fringe these estuaries, leading to decreases in diversity and changes in community composition or in relative abundance (Boyer and Zedler 1999; Crain 2007; Emery *et al.* 2001; Jefferies and Perkins 1977; Suding *et al.* 2005; Tyler *et al.* 2007) as well as changes in plant growth form (Boyer and Zedler 1998; Boyer *et al.* 2001; Levine *et al.* 1998; Valiela and Teal 1974).

Nitrogen may be especially important in saline portions of estuaries, where it is the primary limiting nutrient for plant growth (Boyer *et al.* 2001; Crain 2007; Valiela and Teal 1974; van Wijnen and Bakker 1999). In addition, N plays an important role in mediating salt stress by maintaining internal water balance through the production of compatible organic solutes (Storey and Wyn Jones 1977). These are often N-based (e.g. proline and glycinebetaine), and their production can consume 20% of plant N (Storey *et al.* 1977). N availability can also affect the water use efficiency of photosynthesis, which is important in the drought-like conditions induced by salt stress (Chaves *et al.* 2009; Martin *et al.* 2010).

In addition to increased N input, salinity is changing within estuaries. Agriculture and urban development alter salinity patterns by changing when, where and how much freshwater enters estuaries (Mirza 1998; Peterson *et al.* 1995). Climate change is expected to further complicate salinity patterns within estuaries by altering rainfall, snowmelt and sea level (Barnett *et al.* 2004; Knowles and Cayan 2002; Scavia *et al.* 2002). In addition, discharges from wastewater treatment or desalinization plants can influence salinity within estuaries. Thus on a local scale, salt marshes may experience average seasonal, yearly and decadal changes in salinity in either direction.

The ways in which simultaneous changes in N and salinity affect salt marsh plant productivity and community composition are unclear. While increased N and salinity can both decrease diversity (Clark and Tilman 2008; Crain 2007), the fact that N can help mediate salt stress suggests that simultaneous increases in both factors could have a balancing rather than additive negative effect on species richness. Increased salinity is also associated with a decline in biomass (Carter 1982), but increased N may counteract some of the negative effects of salinity on production. Exactly how these interactions will manifest in plant communities will depend on the characteristics of their member species. Not all plant species rely on N-based compatible solutes for osmotic adjustment to the same degree (Flowers 1985). Thus, the availability of N can differentially influence the capacity of plant species to avoid salt stress. Likewise, the ability to photosynthesize with high water use efficiency (important in the drought-like conditions of a salt marsh) is highly variable. For example, C4 plants utilize

considerably less N for photosynthesis (5–9% of total leaf N) compared to most C3 plants (20–30% of total leaf N) and thus can allocate more for growth (Makino *et al.* 2003). Given the variety of mechanisms of salt tolerance and the presence of both C3 and C4 species in most salt marshes, the direction and magnitude of architectural and compositional changes in response to changing N and salinity are difficult to predict. Furthermore, the response of member species to initial stressors may affect their response to future additive stresses: if community members are able to co-adapt, they may be better situated to cope with additional stress (Vinebrooke *et al.* 2004). The way salt marsh communities respond to N at different salinities may reflect those communities' resilience to additional stressors.

The San Francisco Estuary, the largest on the west coast of the Americas, drains the Central Valley of California, one of the world's most productive agricultural areas and is surrounded by the fifth largest urban area in the USA (U.S. Census Bureau 2000). Estimates of N originating from artificial sources run as high as 70–80% for major watersheds entering the estuary (Hager and Schemel 1992; McKee and Krottje 2005; Sobota *et al.* 2009). Water diversion for agriculture in the Central Valley caused salinity to increase by an average of 2 ppt during peak springtime flows over the latter half of the 20th century (Peterson *et al.* 1995). Furthermore, modeling predicts that earlier onset of snowmelt, combined with sea level rise, will further increase salinities (Knowles and Cayan 2002). On a local scale, input from wastewater, storm drains, and industry mean salinity may either increase or decrease.

In this study, we conducted field and greenhouse experiments to examine how changes in N and salinity, alone and in combination, affect the growth and structure of salt marsh communities. This study focused on the response of the salt marsh dominant of San Francisco Estuary and the US West Coast, *Sarcocornia pacifica* (Standl.) A.J. Scott to these factors and examined its responses alone as well as in a mixed community of other common marsh species. We hypothesized that *S. pacifica* would increase in dominance with increased salinity; however, N inputs could help to maintain other species in the assemblage through use of added N in osmotic adjustment.

MATERIALS AND METHODS
Study species

This study investigated three common plant species that occur over a range of salinities in central California marshes. *Sarcocornia pacifica* (Standley) A.J. Scott, perennial pickleweed, dominates the salt marsh habitat, particularly the marsh plain, along the west coast of North America. *Sarcocornia pacifica* (formerly *Salicornia virginica* L.) *Sarcocornia pacifica* (Standley) A.J. Scott, is a low-growing succulent C3 shrub of the Chenopodiaceae, a family with members common to saline environments (e.g. the genera *Salicornia*, *Sarcobatiu*, *Atriplex* and *Suaeda*; Welsh *et al.* 2003). *Distichlis spicata* (L.) Greene, saltgrass, is a C4 grass (Poaceae) distributed throughout North America in salt and brackish

marshes and alkali flats. *Jaumea carnosa* (Less.) A. Gray, jaumea, is a semi-succulent, C_3 forb of the Asteraceae found on the west coast of the Americas. It spreads laterally via stoloniferous runners, occasionally in monotypic stands. Like *D. spicata*, *J. carnosa* is generally found in high marsh or along channels. *Distichlis spicata* and numerous members of the Asteraceae and Chenopodaceae use N-based compatible solutes to aid in salt tolerance (Flowers 1985; Rhodes and Hanson 1993). *Distichlis spicata*, as a C_4 species, should have lower N demands for photosynthesis than the other species and thus may be especially well suited to using excess N for osmotic adjustment and growth.

Field experiment

The field experiment was conducted at China Camp State Park (38°00′37″N 122°2912″W; Fig. 1) where the relatively unaltered historic marsh is commonly used as a reference site for restoration and management in the region. It is composed of expanses of nearly level marsh plain, dominated by a monoculture of *S. pacifica*

interspersed with channels. The channel edges are composed of a mix of *S. pacifica*, *J. carnosa* and *D. spicata*, with some *Grindelia hirsutula* Hook. and Arn. (gumplant; formerly *Grindelia stricta*) and *Limonium californicum* (Boiss.) A. Heller (sea lavender).

Field plots (60 total) were laid out in a randomized complete block design with five blocks of four 1-m² plots. N and salt additions (each added or not) were crossed to produce four treatments: control, N addition, salt addition and N + salt addition. This design was replicated in three habitats: pure *S. pacifica* stands on the marsh plain (pickleweed plain), pure *S. pacifica* stands adjacent to channels (pickleweed channel) and mixed species plots adjacent to channels (mixed species; Fig. 1). Plots were located either 12 m (pickleweed plain) or 4 m (mixed or pickleweed channel plots) from the nearest channel. The elevation of plots within blocks did not vary > ±2 cm (Topcon laser level). Mixed species plots were chosen to have the three species, *S. pacifica*, *D. spicata* and *J. carnosa*, present.
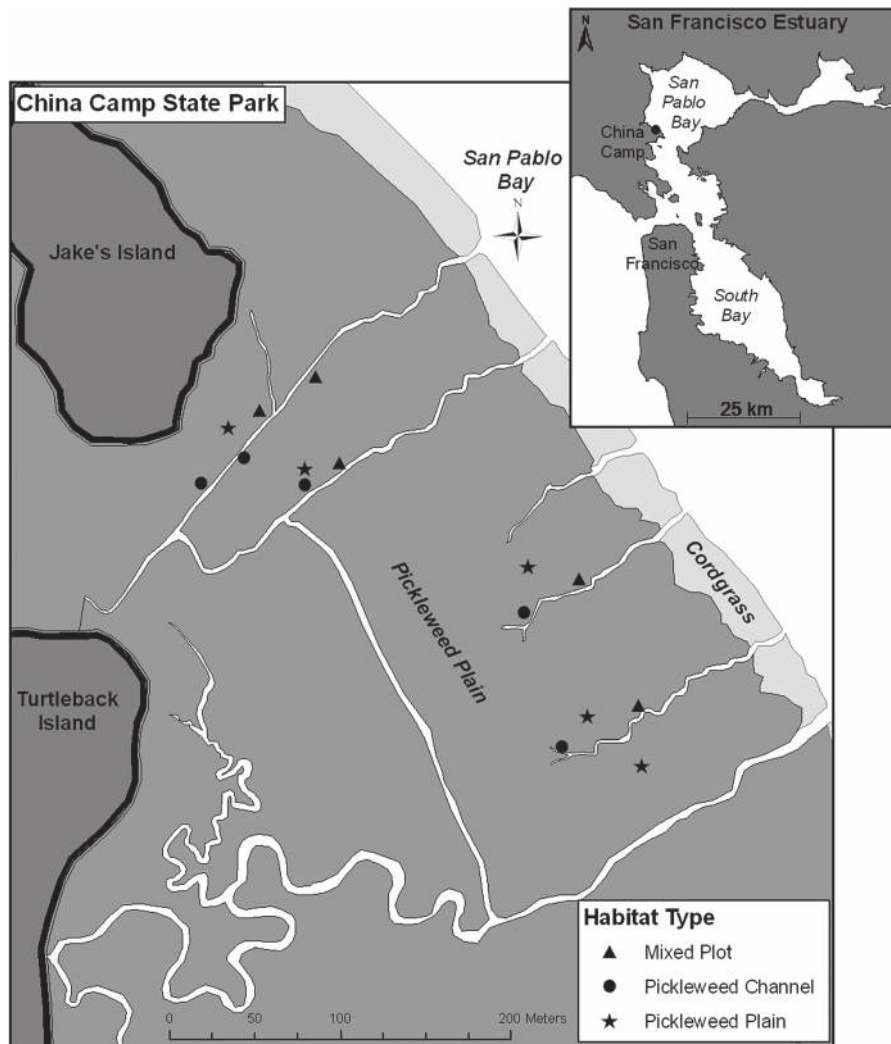
Figure 1: distribution of field research plots, China Camp State Park, San Pablo Bay and San Francisco Estuary.

Treatments began in June 2007 and continued monthly through April 2008 except during plant dormancy (November 2007–January 2008). N addition plots received 33 g of 46-0-0 urea (YARA International ASA, Oslo, Norway) applied monthly as per Boyer *et al.* (2001). Urea increases soil NH_4–N (Valiela and Teal 1974), the form of N most readily taken up by *S. pacifica* (Page 1995). In salt addition plots, 1275 g of Instant Ocean® Sea Salts (Spectrum Brands, Atlanta, GA) were applied monthly during the growing season (rate based on Kuhn and Zedler 1997). Instant Ocean® includes Na, Cl, SO_4, Mg, Ca, P, Br, B, Sr and other constituents at levels found in seawater. N was dissolved into 4 l of water collected from the adjacent channel in a designated N-watering container. Salt treatments were sprinkled evenly over the plots and watered in with 4 l of channel water, with or without dissolved N. Control treatments were also watered but with no N or salt added.

Plant height was determined by averaging 10 measurements taken at predetermined positions. Percent change in height was calculated between the June 2007 and May 2008 sampling dates as $(height_2 - height_1)/height_1$. Percent cover was estimated using a 1 m^2 quadrat with an internal 0.1×0.1 m grid. Each plant species present was noted in every other square (total of 50) to estimate absolute cover (total cover could exceed 100% due to layering). Percent change in cover was calculated between June 2007 and September 2008 as $(cover_2 - cover_1)/cover_1$. Aboveground biomass was clipped within a 15-cm-diameter hoop at a predetermined location in each plot, weighed fresh, then dried at 50°C and reweighed. Samples collected in June 2008 were further separated into living and dead biomass by species. For *S. pacifica*, the number of branches was tallied and represented as total branches per gram dry weight. Dried samples were ground in a Wiley® Mini-Mill to pass through #40 (0.425 mm) mesh and analyzed for percent N by gas chromatography in a Costech (Valencia, CA) ECS elemental analyzer. At times, *Cuscuta salina* Engelm., a native holoparasitic vine, grew in some plots; its cover was estimated when present and biomass determined from June 2008 samples.

At the experiment's beginning and end, soil cores to 10 cm were weighed, dried to a constant mass at 50°C, reweighed to determine bulk density (dry mass/wet volume) and percent moisture and ground to pass through a 2 mm sieve. Soil organic matter was determined by loss on ignition (400°C for 10 h) and relative salinity and pH using saturated soil pastes (Richards 1954). Initial (June 2007) soil N was determined at the Marine Science Institute at the University of California Santa Barbara using a CEC 440HA (Exeter Analytical, Inc., Coventry, UK) organic elemental analyzer (Dumas combustion method).

Greenhouse experiment

Plants were grown in one of six treatments: salinity of 5, 20 and 35 ppt (a range of water salinity typical of coastal marshes) crossed with two levels of N addition (with or without). These were applied to two different diversity levels: 'pickleweed monoculture' (six *S. pacifica* plants per pot) and 'mixed species' (two each of *S. pacifica*, *D. spicata* and *J. carnosa*). Seven randomized blocks included a total of 84 pots.

Plants were started from root divisions (from Rush Ranch, Suisun City, CA) in winter 2007, grown out in flats for 2 months, then transferred to pots of a low nutrient high clay terrestrial soil (organic matter = 4.6%, soil N 0.11%). Pots with holes in the bottom and sides sat in containers holding the assigned salinity of water. Salinities were raised gradually to allow acclimation. Water of the assigned salinity was replaced monthly in the outer pots. Between replacement, water levels were kept constant using freshwater. N was added in the form of 8.3 g (one-fourth of the amount applied in field plots) of 46-0-0 urea at 6-week intervals.

Treatments were applied from June to December 2007. Branching per stem length was calculated in September 2007. At the end of the experiment, aboveground biomass was removed, weighed, dried at 50°C and reweighed, then ground in a Wiley mill as above. Subsamples were burned in a muffle furnace at 500°C for 3 h to obtain percent ash. Tissue N, soil salinity and pH [both from pooled surface (0–2 cm) and 20 cm depth samples] and soil N (from surface samples) were determined as described for the field experiment.

Statistical analyses

We determined overall treatment effects in both field and greenhouse experiments with multivariate analysis of variance (MANOVA) using the SPSS 16.0 (Chicago, IL) general linear model. For each experiment, three MANOVAs were used. The pickleweed MANOVA analyzed the effects of N and salinity on pickleweed responses in all three habitats (field) or both diversity levels (greenhouse). The soil MANOVA analyzed N and salinity effects on soil responses. The mixed species MANOVA used a repeated measures general linear model with species as a within subjects factor and N and salinity as between subjects factors. The response variables used in the MANOVA for both field and greenhouse experiments were pickleweed branching, biomass, percent N, percent ash and percent moisture. In the field, change in average plant height was also included and change in percent cover in the mixed species treatments. Soil response variables were salinity, pH and N (greenhouse only). While all five blocks were used for the mixed species MANOVA, the field experiment pickleweed and soil MANOVA included only four out of five blocks because one set of channel plots and one set of plain sites were aligned differently along the channel due to habitat constraints (Fig. 1).

Data were transformed as needed to meet the assumptions of parametric statistics: greenhouse biomass (log), field biomass (square root) and percent N and percent change in cover (arcsine square root). Live and dead aboveground biomass were combined in the field experiment pickleweed and mixed species MANOVAs to reduce the number of variables.

P values <0.05 were considered significant. Effect size was assessed using partial eta-squared (η_p^2). Significant effects were examined further using *post hoc* ANOVAs with N, salinity and block as factors. In the greenhouse experiment, *post hoc*

ANOVAs examined the effect of diversity level on pickleweed with and without added N.

The effect of parasitic *C. salina* cover was examined via linear regression on *S. pacifica* cover, %N and height. ANOVA tested treatment effects on *Cuscuta* biomass and cover.

RESULTS

Site conditions

Of the three habitats, the pickleweed plain ranked lowest in relative elevation, pH and bulk density and highest in soil salinity, moisture, N and organic matter, though neither elevation nor organic matter showed significant differences (Table 1). Soil salinity was significantly lower in the mixed species channel plots than in the other two habitats (Table 1). Pickleweed channel and mixed species plots were similar in organic matter, moisture, bulk density, N and pH.

Precipitation was lower than average during the study period. The Marin Civic Center, 5 km to the west of the study site, received 25.40 and 56.67 cm of precipitation in 2007 and 2008 compared to the 30-year average between 1971 and 2000 of 87.10 cm (NCDC 2004). The period of February–November 2008 was the driest on record for California (NCDC 2009).

Field experiment

Soils

Salt addition significantly affected soil responses overall (MANOVA response variables of salinity and pH), as did habitat (Table 2). *Post hoc* analyses showed that the significant effect of salt addition on soils in the MANOVA was driven primarily by the slight but significant increase in pH in all three habitats when salt was added (pickleweed plain $P = 0.016$, pickleweed channel $P = 0.002$ and mixed species $P = 0.029$; *post hoc* analyses in Appendix, see online supplementary material). There was no significant effect of N on the measured soils properties (Table 2).

Plants

The effect of added N on plants in the field experiment was highly significant: for both the pickleweed and the mixed species MANOVAs, $P < 0.0005$ (Table 2 and Fig. 2). Species (mixed species MANOVA) and habitat (pickleweed MANOVA) also had highly significant overall effects ($P < 0.0005$; Table 2). Salt tended to influence mixed species plants ($P = 0.051$) but had no overall effect on pickleweed responses ($P = 0.679$) (Table 2).

Adding N increased *S. pacifica* tissue N significantly in all three habitats (for *post hoc* analyses, see Appendix, online supplementary material; Fig. 2). Live biomass increased significantly in N-addition plots in pickleweed channel ($P = 0.001$) and mixed species habitats ($P = 0.010$) and tended to do so on the pickleweed plain ($P = 0.053$). N increased pickleweed height in the mixed species habitat ($P = 0.001$) but not in the other habitats (Fig. 2). Pickleweed branching increased greatly in the pickleweed plain and mixed species habitats and tended to increase in the pickleweed channel habitat ($P = 0.056$; Fig. 2).

Like *S. pacifica*, both *D. spicata* and *J. carnosa* responded to N addition in the mixed species plots, but response varied by species (Fig. 2). N addition increased the total biomass of *D. spicata*, though it had more dead than live biomass. *Jaumea carnosa* height increased significantly with added N ($P = 0.008$; Fig. 2). Tissue N of both *D. spicata* and *J. carnosa* increased with N addition ($P = 0.015$ and $P = 0.003$, respectively).

While salt addition had no overall effect on *S. pacifica* across habitats (Table 2), the trend in the mixed species habitat was driven in part by pickleweed, which had significantly more total biomass ($P = 0.042$) but shorter height ($P = 0.009$) in salt addition plots compared to control and in salt + N plots compared to N only (Fig. 2; for *post hoc* analyses, see Appendix, Table 4, online supplementary material). *Distichlis spicata* also decreased in average height ($P = 0.018$; Fig. 2) and had less biomass of dead *D. spicata* compared to control or N addition plots ($P = 0.043$; see Appendix, online supplementary material). There was no effect of salt on *J. carnosa*.

Sarcocornia pacifica gained in percent cover in all mixed species plots, including controls, and especially in N addition plots (Fig. 2). *Jaumea carnosa*, on the other hand, lost significantly more cover in the N addition plots than in other treatments. *Distichlis spicata* declined significantly in percent cover in all

Table 1: field soil characteristics, from samples taken June 2007 before treatments began

	Habitat			*F*	df	*P*
	PW plain	PW channel	Mixed species channel			
Relative elevation (cm)	4.51 ± 0.33	5.64 ± 0.26	4.98 ± 0.37	3.049	2	0.055
Salinity (ppt)	83.4 ± 5.9[b]	72.2 ± 1.2[b]	47.8 ± 2.5[a]	23.259	2	<0.001
pH	5.40 ± 0.10[a]	5.74 ± 0.09[ab]	5.90 ± 0.10[b]	6.809	2	0.011
Bulk density (g/cm^3)	0.55 ± 0.20[a]	0.72 ± 0.01[b]	0.71 ± 0.01[b]	43.507	2	<0.001
Moisture (%)	58.2 ± 1.0[b]	50.0 ± 0.7[a]	49.8 ± 0.4[a]	41.626	2	<0.001
Organic matter (%)	11.8 ± 0.6	10.6 ± 0.5	10.9 ± 0.4	1.344	2	0.269
N (%)	0.348 ± 0.044	0.280± 0.014	0.297± 0.014	1.627	2	0.237

Different letters (a or b) indicate significant differences among habitats in *post hoc* Tukey tests following ANOVA. Abbreviations: PW = pickleweed, df = degrees of freedom.

Table 2: MANOVA results of field treatment effects on soil measures and plant characteristics of pickleweed and mixed species plots

Field experiment factor	F	df	P	η_p^2
Soil Measures				
Nitrogen	1.700	2	0.197	0.089
Salt	13.858	2	<0.0005	0.442
Habitat	15.421	4	<0.0005	0.461
Nitrogen × salt	0.210	2	0.811	0.012
Habitat × nitrogen	1.274	4	0.288	0.066
Habitat × salt	0.189	4	0.943	0.010
Habitat × nitrogen × salt	1.307	4	0.276	0.068
Pickleweed				
Nitrogen	17.496	6	<0.0005	0.772
Salt	0.665	6	0.679	0.114
Habitat	4.376	12	<0.0005	0.451
Nitrogen × salt	1.088	6	0.391	0.174
Habitat × nitrogen	1.773	12	0.072	0.249
Habitat × salt	1.015	12	0.446	0.160
Habitat × nitrogen × salt	0.618	12	0.819	0.104
Mixed species				
Nitrogen	17.431	6	<0.0005	0.905
Salt	3.073	6	0.051	0.626
Nitrogen × salt	0.646	6	0.693	0.261
Species	76.257	12	<0.0005	0.995
Species × nitrogen	1.312	12	0.406	0.759
Species × salt	0.294	12	0.962	0.414
Species × nitrogen × salt	0.330	12	0.946	0.442

Pickleweed and soil MANOVAs include measures from all three habitats; mixed species tests were conducted on the three species within that habitat. Pickleweed and mixed species response variables were dry biomass, height change, percent N, percent ash and percent moisture. The pickleweed MANOVA also included pickleweed branching, while change in percent cover was included in the mixed species MANOVA. Soil response variables were salinity and pH. The F-statistic is Pillai's Trace. Abbreviation: df = degrees of freedom.

treatments ($P < 0.001$—*post hoc* paired samples t-test compared to Spring 2007) with no treatment effects (Fig. 2). The overall increase in *S. pacifica* and decrease in *D. spicata* cover may reflect the extremely dry conditions of the two growing seasons of this study.

While the presence of the parasitic *C. salina* presumably had some influence on its hosts, we found no effect on host height or percent cover. Neither cover nor biomass of *C. salina* was significantly affected by salinity or N treatment (see Appendix, online supplementary material).

Greenhouse experiment

Soils

N and salinity had significant effects on soil variables overall ($P < 0.0005$) and a significant interactive effect ($P = 0.003$; Table 3 and Fig. 3).

N addition led to significant increases in soil salinity in both the pickleweed ($P < 0.001$) and the mixed species pots ($P < 0.0005$; Fig. 3). Salinities in mixed species soils averaged slightly lower than in pickleweed monocultures, by 2–4 ppt depending on N treatment. All treatments in the greenhouse developed higher soil salinities than intended, as in other sub-irrigated greenhouse experiments (Bonin and Zedler 2008; Kuhn and Zedler 1997), but salinities remained in the range of field measures (Table 1).

Despite the strong growth response of plants to N addition (see below), fertilized pots had more than double the N content of unfertilized soils (Fig. 3), and effects were significant in both pickleweed monoculture and mixed species treatments ($P < 0.0005$ for both).
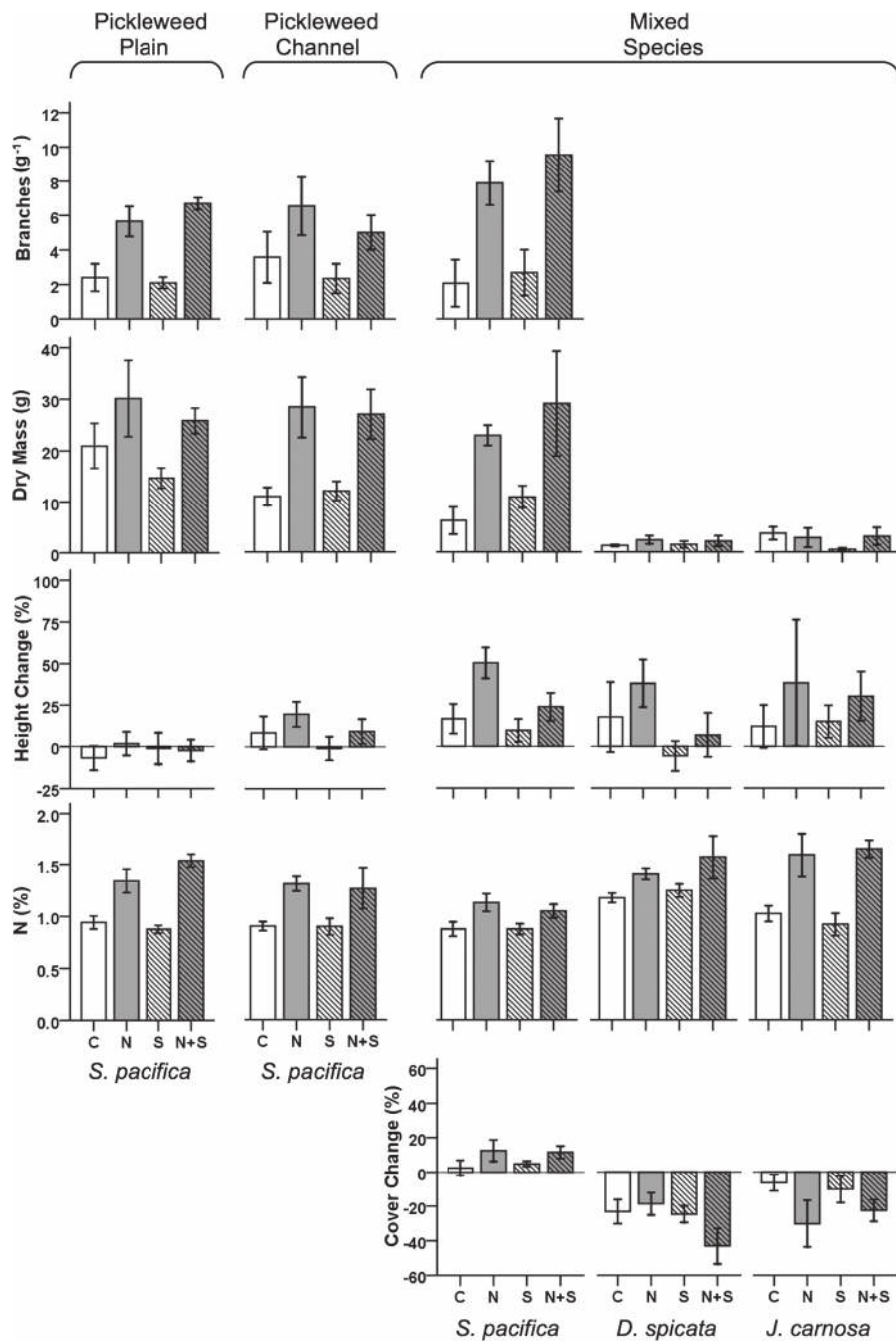
Plants

Overall, N and salinity both had highly significant effects on pickleweed responses ($P < 0.0005$; Table 3 and Fig. 4). Diversity level (i.e. growing in monoculture or mixture) also had strong effects on pickleweed ($P = 0.001$). Significant interactions indicate that N response varied with both salinity ($P = 0.014$) and diversity level ($P < 0.0005$). The effect size (η_p^2) of N addition was the largest among the factors, followed by the N × diversity interaction (Table 3).

In the mixed species MANOVA, N, salinity, species and the species × N interaction all had highly significant effects ($P < 0.0005$; Table 3). The effect size of N was largest, but species and species × N interaction effects were nearly as large. N × salinity and species × salinity interactions were significant.

In *post hoc* analyses (see Appendix, online supplementary material), N addition increased all measured plant response variables (aboveground biomass, branching, tissue percent N) in both the pickleweed and the mixed species pots (Fig. 4). Salinity treatment affected tissue percent N in the pickleweed monocultures but not in the mixed species pots. N and salinity had a significant interactive effect on tissue N ($P = 0.001$) in the monocultures but only a trend ($P = 0.095$) in mixed species pots.

Sarcocornia pacifica responded to competition from other species differently depending on whether N was added (Fig. 4). The pickleweed MANOVA showed a significant diversity (i.e. growing alone or in mixture) × N interaction (Table 3). *Post hoc* analyses of diversity effects on *S. pacifica* separately in fertilized and unfertilized pots showed significantly lower biomass and tissue N in unfertilized pots when it was grown in mixture than in monoculture ($P < 0.0005$ for both). When pots were fertilized, however, there was no effect of diversity on any of the measured pickleweed response variables.

Distichlis spicata dominated the lower salinity treatments of the mixed species experiments, especially when N was added (Fig. 4). N addition significantly increased biomass and percent N ($P < 0.0005$ for both). N and salinity interactions were also significant for these measures. Higher salinity treatments significantly decreased *D. spicata* biomass but increased percent N in the tissue ($P < 0.0005$ for all three). While *D. spicata*

Figure 2: field experiment plant branching (*Sarcocornia pacifica* only), biomass, height change, percent N and percent cover change (mixed species only) by habitat and species. Error bars represent ±1 SE.

demonstrated a drop in shoot biomass with increasing salinity whether or not N was added, the decline was more pronounced with N addition (Fig. 4).

N significantly increased *J. carnosa* biomass ($P = 0.025$), branching ($P = 0.022$) and percent N ($P < 0.0005$; Fig. 4). Increased salinity caused a significant decline in *J. carnosa* biomass ($P = 0.004$). In both treatments, *J. carnosa* had less biomass at 20 or 35 ppt than at 5 ppt ($P = 0.001$).

DISCUSSION

Overall, our investigations into the interactive effects of nitrogen and salinity on Pacific US salt marsh species confirmed that both factors influence the structure of salt marsh plant communities, particularly the relative abundance and dominance of *S. pacifica*. The response of each species to N reflects competition between species to capture N and the competition within

Table 3: MANOVA results of greenhouse treatment effects on pickleweed, mixed species and soil measures

Greenhouse experiment factor	F	df	P	Partial η^2
Soil measures				
Nitrogen	41.706	3	<0.0005	0.641
Salinity	17.908	6	<0.0005	0.431
Diversity	2.667	3	0.054	0.103
Nitrogen × salinity	3.532	6	0.003	0.130
Diversity × nitrogen	0.495	3	0.687	0.021
Diversity × salinity	0.975	6	0.444	0.040
Diversity × nitrogen × salinity	0.884	6	0.508	0.036
Pickleweed				
Nitrogen	895.123	5	<0.0005	0.985
Salinity	4.190	10	<0.0005	0.233
Diversity	4.992	5	0.001	0.269
Nitrogen × salinity	2.331	10	0.014	0.145
Diversity × nitrogen	6.362	5	<0.0005	0.319
Diversity × salinity	1.461	10	0.160	0.096
Diversity × nitrogen × salinity	0.959	10	0.482	0.065
Mixed species				
Nitrogen	216.879	4	<0.0005	0.976
Salinity	5.010	8	<0.0005	0.477
Nitrogen × salinity	2.746	8	0.015	0.333
Species	59.764	8	<0.0005	0.966
Species × nitrogen	49.780	8	<0.0005	0.959
Species × salinity	2.574	16	0.009	0.534
Species × nitrogen × salinity	1.686	16	0.096	0.428

Pickleweed and soil MANOVAs include measures from both diversity levels; mixed species tests were conducted on the three species within those pots. Pickleweed and mixed species response variables were dry biomass, height change, percent N, percent ash and percent moisture. The pickleweed MANOVA also included pickleweed branching. Soil response variables were salinity, pH and N. The F-statistic is Pillai's Trace. Abbreviation: df = degrees of freedom.

each plant between the allocation of N for growth and for intercellular processes, such as water balance and photosynthesis. The outcomes of both internal and external competition for N are in part a function of the salinity stress to which a species is exposed and its mechanisms of stress tolerance. Our findings suggest that if both salinity and N continue to increase, the outcome may be detrimental to plant community diversity.

Soils

Our finding of significantly saltier soils in the greenhouse N addition treatments may reflect salt contributed by the urea fertilizer as well as increased water demand associated with increased growth; i.e. more salt could have been drawn into the soil column by rapidly growing plants, yet largely excluded from uptake at the roots. The higher soil salinities in pickleweed monocultures relative to the mixed species pots may have been due to several possible mechanisms. Though the succulent pickleweed stores more salt per unit mass than
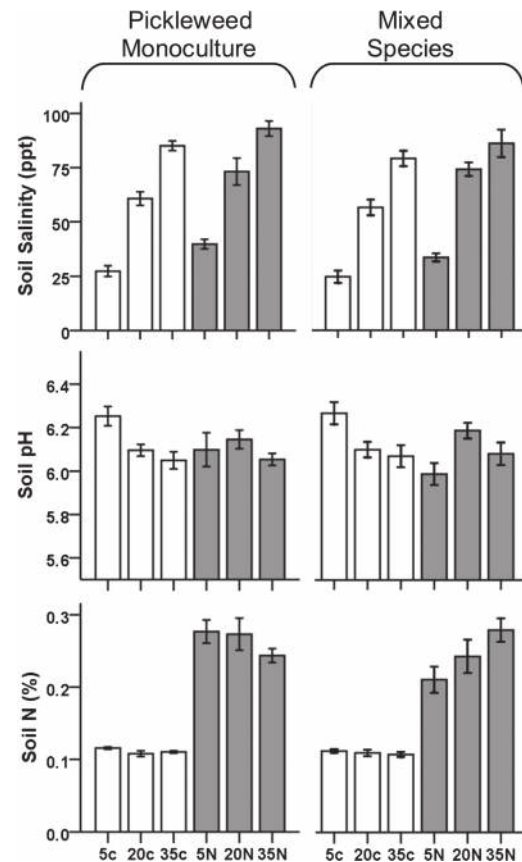

Figure 3: greenhouse response to treatments in soil percent N, salinity and pH. Treatments indicate salinity (5, 20 or 35 ppt) and whether N addition (N) or control (c). Error bars represent ±1 SE. n = 7 for salinity and pH and n = 5 for soil N. Salinity and pH are average between surface (0–2 cm) and 20 cm depth samples and N values are from surface samples.

D. spicata, in the lower salinity treatment, mixed species had far greater total biomass than the pickleweed monocultures, so may have taken up more total salt. This hypothesis is supported by greater total mass of ash in the mixed species treatment (data not shown; see Ryan 2009). In addition, C_4 grasses such as *D. spicata* have higher water use efficiency (Makino *et al.* 2003), so saltgrass may have drawn less saltwater up into the pots than the succulent *S. pacifica*.

Soil pH was slightly more acidic in greenhouse fertilization treatments as can occur with urea additions (Brady and Weil 1999) and may indicate excess N availability (Wallace 1994).

Concurrent changes to soil salinity along with N may have other implications for the availability of N in the soils regardless of the total amount. A Gulf Coast study found increases in marsh salinity increased microbial diversity but reduced enzyme activity associated with N and P mineralization (Jackson and Vallaire 2009). Thus, plants in high salinity environments may need nitrogen more, but have less access to it. Increases in N have also been found to change soil bacterial assemblages (Jackson and Vallaire 2009); thus, changes in both N and salinity could significantly alter nutrient cycling in the soils.
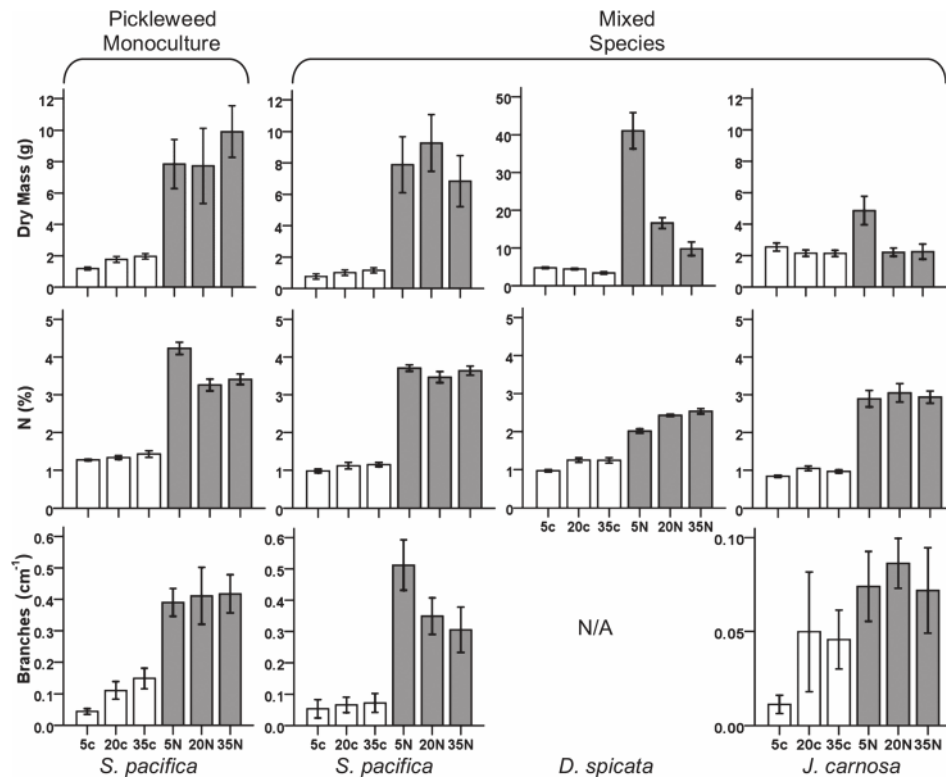
Figure 4: greenhouse dry biomass per plant, percent N and branching (*Jaumea carnosa* and *Sarcocornia pacifica* only) by diversity level and species. Note the difference in scale for mass of *Distichlis spicata*. Treatments as in Fig. 3. Error bars represent ±1 SE.

Additionally, increased salinity has been found to change the ratio of available N forms, leading to an increase in ammonium and a decrease of nitrates in the soil (Giblin *et al.* 2010). *Sarcocornia pacifica* appears to preferentially uptake ammonium (Page 1995) and it is possible that *S. pacifica* benefited competitively in higher salt treatments partly due to increased availability of the form of N it is best adapted to take up.

Plants

In both field and greenhouse experiments, *S. pacifica* responded positively to N, increasing in shoot biomass, branching and tissue N, regardless of salinity or presence of associated species in culture or field plots. The response of *S. pacifica* to N addition in our experiment echoes the findings of other investigators (Boyer *et al.* 2001; Covin and Zedler 1988; Pennings *et al.* 2002). As in this experiment, Boyer *et al.* (2001) found that N addition, even in an environment rich in N, increased biomass, branching and root and shoot N content.

Increased salinity had no discernable negative effects on *S. pacifica* growth. In the greenhouse experiment, *S. pacifica* shoot biomass was highest at the mid and high salinities. Other studies have found a peak in *S. pacifica* biomass at mid range salinities, primarily due to an increase in shoot ash content (Pearcy and Ustin 1984) or no effect of salinity on biomass

except a slight decline in woody tissue biomass at the highest salinity (Mahall and Park 1976). The ratio of woody and fresh tissue was not measured in this study, but a decrease in woody tissue is a possible explanation for why *S. pacifica* in the saltier pickleweed plain habitat (Table 1) did not increase in height when biomass increased with N addition (Fig. 2).

In the greenhouse, *S. pacifica* tissue N content increased with salinity in the unfertilized pots, perhaps indicating it was used in salinity tolerance mechanisms, but in fertilized monoculture pots, tissue N was highest in the low salinity treatment. Because the 5 ppt treatment should be the least stressful, one might expect allocation to growth rather than increased tissue N. Biomass did not differ in the fertilized treatments nor did overall N mass (%N × biomass), suggesting that the high N in the fertilized 5 ppt treatment may represent luxury uptake (Chapin 1980; Lipson *et al.* 1996).

There was no overall effect of salt addition on plants in the field experiment, though the additions were sufficient to cause changes in the soil (Table 2). The higher fraction of dead biomass in the plain habitat and total biomass in the mixed species habitat may indicate increased shedding of shoot tips when salt is added and warrants further investigation. Salt addition was associated with shorter stature in mixed species plots only. The overall lack of response of pickleweed to salinity treatments may simply reflect that this species is highly adapted to conditions of extreme salinity.

In our field experiment, *S. pacifica* was the superior competitor over the course of the experiment, increasing cover in all treatments, but especially those in which N was added. In the greenhouse, where the response of *S. pacifica* to intraspecific and interspecific competition can be compared, N availability clearly affected competitive dynamics. In unfertilized pots, interspecific competition exerted strong inhibitory effects on *S. pacifica*. Per plant dry weight was 44% lower in mixture as compared to monoculture. Tissue percent N was also significantly lower. However, when N was added, *S. pacifica* growth and N uptake were unaffected by competition. Similarly, a southern California study found that pickleweed was unaffected by competition with *Spartina foliosa* in fertilization treatments but had significantly less biomass when growing unfertilized with *S. foliosa* than alone (Covin and Zedler 1988). However, in a study involving the interactions of *S. pacifica* and another cordgrass, *Spartina alterniflora*, *S. pacifica* was found to be outcompeted by the exotic when nitrogen was added (Tyler *et al.* 2007). In a review of multiple N addition experiments on the US East Coast, *S. pacifica* was found to respond favorably to N in 50% of 12 studies (Pennings *et al.* 2005), whereas *S. alterniflora* responded positively in 100% of experiments. That study was unable to distinguish any pattern in *S. pacifica* response based on geographic location, plant community or environmental factors, but salinity was not among the variables considered. In our greenhouse study, although pickleweed was not measurably affected by competition in the fertilized treatments, the relative biomass of *D. spicata* was far greater than *S. pacifica* in the low salinity treatments. The competitive response of pickleweed to N addition may be more reliably positive in high salinity environments then low. Data on the salinity of the environment in question, and not just the identity of competitors, may be needed to adequately predict outcomes of competition between species for N.

Distichlis spicata appears to be strongly affected by salinity. In the greenhouse experiment, despite its dominance in the fertilized low salinity pots, *D. spicata* sharply declined in biomass at higher salinities, a finding reflected in earlier studies of *D. spicata* grown in monoculture (Barbour and Davis 1970; Smart and Barko 1980; Warren and Brockleman 1989). Tissue N did increase with salinity in *D. spicata*, perhaps indicating N use in salinity tolerance, but overall N mass (%N × dry mass) was 72% lower at 35 ppt compared to 5 ppt. Several studies have found that in some plants salinity can reduce N uptake or availability, either by interfering with transport mechanisms (Helal *et al.* 1975; Rubinigg *et al.* 2003) or by causing less N to be mineralized (Jackson and Vallaire 2009). Likewise, the total shoot N mass represented by all three species (but driven by *D. spicata* and *J. carnosa*) was lower in the 35 ppt fertilized treatment compared with 5 ppt treatments, while soil N was highest at this salinity, suggesting a reduction in N availability by one mechanism or another.

A recent study in San Francisco Bay that resurveyed plots originally established 30 years ago found a significant overall decline in *D. spicata* correlated to sea level rise and not salinity (Watson and Byrne 2009). Our data, however, suggest that salinity increases could indeed play a role in *D. spicata* decline. *Distichlis spicata* decreased in cover in all of our plots, possibly due to the extremely high ambient soil salinities during the study (Table 1), which occurred during 2 drought years. In addition to the general decrease in cover, *D. spicata* was significantly shorter in the salt addition treatments and produced less thatch (i.e. dead biomass) than other treatments, perhaps due to slowed growth earlier in the year leading to less dead biomass later. A 17-year-long study in Chesapeake Bay associated decline in biomass of the C_4 grasses *D. spicata* and *Spartina patens* with both increases in sea level and salinity (Erickson *et al.* 2007), supporting the conclusion that changes in salinity as well as sea level may impact *D. spicata* in coming years.

In the field experiment, N addition had no significant effect on *D. spicata* cover, biomass or height but did lead to an increase in tissue N content. Pennings *et al.* (2005) found that *D. spicata* biomass or percent cover responded favorably to N addition in 7 of 10 field studies. It is possible that the stressful field conditions that led to a general dieback of *D. spicata* on the marsh masked or overwhelmed any growth response to N, and the high tissue N content in fertilized plots suggests *D. spicata* may have allocated N to alleviating salt stress rather than growth.

Though *D. spicata* responded very positively to fertilization, that response was heavily tempered by salinity. Our greenhouse experiment found that the percentage of pot biomass occupied by *D. spicata* dropped at each successive increase in salinity and the relative biomass of *S. pacifica* increased. This effect was more, not less, pronounced in the fertilized treatments. In addition, while fertilized pots had higher *D. spicata* tissue N content than unfertilized pots, N content did not increase with salinity, suggesting that *D. spicata* has a limited ability to adjust to increasing salinity, even in conditions of high N.

Salinity treatments had no effect on *J. carnosa* in the field. As with the other species, the effects of salinity were more pronounced in the controlled environment of the greenhouse. In the greenhouse experiment, *J. carnosa* declined in biomass at higher salinity levels, a finding also consistent with earlier studies (Barbour and Davis 1970; St. Omer and Schlesinger 1980).

In those greenhouse treatments in which no N was added, *J. carnosa* tissue N increased at higher salinity, suggesting that in the unfertilized pots, N-based compounds may have been playing an important role in salt tolerance. When fertilized, *J. carnosa* tissue N was much higher but did not increase with salinity. As was the case with *D. spicata*, decrease in tissue N in the N addition treatments did not correspond with an increase in biomass, meaning that total N decreased in both species at higher salinities, perhaps reflecting a stress-mediated decline in competitive ability of these two species. In the field experiment, N addition increased the shoot N content and average height of *J. carnosa* but decreased its percent cover. At the same

time, *S. pacifica* increased in cover, suggesting that *S. pacifica* is a superior competitor.

Research implications

The human-induced increases in ambient salinity over the last century already seem to favor *S. pacifica*. Sediment cores from China Camp show that pollen from the family Chenopodiaceae, assumed to be *S. pacifica*, has increased in the last 60 years (Goman *et al.* 2008), corresponding to a 40% drop in mean freshwater flows through the Sacramento/San Joaquin river deltas (Nichols *et al.* 1986). Marsh transects across the salinity gradient in San Francisco Estuary found *S. pacifica* expansion associated with increased salinity and a general shift in salt-tolerant species up-estuary (Watson and Byrne 2009).

Increases in salinity will also likely impact seedling establishment. Another China Camp study reported strong correlation between seedling mortality and seasonal increases in salinity—even for *S. pacifica*, though at higher salinity than other species (Rankin 1992). Our finding of higher soil salinities in fertilized pickleweed treatments suggests that N increases could magnify salinity induced declines in seedling recruitment. While many clonal salt marsh species, including *D. spicata*, have been shown to be able to colonize sites too stressful for seedling establishment, via rhizomatous or stoloniferous growth (Shumway 1995), the colonization of non-adjacent sites may be increasingly limited.

Increasing salinity is often associated with decreased species richness (Crain *et al.* 2004; Odum 1988), but some work suggests that when an estuary is urbanized these decreases may be more precipitous. In two US East Coast estuaries, Sharpe and Baldwin (2009) found oligohaline marshes had species richness as high, or higher, than upstream freshwater marshes in a relatively undeveloped estuary, but species richness decreased by one-third at similar salinities in an urban estuary. An increased level of nutrients in the water column is among the factors that often differentiate urbanized estuaries from relatively pristine ones.

While plants can show remarkable resilience to one stressor, multiple stressors can reduce their ability to adapt (Day *et al.* 2008; Mendelssohn and Morris 2000). Vinebrooke *et al.* (2004) put forth a conceptual model in which both biodiversity and ecosystem function depend on whether members of a community show positive or negative co-tolerance for stressors. Our study found that adding N did not noticeably counteract the effects of increased salinity for less common species. Rather, additions of both N and salinity appeared to favor the marsh dominant *S. pacifica* at the expense of less common species.

Changes to salinity and N have already occurred within our study system and within many estuaries worldwide (Billen *et al.* 2009; Cloern 2001; Mirza 1998; Peterson *et al.* 1995). Future increases to these factors are likely to be coupled with simultaneous changes in CO_2, sea level and temperature. Our study suggests that increases in N and salinity have negative implications for community diversity, and such changes may undermine community resilience (Vinebrooke *et al.* 2004). For example, one study on the east coast of the USA found that increasing salinity and N may interact negatively with increasing CO_2 and water-logging to counteract the ability of marshes to keep pace with sea level rise (Langley *et al.* 2009).

As scientists, land managers and policy makers prepare for continuing environmental change, the importance of considering how multiple factors interact to affect natural systems is increasingly apparent (Seastedt *et al.* 2008; Williams and Jackson 2007). In order to maximize the resilience of ecosystems to climate and other changes, we need to understand not only how multiple factors interact but also to identify the factors for which management is possible.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

FUNDING

ACKNOWLEDGEMENTS

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