



Salinity tolerance and the decoupling of resource axis plant traits

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Nomenclature

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Introduction

The resource axis (Díaz et al. 2004) describes a syndrome of plant traits associated with nutrient capture and turnover. Also known as the leaf mass per area–leaf life span spectrum (Westoby et al. 2002) and the leaf economics axis (Wright et al. 2004), this concept quantifies the CR-S spectrum of Grime's (1977) scheme based on a few traits, including specific leaf area (SLA) and leaf nitrogen (N) concentration on a mass basis (leaf N_{mass}), which are positively correlated. This axis has proven robust in a variety of environments (Díaz et al. 2004; Wright et al. 2004) and is linked to predictable plant trait responses (Aerts 1999; Baltzer & Thomas 2010; Marino et al. 2010). However, an unresolved issue is how

Abstract

Questions: What ecophysiological relationships underlie the sorting of species from freshwater to inland salt marsh communities? Which plant functional trade-offs are present along a gradient of low to high salinity?

Location: New York State, USA.

Methods: We evaluated changes in species composition and plant functional traits along resource and salinity gradients at three sites using principal components, correlation and fourth-corner analyses.

Results: Analyses of plant traits suggested an axis of specialization that was related to salinity tolerance and distinct from a resource axis describing a spectrum of plant traits associated with resource acquisition and retention. Plant species with low scores on the salinity axis had highest abundance in the most saline plots and tended to have small leaf size, high leaf nitrogen per area (N_{area}), C_4 photosynthesis and succulence. Whereas C_4 photosynthesis, leaf size and leaf succulence were each linked to patterns of species presence and relative cover along soil Na:K and electrical conductivity (EC) gradients, leaf N_{area} was most positively correlated with salinity tolerance. Leaf size also played a key role in species sorting along each of the environmental gradients examined in this study (soil EC, Na:K, flooding duration, soil extractable calcium and magnesium and soil N concentrations).

Conclusions: Salinity tolerance is an alternative axis of plant specialization. The overall trade-off underlying salinity tolerance is inefficient N use in exchange for efficient water use and tolerance of toxic soil salinity levels. This research further elucidates the processes of community assembly along environmental gradients, trade-offs associated with salinity tolerance, and provides baseline information for wetland restoration.

specific, abiotically mediated selective pressures (e.g. soil salinity) alter resource axis trade offs and may result in alternative axes of specialization. For example, water and soil phosphorus availability have driven alternative axes of specialization and plant trait responses in chaparral (Ackerly 2004) and rain forest (Baltzer & Thomas 2010) ecosystems, respectively. Soil salinity is another abiotic stressor that exerts selective pressure on plants, and has the potential to alter how they use nutrients. In order to expand the trait-based conceptual framework and develop a better understanding of plant salinity tolerance in general, we evaluated how soil salinity drives plant trait expression.

Elevated soil salinity causes osmotic and ion toxicity stresses in plants. Halophytes have a variety of mechanisms

that result in tolerance to these stresses, principally the uptake and compartmentalization of mineral solutes (e.g. Na^+ and Cl^-) and the production of compatible organic solutes (e.g. Flowers et al. 1986; Glenn et al. 1999). In general, these adaptations are costly, because they require N investments in proteins and compatible osmotica that do not contribute to photosynthesis (Storey et al. 1977; Jefferies 1981; Flowers 1985; Flowers et al. 1986; Casas et al. 1992; Glenn et al. 1999; Kant et al. 2006; Radyukina et al. 2007; Flowers & Colmer 2008), and N is often limiting in terrestrial ecosystems, particularly salt marshes (Valiela et al. 1974; Vitousek & Howarth 1991).

Because adaptations to elevated salinity often have a high N demand, natural selection in such environments may prioritize N allocation to salinity tolerance as opposed to growth. As a result, the resource axis link between SLA ($\text{mm}^{-2}\cdot\text{mg}^{-1}$) and N_{mass} (%) may become uncoupled in these settings. Due to the N investments required for salinity tolerance, this uncoupling should manifest itself as an elevated N_{mass} at a given SLA, i.e. an increased [N] per unit leaf area, N_{area} ($\text{g N}\cdot\text{m}^{-2}$).

We conducted a study within the three extant inland salt marshes in central New York State (USA) in order to test whether the otherwise tight coupling of SLA and N_{mass} within plant communities breaks down in saline settings. Specifically, we predicted that (1) principal components analysis (PCA) of plant traits will identify an axis on which leaf N_{area} loads heavily, and along which SLA and leaf N_{mass} do not co-vary (i.e. eigenvector scores will not share similar value and sign); (2) trait values typically associated with salinity tolerance or tolerance to osmotic stress, such as low SLA, small leaf size (Givnish et al. 1984; Lambers et al. 2008), high leaf N_{area} (Ackerly 2004), annual life span (Burchill & Kenkel 1991), C_4 photosynthesis (Sage 2004) and succulence (Glenn et al. 1999), will co-vary along this axis; and (3) this axis will be strongly correlated with species presence and relative abundance along gradients of soil salinity.

Methods

Study site

We surveyed the vegetation of three inland salt/freshwater marsh sites near Montezuma, NY: Carncross (43.08° N, 76.71° W), Howland Island (43.07° N, 76.70° W) and Fox Ridge (43.05° N, 76.70° W). In terms of number and area, these sites represent most of the naturally occurring inland salt marshes in the northeastern USA. The inland salt marshes ranged in size from 0.15 to 3.00 ha, and were within freshwater marshes ranging from 3 to 40 ha. Average annual precipitation at a nearby weather station (Syracuse, NY) was 102 cm, distributed consistently throughout the year. Monthly average temperatures ranged from -5°

C in January to 22°C in July (National Water & Climate Center/Climatic Data Access Network 2002). At Carncross and Howland Island the soils were Martisco muck and Warners loam, respectively, and both had layers of marl within 1.5 m of the soil surface. The soils at Fox Ridge were Madalin silt loam and Fonda mucky silt loam (Soil Survey Staff 2006–2007).

Field data collection

In order to sample vegetation with equal effort in saline and non-saline communities, we used an adaptive cluster sample approach (Thompson 1990) that resulted in 133, 1.0-m^2 plots across the three sites. Vascular species presence was recorded and percentage cover was estimated in these plots in August 2007. Because native and non-native genotypes of *Phragmites australis* exist at the sites, cover estimates for each type were performed using the diagnostics of Blossey (2002). For 42 of the 49 species observed in the vegetation survey, we collected field- and literature-based data related to leaf traits, growth form and life span (Table 1). We measured the size of leaves collected from the upper canopy (one leaf per species per plot) by scanning at 800 dpi and processing with an auto-thresholding and pixel counting software that had 2% accuracy against known samples (ITT Visual Information Solutions, Boulder, CO, US). Leaves were dried at 60°C , weighed, ground and analysed for C and N concentration in an elemental analyser (Carlo Erba NC2500; Carlo Erba, Valencia, CA, US).

For each plot, we calculated flooding duration as the percentage of times each plot was flooded during bi-weekly monitoring from 22 May to 22 August 2007 and from 14 May to 30 October 2008. During June 2007, 0–10-cm depth soil samples were collected adjacent to all vegetation plots, transported in coolers, and stored in a refrigerator at 4°C . Within 2 d of collection the samples were extracted and analysed for NO_3^- and NH_4^+ (Keeney et al. 1982; BRAN + LUEBBE Method Nos. US-696F-82W and US-696D-82X for NO_3^- and NH_4^+ , respectively). The remainder of each soil sample was processed by air drying, grinding with a mortar and pestle and sieving (2 mm). Subsamples of the processed samples were homogenized with a wig-l-bug and analysed for total N with a Thermo Flash EA 1112 (Thermo Fisher Scientific; Waltham, MA, US). An additional set of 0–10 cm soil samples was collected in August 2007, processed as described above, and the following analyses were performed on separate subsamples: electrical conductivity (EC; Janzen & Carter 1993), extractable cation concentration (Ca^{2+} , Mg^{2+} , Na^+ and K^+ ; Hendershot et al. 1993) and pH (Thomas et al. 1996). Concentrations of cations, total N, NH_4^+ and NO_3^- were corrected to soil dry weights of 105°C .

Table 1. Traits observed on 42 species from the field sites.

Trait	Type of Variable (units)	Range	Source
Leaf size ¹	Continuous (mm ²)	9.2–14252.1	Field
Specific leaf area (SLA) ¹	Continuous (mm ² .mg ⁻¹)	3.8–56.9	Field
Leaf N concentration (N _{mass}) ¹	Continuous (%)	0.9–4.8	Field
Leaf N concentration (N _{area}) ¹	Continuous (g N.m ⁻² leaf area)	0.7–5.7	Field
Leaf C:N ¹	Continuous	8.9–47.1	Field
Succulence	Binary (1 = yes, 0 = no)	0, 1	Field
Graminoid growth form	Binary (1 = yes, 0 = no)	0, 1	Field
Rhizomatous growth	Binary (1 = yes, 0 = no)	0, 1	Field
Mature height ¹	Continuous (cm)	5.0–400.0	Literature ^{2,3,4}
Perennial life span	Binary (1 = yes, 0 = no)	0, 1	Literature ^{2,3,4}
C ₄ photosynthetic pathway	Binary (C ₄ = 1, C ₃ = 0)	0, 1	Literature ⁵

¹Variable log transformed prior to PCA, fourth-corner and correlation analyses.

²Flora of North America Editorial Committee (1993).

³Grass manual on the web (2002).

⁴Gleason & Cronquist (1991).

⁵Numerous references; please see Appendix S1.

Data analysis

We used PCA to identify groups of traits (Table 1) that describe axes of specialization related to nutrient resource use and salinity tolerance. Binary variables were included

in the PCA by following the approach suggested in Hill & Smith (1976), i.e. coded as zero and one and treated as continuous variables. Input data were species means of the field-based traits and the reported literature-based values (Appendix S1). The variable correlation matrix was used in the PCA.

Pearson correlation was used to test for association between species scores on PCA axes, species traits and salinity tolerance, which was indicated by the 95th percentile soil EC level at which a species was present in field plots. This percentile was used in lieu of the maximum soil EC level in order to limit the likelihood of rare phenomena driving results.

We used fourth-corner analyses (Legendre et al. 1997; Dray & Legendre 2008) to evaluate linkages between species traits or axes of specialization (represented by species PC scores), species presence or relative abundance and environmental gradients (summarized in Table 2 and Appendices S2 and S3). Fourth-corner analysis combines three matrices: **L** (plots*species), **R** (plots*environmental variables) and **Q** (species*traits), and measures the association between each column in **R** and **Q** (i.e. vectors of each environmental and trait variable) by way of species abundances (or, alternatively, by species presence/absence) in **L**. The type of association statistics calculated depends on the types of variables involved. When both environmental and trait variables are qualitative, the analysis computes a chi-squared statistic; if two quantitative variables are tested, a Pearson correlation coefficient is calculated; and if one of each type is tested, the analysis computes a correlation ratio (Dray & Legendre 2008). We tested the significance of each association by permuting **L** according to the recommendations of Dray & Legendre (2008; ade4 package of Dray & Dufour 2007). The species trait data (**Q**) included mean trait values, literature-based values and species scores on important PCA axes. Separate

Table 2. Summaries of environmental variables used in the fourth-corner analyses. Frequency distributions and correlations among these variables are presented in Appendices S2 and S3, respectively.

Variable	Unit	Minimum	Median	Mean	Maximum
Soil Variables					
EC ¹	dS m ⁻¹	1.1	12.6	21.7	106.2
Ca ¹	cmol _c kg ⁻¹	8.4	34.2	35.7	69.4
Mg ¹	cmol _c kg ⁻¹	0.9	3.0	3.5	9.6
Na:K ¹	cmol _c cmol _c ⁻¹	1.5	51.3	76.5	333.2
pH		3.6	6.6	6.5	8.6
Total N ¹	%	0.2	1.1	1.3	3.2
NO ₃ ⁻	mg kg ⁻¹	0.1	0.8	3.7	37.4
NH ₄ ⁺	mg kg ⁻¹	2.7	9.8	17.3	85.3
Water Variable					
Flooding Duration ¹	% of sampling events	0	0	3.3	26.7

¹Variable log transformed prior to PCA and fourth-corner analyses.

fourth-corner analyses were run with relative cover and presence/absence data in **L** in order to describe processes of plant presence vs competitive dominance (e.g. Keddy 1992; Cingolani et al. 2007).

Results

Four PCA axes explained 77% of the variation in the plant traits data set, with axes one through four explaining 35%, 18%, 14%, and 10%, respectively (Table 3). The first PCA axis (PC1) was consistent with the resource/leaf economics axis (Díaz et al. 2004; Wright et al. 2004). Traits related to resource acquisition and turnover were highly correlated with this axis, such as SLA (0.43), leaf C:N (−0.43) and N_{mass} (0.40; Table 3). Forbs and annuals had high scores on PC1 (Table 3, Fig. 1). Invasive species such as *P. australis*, *Lythrum salicaria*, *Phalaris arundinacea*, *Typha × glauca* and *Iris pseudacorus* had low scores on PC1, suggesting that their widespread dominance in wetlands is not related to nutrient capture as expressed along a resource axis (i.e. PC1). Most of these invasive species scored low on PC3, which represents an axis negatively correlated with leaf size and plant height (Table 3).

The second principal component, PC2, was most highly correlated with leaf size (0.43), leaf N_{area} (−0.37), C_4 photosynthesis (−0.37) and succulence (−0.36). While SLA was marginally correlated (0.28) with PC2, leaf N_{mass} was not (0.01). These results are consistent with our first and second predictions related to a salinity axis, along which SLA and leaf N_{mass} do not co-vary. Species scoring low on PC2 are known to persist in moderate to highly saline environments [e.g. *Leptochloa fusca* subsp. *fascicularis* (Qadir et al. 1996), *Spergularia salina*, *Puccinellia distans* and *Atriplex patula* (Ungar 1996; Piernik 2005)]. Although leaf N_{area} loaded heavily in comparison to other traits on PC2 and PC3, a key difference between these axes is that leaf

N_{area} and leaf size vary in opposite directions on PC2 and in same direction on PC3 (Table 3).

Correlations between species PC scores and 95th percentile values of soil EC suggest that species location on PC2 was the best multivariate indicator of salinity tolerance as measured in the field (Table 4), partially confirming our third prediction. Among the plant traits evaluated, leaf N_{area} had the strongest correlation (0.64) with 95th percentile soil EC values. The importance of leaf N_{area} for salinity tolerance and subsequent impacts on plant community characteristics is illustrated by the convergence to relatively high community mean leaf N_{area} values as soil EC increases (Fig. 2). SLA and species score on PC1 were also negatively correlated with these EC percentile values (Table 4).

The fourth-corner analyses suggest that species–environment associations are driven primarily by leaf size, mature height and salinity tolerance (Table 5). The results suggest that tall species with large leaves are excluded from stressful edaphic environments (i.e. elevated soil pH, EC, Na:K, flooding duration) but favoured in those with high soil Mg^{2+} , total N, NO_3^- and NH_4^+ concentrations. Correlation analyses among soil variables (Appendix S3) show that many of these fertility-related variables co-vary across our study sites, and that soil EC and Na:K are part of a separate gradient corresponding with soil total N levels. Low species scores on PC3 were associated with high soil NO_3^- and NH_4^+ concentrations (Table 5). Species scoring high on the salinity axis (PC2) or those without leaf succulence were less likely to be present in settings with elevated soil pH, EC and Na:K, and flooding duration, but were more likely to be present in fertile soils (Table 5). Photosynthetic pathway did not constrain species presence in plots; however, relative cover of C_4 species increased as soil Na:K and flooding duration increased and as soil total N decreased. Alternatively, most traits (particularly leaf size, mature height, succulence and salinity axis score) were more frequently a constraint on species presence along environmental gradients than on relative cover. Although SLA and location on the resource axis (i.e. species score on PC1 in our study) have been the focus of substantial interest in plant trait research, these variables were not significant in controlling species patterns across any of the environmental gradients (Table 5).

Discussion

Soil salinity gradients and species salinity tolerance are key variables driving species patterns in saline ecosystems (Rozema et al. 1985; Partridge & Wilson 1988; Brewer & Grace 1990; Burchill & Kenkel 1991; Wilson et al. 1996). Despite the fact that the physiological attributes of salinity tolerance have been thoroughly studied (Flowers 1985;

Table 3. Eigenvector scores of plant traits on principal components one through four.

Trait	PC1	PC2	PC3	PC4
Specific leaf area	0.43	0.28	0.13	−0.17
Leaf N_{mass}	0.40	0.01	−0.30	−0.38
Succulence	0.17	−0.36	0.09	0.53
Leaf size	−0.01	0.43	−0.52	0.10
C_4	−0.13	−0.37	−0.25	−0.25
Mature height	−0.23	0.22	−0.55	0.25
Graminoid	−0.26	−0.29	0.03	−0.54
Leaf N_{area}	−0.29	−0.37	−0.37	−0.01
Rhizomatous	−0.31	0.33	0.15	−0.18
Perennial	−0.35	0.31	0.17	−0.20
Leaf C:N	−0.43	0.05	0.23	0.25
% variance explained	35	18	14	10
Cumulative % variance explained	35	53	67	77

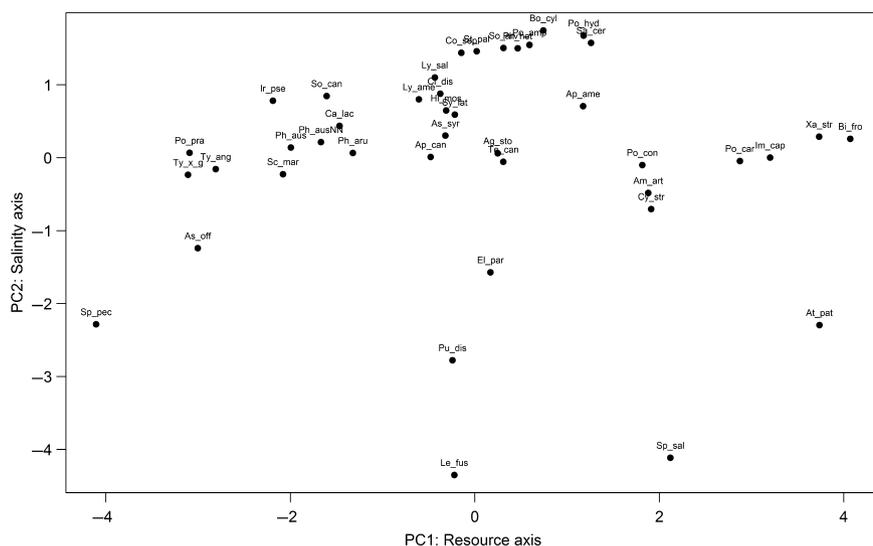


Fig. 1. Species scores on PCA axes 1 and 2. Points are labelled with species abbreviations defined in Appendix S1. The ordination is based on 42 species and 11 traits (Tables 1 and 3).

Table 4. Pearson correlation coefficients between species trait values or PC axis scores and species 95th percentile soil EC values corresponding with presence ($n = 42$). Percentile values were taken as an indicator of species tolerances of soil salinity and were used in lieu of maxima in order to reduce the likelihood of rare, extreme values dominating the analysis.

Trait	95th percentile soil EC value
Leaf size	-0.42**
Specific leaf area	-0.52***
Leaf N_{mass}	-0.07
Leaf N_{area}	0.64***
Leaf C:N	0.02
Mature height	-0.02
PC1 – resource axis	-0.32*
PC2 – salinity axis	-0.60***
PC3	-0.03
PC4	-0.18

P-values of the associations are indicated as: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Flowers et al. 1986; Glenn et al. 1999; Flowers & Colmer 2008), a whole-plant resource economics understanding of it has been lacking (e.g. Ogburn & Edwards 2010). Here we take a resource economics perspective by evaluating salinity tolerance as a function of SLA, leaf N_{mass} and N_{area} because these traits may provide a conceptual link to a well-known spectrum of plant trait responses: the resource axis.

In this study of inland salt and freshwater marsh plant communities in central New York, we found that tolerance of elevated salinity is conferred by decoupling of resource axis co-variation between SLA and leaf N_{mass} , and that this decoupling is indicated by an elevated leaf N_{area} . Lower-

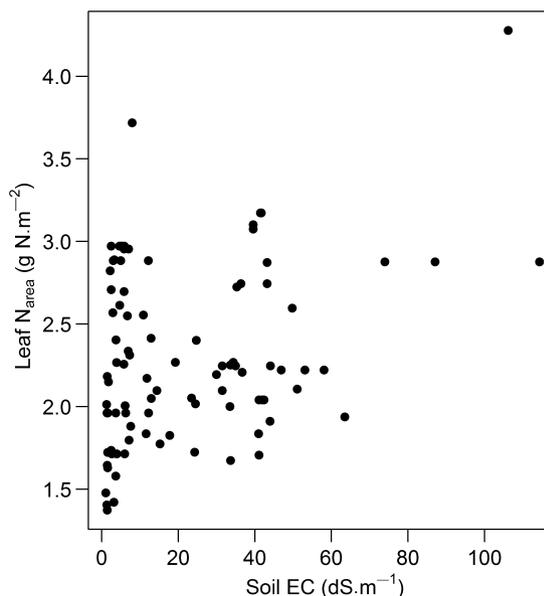


Fig. 2. Community mean leaf N_{area} ($\text{g N}\cdot\text{m}^{-2}$) across soil EC ($\text{dS}\cdot\text{m}^{-1}$).

envelope community mean leaf N_{area} values consistently increased with soil salinity, to the extent that at the highest levels of salinity, only species with elevated leaf N_{area} were present (Fig. 2). This trait was the strongest correlate with our field-based measure of salinity tolerance (Table 4) and was highly correlated with the salinity axis, likely because this trait indicates a variety of ways by which plants tolerate salinity, such as compartmentalization of salt in vacuoles and the production of compatible organic solutes

Table 5. Results from fourth-corner analyses. Black cells represent significant positive associations between a given trait or PCA axis and a given environmental variable (grey, negative). Analyses using presence/absence and relative cover data are summarized separately.

Trait	pH	EC	Na:K	Flooding	Ca	Mg	Total N	NO ₃	NH ₄
Presence/absence									
Perennial				**			*		
Rhizomatous									
Graminoid									
C ₄							*		
Succulent		**	**				*		
Leaf size	***	*	*		*	**	***	**	***
SLA									
Leaf N _{mass}					*				
Leaf N _{area}									
Leaf C:N					*				
Mature height				**	*	*	**	*	**
PC1 – resource axis									
PC2 – salinity axis	**	***	***	***		***	***		*
PC3								*	*
PC4									
Relative cover									
Perennial				*					
Rhizomatous									
Graminoid									
C ₄			*	*			*		
Succulent									
Leaf size	**					*	*		*
SLA									
Leaf N _{mass}					*				
Leaf N _{area}									
Leaf C:N									
Mature Height.									
PC1 – resource axis									
PC2 – salinity axis	*	*	*				*		
PC3				*					
PC4								*	

P-values of the associations are indicated as: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

(Glenn et al. 1999), which helps isolate toxic ions and maintain favourable osmotic gradient in saline soils; production and maintenance of glands that expel salt from plants (Munns & Tester 2008); tight packing of photosynthetic proteins and/or reduced cellular expansion rates, associated with increased water use efficiency (Wright et al. 2003; Munns & Tester 2008) and tolerance of osmotic stress (Reich et al. 1999; Wright et al. 2001, 2003; Ackerly 2004; Cornwell & Ackerly 2009). Whereas these mechanisms are important for salinity tolerance, they also come at a nutrient cost. This cost is expressed as a decoupling of N_{mass} and SLA because these mechanisms of salinity tolerance require N investments that are not related to resource axes phenomena (e.g. resource uptake and turnover). Hence, the overall trade-off associated with persisting in highly saline settings appears to be copious N use (relative to SLA) in exchange for efficient water use and tolerance of potentially toxic soil salinity levels (*sensu* Wright et al. 2003).

Although leaf N_{area} was the strongest correlate with salinity tolerance, it was not detected in the fourth-corner analyses as a driver of species patterns across soil EC and Na:K gradients. This lack of detection may be a result of this trait only being linked to species patterns at elevated soil EC levels. That is, community mean leaf N_{area} varies widely at low soil EC, but converges on high values at high soil EC, indicating that species with low leaf N_{area} are excluded as soil EC increases (Fig. 2). On the other hand, the salinity axis was linked to species patterns across soil EC and Na:K gradients, suggesting that the overall accumulation of trade-offs associated with the other salinity axis traits measured in this study – leaf size, C₄ photosynthesis and succulence – underlie the sorting of species along these gradients. Further, each of these traits was independently detected as an important driver of species sorting along soil EC or Na:K gradients (Table 5).

C₄ plants composed a larger part of the community on a relative cover basis in plots with high soil Na:K. However,

this association was absent when species presence data were used in lieu of cover, suggesting that C_4 photosynthesis is not required for establishment in high Na settings but may confer a competitive advantage there (Kalapos et al. 1997; Wang 2002a,b, 2004; Sage 2004; Wang et al. 2006). Typically C_4 species are not favoured in cool temperate regions such as the location of our field site, where in the absence of elevated soil salinity they are subject to early season preemption by C_3 species (e.g. Pearcy & Ehleringer 1984; Bertness 1991). C_4 photosynthesis is not considered to be an adaptation specifically to elevated soil salinity, but several associated processes serve to increase energy returns on investment in saline settings. These include reduced photorespiration with salt-induced stomatal closure (Sage 2004) and low transpiration rates that reduce Na loading to leaves, increase leaf life span and reduce energy expenditure in salt excretion (Adam 1990). In addition, the high N use efficiency of C_4 photosynthesis may allow for increased allocation of N to salinity tolerance (Adam 1990).

Leaf succulence may also serve to improve energy returns on leaf investment by replacing expensive C structures with water (*sensu* Shipley 1995), and therefore allowing for increased C investment in mechanisms of salinity tolerance. The fourth-corner analysis showed a strong relationship between saline settings and presence of plants with succulent leaves (Table 5), although not increased relative cover of succulent plants. This energy economics perspective expands upon the current view of succulence, namely, that it is a function of vacuolar expansion during the compartmentalization of mineral solutes (Munns & Tester 2008).

Of the plant traits considered in this study, leaf size was most frequently linked to environmental gradients, although it was more frequently a constraint on species presence than relative cover (Table 5). Species with small leaves were more likely to be present in settings with elevated soil EC or Na:K and in settings with low soil Mg, total N, NH_4^+ or acidity. Leaf size tends to correlate negatively with soil water scarcity because the C gain of large leaves is outweighed by the below-ground C costs required for adequate water supply (Givnish et al. 1984; Fonseca et al. 2000; Ackerly 2004). An analogous C trade-off occurs along fertility gradients, in which leaf size is limited in infertile settings (Givnish et al. 1984; Fonseca et al. 2000).

Although SLA and the resource axis have been the subject of much attention in functional ecology, and in our study were linked to salinity tolerance (Table 4), these variables were unimportant in driving species patterns along the environmental gradients examined in this study (Table 5). Particularly surprising was the lack of a correlation between measures of mineral N availability (NO_3^- , NH_4^+) and species SLA or resource axis scores. One

explanation for this lack of detection is that plant N availability was not adequately measured in this study, limiting our ability to detect an impact of SLA on species patterns. Another possible explanation for the lack of detection may be the interplay of SLA and leaf size, in that the additional structural investments typically needed to deploy large leaves result in leaf mass increasing more rapidly than leaf size (Shipley 1995; Milla & Reich 2007; Niklas et al. 2007). Hence, the apparent selection for large leaves in settings with low soil EC, Na:K and pH, and high levels of soil nutrients, particularly mineral N [Table 5; see also Givnish (1987)], may have driven species with large leaves down the resource axis via reduced SLA values. However, additional work evaluating the relative strength of selection on leaf size vs specific leaf area along wetland stress and resource gradients is needed to better support this explanation.

In summary, this paper reveals the numerous functional underpinnings of species assembly along environmental gradients spanning from freshwater to saline ecosystems, and that adaptations to one abiotically mediated selective pressure (i.e. soil salinity) alter resource axis trade-offs resulting in an alternative axis of specialization. Further research evaluating the salinity axis and its correspondence with other halophyte traits should be conducted in other saline ecosystems to better describe this axis. Additionally, discrepancies between trait-based controls over species presence and relative abundance warrants further evaluation to better understand mechanisms of community assembly along environmental gradients (Cingolani et al. 2007), and to understand the contexts within which discrepancies may be expected. Although leaf N_{area} was the strongest correlate with salinity tolerance, species with relatively high values for this trait were not necessarily excluded from, and can be dominant in, non-saline settings (e.g. *Phragmites australis*, *Typha* × *glauca*; Appendix S1), suggesting that additional research is warranted to better understand the role of leaf N_{area} in community assembly and the ways in which the trade-offs associated with high leaf N_{area} can be overcome by other traits.

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References

- Ackerly, D. 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs* 74: 25–44.
- Adam, P. 1990. *Salt marsh ecology*. Cambridge University Press, New York, NY, US.
- Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant–soil feedbacks. *Journal of Experimental Botany* 50: 29–37.
- Baltzer, J.L. & Thomas, S.C. 2010. A second dimension to the leaf economics spectrum predicts edaphic habitat association in a tropical forest. *PLoS ONE* 5: e13163.
- Baskin, J.M., Baskin, C.C. & Chester, E.W. 1999. The Big Barrens region of Kentucky and Tennessee. In: Anderson, R.C., Fralish, J.S. & Baskin, J.M. (eds.) *Savannas, barrens, and rock outcrop plant communities of North America*. pp. 190–205. Cambridge University Press, New York, NY, US.
- Bertness, M.D. 1991. Interspecific interactions among high marsh perennials in a New England salt marsh. *Ecology* 72: 125–137.
- Blossey, B. 2002. *Morphological differences between native and introduced genotypes*. Cornell University Ecology and Management of Invasive Plants Program. Available at: <http://invasiveplants.net/phragmites/morphology.htm>.
- Brewer, J.S. & Grace, J.B. 1990. Plant community structure in an oligohaline tidal marsh. *Vegetatio* 90: 93–107.
- Burchill, C.A. & Kenkel, N.C. 1991. Vegetation–environment relationships of an inland boreal salt pan. *Canadian Journal of Botany* 69: 722–732.
- Casas, A.M., Nelson, D.E., Raghothama, K.G., D'Urzo, M.P., Singh, N.K., Bressan, R.A. & Hasegawa, P.M. 1992. Expression of osmotin-like genes in the halophyte *Atriplex nummularia* L. *Plant Physiology* 99: 329–337.
- Cingolani, A.M., Cabido, M., Gurruchaga, D.E., Renison, D. & Díaz, S. 2007. Filtering processes in the assembly of plant communities: are species presence and abundance driven by the same traits? *Journal of Vegetation Science* 18: 911–920.
- Cornwell, W.K. & Ackerly, D.D. 2009. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs* 79: 109–126.
- Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C., Jalili, A., Montserrat-Martí, G., Grime, J.P., Zarrinkamar, F., Asri, Y., Band, S.R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Perez-Harguindeguy, N., Perez-Rontome, M.C., Shirvany, F.A., Vendramini, F., Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., de Torres-Espuny, L., Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martinez, M., Romo-Díez, A., Shaw, S., Siavash, B., Villar-Salvador, P. & Zak, M.R. 2004. The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science* 15: 295–304.
- Dray, S. & Dufour, A.B. 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22: 1–20.
- Dray, S. & Legendre, P. 2008. Testing the species traits–environment relationships: the fourth-corner problem revisited. *Ecology* 89: 3400–3412.
- Flora of North America Editorial Committee. 1993. *Flora of North America north of Mexico*. 14 + vols. New York and Oxford. Available at: <http://fna.org>.
- Flowers, T.J. 1985. Physiology of halophytes. *Plant and Soil* 89: 41–56.
- Flowers, T.J. & Colmer, T.D. 2008. Salinity tolerance in halophytes. *New Phytologist* 179: 945–963.
- Flowers, T.J., Hajibagheri, M.A. & Clipson, N.J.W. 1986. Halophytes. *The Quarterly Review of Biology* 61: 313–337.
- Fonseca, C.R., Overton, J.M., Collins, B. & Westoby, M. 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. *Journal of Ecology* 88: 964–977.
- Givnish, T.J. 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106: 131–160.
- Givnish, T.J. 1984. Leaf and canopy adaptations in tropical forests. In: Medina, E., Mooney, H.A. & Vázquez-Yanes, C. (eds.) *Physiological ecology of plants of the wet tropics*. pp. 51–84. Dr. W. Junk, The Hague, NL.
- Gleason, H.A. & Cronquist, A. 1991. *Manual of vascular plants of northeastern United States and adjacent Canada*. New York Botanical Garden, Bronx, NY, US.
- Glenn, E.P., Brown, J.J. & Blumwald, E. 1999. Salt tolerance and crop potential of halophytes. *Critical Reviews in Plant Sciences* 18: 227–255.
- Grass manual on the web. 2002. Utah State Intermountain Herbarium. Available at: <http://herbarium.usu.edu/web-manual>.
- Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* 111: 1169–1194.
- Hendershot, W.H., Lalande, L. & Duquette, M. 1993. Ion exchange and exchangeable cations. In: Carter, M.R. (ed.) *Soil sampling and methods of analysis*. pp. 167–176. Lewis, Boca Raton, FL, US.
- Hill, M.O. & Smith, A.J.E. 1976. Principal components analysis of taxonomic data with multi-state discrete characters. *Taxon* 25: 249–255.

- Iverson, L.R., Ketzner, D. & Karnes, J. 1999. Illinois plant information network. Illinois Natural History Survey and USDA Forest Service. Available at: <http://fs.fed.us/ne/delaware/ilpin.html>.
- Janzen, H.H. 1993. Soluble salts. In: Carter, M.R. (ed.) *Soil sampling and methods of analysis*. pp. 161–166. Lewis, Boca Raton, FL, US.
- Jefferies, R.L. 1981. Osmotic adjustment and the response of halophytic plants to salinity. *BioScience* 31: 42–46.
- Kalapos, T., Balogh -Nyakas, A. & Csontos, P. 1997. Occurrence and ecological characteristics of C₄ dicot and Cyperaceae species in the Hungarian flora. *Photosynthetica* 33: 227–240.
- Kant, S., Kant, P., Raveh, E. & Barak, S. 2006. Evidence that differential gene expression between the halophyte, *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na⁺ uptake in *T. halophila*. *Plant, Cell and Environment* 29: 1220–1234.
- Kantrud, H.A. 1996. *The alkali (Scirpus maritimus L.) and salt-marsh (S. robustus Pursh) bulrushes: a literature review*. pp. 1–76. U.S. Department of the Interior National Biological Service, Denver, CO, US.
- Kao-Kniffin, J. & Balsler, T.C. 2007. Elevated CO₂ differentially alters belowground plant and soil microbial community structure in reed canary grass-invaded experimental wetlands. *Soil Biology and Biochemistry* 39: 517–525.
- Keddy, P.A. 1992. Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science* 3: 157–164.
- Keeney, D.R. & Nelson, D.W. 1982. Nitrogen-inorganic forms. In: Page, A.L., Miller, R.H. & Keeney, D.R. (eds.) *Methods of soil analysis part 2*. pp. 643–698. Soil Science Society of America, Inc, Madison, WI, US.
- Lambers, H., Chapin, F.S. & Pons, T.L. 2008. *Plant physiological ecology*. Springer, New York, NY, US.
- Legendre, P., Galzin, R.G. & Harmelin-Vivien, M.L. 1997. Relating behavior to habitat: solutions to the fourth-corner problem. *Ecology* 78: 547–562.
- Marino, G., Aqil, M. & Shipley, B. 2010. The leaf economics spectrum and the prediction of photosynthetic light-response curves. *Functional Ecology* 24: 263–272.
- Milla, R. & Reich, P.B. 2007. The scaling of leaf area and mass: the cost of light interception increases with leaf size. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 274: 2109–2115.
- Montemayor, M.B., Price, J.S., Rochefort, L. & Boudreau, S. 2008. Temporal variations and spatial patterns in saline and waterlogged peat fields: 1. Survival and growth of salt marsh graminoids. *Environmental and Experimental Botany* 62: 333–342.
- Munns, R. & Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651–681.
- National Water & Climate Center/Climatic Data Access Network. 2002. WETS Station: Syracuse WSO AP, NY8383. Available at: <http://www.wcc.nrcs.usda.gov/climate/wetlands.html> Accessed 14 August 2009.
- Niklas, K.J., Cobb, E.D., Niinemets,  ., Reich, P.B., Sellin, A., Shipley, B. & Wright, I.J. 2007. “Diminishing returns” in the scaling of functional leaf traits across and within species groups. *Proceedings of the National Academy of Sciences of the USA* 104: 8891–8896.
- Ogburn, R.M., & Edwards, E.J. 2010. The ecological water-use strategies of succulent plants. In: Kader, J. & Delseny, M. (ed.) *Advances in botanical research*. pp. 179–225. Academic Press, New York, NY, US.
- Partridge, T.R. & Wilson, J.B. 1988. The use of field transplants in determining environmental tolerances in salt marshes of Otago, New Zealand. *New Zealand Journal of Botany* 26: 183–192.
- Pearcy, R.W. & Ehleringer, J. 1984. Comparative ecophysiology of C₃ and C₄ plants. *Plant, Cell and Environment* 7: 1–13.
- Piernik, A. 2005. Vegetation–environment relations on inland saline habitats in Central Poland. *Phytocoenologia* 35: 19–37.
- Qadir, M., Qureshi, R.H., Ahmad, N. & Ilyas, M. 1996. Salt-tolerant forage cultivation on a saline-sodic field for biomass production and soil reclamation. *Land Degradation and Development* 7: 11–18.
- Radyukina, N.L., Kartashov, A.V., Ivanov, Y.V., Shevyakova, N. I. & Kuznetsov, V.V. 2007. Functioning of defense systems in halophytes and glycophytes under progressing salinity. *Russian Journal of Plant Physiology* 54: 806–815.
- Reich, P.B., Ellsworth, D., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C. & Bowman, W.D. 1999. Generality of leaf trait relationships: a test across six biomes. *Ecology* 80: 1955–1969.
- Rozema, J., Bijwaard, P., Prast, G. & Broekman, R. 1985. Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. *Vegetatio* 62: 499–521.
- Sage, R.F. 2004. The evolution of C₄ photosynthesis. *New Phytologist* 161: 341–370.
- Shipley, B. 1995. Structured interspecific determinants of specific leaf area in 34 species of herbaceous angiosperms. *Functional Ecology* 9: 312–319.
- Shipley, B. & Lechowicz, M.J. 2000. The functional co-ordination of leaf morphology, nitrogen concentration, and gas exchange in 40 wetland species. *Ecoscience* 7: 183–194.
- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. 2006–2007. Web Soil Survey. Available at: <http://websoilsurvey.nrcs.usda.gov>. Accessed 13 October 2009.
- Storey, R., Ahmad, N. & Wyn Jones, R.G. 1977. Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds in plants. *Oecologia* 27: 319–332.
- Thomas, G.W. 1996. Soil pH and acidity. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T. & Sumner, M.E. (eds.) *Methods of soil analysis part 3*. pp.445–490. Soil Science Society of America, Inc, Madison, WI, US.
- Thompson, S.K. 1990. Adaptive cluster sampling. *Journal of the American Statistical Association* 85: 1050–1059.
- Ungar, I.A. 1996. Effect of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaceae). *American Journal of Botany* 83: 604–607.

- USDA (United States Department of Agriculture), NRCS (Natural Resources Conservation Service). 2010. *The PLANTS Database*. National Plant Data Center, Baton Rouge, LA 70874-4490, US. Available at: <http://plants.usda.gov> Accessed 12 February 2010.
- Valiela, I. & Teal, J.M. 1974. Nutrient limitation in salt marsh vegetation. In: Reimold, R.J. & Queen, W.H. (eds.) *Ecology of halophytes*. pp. 547–563. Academic Press, New York, NY, US.
- Vitousek, P.M. & Howarth, R.W. 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13: 87–115.
- Wang, R.Z. 2002a. Photosynthetic pathways and life forms in different grassland types from North China. *Photosynthetica* 40: 243–250.
- Wang, R.Z. 2002b. Photosynthetic pathways, life forms, and reproductive types for forage species along the desertification gradient on Hunshandake desert, North China. *Photosynthetica* 40: 321–329.
- Wang, R.Z. 2004. Plant functional types and their ecological responses to salinization in saline grasslands, Northeastern China. *Photosynthetica* 42: 511–519.
- Wang, R.Z., Liu, X.Q. & Bai, Y. 2006. Photosynthetic and morphological functional types for native species from mixed prairie in Southern Saskatchewan, Canada. *Photosynthetica* 44: 17–25.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.
- Wilson, J.B., King, W.M., Sykes, M.T. & Partridge, T.R. 1996. Vegetation zonation as related to the salt tolerance of species of brackish riverbanks. *Canadian Journal of Botany* 74: 1079–1085.
- Wright, I.J., Reich, P. & Westoby, M. 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology* 15: 423–434.
- Wright, I.J., Reich, P.B. & Westoby, M. 2003. Least-cost input mixtures of water and nitrogen for photosynthesis. *The American Naturalist* 161: 98–111.
- Wright, I.J., Reich, P., Westoby, M., Ackerly, D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.

Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Mean trait values and literature-based trait values for 42 of the 49 species observed in vegetation plots.

Appendix S2. Histograms of environmental data measured in this study.

Appendix S3. Pearson correlation coefficients among environmental variables included in fourth-corner analyses.

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