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RESEARCH ARTICLE

Phylogenetic relatedness, ecological strategy, and stress determine interspecific interactions within a salt marsh community

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Abstract Species interactions in nature can be positive or negative. The stress gradient hypothesis (SGH) states that the strength of positive interactions increases with increasing stress. The phylogenetic limiting similarity hypothesis (PLSH) states that competition intensity is likely to be greater between closely related species than between distantly related species. Testing the SGH, the PLSH and determining the factors that influence species interactions with changing stress levels are important for ecosystem conservation and restoration. In the following study we conducted experiments to investigate the effects of salinity stress, phylogenetic relatedness (i.e., the sum of branch lengths separating species on a phylogenetic tree), and species ecological strategy on interspecific interactions using 11 species found with in a salt marsh located in the Yellow River Delta, China. We found most of the species interactions across increasing salinity levels to be inconsistent with the SGH. The net outcomes of interspecific interactions were significantly affected by multiple factors, including salinity stress, phylogenetic distance, ecological strategy, and the interaction between salinity and phylogenetic distance. Importantly, with increasing phylogenetic distance separating a pair species, the likelihood of facilitative interactions was increased and the likelihood of competitive interactions was reduced; this relationship was especially strong at medium and high salinities and supports the PLSH.

Introduction

Facilitation and competition between species occur simultaneously in nature, and the net outcome of these interspecific interactions can be either positive or negative. Species facilitation commonly occurs within ecosystems with harsh conditions, such as salt marshes, cobble beaches, arid ecosystems, marine ecosystems, alpine ecosystems, and deserts (Bruno et al. 2003; Zhang and Shao 2013).

Stress plays an important role in shaping plant community assemblages (Bornette and Puijalon 2011; Zhang and Wang 2016), and the outcome and strength of species interactions influencing community assemblages may be related to the intensity of abiotic and biotic stress. The stress gradient hypothesis (SGH) states that the frequency or intensity of facilitative and competitive interactions between species is inversely related to abiotic or biotic stress levels (Bertness and Callaway 1994; He et al. 2013; Lortie and Callaway 2006; Silliman et al. 2015). This influence of stress on interspecific interactions is thought to be due to mitigation of the stress effects by stress-tolerant species within the community, which enhances the fitness of neighboring species. However, the prevalence of the SGH occurring within plant communities is still under debate and requires testing across a diverse range of ecosystem types. Maestre et al. (2009) argue that outcomes arising from species

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interactions also depend on the ecological strategies of the individual species and types of stressors experienced. Species develop specific strategies to deal with ecological abiotic or biotic stresses (e.g., competition for resources from surrounding species) through natural selection for multiple key functional traits (Grime 1977; Reich et al. 2003). Thus the ecological strategies employed by individual species may be indicators of the outcomes arising from interactions between these species and their neighbors.

Phylogenetic relatedness between two species reflects their evolutionary relationship, and is also thought to influence outcomes arising from species interactions. The phylogenetic limiting similarity hypothesis (PLSH) proposes that competition intensity is likely to be greater between closely related species than between distantly related species because phylogenetically-close species may have more similar niches, due to niche conservatism. In contrast, the PLSH states that facilitation tends to occur more frequently or strongly between distantly related species, and thus, distantly related species are more likely to coexist stably within the community (Burns and Strauss 2011; Castillo et al. 2010; Soliveres et al. 2012; Verdu et al. 2009; Violle et al. 2011).

However, support for the PLSH is equivocal (Alexandrou et al. 2015; Cahill et al. 2008; Fritschie et al. 2014; Narwani et al. 2013; Venail et al. 2014). For example, both Cahill et al. (2008) and Verdu et al. (2012) performed meta-analyses on the relationships between phylogenetic distances and plant species interactions. Cahill et al. (2008) found that the relationship between phylogenetic distance and strength of species competition was not significant among vascular plants. Conversely, Verdu et al. (2012) showed increasing phylogenetic distance and life-form disparity between nurse plants and target plants to promote successful nurse plant restoration. Thus, the relationship between phylogenetic distance and species interactions remains uncertain. One possible reason to explain this discrepancy is that the species studied by Cahill et al. (2008) and Verdu et al. (2012) originated from different environmental conditions (i.e., benign and harsh conditions, respectively). The environmental conditions the species have acclimated and adapted to may influence the relationship between phylogenetic relatedness and species interactions. A recent meta-analysis found species interactions between more distantly related species within relatively harsh coastal ecosystems to be more likely to increase facilitation or reduce competition than for more closely related species (Zhang et al. 2016).

To the best of our knowledge, no experimental study has evaluated the relationships between stress levels, phylogenetic relatedness, and species ecological strategies by direct measures of the outcomes arising from species interactions. We aimed at testing the following four hypotheses. Firstly,

according to the SGH, species competition is stronger at low stress than that at high stress. Secondly, the ecological strategies of interacting species influence the pattern of species interactions along a salinity gradient. Thirdly, according to the PLSH, interactions between phylogenetically distant species are more likely to result in facilitation or reduction of competition. Finally, stress levels interact with phylogenetic relatedness to influence species interactions.

Salt marsh plant communities provide excellent model systems for testing ecological principles because plant species diversity tends to be relatively low in salt marshes and there is often a well-defined salinity gradient from the sea to the upland (Bertness 1991). In order to examine the SGH and PLSH and the hypothesis that stress levels interact with phylogenetic relatedness to influence species interactions, we assessed the outcomes arising from interactions between common species within a salt marsh. The influences of salinity level, phylogenetic distance, and species ecological strategy on these outcomes were then analyzed. This study will advance our understanding of species interactions within ecosystems, improving our ability to plan for successful conservation and restoration of degraded plant communities, particularly within coastal wetlands.

Materials and methods

Study site

The experiments were conducted in the Yellow River Delta Ecology Research Station of the Coastal Wetland run by the Chinese Academy of Sciences. Plant species used in the experiments came from Yellow River Delta National Reserve located in Shandong Province, northeast China (37°40′N–38°10′N, 118°41′E–119°16′E). The climate of this region is warm-temperate. Mean annual temperature of the study site is 12.1 °C; average precipitation is 551 mm/year, with most rainfall occurring in the summer; average evaporation is 1962 mm/year. Tidal fluctuations are irregularly semidiurnal. The high evaporation and tidal fluctuation lead to high salinities in the soil of this salt marsh. The salt marsh vegetation varies with plant zones along a salinity gradient. *Suaeda salsa* dominates lower elevation zones; *S. salsa, Phragmites australis*, and *Tamarix chinensis* dominate the terrestrial border zones; and *P. australis* dominates the upland zones.

Experiments

Experiments assessed interactions between target (individuals of the focal species surrounded by neighbor plants) and neighbor plants. The neighbor species (*S. salsa*) was chosen for two reasons: Firstly, *S. salsa* is one of the

dominant species within the salt marsh and, therefore, is a realistic neighbor species within the salt marsh plant community. Secondly, *S. salsa* is tolerant of high salinities and thus can survive in the highest experimental salinity treatment. The ten target species were chosen because they are all common within and important to the salt march ecosystem (Appendices Table S1). Additionally, the phylogenetic distance between these ten species and *S. salsa* ranged from 29 Myr (million years) to 163 Myr (see "Results"), which is an ideal degree of variation for investigation of the influence of phylogenetic relatedness on species interactions.

Similar sized seedlings of the target and neighbor species for experiments were obtained from the Yellow River Delta salt marsh in early spring. For the first two weeks of transplant periods, pots were watered with fresh water from the Yellow River every other day to avoid transplant shock, and dead seedlings were replaced with new seedlings of the same species. Survival of seedlings (number of days) transplanted into pots that were placed in a flat and bare field with homogeneous light condition along a salinity gradient for the 4 months growing season (May–August) was recorded. At the end of the growing season, we determined aboveground and belowground dried biomass of the surviving plants. The plants were harvested and roots were cleaned over a sieve to prevent loss of smaller fragments, then oven dried at 50 °C for 72 h before mass measurements.

Salinity treatments were applied by placing pots into a plastic basin with a shallow layer (~5 cm) of standing water adjusted to the treatment salinity. Salinity of standing water was monitored using a conductivity meter and adjusted daily by adding fresh water or sea salt to the standing water.

Competition experiment

In order to investigate the competitive ability of the target species, target species seedlings were transplanted into pots (one plant per pot; 8 pots per species; caliber: 33.0 cm, bottom diameter: 20.0 cm, height: 25.0 cm) filled with low-salinity fertile soil (mean electronic conductivity: 0.87 ± 0.05 ms/cm, n = 80) from the Yellow River estuary. All pots planted with the same target species were grouped in pairs, with one assigned to a 'with-neighbors' treatment and the other assigned to a 'without-neighbors' treatment. In the with-neighbors pots, four *S. salsa* seedlings were transplanted around the target plant, with an average distance of 12 cm between each seedling. The without-neighbors pots contained only the one target species seedling. There were four replicates of treatment pairs for each species, making a total of 80 pots in this experiment. After the first 2 weeks (avoiding transplant shock), pots were watered with fresh water every 4 days.

Salinity tolerance experiment

In order to compare salinity tolerances among the ten target species and *S. salsa*, we transplanted seedlings from all 11 species into separate pots (one seedling per pot; caliber: 21.0 cm, bottom diameter: 15.0 cm, height: 18.5 cm), which were filled with the same soil type as described for the competition experiment. After 2 weeks, the pots were assigned salinity treatments of 0, 20, 40, 60, or 80 PSU (Practical salinity units, ‰). Salinity was gradually increased, at a rate of 20 PSU every 3 days, to the treatment salinity over 2 weeks. Each salinity treatment was replicated four times, and there were a total of 220 pots. However, at the end of the experiment, only three replicates of *Scorzonera mongolica* at salinity 40 PSU remained because one seedling died from transplant shock at the end of the two week transplant acclimation period, leaving insufficient time to transplant a replacement replicate.

Species interactions across a salinity gradient experiment

In order to investigate salinity effects on interactions between target and neighbor species, we transplanted seedlings of the ten target species into separate pots (one seedling per pot; caliber: 33.0 cm, bottom diameter: 20.0 cm, height: 25.0 cm), which were filled with the same soil types as in the competition experiment. Also as in the competition experiment, all pots containing seedlings of the same species were grouped into pairs (with- or without-neighbors), and each pair was assigned to one of three salinity treatments (low salinity: 0, medium salinity: 15 PSU, and high salinity: 30 PSU). Each treatment pair was replicated four times per species, making a total of 240 pots at the beginning of the experiment. Replicates were lost during the experiment due to damage incurred by a strong typhoon in July, resulting in only two to three replicates for some species' treatments by the end of the experiment.

Data analysis

Interactions between target and neighbor species were assessed by comparing biomass production of 'with-neighbors' target seedlings with their paired 'without-neighbors' target seedlings. The Relative Interaction Intensity (RII) index was calculated as (Armas et al. 2004):

$$RII = \frac{B_{+N} - B_{-N}}{B_{+N} + B_{-N}},$$

where B_{+N} is the total biomass (sum of aboveground and belowground biomass) of the with-neighbors target species plant, and B_{-N} is the total biomass of the target species plant in the corresponding without-neighbors pot. The

RII values fall on a scale of $+1$ to -1. A negative RII value denotes interspecific competition, and a positive RII value indicates interspecific facilitation. In the species interactions across salinity gradient experiment, all *Sonchus oleraceus* plants died across all salinity levels; therefore, in order to investigate salinity gradient effects on interactions between *S. oleraceus* and *S. salsa*, survival duration (days), instead of biomass, was quantified and applied to the RII calculation.

We estimated phylogenetic distances between the ten target species and the neighbor species (*S. salsa*) using the web application TimeTree, which is a public database of divergence times (Hedges et al. 2006; Verdu et al. 2012). However, in the TimeTree application, we did not find the phylogenetic distance between *Suaeda glauca* and *S. salsa*, so we reconstructed a phylogenetic tree of the ten target species employing the Phylomatic program (Webb and Donoghue 2005). In this program, the family names of these ten species were matched with a megatree, which was made by the Angiosperm Phylogeny Group III system (Bremer et al. 2009). Then, a phylogenetic tree was created for these ten species. The nodes of the phylogenetic tree were dated using the bladj algorithm, based on the ages from Wikstrom et al.'s database (Wikstrom et al. 2001), and using Phylocom software (Webb et al. 2008). In agreement with the findings of Verdu et al. (2012), we found a linear relationship between the phylogenetic distances obtained by Phylocom and Timetree (Table S2). We used this linear relationship to estimate the phylogenetic distance between *S. glauca* and *S. salsa* (Verdu et al. 2012).

We employed the Shapiro–Wilk normality test to confirm the distribution of the residuals was normal and Levene's Test to confirm the homogeneity of variance. We performed a one-way ANOVA to analyze the relationships between RII values and salinity levels. Post-hoc Tukey HSD tests were used to compare the mean RII values of the different salinity treatments.

We classified target species' ecological strategies according to the competition experiment and the salinity tolerance experiment. The four ecological strategy classifications were: high salinity tolerance-weak competitive ability (HS-WC); Low salinity tolerance-strong competitive ability (LS-SC); low salinity tolerance-weak competitive ability (LS-WC); high salinity tolerance-strong competitive ability (HS-SC). Then we employed regression linear models to determine which factors significantly influenced the RII values. In the model, the RII values were set as the response variable, and salinity levels (low, medium, and high salinity as factors), phylogenetic distance (log-transformed), target species' ecological strategies (LS-SC, HS-SC, LS-WC, and HS-SC), the interaction between salinity and phylogenetic distance, and the interaction between phylogenetic distance and species' ecological

strategy were independent variables. We also analyzed the linear relationship between interspecific interactions and phylogenetic distance at different salinity levels. We performed statistical analyses using R 2.15.3 (R Development Core Team).

Results

Phylogenetic relatedness and species ecological strategies

Phylogenetic distance between the ten target species and *S. salsa* ranged from 29 to 163 Myr (Table 1). Salinity tolerances of the 11 study species (Fig. 1) were used to assign each species into a high salinity tolerant (survived more than 60 days at salinity 40 PSU) or low salinity tolerant (survived fewer than 50 days at salinity 40 PSU) group. The high salinity tolerant group included *S. salsa*, *Aeluropus pungens*, *Limonium sinense*, *T. chinensis*, and *S. glauca*; and the low salinity tolerant group included *Apocynum lancifolicum*, *Cynanchum chinense*, *Tripolium vulgare*, *Scorzonera mongolica*, *P. australia*, and *Sonchus oleraceus*. As we expected, *S. salsa* was tolerant of high salinities and was well suited as the neighbor species for the experiments investigating species interactions.

The RII values from the competition study, which was performed at low salinity, were used to classify each of the ten target species as strongly (RII <-0.25) or weakly (RII >-0.25) competitive (Fig. 2; Table S3). The strongly competitive group, included *T. chinensis*, *A. pungens*, *S. glauca*, *T. vulgare*, and *A. lancifolicum*. The weakly competitive group included *P. australis*, *S. oleraceus*, *L. sinense*, *C. chinense* and *S. mongolica*. He et al. (2012) reported a greater salinity tolerance and weaker competitive ability for

Table 1 Phylogenetic distance between target and neighbor species obtained from TimeTree

Target species	Neighbor species	Phylogenetic distance (Myr)
Suaeda glauca	*Suaeda salsa*	29
Tamarix chinensis	*S. salsa*	76
Limonium sinense	*S. salsa*	84.8
Cynanchum chinense	*S. salsa*	110.8
Tripolium vulgare	*S. salsa*	110.8
Sonchus oleraceus	*S. salsa*	110.8
Apocynum lancifolicum	*S. salsa*	110.8
Scorzonera mongolica	*S. salsa*	110.8
Phragmites australis	*S. salsa*	163
Aeluropus pungens	*S. salsa*	163

The unit of phylogenetic distance is Myr (million years)

Fig. 1 Survival duration (days) of the neighbor species (*Suaeda salsa*) and ten target species (*Aeluropus pungens, Limonium sinense, Tamarix chinensis, Suaeda glauca, Apocynum lancifolium, Cynanchum chinense, Tripolium vulgare, Scorzonera mongolia, Phragmites australis, Sonchus oleraceus*) at different salinity treatments (0, 20, 40, 60, or 80 PSU). *Error bars* depict ±SD, and n=4, except the for *S. mongolica* at salinity 40 PSU, which had an n of 3. PSU is practical salinity units (‰)
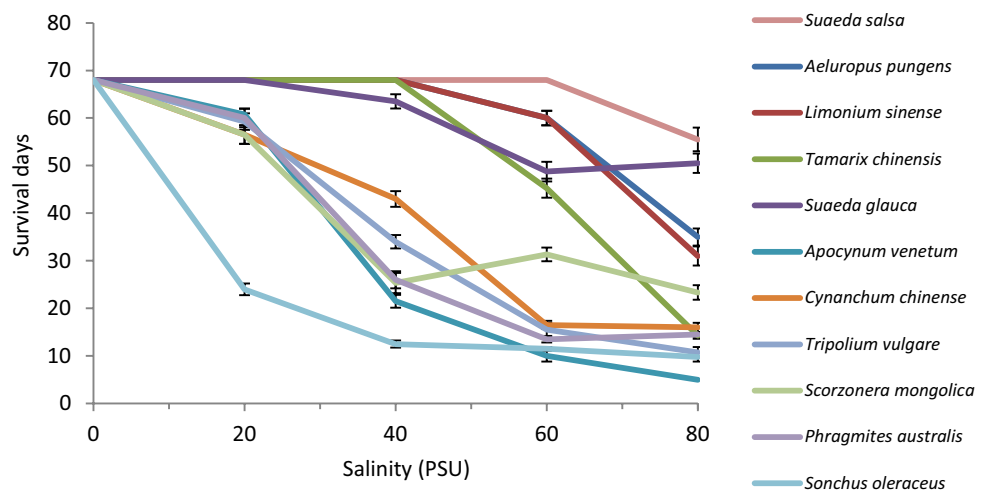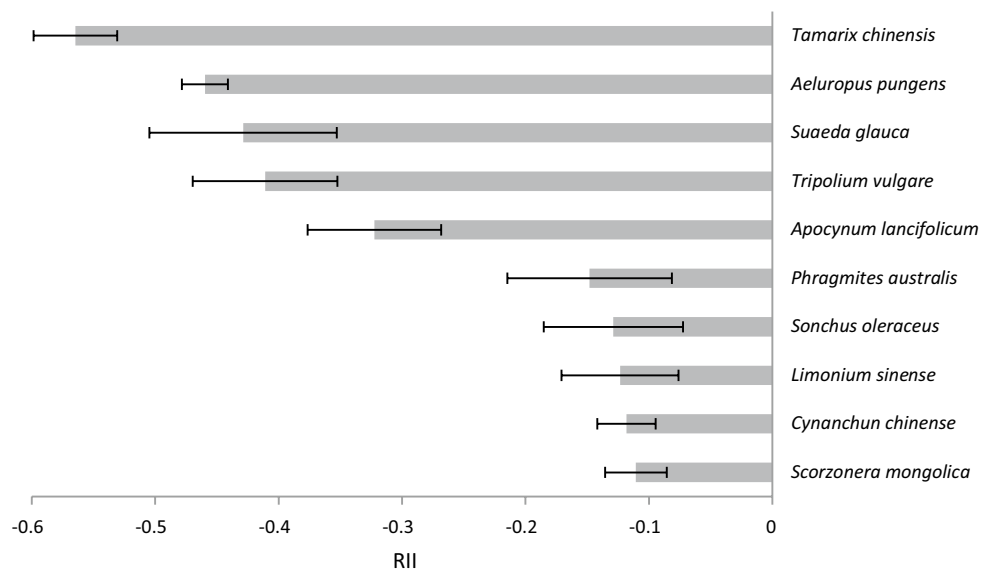

Fig. 2 Competitive ability of the ten target species (*P. australis, S. mongolia, A. pungens, C. chinense, L. sinense, S. glauca, A. lancifolium, T. vulgare, T. chinensis, S. oleraceus*). RII refers to relative interaction intensity index; the smaller the RII value, the weaker the competitive ability. *Error bars* depict ±SD, and n=4

S. salsa relative to *S. glauca*. Their experiment was conducted with the same method in the same study site as ours. Finally, we classified each of the 11 species studied here as employing one of four ecological strategies, according to salinity tolerance and competitive ability (see details in methods): *S. salsa, T. chinensis, S. glauca*, and *A. pungens* were classified as HS-WC; *C. chinense, S. oleraceus, P. australis*, and *S. mongolica* were classified as LS-SC; *T. vulgare* and *A. lancifolium* were classified as LS-WC; *L. sinense* was classified as HS-SC.

Species interactions across a salinity gradient (Table 2)

The only species to show evidence of facilitative interactions with *S. salsa* across low, medium, and high salinity treatments were the LS-SC species *S. oleraceus* (for

which the RII value was calculated with survival data), *P. australis*, and *S. mongolica*. For all three of these LS-SC species, competition with *S. salsa* declined as the salinity increased and species facilitation was greatest (RII >0) at the highest salinity tested. For *A. pungens*, competition with *S. salsa* at the high salinity was stronger than that at the medium salinity, and was weaker at high/medium salinities than at the low salinity. Competition between *C. chinense* and *S. salsa* was strongest at the medium salinity, and declined from medium to low salinity. Competition between *L. sinense* and *S. glauca* and *S. salsa* was significantly stronger at the medium and high salinities than at the low salinity. Competition between *A. lancifolium, T. chinensis*, and *T. vulgare* and *S. salsa* did not significantly vary with salinity.

Table 2 Species interactions between the ten target species and *S. salsa* across low, medium, and high salinity levels

Strategy groups	Species	Plant performance	RII values		
			Low salinity	Medium salinity	High salinity
HS-WC	*T. chinensis*	Growth	-0.56 ± 0.03^a (n=4)	-0.43 ± 0.13^a (n=4)	-0.40 ± 0.16^a (n=4)
	S. glauca	Growth	-0.43 ± 0.08^a (n=4)	-0.64 ± 0.04^b (n=4)	-0.61 ± 0.04^b (n=4)
	A. pungens	Growth	-0.46 ± 0.02^c (n=4)	-0.24 ± 0.09^a (n=4)	-0.30 ± 0.13^b (n=4)
LS-SC	*C. chinense*	Growth	-0.12 ± 0.02^a (n=4)	-0.51 ± 0.09^b (n=3)	NA
	S. oleraceus	Survival	0 (n=4)	-0.04 ± 0.03^b (n=4)	0.11 ± 0.05^a (n=4)
	P. australis	Growth	-0.15 ± 0.07^b (n=4)	-0.09 ± 0.03^b (n=2)	0.05 ± 0.01^a (n=4)
	S. mongolica	Growth	-0.11 ± 0.02^b (n=4)	-0.02 ± 0.10^b (n=4)	0.19 ± 0.08^a (n=4)
LS-WC	*T. vulgare*	Growth	-0.41 ± 0.06^a (n=4)	-0.39 ± 0.16^a (n=3)	-0.39 ± 0.14^a (n=3)
	A. lancifolicum	Growth	-0.32 ± 0.05^a (n=4)	-0.34 ± 0.12^a (n=4)	NA
HS-SC	*L. sinense*	Growth	-0.12 ± 0.05^a (n=4)	-0.44 ± 0.11^b (n=4)	-0.51 ± 0.03^b (n=4)

The ten target species are divided into four ecological strategy groups. Letters indicate the post-hoc test results, where values with no common letters are significantly different from each other (p value < 0.05). RII is the relative interaction intensity, which denotes the intensity of species interactions. "HS-WC" means species with high salinity tolerance and weak competitive ability; "LS-SC" denotes species with low salinity tolerance and strong competitive ability "LS-WC" indicates species with low salinity tolerance and weak competitive ability; and "HS-SC" denotes species with high salinity tolerance and strong competitive ability. "NA" means no data. RII values are shown as mean ± SD

Factors that influenced species interactions

The significant independent variables influencing the RII index, according to regression analysis were salinity, phylogenetic distance, and the interaction between salinity and phylogenetic distance (Table 3). Of all significant variables, phylogenetic distance had the largest mean square. The interaction between phylogenetic distance and ecological strategy did not significantly influence species interactions. The independent variables included in the model explained a large proportion of the variation in RII values (adjusted R-square = 0.603, F value = 17.22, p value < 0.001).

We also analyzed the linear relationship between RII values and phylogenetic distances at different salinity levels (Fig. 3). From the slope coefficients of these linear models, we found that with increasing phylogenetic distance, species competition tended to decline at all salinity levels (the slope coefficient at low salinity was 0.260; the slope coefficient at medium salinity was 0.603; and the slope coefficient at high salinity was 0.740). The effect of phylogenetic distances on species interactions was much stronger at medium and high salinities than that at the low salinity.

Discussion

The interactions between the ten target species with *S. salsa* across three salinity levels varied with species and salinity, with only a few species showing trends consistent with the SGH. Specifically, competitive interactions between the target species *P. australis* and *S. mongolica* and the neighbor species, *S. salsa*, gradually declined with increasing salinity stress, as is consistent with the SGH. One plausible explanation for this variability in stress effects on species interactions is that interactions may be highly species-specific (Callaway 1995, 1997), with some species requiring

Table 3 Results of the Relative Interaction Intensity (RII) index regression model. The response variable of model was the RII index, and the independent variables included salinity (Low, Medium, and High salinity), phylogenetic distance (log-transformed), target species' ecological strategy, the interaction between salinity and phylogenetic distance, and the interaction between phylogenetic distance and species' ecological strategy

Source of variation	Df	Sum of squares	Mean square	F value	p value
Salinity	2	0.190	0.095	4.827	<0.05*
Phylogenetic distance	1	1.317	1.317	67.011	<0.001***
Species ecological strategy	3	1.303	0.434	22.094	<0.001***
Salinity: Phylogenetic distance	2	0.235	0.118	5.987	<0.001***
Phylogenetic distance: Species ecological strategy	1	0.001	0.001	0.072	0.789
Residuals	87	1.007	0.020		

'*' indicates p value < 0.05 and '***' denotes p value < 0.001

(a) Low salinity

y = 0.260x - 0.799
R² = 0.094
Adjusted R² =0.070

(b) Medium salinity

y = 0.603x - 1.551
R² = 0.441
Adjusted R² =0.422

(c) High salinity

y = 0.740x - 1.719
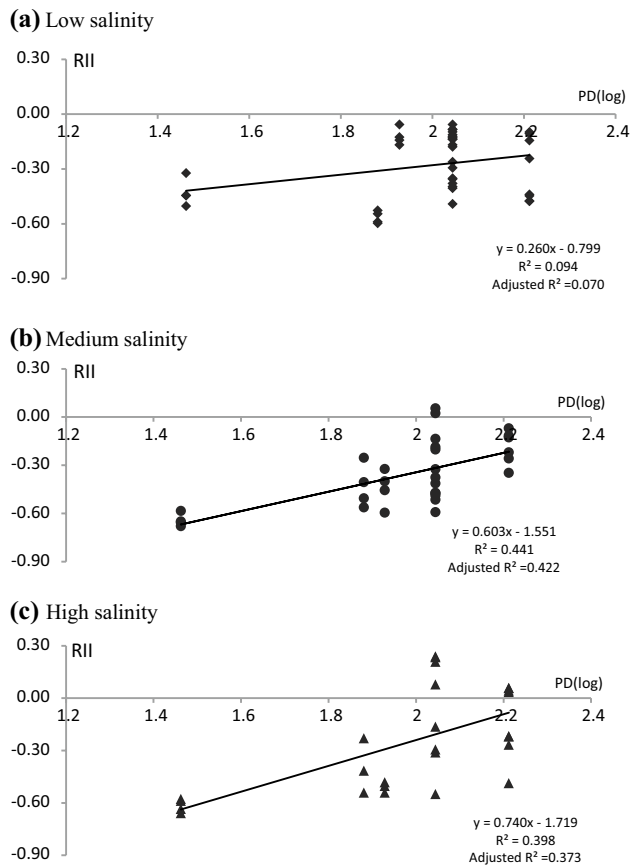R² = 0.398
Adjusted R² =0.373

Fig. 3 The linear relationship between species interactions and phylogenetic distance (log-transformed) at different salinity levels. The *Y* axis plots RII (the relative interaction intensity), and the *X* axis plots the log-transformed phylogenetic distance (PD)

interactions with a specific neighbor species for facilitation of survival or growth under high stress environments. Additionally, the SGH may be sensitive to the specific salinity levels tested. For example, the interactions between *C. chinense* and *S. salsa* support the SGH at medium and high salinities but not at the low salinity treatment. Therefore, future experiments incorporating more salinity increments over a more narrow salinity range, as suggested by Silliman et al. (2015), may be more informative when testing the SGH.

Previous studies have classified the ecological strategies of species as either competitive or stress-tolerant because of the general belief that there is a trade-off between competitive ability and stress tolerance (Crain et al. 2004; Liancourt et al. 2005). We also find evidence of a trade-off between stress tolerance and competitive ability in 8 out of the 11 tested species (*S. salsa, T. chinensis, S. glauca, A. pungens, C. chinense, S. oleraceus, P. australis* and *S. mongolica*). The remaining three species we tested (*T. vulgare, A. lancifolium* and *L. sinense*) showed no signs of this trade-off. Grime (1977) suggested

the ruderal strategy as a third ecological strategy, where species are tolerant of high intensity disturbances, but only low intensity stress. In this study, we classified the 11 study species into one of four ecological strategy types (LS-WC, HS-WC, HS-SC, and LS-WC) according to salinity-tolerance and competitive ability. We found a large degree of variation in the ecological strategies followed by these plant species from the Yellow River Delta salt marsh communities, and suggest this variation may result from different functional traits related to competitive ability or salinity tolerance. Unfortunately, it is very difficult to identify these functional traits. Therefore, identification of the ecological strategies employed by species may be a practical tool for predicting the outcomes of species interaction.

Maestre et al. (2009) tried to combine species ecological strategies with the SGH to form a new testable hypothesis. Their hypothesis predicts that facilitation would increase more rapidly if the interaction occurs between a competitive and a stress-tolerant species, relative to an interaction between two stress-tolerant species, when the stress is not a resource limitation (Maestre et al. 2009). In our experiment, the neighbor species, *S. salsa*, is a stress-tolerant species and the stress exposure was not a resource limitation. The species interactions observed in our study did not support the hypothesis proposed by Maestre et al. (2009) for stress-tolerant species or competitive target species (Table 2). Therefore, we suggest that species interactions across a stress gradient may be influenced by multiple factors, and the outcomes of these interactions may not be simply predicted by one or two factors.

Species interactions in our experiments were significantly affected by multiple factors: salinity, phylogenetic distance, ecological strategy, and interactive effects of salinity and phylogenetic distance. Salinity stress is one of a number of important factors limiting the distribution and diversity of species within coastal marshes (Bertness et al. 1992; Bertness and Hacker 1994; Hacker and Bertness 1999; Pennings et al. 2005). Although the salinity responses of only a few pairs of species supported the SGH in this study, the effect of salinity on species interactions is clear. Phylogenetic distance had a slightly positive effect on species interactions at the low salinity, and a strongly positive effect at medium and high salinities, which supports the PLSH. These trends also suggest that larger phylogenetic distances between interacting species are more likely to result in facilitative interactions or reductions of species competition under high stress conditions. However, the interactive effect of phylogenetic distance and ecological strategy did not significantly influence species interactions, suggesting that, under this study system, phylogenetic distance did not reflect the ecological strategies of the tested species.

Our findings are consistent with those of a meta-analysis on the relationship of phylogenetic relatedness and species interactions under the harsh conditions of coastal ecosystems (Zhang et al. 2016) which concluded that, when the phylogenetic relatedness of target and neighbor species is more distant, their interactions are more likely to facilitate growth of the target species. In contrast, other studies have found no relationship between phylogenetic relatedness and strength of competition (Alexandrou et al. 2015; Fritschie et al. 2014; Venail et al. 2014). These studies, however, were performed under relatively benign environments relative to our study. Less harsh ecosystems may not exert selective pressures that drive species to evolve specialized traits (phylogeny is conserved or convergent) that influence species interactions (Bertness and Hacker 1994; Callaway and Pennings 2000).

In summary, the interactions between most of the ten target species and *S. salsa* studied here across three salinity levels did not support the SGH, regardless of ecological strategy. We found multiple factors (i.e., salinity, phylogenetic distance, ecological strategy, and the interactive effect between salinity and phylogenetic distance) to significantly influence species interactions. Importantly, we observed that, as the phylogenetic distance separating a pair of species increased, facilitative interactions increased and competitive interactions decreased. This pattern was especially strong at the medium and high salinities. Unfortunately, due to destruction of replicates by a typhoon, our findings, in some cases, are based on low replication. Therefore, further research is required to confirm these relationships. Moreover, the relationships observed in this study should be tested in other salt marshes and other ecosystems with harsh environmental conditions. With replication of these findings throughout a diverse range of harsh ecosystems, they could be used to guide the management and restoration of salt marshes.

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