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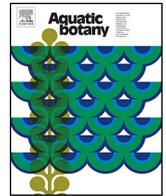


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Interactions between transplants of *Phragmites australis* and *Juncus acutus* in Mediterranean coastal marshes: The modulating role of environmental gradients



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ABSTRACT

Interactions between two coastal marsh plants (*Phragmites australis* and *Juncus acutus*) were investigated along three natural gradients of salinity, water table and soil texture, variously combined in a microtidal Mediterranean coastal marsh. Our aim was to clarify to what extent plant interactions explain the occurrence of stands of both species, since they are not solely due to their tolerance to environmental conditions. We used a replacement series design of field transplants. Mortality and relative yield index of aerial biomass were analyzed to assess the outcome of interactions using Generalized Linear Mixed Models. Results show that mortality was entirely driven by natural gradients. Specifically, higher salinity and soil clay contents increased mortality in both species, whereas high water table increased survival only in *P. australis*. Growth was controlled by plant interactions and by natural gradients. According to these results *P. australis* can suppress *J. acutus* in waterlogged and non-saline conditions. Where water table is deeper, *J. acutus* can suppress *P. australis* along a wide range of saline conditions. In the driest situations both species exhibited mutual interference and, in some cases, *P. australis* can again suppress *J. acutus*. Facilitation occurred along the salinity gradient in situations that correspond to low, medium or high stress for the beneficiary species, pointing that exceptions to the stress gradient hypothesis may occur in systems including multiple stress gradients. Our results suggest that competitive interactions and environmental gradients are not fully responsible for plant distribution in microtidal coastal marshes.

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1. Introduction

Although interactions among plants are a dominant driver of the dynamics and structure of natural plant populations and communities (Malkinson and Tielbörger, 2010) there is a lack of knowledge about how they change with biotic or abiotic factors (Agrawal et al., 2007). Based on the classical Gause's competitive exclusion principle, ecological research has traditionally focused on negative interactions. However, since the stress gradient hypothesis (hereafter SGH) was formulated (Bertness and Callaway, 1994), there has been a growing acknowledgement of the importance of facilitative interactions. This hypothesis suggests that in communities growing under high abiotic stress or high herbivory pressure competition is less intense and plant–plant interaction becomes facilitative. Over

the last two decades, several empirical studies carried out in stressful environments such as coastal marshes (Bertness and Ewanchuk, 2002), alpine areas (Callaway et al., 2002), dry calcareous grasslands (Liancourt et al., 2005) or arid steppes (Pugnaire and Luque, 2001) support the SGH (see He et al., 2013 for a detailed review). Some studies mainly carried out in arid environments suggest that facilitative interactions cease at the limits of the stress gradients. (e.g., Tielbörger and Kadmon, 2000; Maestre and Cortina, 2004; Maestre et al., 2005). Despite the fact that these studies involve some methodological problems and cannot be considered a robust demonstration of an exception to the SGH (see He et al., 2013 and He and Bertness, 2014 for detailed explanations) their conclusions have motivated different theoretical reformulations of the SGH (Maestre et al., 2009; Holmgren and Scheffer, 2010; Malkinson and Tielbörger, 2010). According to He and Bertness (2014), the SGH keeps being a general applicable rule of thumb to predict general trends in species interactions under stress conditions, and exceptions may appear where the stress gradient considered is outside

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of the realized niche of the species, or where multiple co-occurring stress gradients not spatially correlated are found.

Plant communities in coastal marshes are commonly species-poor and dominated by a single or a few species (Adam, 1993; Day et al., 1988; Weiher and Keddy, 1995). Distribution of coastal marsh species is mainly consequence of two interacting factors: physiological tolerance of species to environmental constraints imposed by salinity and waterlogging (Adam, 1993; Pennings et al., 2005; Erfanzadeh et al., 2010) and by nutrient shortage (Levine et al., 1998); and biotic interactions, mainly between plants (Bertness, 1991; Pennings and Callaway, 1992; He et al., 2011) but also involving plant consumers (Costa et al., 2003; Crain, 2008). Past community ecology studies in macrotidal coastal marshes focused on the role of competitive interactions. The emerging paradigm proposed a clear niche segregation along environmental gradients, where the most competitive plants would occupy the least stressful zone of the coastal marsh, displacing the least competitive plants to the most stressful zones (Pennings et al., 2005). This paradigm agrees with the Grime's hypothesis previewing an inverse relationship between competitive ability and stress tolerance (Pennings et al., 2005). Moreover, since coastal marshes are stressful habitats for plant life, positive interactions according to the SGH were early detected in these habitats (Bertness et al., 1994). Further specific research demonstrated that facilitation determines secondary succession (Castellanos et al., 1994), plant diversity (Bertness et al., 1994) and distribution of non dominant plants (Crain, 2008; He et al., 2011) in macrotidal coastal marshes. In contrast, as far as we know, only Costa et al. (2003) have paid attention on the effect of competitive exclusion and the SGH in the case of microtidal (tidal range <2 m) coastal marshes. In Mediterranean microtidal marshes waterlogging dynamics is primarily driven by variations in rainfall and wind direction, therefore salinity and water table level are not

easily predictable (Curcó et al., 2002). Moreover, salinity and waterlogging gradients are not mutually dependent. In summer, upper marshes may be more saline than lower ones because the high evapotranspiration cause salt ascension to top soil (Álvarez-Rogel et al., 2001). This situation may reverse by autumn storms, which flood the upper marshes with fresh water while causing meteorological tides that flood low marshes with marine water (Curcó et al., 2002). This seasonal pattern may be extremely complex because marine and freshwater inputs are highly variable within and among years.

The present study is focused on *Juncus acutus* and *Phragmites australis*, two herbaceous perennial species that form vast, dense stands in coastal Mediterranean marshes. Reed (*P. australis* s.l.) is a cosmopolitan rhizomatous grass able to grow in a wide range of ecologic conditions (Romero et al., 1999) and usually forming almost mono specific stands due to its high competitiveness (Wang et al., 2006; Wang et al., 2006). Clonal growth is the main competitive mechanism of reed, which creates a dense canopy and a large, branched rhizome system that prevents establishment of other marsh plants. It also modifies its habitat by increasing the accumulation of plant litter and altering the soil physico-chemical conditions (Meyerson et al., 2000; Minchinton et al., 2006). *J. acutus* is a perennial tussock rush with a broad geographic distribution, found in a wide range of ecologic conditions within coastal marshes. In Australia it performs an invasive behavior, being able to exclude other native *Juncus* species (Greenwood and MacFarlane, 2009). *J. acutus* can thrive better under dry and saline conditions than *P. australis*, whereas the latter tolerates waterlogging better than the former.

The fundamental aim of this study is to clarify the role of reciprocal interactions on the distribution of *P. australis* and *J. acutus* in Mediterranean microtidal marshlands. This is in line with Batriu



Fig. 1. Experimental locations within the Llobregat delta marshes. White points mark the experimental locations. Top-left locations, which were set up in meso-haline marshes, were caged in order to prevent rabbit predation.

et al. (2011), which show that distribution of these species is not solely explained by niche segregation along environmental gradients, and suggest that interspecific interactions could play an important role. Secondly, we have the aim to improve the present SGH knowledge through clarifying to which extent it may be applied to the microtidal Mediterranean coastal marshes. According to Brooker et al. (2008), Malkinson and Tielbörger (2010), He et al. (2013) and Mod et al. (2014), studies dealing with the SGH must account for several co-occurring stress factors and for varying levels within each stress factor. As suggested by He and Bertness (2014) SGH studies must quantitatively define the fundamental niche and at least four stress situations (low, medium, high or extreme). To fulfill these requirements, we have examined the effect of plant interactions on survival and growth of *P. australis* and *J. acutus*, planted along three natural gradients of various combinations of salinity, water table depth, and soil texture. We used a methodological approach suggested by Williams and McCarthy (2001), based on a replacement series design within multivariate gradients. In accordance with the species' niche differences reported in Batriu et al. (2011), we expect that *J. acutus* outcompete *P. australis* in dry and/or saline situations, and the opposite trend in moist, non saline situations. These results can be, however, modulated by soil clay contents, which would increase competitiveness of *J. acutus* and *P. australis* under similar salinity and water table levels, respectively.

2. Materials and methods

2.1. Study site

The study was performed in the delta of the Llobregat river, a microtidal wetland area (maximum astronomical tidal range of about 0.25 m) of 7000 ha in the Barcelona metropolitan area (Fig. 1). The climate is typically Mediterranean with maritime influence, showing a mean annual rainfall of 640 mm with two drought seasons (summer and winter), and a mean annual temperature of 15.5 °C with hot summers and mild winters (data obtained from the International Airport of Barcelona, located in the delta, for the 1971–2000 period).

The Llobregat delta microtidal system is formed by a complex mosaic of coastal marsh communities that are not arranged in the typical zonation of macrotidal systems due of the lack of a clear tidal waterlogging gradient. Instead, the overall communities distribution is controlled by a combination of local factors such as topography and salinity. Moreover, the Llobregat delta has undergone major land transformations over the last 50 years, with an overall expansion of urban and industrial areas together with the abandonment of former agricultural areas. Part of these abandoned fields experienced spontaneous restoration, and forms the present marsh mosaic. The most abundant marsh plant communities are reedbeds, rushbeds, and halophytic scrubs.

2.2. Experimental design

We performed a field competition experiment based on replacement series (de Wit, 1960), using 20 different locations that represented a complex of natural gradients due to waterlogging, salinity and soil texture (Fig. 2). The locations were selected from a data set of 45 natural marsh stands in the Llobregat delta from which some edaphic parameters from the upper 20 cm (texture, percentage of organic matter, etc.), as well as the hydrologic conditions over the previous three years (conductivity and level of water table) were known (for more information see Batriu et al., 2011). From this data set, we selected 10 locations encompassing a wide gradient of three environmental variables: (i) mean water table

level, (ii) mean conductivity of the water table, and (iii) soil texture. An additional set of 10 locations were randomly selected from the remaining stands. Each location was equipped with a buried vertical PVC pipe (1.5 m long) in order to monitor the water table level during the experiment.

In each experimental location we set five squares of 50 cm × 50 cm, surrounding the point where water table was monitored (Fig. 3). In each square we planted six plant cuttings (short segments of rhizome with developing leaves), in a regular pattern and each separated 15 cm from its nearest neighbors. This distance was similar to that used in other competition experiments done with *P. australis* (Rickey and Anderson, 2004; Wang et al., 2006). The replacement series consisted of one pure square per species, and three squares with 2, 3 and 4 cuttings (i.e., 33%, 50% and 67%) of each species. Thus, in each location there were five competition treatments (two squares with only one of each species, and three squares with both species) and 30 plants (15 of each species).

During the spring of 2007 we cut off all the vegetation within a 1.5 m radius circle around the pipe, in each location. Also, in each treatment square all the rhizomes and regenerative roots from the top 25 cm of soil were removed.

In winter 2007, approximately 600 young rhizome segments of each species were collected from a mesohaline Llobregat delta marsh. We obtained 4–8 cm long rhizome segments of *P. australis* each with two buds from which new shoots could emerge to originate a new plant. In *J. acutus* we obtained shorter (2–4 cm) rhizome segments, including two buds and two photosynthetic stems each. These rhizome fragments were grown in pots with standard organic substrate in a greenhouse, during spring 2007. In June 2007, 300 living plants of each species were randomly selected and transferred to the 20 experimental locations. We watered the plants once a week for one month to minimize the transplant shock. During this period, plants showing signs of either herbivory (mainly by rabbits) or transplant shock were replaced with plants from the greenhouse. In addition, locations with some replaced plant due to herbivory or showing any evidence of herbivory activity (e.g., grazed surrounding vegetation, excrements, etc.), were fenced with an open top iron cage in order to prevent further herbivory effects; they correspond to 8 locations sited in two meso-haline marshes. The other locations were not fenced because they were situated in more halophilous marshes where rabbits and rodents are scarce or absent (see Fig. 1).

Every two months, we monitored the water table level and conductivity in each location, from August 2007 to October 2008. For conductivity measurements, a WTW TetraCond 325 sensor was used. During this period, each experimental square was kept clean of plants other than those transplanted, and the surrounding vegetation was periodically cut off in order to prevent light reduction. In October 2008 both mortality and growth were assessed. Mortality of plants was calculated as the proportion of dead individuals at the end of the study relative to the planted individuals, in each treatment. To assess growth, all the above ground plant biomass was collected and oven-dried at 60 °C until constant weight. The below ground biomass was not collected due to technical difficulties in root and rhizome extraction in the field, as discussed by Cahill (2002).

Clay contents of the 20 cm top soil (hereafter clay) was obtained from Batriu et al. (2011) data, assuming that it does not change over short periods of time. Clay content ranged from 47.7% to 4%. Both mean water table level (hereafter water table) and mean water table conductivity (hereafter conductivity) were calculated using the current monitoring data set (from August of 2007 to October of 2008). Water table ranged from 5.5 cm below ground to 61.3 cm below ground, and conductivity ranged from 3900 $\mu\text{S}/\text{cm}$ (2.08 ppt) to 56,000 $\mu\text{S}/\text{cm}$ (37.24 ppt).

To evaluate the fundamental niche of each species and to quantify the stress effects we used the mortality values of the treatments

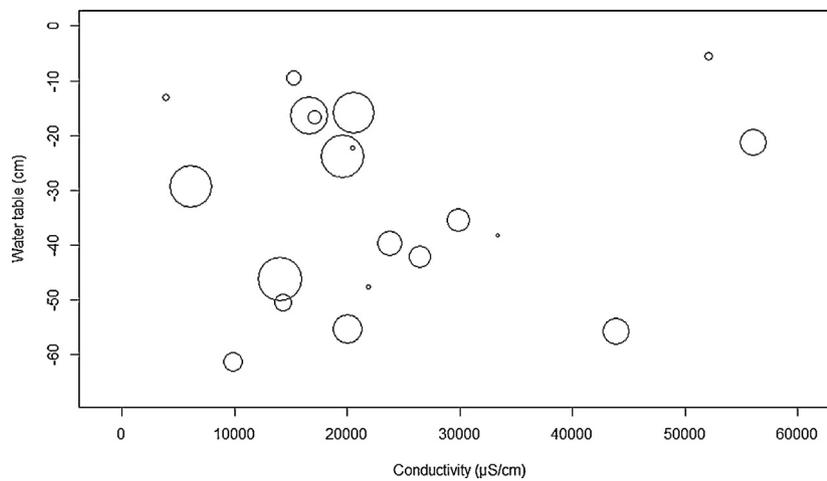


Fig. 2. Mean conductivity and water table level in the 20 experimental locations. Circle diameters are proportional to the clay content of the local soil, according to Batriu et al. (2011).

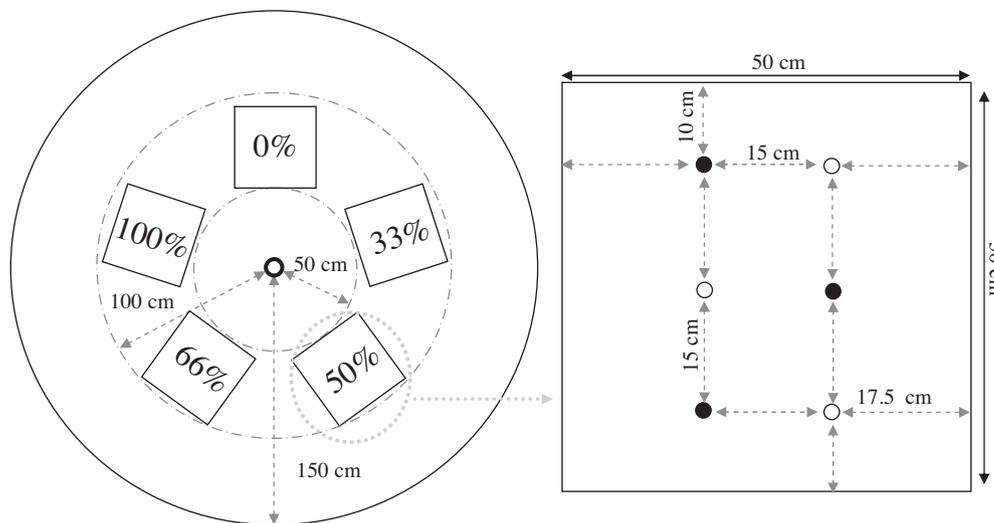


Fig. 3. Experimental design of the replacement series. Left figure shows the structure of one experimental location, with the five competition treatments and the central point with the sunken pipe used to monitor water table. Right figure details the transplant distribution in a given treatment (50%). Black and white points correspond, respectively, to *J. acutus* and *P. australis* transplants.

with no interspecific competition, following the recommendations by He and Bertness (2014). Four categories of stress were considered (low, <20% mortality; medium, 21–50% mortality; high, 51–80% mortality; and extreme, >81% mortality).

2.3. Statistical analyses

The effects of the environmental gradients and the competition treatment on mortality and dry aboveground biomass of transplanted individuals were tested separately using a used a GLMM (Generalized Linear Mixed Model) because the data were nested, with 20 sampling points within five competition treatments. Previously, the possible effects of fencing upon mortality and biomass were assessed by means of a Kurskal–Wallis test comparing fenced and non-fenced localities. Significant differences were detected in *P. australis* mortality (Chi-sq = 5.32 and $p = 0.021$) and relative yield of *J. acutus* (Chi-sq = 5.88 and $p = 0.015$). Therefore, we decided to include fencing in all the models. GLMM analysis were done using environmental variables (water table, conductivity, and clay) and competition (expressed as competition treatment proportions 0.33, 0.5, 0.66 and 1) as quantitative continuous explanatory variables; fencing as a dummy explanatory variable (i.e., fenced versus non-

fenced) and location as a random variable. Mortality data were processed as a contingency table and therefore a binomial error distribution was used. Models were simplified using a manual stepwise backward selection, and then were compared using a likelihood ratio test, as Crawley (2007) recommends. For each variable in the model, p values were obtained comparing the likelihood ratio with and without the given variable.

To test the effects of gradients and competition treatment on dry biomass we used a relative yield index (hereafter RY). According to Williams and McCarthy (2001), RY is a very versatile index, it is meaningful, and it can be plotted on a RY space to illustrate the outcome of competitive interactions. To calculate RY we could only use 13 locations, since in the rest of locations (i.e., seven) all the individuals of one of the study species died in at least one treatment. RY was calculated following Williams and McCarthy (2001), as expressed below:

$$Y_{\text{alive}} = \frac{Y_{\text{total}}}{N_{\text{alive}}}$$

$$RY = \frac{Y_{\text{alive mix}}}{Y_{\text{alive mono}}}$$

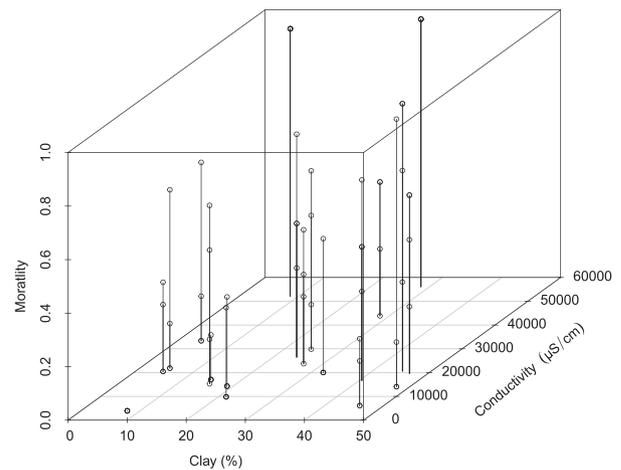
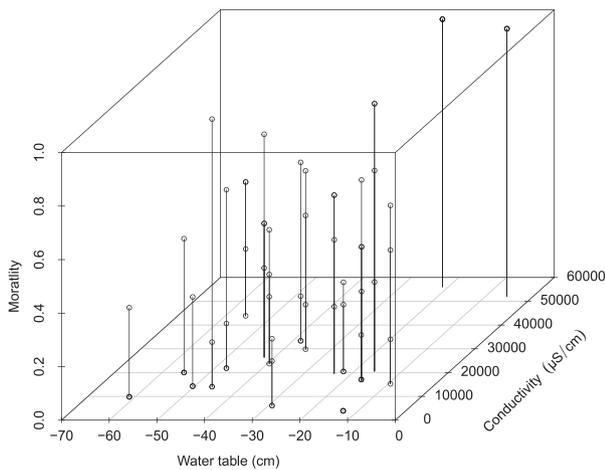
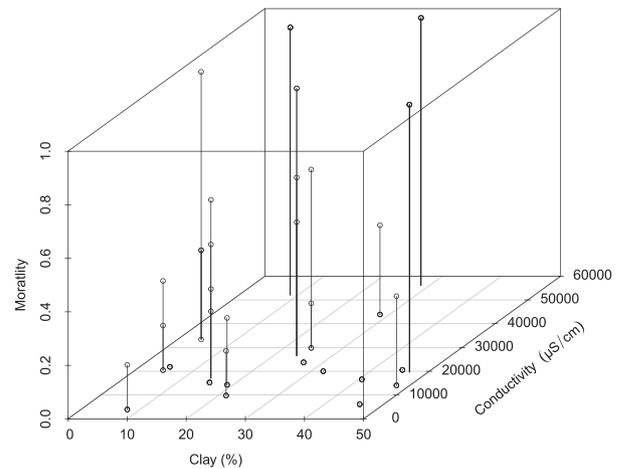
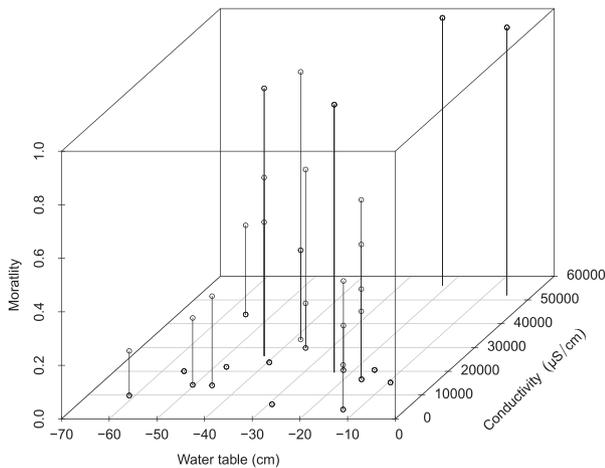
Juncus acutus*Phragmites australis*

Fig. 4. Mean values for each location of mortality of *P. australis* and *J. acutus* plotted against water table level, water table conductivity and clay content in soil.

Y = yield; Y_{alive} = yield per living individual; N_{alive} = number of living individuals per competition treatment; Y_{total} = yield per competition treatment; Y_{alivemix} = yield per living individual in mixture competition treatment; $Y_{\text{alivemono}}$ = yield per living individual in monoculture competition treatment; RY = relative yield index.

For species A competing with species B, RY_A is higher than, equal to, or lower than 1, when intraspecific competition is stronger than, equal to, or weaker than interspecific competition, respectively. According to Williams and McCarthy (2001), the combination of the indexes of species A and B determines the outcome of interactions as follows: if $RY_A < 1$ and $RY_B > 1$, species A is competitively suppressed; if $RY_A > 1$ and $RY_B < 1$, species B is competitively suppressed; where, $RY_A < 1$ and $RY_B < 1$, both species are suppressed in a mixture crop; and if $RY_A > 1$ and $RY_B > 1$, then both species exhibit facilitation, as they perform better in mixture plots.

RY was assessed using GLMM simplified as previously mentioned, but using a Gaussian error distribution. For each variable in the model, p values were obtained comparing the likelihood ratio with and without the given variable. To calculate the thresholds between $RY < 1$ and $RY > 1$, that is, the point where intraspecific competition exceeds interspecific competition, we used the model

predictions. All the statistical analyses were performed using R 2.4.1 (R Development Core Team, 2012). To perform GLMM we used the package lme4 version 0.99375-31 (Bates et al., 2009).

3. Results

Transplant mortality was strongly associated to extreme environments. All the individuals of both study species died in two extremely saline locations ($>50,000 \mu\text{S}/\text{cm}$ in the water table) with contrasting soil texture: one with sandy soil (8.7% of clay) and another with rich clay soil (46.8%; Fig. 4 and Table 1). Moreover, all the *P. australis* individuals died in a location with rich clay soil (46.8%) but with not extremely saline water table (19,600 $\mu\text{S}/\text{cm}$). For both species, quantified stress based on intraspecific treatment mortality ranged from low to extreme (Table 1).

In addition, no differential effects between interspecific interactions and intraspecific interactions were observed on the survival of either *J. acutus* or *P. australis*. In both species, mortality increased with increasing conductivity and soil clay content ($p < 0.025$), and it was significantly affected ($p < 0.025$) by the interaction of conductivity and level of water table (Table 2). In consequence,

Table 1
Mortality of *J. acutus* and *P. australis* measured in intraspecific competence treatments as approach to species fundamental niche. Stress categories: Low <20% mortality; Medium, 21–50% mortality; High 51–80% mortality; and Extreme, >81% mortality.

Environmental conditions			<i>J. acutus</i>		<i>P. australis</i>	
Conductivity ($\mu\text{S}/\text{cm}$)	Water table level (cm)	Clay (%)	Mortality	Stress	Mortality	Stress
3936	-13.1	7.84	0	Low	0.17	Low
6127	-29.3	45.94	0.17	Low	0	Low
9825	-61.3	21.29	0.33	Medium	0.17	Low
14079	-46.3	47.74	0.17	Low	0	Low
14301	-50.4	18.96	0.33	Medium	0	Low
15278	-9.5	15.47	0.17	Low	0	Low
16623	-16.3	40.47	0.5	High	0	Low
17074	-16.6	14.70	0.17	Low	0.67	High
19599	-23.7	46.87	0.67	High	1	Extreme
20041	-55.4	32.04	0	Low	0	Low
20478	-22.3	4.70	0	Low	0.17	Low
20568	-15.8	45.14	1	Extreme	0	Low
21863	-47.6	5.09	0.17	Low	0	Low
23766	-39.6	26.65	0	Low	0	Low
26450	-42.2	23.99	0.83	Extreme	1	Extreme
29825	-35.4	24.56	0.17	Low	0.17	Low
33375	-38.4	3.99	0.17	Low	0.33	Medium
43875	-55.8	28.40	0.5	High	0.33	Medium
52050	-5.5	8.69	1	Extreme	1	Extreme
56063	-21.3	28.57	1	Extreme	1	Extreme

Table 2
Simplified GLMM models of *J. acutus* and *P. australis* mortality.

Variable	<i>J. acutus</i> mortality			<i>P. australis</i> mortality		
	Estimate	Std. error	z value	Estimate	Std. error	z value
(Intercept)	-5.412	1.652	-3.276 ns	-13.65	3.413	-3.999 ns
Cond	0.000219	0.000075	2.908***	0.000567	0.000159	3.554***
Wt	-0.02928	0.03592	-0.815 ns	-0.1668	0.0634	-2.631*
Clay	0.04917	0.01765	2.786**	0.05918	0.02299	2.574**
Cond \times Wt	0.000003	0.000002	1.983**	0.00001	0.000003	3.055***

Wt, mean water table level; Cond, mean water table conductivity; Clay, soil clay content; \times , interaction; significance of z value: ns $p \geq 0.05$, * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

the negative effects of conductivity increased dramatically with increasing either water table or soil clay content (Fig. 4). Water table determined the differences between the *J. acutus* and *P. australis* mortality models. While an increase in water table level significantly ($p < 0.025$) reduced mortality in *P. australis*, this effect was not significant in *J. acutus* ($p > 0.05$; Table 2). Finally, it should be noted that fencing did not have any significant effect on the mortality of the studied species.

RY of *J. acutus* was modulated by conductivity and water table gradients. Conductivity had a positive significant effect ($p < 0.05$) on RY. The effect of water table level was not significant, while the interaction of water table and conductivity was significant ($p < 0.05$; Table 3, Figs. 5 and 6). This means that the combination of conductivity and water table determines the threshold between $\text{RY} < 1$ and $\text{RY} > 1$, that is, the point where intraspecific competition exceeds interspecific competition. No significant effects of competition were detected, and the initial proportion of *J. acutus* planted did not affect the outcome of competition. Clay content had no significant effect on RY of *J. acutus*. Like in mortality, fencing did not have any significant effect on RY of *J. acutus*.

RY of *P. australis* was also modulated by conductivity and water table gradients, like RY of *J. acutus*, but also for the competition treatment proportion (i.e., the proportion of *P. australis*) (see Table 3, Figs. 5 and 6). Therefore, the combination of conductivity, water table level and proportion of *P. australis* determines the threshold between $\text{RY} < 1$ and $\text{RY} > 1$. The RY of *P. australis* was significantly ($p < 0.001$) affected by water table. Conductivity had not a significant negative effect, while its interaction with water table level was significant ($p < 0.0001$). Moreover, *P. australis* proportion had a significant positive effect ($p < 0.025$) as well as the interac-

tion of this factor with conductivity ($p < 0.001$). Clay content did not significantly affect the RY of *P. australis*. Like in mortality, fencing did not have any significant effect on RY of *P. australis*.

The RY values of both species plotted against conductivity and water table (Fig. 7) highlight the outcome of species' interactions along these environmental gradients. Three types of interactions (sensu Williams and McCarthy, 2001) may occur: suppression, interference or facilitation. Specifically, *P. australis* can competitively suppress *J. acutus* when water table is shallower than 20 cm or deeper than 55 cm, while the opposite pattern is expected when the water table level is between 20 and 50 cm deep. Interference occurs when water table is around 50 cm deep and conductivity is between 15,000 $\mu\text{S}/\text{cm}$ and 20,000 $\mu\text{S}/\text{cm}$, while facilitation occurs when water table is shallower than 20 cm and conductivity is higher than 15,000 $\mu\text{S}/\text{cm}$, or when the water table level is around 40 cm deep and conductivity is around 35,000 $\mu\text{S}/\text{cm}$. According to the results of mortality (Table 1), the environmental conditions promoting facilitation correspond to low stress situations for *J. acutus* and low, medium or high stress situations for *P. australis*.

4. Discussion

4.1. Plant interactions

In coastal marshes, most of the studies dealing with mortality focus on nurse effects (Castellanos et al., 1994; Egerova et al., 2003), while studies centered in juveniles of perennial species are scarce (but see He et al., 2013). One of these scarce studies shows that there are no differences between intraspecific and interspecific interac-

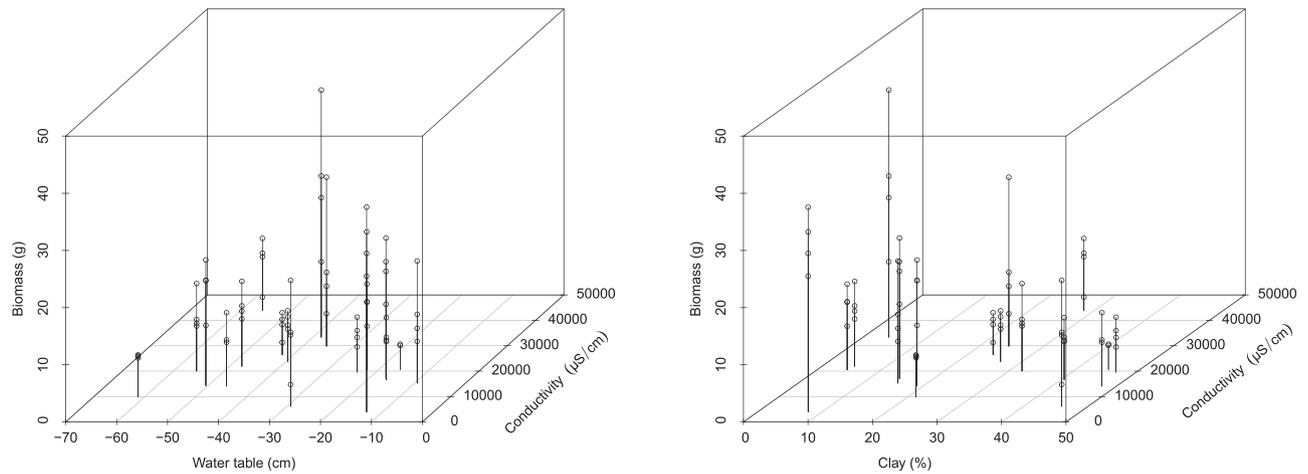
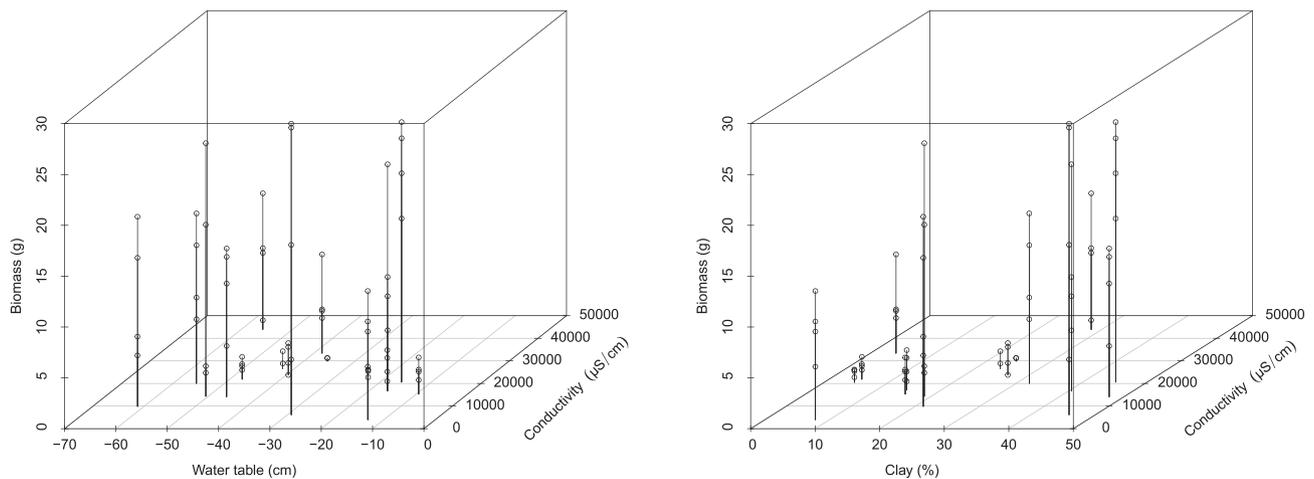
Juncus acutus*Phragmites australis*

Fig. 5. Mean values for each location of biomass per alive individual of *P. australis* and *J. acutus* plotted against water table level, water table conductivity and clay content in soil.

Table 3
GLMM models on relative yield per living individual of *J. acutus* and *P. australis*.

Variable	<i>J. acutus</i> relative yield			<i>P. australis</i> relative yield		
	Estimate	Std. Error	<i>t</i> value	Estimate	Std. Error	<i>t</i> value
(Intercept)	0.6845	0.379	1.806 ns	1.82	−1.974	0.922 ns
Cond	0.000051	0.000024	2.12*	−0.000066	0.000118	−0.562 ns
Wt	−0.004605	0.00887	−0.519 ns	0.1177	0.0395	2.978**
Prop				4.924	2.061	2.387*
Cond × Wt	0.000001	0.000001	1.945*	−0.000008	0.000002	−3.423**
Cond × Prop				0.000331	0.000098	−3.219**

Wt, mean water table level; Prop, proportion of *P. australis*; Cond, mean water table conductivity; Clay, soil clay content; ×, interaction; ns $p \geq 0.05$, * $p < 0.05$, ** $p \leq 0.01$.

tions in seedling survival of perennial species growing in coastal marshes (Bertness and Yeh, 1994), which agrees with our results.

Although tolerance of *P. australis* to salinity varies greatly at local and regional scales throughout the world (Lissner and Schierup, 1997), negative effects of salinity on reed growth, seedling survival, and seed germination have been widely documented (Burdick et al., 2001; Greenwood and MacFarlane, 2006; Hootsmans and Wiegman, 1998). Recent works show that *J. acutus* does not tolerate

high salinity (Greenwood and MacFarlane, 2009; Martínez-Sánchez et al., 2006), though its capacity to overcome saline stress exceeds that of most *P. australis* clones (Batriu et al., 2011). Accordingly, we expected that *J. acutus* would outcompete *P. australis* under high water conductivity, independently of the water table level. However, we were not able to verify this effect since most of the studied situations with high water table conductivity caused high mortality of *P. australis* – which did not allow RY calculations (see Table 1). The

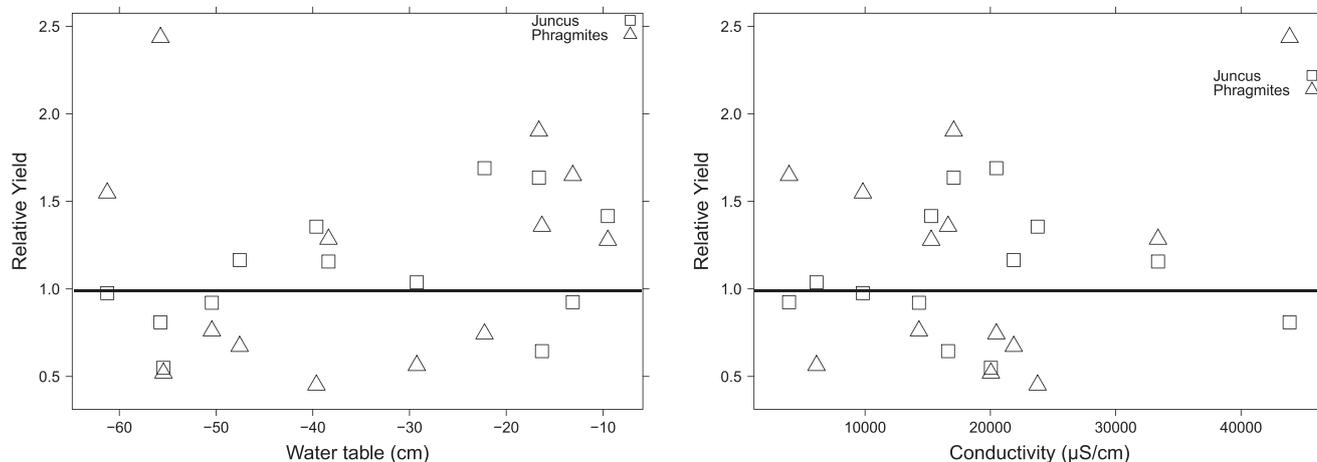


Fig. 6. Mean Relative Yield (RY) values for *P. australis* and *J. acutus* in each location plotted against water table level and conductivity. Black line marks the threshold of RY = 1.

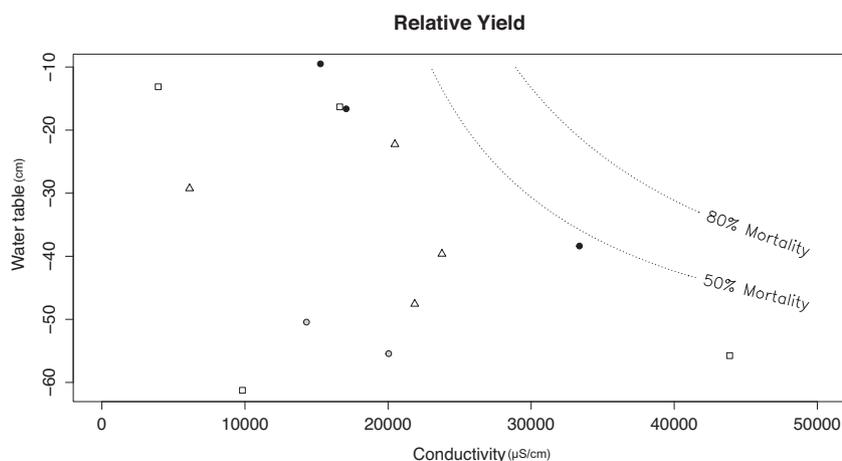


Fig. 7. Response model based on the balance of relative yield between mean RY values of *P. australis* and *J. acutus* in each location plotted against water table level and water table conductivity. Dotted lines mark mortality thresholds of *P. australis* obtained in the GLM model with a 15% soil clay. Point codes: □, *P. australis* suppresses *J. acutus* (*J. acutus* RY < 1 and *P. australis* RY > 1); △, *J. acutus* suppresses *P. australis* (*J. acutus* RY > 1 and *P. australis* RY < 1); ●, facilitation (*J. acutus* RY > 1 and *P. australis* RY > 1); ◐, interference (*J. acutus* RY < 1 and *P. australis* RY < 1).

locations where RY was calculated correspond to situations where facilitation (*sensu* Williams and McCarthy, 2001) occurred.

Results from replacement series in plant competition studies reflect both interspecific and intraspecific interactions, but they do not express the specific magnitude of each of them (Jolliffe, 2000), despite they can be successfully used to explore facilitative and competitive interactions (e.g., Greenwood and MacFarlane, 2009). Wang et al. (2006) reported that *Spartina alterniflora* enhances *P. australis* performance under high salinity conditions, and they argued that it might reduce the interstitial salinity of the rhizosphere of *P. australis* through selective salt absorption. *J. acutus* is also known to absorb salt through the roots (Boscaiu et al., 2007) and, therefore, it might enhance the environmental conditions for *P. australis* under moderate salinity stress, like that found in some of our locations. We must remark that water table conductivity values are only a good surrogate for rhizosphere salinity if water table is high (Álvarez-Rogel, 2001). Therefore, in some location when water table was low (below 55 cm) competitively suppress *J. acutus* (see Fig. 7).

Concerning water table, the prediction that common reed would competitively suppress *J. acutus* under low saline waterlogged conditions was corroborated. We also expected that *J. acutus* would outcompete *P. australis* when water table was deep and non-saline, since previous information indicates that *J. acutus* is less drought

sensitive than *P. australis* (Batriu et al., 2011). This effect was partially verified in middle ranges of water table level but, surprisingly, the study species showed interference, or even *P. australis* could competitively suppress *J. acutus*, in deeper water table situations. Suppression of *J. acutus* seems to be more likely in fertile soils (i.e., low values of organic C/N; see below), as *P. australis* is well adapted to nutrient-rich locations (Nakamura et al., 2010; Romero et al., 1999) where it can grow much faster than *J. acutus*.

Some greenhouse studies have shown that soil texture may affect competition between coastal marsh species (Huckle et al., 2000; Wang et al., 2006). But according to Barbour et al. (1998), experimental results obtained under highly controlled greenhouse conditions are often difficult to reproduce in the field. Properties of substrates used in greenhouse experiments are tightly controlled, while natural soils are complex systems with properties that are mutually correlated and often affected by historical factors. In our case, soil clay content is related to soils with high nitrogen content with respect to the organic carbon (Batriu et al., 2011) that should be related to the past agricultural use of some areas in the Llobregat delta. A priori we expected that the combination between saline conditions and clay abundance in soil would give *J. acutus* a competitive advantage, but common reed had higher salinity tolerance than expected. Our hypothesis is that soil fertility could have caused this unexpected effect. In most of the clayish locations conductivity

did not exceed the limits that produce a reduction in *N* acquisition of *P. australis* (Lissner et al., 1999). Thus, plants growing in clay fertile soils could tolerate salinity better than we initially expected. We also found that the combination of waterlogging and clay did not give a clear competitive advantage to *P. australis*, contrarily to our expectations. In coastal marsh plants, a general mechanism to overcome waterlogging is to produce adventitious roots (Colmer and Flowers, 2008). Such roots have been observed in *J. acutus* during this study, and are well known for closely related species like *Juncus maritimus* (Schat, 1984). As far as we know, the physiology and regulation mechanisms of adventitious roots production in *J. acutus* have not been studied, but they might be favored by improved nutrition conditions.

4.2. Plant zonation in microtidal coastal marshes

Shoot biomass growth is a good proxy for long-term plant performance but results of this studies must be interpreted with caution (Cahill, 2002). Plant interactions may vary through life stages of the interacting species (Callaway and Walker, 1997) and even abrupt changes on these interactions may occur following rare environmental events (Rebele, 2000). However plant interactions inferred from short-term transplant experiments that only account on shoot growth satisfactorily explain actual vegetation patterns in macrotidal coastal marshes (e.g., Pennings et al., 2005). In our case, plant interactions and differences of fundamental niche partially explain the field distribution of *J. acutus* and *P. australis*. Along the waterlogging gradient considered here, monospecific stands of common reed and *J. acutus* occurred in more and less waterlogged sites, respectively, due to competitive suppression phenomena. In contrast, mixed stands occurred in drier sites due to species interference. This partially corresponds to field stands where common reed dominates but *J. acutus* was also found. However, our study indicates that *P. australis* can suppress *J. acutus* in drier sites, which is opposite to the observed field pattern. In fact, the experimental locations from which we obtained these results were initially *J. acutus* stands. Along the salinity gradient, the study indicates that *J. acutus* stands occur in more saline conditions (water table shallow and with high to medium conductivity levels) than *P. australis* stands do, through higher mortality of reed juveniles – and this agrees with our field observations. Finally, in less saline sites mixed stands would occur due to facilitation interactions, a pattern which was not observed in the field. Differences between present and predicted patterns point the importance of other factors such as past herbivory or founder effect, especially in situations where facilitation occurs. In any case, our results support those by Costa et al. (2003) in arguing that competitive interactions in microtidal coastal marshes are not so relevant than they are in macrotidal marshes. Therefore, additional specific research would be desirable to clarify the processes determining the complex plant distribution in microtidal systems.

“Stress” is species-specific and relative, the higher extreme of a stress gradient being defined as the harshest environment that a given organism can tolerate, or the physical limit of its fundamental niche over a specific study area/habitat type (Bruno et al., 2003; Maestre et al., 2009). In our study, the tolerance limits of stress were reached for both *P. australis* and *J. acutus* (Batriu et al., 2011). Results regarding mortality patterns suggest that the study species partly share their fundamental niche along the studied gradients, although *P. australis* was more sensitive to salinity than *J. acutus*. In relation to the predictions of the SGH, this study indicates that facilitation may occur at contrasting stress levels for different species, specifically low for *J. acutus* but indifferent for *P. australis*. These results highlight that the predictions of the SGH are not fulfilled where plant interactions are driven by distinct stress gradients that are not spatially correlated (He and Bertness, 2014;

Mod et al., 2014). This is especially relevant in microtidal marshes, where salinity and waterlogging are in many cases not spatially correlated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquabot.2015.03.003>.

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