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Mycorrhizal fungi determine salt-marsh plant zonation depending on nutrient supply

Pedro Daleo[1,2]*, Juan Alberti[1,2], Alejandro Canepuccia[1], Mauricio Escapa[2,3], Eugenia Fanjul[1,2], Brian R. Silliman[4], Mark D. Bertness[5] and Oscar Iribarne[1,2]

[1]*Laboratorio de Ecología, Departamento de Biología (FCEyN), UNMdP, CC 573 Correo Central, B7600WAG, Mar del Plata, Argentina,* [2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina,* [3]*Instituto Argentino de Oceanografía (IADO), CC 804, Correo Central, 8000, Bahía Blanca, Argentina,* [4]*Department of Zoology, University of Florida, Gainesville, FL 32611, USA, and* [5]*Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912, USA*

Summary

1. Arbuscular mycorrhizal fungi (AMF) can affect nutrient uptake of associated plants and can vary in function from mutualism to parasitism as nutrient availability increases; thus they may interact with nutrient availability to influence plant community structure.

2. We experimentally investigated the hypotheses that AMF can affect the community structure of salt marshes by affecting plant competitive ability. We focused on: *Spartina densiflora*, that dominates physically benign high marsh habitats and *S. alterniflora*, which dominates more stressful low marsh habitats.

3. Colonization by AMF increased *S. densiflora* growth at low nutrient levels, but reduced growth at high nutrient levels. *Spartina alterniflora* was not colonized by AMF and showed increased growth only with nutrient amendment. Nutrient or fungicide additions resulted in *S. alterniflora* migrating to higher marsh elevations, displacing *S. densiflora*. When nutrient and fungicide additions were made together, however, dominance of *S. densiflora* was maintained in the high marsh.

4. *Synthesis.* These results show that AMF can affect the competitive ability of plants and can have a large impact on plant community structure. The community impacts of these symbioses may be especially sensitive to human-induced eutrophication, given that nutrient supply can modulate whether AMF positively or negatively affect associated plants.

Key-words: competition, community structure, mycorrhizal fungi, nutrients, salt marsh, *Spartina*, zonation

Introduction

Trade-offs between stress toleration and competitive ability have been found in a variety of environments (e.g. marine, Connell 1975; grasslands, Gurevitch 1986; alpine, del Moral 1983; floodplains, Jansen *et al*. 2005; salt marshes, Bertness 1991), and are known to generate zonation patterns where superior competitors dominate less stressful habitats, displacing subordinate, stress tolerant species, to stressful habitats (del Moral 1983; Wilson & Keddy 1986; Bertness 1991). Under low nutrient levels, nutrient uptake efficiency (Tilman 1982) or resource allocation (Grime 1977) commonly determines plant competitive interactions. Because mycorrhizal symbioses can affect nutrient uptake and require carbon investment (Smith & Read 1997), they can potentially affect competitive hierarchies and influence community structure.

*Correspondence author. E-mail: pdaleo@mdp.edu.ar

Arbuscular mycorrhizal fungi (AMF) form close associations with plants, in which fungi provide mineral nutrition to hosts while hosts provide fixed carbon to fungi (Smith & Read 1997). AMF are important in terrestrial ecosystems and can affect plant species diversity and productivity (van der Heijden *et al*. 1998; Smith *et al*. 1999; Klironomos *et al*. 2000), and community structure (e.g. van der Heijden *et al*. 1998; Hart *et al*. 2003). AMF can influence the outcome of competition depending on the level of mycorrhizal dependence and the competitive hierarchies of different plant species. For example, if a superior competitor is more dependent, AMF removal can promote coexistence, or even exclusion, by reducing its competitive ability (Umbanhowar & McCann 2005). Nutrient availability is also predicted to determine whether AMF affect the outcome of competition (Hart *et al*. 2003). At low nutrient levels, AMF may positively affect competitive ability by increasing nutrient uptake. At high nutrient levels, in contrast, mycorrhizal plants may be selected against because the

costs of symbiosis (i.e. large carbon removal) outweigh the benefits (Johnson *et al*. 1997; Hart *et al*. 2003; Olsson & Tyler 2004). Although the evidence suggests that mycorrhizal fungi can have a large impact on community structure, the effect of mycorrhizal fungi in determining species distributions and the importance of nutrient levels in determining the impact of mycorrhizal fungi on natural community structure remains largely unknown.

Plant zonation in salt marshes is believed to be the result of a trade-off between nutrient competition and flooding-related stress toleration (Bertness 1991; Pennings & Callaway 1992). Lower, more stressful habitats are characterized by stress tolerant, competitively inferior species, while the higher habitats are dominated by less tolerant, competitively superior species (Bertness 1991). Increases in nutrient availability, however, greatly alter this scenario. Under high nutrient conditions, above-ground competition for light dictates the outcome of species interactions (Levine *et al*. 1998; Emery *et al*. 2001). This shift leads to a change in species dominance, with stress tolerators becoming the dominant competitors (Levine *et al*. 1998; Emery *et al*. 2001). The mechanism for this switch may be, in part, a result of the degree of species-specific interactions with mycorrhizal fungi and how those interactions change with nutrient addition.

AMF fungi are commonly associated with plant species, including wetland and salt marsh species (see Carvalho *et al*. 2001; Burke *et al*. 2002; McHugh & Dighton 2004), but plant species differ in AMF colonization. Species found at lower elevations are usually less colonized or devoid of colonization (McHugh & Dighton 2004), while plants from higher elevations commonly show AMF colonization (Hoefnagels *et al*. 1993; Carvalho *et al*. 2001). The smooth cordgrass *Spartina alterniflora*, a common dominant at low marsh elevations in North and South America (Bertness 1999) is non-mycorrhizal (McHugh & Dighton 2004), while the species that occupy the upper edge of salt marshes usually have fungal root symbionts (*S. patens* in NW Atlantic coasts, Burke *et al*. 2002; *S. densiflora* in SW Atlantic marshes, Daleo *et al*. 2007). Thus, salt-marsh plant communities are good systems to evaluate questions about the effect of mycorrhizal fungi on plant community structure. In this paper, we experimentally investigate the hypothesis that AMF, in combination with nutrients, can affect the plant community structure of salt marshes.

Methods

The effects of AMF and nutrient levels on salt-marsh plant zonation were investigated by conducting factorial experiments in a marsh located on the eastern portion of the Bahía Samborombón (36°22′ S, 56°45′ W, Argentina).

A field experiment was conducted from 6 December 2005 to 8 April 2007. Forty 0.7 × 0.7 m quadrats were selected on a naturally occurring *S. alterniflora–S. densiflora* species border. Each plot was initially placed so that it contained *c.* 50% of each species (following Emery *et al*. 2001) and borders were marked with 8 mm diameter stakes. Plots were then randomly assigned to one of four treatments: (i) fungicide application (AMF removal), (ii) nutrient addition, (iii) fungicide application and nutrient addition, and (iv) control. In the fungicide application plots, 20 g (= 0.1 g kg soil^{-1}; see Hartnett & Wilson 1999) of the fungicide Benomyl (I.Q.A. Chemical Industries, Buenos Aires, Argentina) was added to the soil every 30 days (see Hartnett & Wilson 1999; Kahiluoto *et al*. 2000; Daleo *et al*. 2007). Benomyl was added dissolved in 10 L of water obtained from the nearest channel during low tide. In the remaining 20 plots (i.e. nutrient addition and control treatments), 10 L of channel water was added every 30 days as a procedural control. Nutrient addition plots received, every 2 weeks, 30 g of the pelletized fertilizer Ferticare (Kemira Growhow, Finland) containing 29% N, 5% P and 5% K. This application frequency is similar to fertilization treatments used in other nutrient addition studies in salt marshes (Levine *et al*. 1998; Emery *et al*. 2001). To independently examine treatment effects on each target plant species, an additional 40 monoculture plots of each species were randomly selected within 2 m of the zonal border and an experiment identical to the one described for the border between species was carried out in each zone. Statistical analyses for the border experiment and monocultures were carried out separately.

To evaluate the effectiveness of the fungicide, root samples from four plots of each treatment were taken at the end of the experiment, and the proportion of root tissue colonized by AMF was estimated. After rinsing with tap water, three random fine roots per plot (from *c.* 0.1 m depth) were cleaned for 20 min in a 10% KOH solution at 90 °C, placed in 1% HCl solution for 10 min and then stained with lactophenol–trypan blue (0.05%) at 90 °C for 20 min (Burke *et al*. 2002). Stained root samples were mounted on slides and examined at 400×. Quantification of root colonization by AMF was carried out by sampling a 1-mm field and determining presence or absence of mycorrhizal associations (using categories of hyphae, arbuscules, vesicles, hyphal coils, unidentified fungal tissue or absence of fungal tissue) in this field. At least 30 fields per slide were observed (at least 90 per plot). In this way, the proportion of potential root tissue occupied by AMF was estimated by averaging the data obtained in the three subsamples from each sample. The null hypothesis of no differences in proportion of potential root tissue occupied by AMF between treatments was evaluated with two-way ANOVA (with fungicide and nutrient as main factors). To evaluate possible non-target effects of the fungicide on other soil microbes, soil samples from control and fungicide addition plots were taken. Colony forming units (thereafter CFU) and colony diversity (by colony morphology differentiation) were determined after 96 h culture in a general bacterial medium (nutritive agar; 5 g L^{-1} peptones, 5 g L^{-1} beef extract, 15 g L^{-1} agar) at ambient temperature. To evaluate the effectiveness of nutrient addition, three soil samples per treatment were taken after 8 months and analyzed after the elimination of Cl^- by ion-chromatography (Universal Anion® column 150 mm, HCO_3^-/CO_3^{-2} mobile phase 2.2/2.8 mm, suppressed conductivity detection) for pore water nitrate concentration.

For the border experiment, percent coverage of each species was measured by placing a 0.5 × 0.5-m quadrat divided into 25 squares of 0.1 × 0.1 m over each plot and counting the number of squares containing each species. The measurements were performed at 1, 4, 8 and 16 months after the beginning of the experiment. For both monoculture experiments and the border experiment, after 16 months, the number of tillers, the mean height of five randomly selected tillers and above-ground biomass were measured in a 0.5 × 0.5-m square cantered in each plot. In the species border plots, this procedure was used for each species separately and the maximum distance that a tiller of each species had advanced from their border to the other species' zone was measured. Biomass was quantified by clipping all tillers in the square sample and drying to a constant weight. At the end of the experiment, one replicate of fungicide

application, one of nutrient addition, and one of fungicide application and nutrient addition of the *S. densiflora* monoculture were lost and no data was taken from these three plots.

The null hypothesis of no effect of nutrient and fungicide addition on species cover at different sampling dates was analyzed with repeated measures ANOVA (Zar 1999) with fungicide and nutrients as main factors, date as repeated factor and relative cover of *S. alterniflora* (i.e. [*S. alterniflora* cover/(cover of *S. alterniflora* + *S. densiflora*)]) as the dependent variable. The distance that a species moved from its own border into the other species zone was analyzed with three-way ANOVA (Zar 1999) with nutrient, fungicide and species as main factors. For the border experiment, and each monoculture experiment, tiller density, height and above-ground biomass of each species were analyzed with two-way ANOVA with nutrient and fungicide as main factors (Zar 1999). Normality of model residuals and homogeneity of variance between groups were checked for each analysis, and original variables were logarithmically transformed when necessary.

Results

Only *S. densiflora* showed AMF colonization. Observed mycorrhizal structures were mainly characteristic longitudinal AMF hyphae between cortex cells, with a few arbuscules and vesicles. Fungicide application reduced the proportion of AMF colonization by nearly 90% (0.48 ± 0.13 without fungicide application, compared to 0.046 ± 0.033 with fungicide application; $F_{1,12} = 77.46$, $P < 0.001$, see Methods section), while nutrient additions ($F_{1,12} = 0.6$, $P = 0.8$) or the interaction between nutrient and fungicide addition ($F_{1,12} = 0.77$, $P = 0.45$) did not affect mycorrhizal colonization. Fungicide application did not affect bacterial abundance (i.e. CFU; $t = 0.49$, d.f. = 18, $P = 0.4$) or diversity ($t = 0.063$, d.f. = 18, $P = 0.95$). Only nutrient addition affected soil nitrate concentrations (Nutrient: $F_{1,20} = 72.4$, $P = 0.01 \times 10^{-6}$; Fungicide: $F_{1,20} = 0.9$, $P = 0.34$; N × F: $F_{1,20} = 0.13$, $P = 0.73$) increasing nitrates by more than an order of magnitude (1.37 ± 0.14 μM without nutrient addition vs. 85.24 ± 24.28 μM with nutrient addition).

Both nutrient and fungicide addition increased the cover of *S. alterniflora* during the experiment (Table 1). At the end of the experiment, in plots without nutrient addition, fungicide application negatively affected *S. densiflora* stem height (from 47 ± 6.1 to 43 ± 8.7 cm), density (from 146.9 ± 26.5 to 90.2 ± 48.2 stems m^{-2}) and biomass (from 329.6 ± 103.8 to 232.3 ± 124.6 g m^{-2}). In combination with nutrient addition, in contrast, fungicide positively affected *S. densiflora* stem height (from 55 ± 11.3 to 70.1 ± 9.6 cm), density (from 95.5 ± 86.5 to 179.1 ± 157.3 stems m^{-2}) and biomass (from 264.9 ± 232.8 to 735.8 ± 532.6 g m^{-2}; Table 2, Fig. 1). Both nutrient and fungicide addition increased *S. alterniflora* height (from 61.7 ± 10.2 to 75.5 ± 10, and to 63.1 ± 5.7 cm, respectively), density (from 22.7 ± 9.3 to 56.3 ± 17.2, and to 35.2 ± 21.6 stems m^{-2}) and biomass (from 256.9 ± 103.7 to

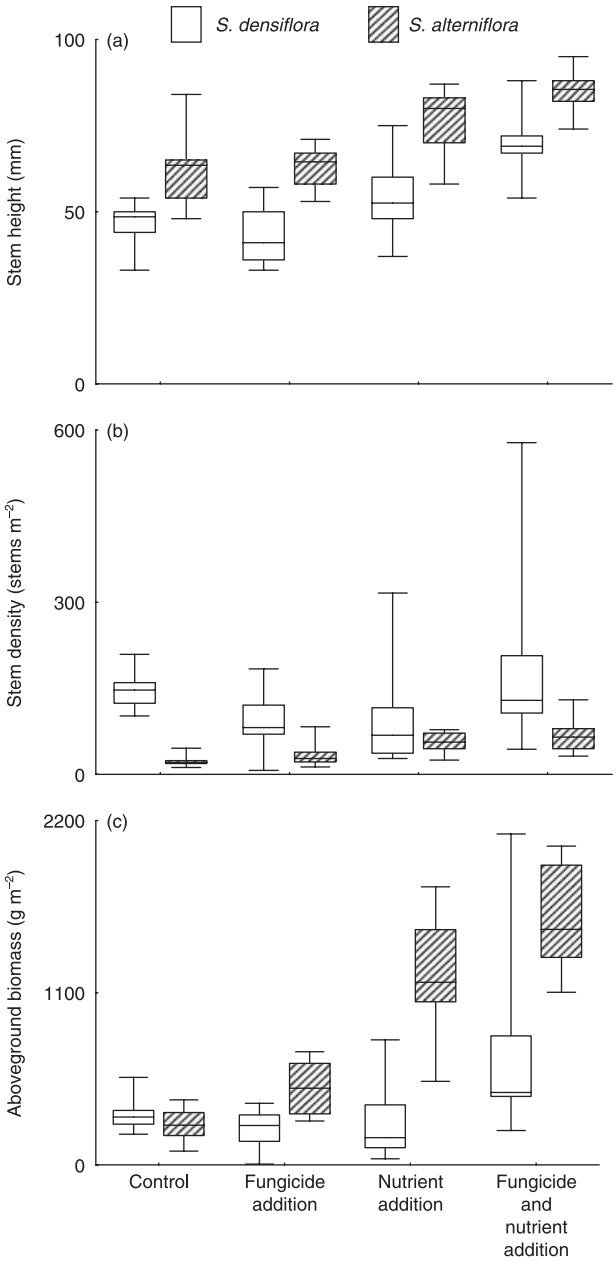

Fig. 1. The effect of mycorrhizal fungi and nutrient levels on (a) stem height; (b) stem density; and (c) above-ground biomass of *S. densiflora* and *S. alterniflora* after 16 months of a field experiment at natural borders between these two plant species. Here and thereafter, boxes represent 25th and 75th percentiles, vertical lines represent 1st and 99th percentiles, and horizontal lines inside boxes represent the median.

Table 1. Repeated measures ANOVA of the effect of nutrient addition (Nutrient effect) and mycorrhizal removal (Fungicide effect) on *Spartina alterniflora* relative cover at four sampling dates (Time) on a *S. densiflora–S. alterniflora* border zone

Source	d.f.	MS	*F*	*P*
Nutrient effect (N)	1	1.085	29.80	< 0.0001
Fungicide effect (F)	1	0.190	5.22	< 0.05
N × F	1	0.139	3.83	0.058
Error	36	0.036	–	–
Time (T)	3	0.345	35.91	< 0.0001
T × N	3	0.07	7.29	0.11
T × F	3	0.019	2.04	< 0.001
T × N × F	3	0.01	1.06	0.37
Error	108	0.0096	–	–

Table 2. Two-way factorial ANOVA of the effect of nutrient addition and mycorrhizal removal on a *Spartina densiflora–S. alterniflora* border zone

Source	d.f.	MS	*F*	*P*
Spartina densiflora				
Height				
Nutrient effect (N)	1	3080.00	36.88	< 0.0001
Fungicide effect (F)	1	319.20	3.82	0.06
N × F	1	893.00	10.69	< 0.005
Error	36	83.50	–	–
Density (log-transformed)				
N	1	0.07	0.01	0.9
F	1	0.11	0.02	0.88
N × F	1	4.43	8.88	< 0.01
Error	36	0.50	–	–
Above-ground biomass (log-transformed)				
N	1	1.53	2.20	0.15
F	1	0.56	0.81	0.37
N × F	1	8.03	11.55	< 0.005
Error	36	0.69	–	–
Spartina alterniflora				
Height				
N	1	3 204	46.17	< 0.0001
F	1	302.5	4.36	< 0.05
N × F	1	168	2.42	0.13
Error	36	69.4	–	–
Density				
N	1	181 710	25.37	< 0.0001
F	1	25 402	3.55	0.07
N × F	1	2	0.00	0.99
Error	36	7 163	–	–
Above-ground biomass				
N	1	10 270 805	137.90	< 0.0001
F	1	803 665	0.80	< 0.001
N × F	1	28 573	0.38	0.54
Error	36	74 428	–	–

Table 3. Two-way factorial ANOVA of the effect of nutrient addition and mycorrhizal removal on *Spartina densiflora* and *S. alterniflora* monocultures

Source	d.f.	MS	*F*	*P*
Spartina densiflora				
Height				
Nutrient effect (N)	1	5646.8	29.930	< 0.0001
Fungicide effect (F)	1	0.2	0.001	0.97
N × F	1	1491.4	7.904	< 0.01
Error	33	188.7	–	–
Density (log-transformed)				
N	1	3.27	29.00	< 0.0001
F	1	0.14	1.23	0.27
N × F	1	1.02	9.00	< 0.01
Error	33	0.11	–	–
Above-ground biomass				
N	1	19 819 651	80.58	< 0.0001
F	1	470 870	1.91	0.18
N × F	1	5 599 986	22.77	< 0.0001
Error	33	245 957	–	–
Spartina alterniflora				
Height				
N	1	15132.1	108.240	< 0.0001
F	1	0.4	0.003	0.96
N × F	1	0.1	0.001	0.98
Error	36	139.8	–	–
Density				
N	1	178 490	48.320	< 0.0001
F	1	314	0.080	0.77
N × F	1	6	0.002	0.97
Error	36	3 694	–	–
Above-ground biomass				
N	1	27 643 172	255.71	< 0.0001
F	1	16 662	0.15	0.69
N × F	1	36 544	0.34	0.56
Error	36	108 104	–	–

1216.9 ± 389.3, and to 486.9 ± 166.7 g m^{-2}); no interactions occurred between factors (Table 2, Fig. 1). At natural low nutrient conditions, fungicide application increased the relative biomass of *S. alterniflora* (i.e. *S. alterniflora*/(*S. alterniflora* + *S. densiflora*) dry weight) from 42% of control plots to 70% with fungicide, but decreased it from 83% to 70% with nutrient addition (Nutrient × Fungicide: $F_{1,36} = 22.07$, $P < 0.0001$; see Fig. 1). Clonal advancement into the other species' zone (distance gained from the original border into the other species habitat) was species dependent. While *S. densiflora* showed no colonization of the other species zone in any treatment (and the border actually move backward with nutrient as well as with fungicide addition), *S. alterniflora* increased colonization with nutrient as well as with fungicide addition (species × nutrients: $F_{1,72} = 4.93$, $P = 0.03$; species × fungicide: $F_{1,72} = 4.88$, $P = 0.03$).

In *S. densiflora* monocultures, as in the border experiment, the effect of fungicide application depended on the presence of nutrient addition; fungicide decreased stem height (from 70.78 ± 14.69 to 58.22 ± 14.65 cm), density (from 1245.78 ± 337.35 to 1066.67 ± 497.5 stems m^{-2}) and above-ground biomass (from 1039.8 ± 470.7 to 486.8 ± 256.3 g m^{-2}) at natural levels, but increased stem height (from 82.8 ± 15.57 to 95.67 ± 8.66 cm), stem density (from 1632 ± 477.9 to 2604.4 ± 936.7 stems m^{-2}) and above-ground biomass (from 1726.2 ± 378.9 to 2730.9 ± 752.2 g m^{-2}) when nutrients were added (Table 3, Fig. 2). In *S. alterniflora* monocultures, in contrast, nutrient addition greatly increased stem height (from 60.4 ± 12.1 to 99.3 ± 10.9 cm), stem density (from 133.6 ± 45.9 to 267.2 ± 70 stems m^{-2}) and above-ground biomass (from 387 ± 122.8 to 2049.6 ± 438.8 g m^{-2}). There were neither fungicide effects nor interactions between the factors with *S. alterniflora* (Table 3; Fig. 2).

Discussion

Our results show that AMF can determine the outcome of competitive interactions between *S. densiflora* and *S. alterniflora* in South American salt marshes. Nutrient addition, as in other salt marshes (see Emery *et al*. 2001), reversed this outcome, suggesting that, at low nutrient levels, competitive hierarchies may be set by below-ground competitive ability. Fungicide addition also reversed this outcome, indicating that at least part of the competitive advantage of *S. densiflora* over *S. alterniflora* is due to AMF symbiosis. Results also show that

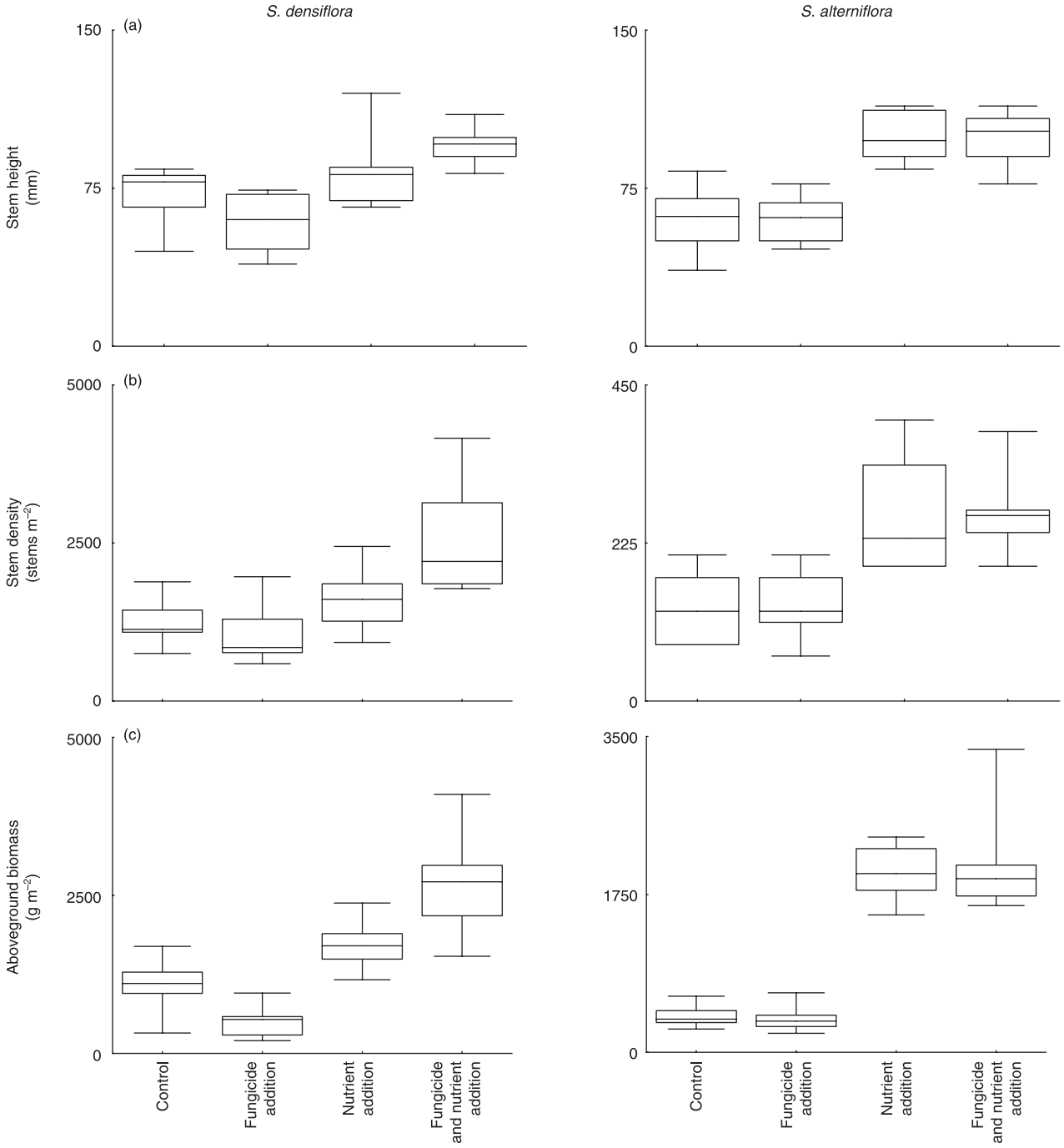

Fig. 2. The effect of mycorrhizal fungi and nutrient levels on (a) stem height; (b) stem density; and (c) above-ground biomass of *S. densiflora* and *S. alterniflora* after 16 months of field experiment at monocultures of each plant species.

as nitrogen availability increases, the effect of AMF removal on *S. densiflora* switches from negative to positive. These results suggest that the structure of salt-marsh plant communities is determined in large part by nutrient levels and AMF.

Benomyl application, the mycorrhizal suppression method used, can also suppress pathogenic and detritivorous fungi (Callaway *et al*. 2004) as well as other soil organisms, like root-feeding nematodes (see van der Putten *et al*. 1990). Unfortunately, there is no method that only allows the elimination of AMF in a field setting. However, Benomyl application is the best option to suppress AMF in the field, because it has, compared with other methods like irradiation, the lowest non-target effects (Hartnett & Wilson 1999; see also Daleo *et al*. 2007) and decreases the abundance of AMF relative to other soil fungi (Helgason *et al*. 2007). In our monocultures, fungicide application only affected *S. densiflora*, and had no effect on the non-mycorrhizal *S. alterniflora*, as expected if the main effect is mycorrhizal suppression. In addition, its

effect on *S. densiflora* growth was negative, but cancelled by nutrient application. These results indicate that, in our experiments, the main effect of Benomyl application was AMF suppression. We also show no fungicide effect on the soil bacterial community, which also suggests minimal experimental artefacts of Benomyl application. Thus, although fungicide application may reduce other soil, pathogenic or endophytic fungi, we are confident that our results are actually due to AMF suppression.

Trade-offs between stress tolerance and nutrient competition are a major driving force in the spatial structuring or zonation of salt-marsh plant communities (Bertness & Ellison 1987) and it has been postulated that high root biomass allocation (Levine *et al*. 1998; Emery *et al*. 2001) and/or optimal root strategies (Bouma *et al*. 2001) are responsible for the competitive advantage of high elevation, low stress species. At the same time, the trade-off between above- and below-ground competitive abilities determines the reversal of competitive outcomes at high nutrient levels (i.e. high biomass allocation to roots is an advantage at low nutrient availability, but a disadvantage if the limiting factor is light; Levine *et al*. 1998). Although we do not measure below-ground biomass allocation, in our case, competitive advantage of *S. densiflora* at low nutrient levels may depend on the investment of carbon into below-ground parts (tissue and/or AMF subsidy), but this species may lose above-ground competition at high nutrient levels because energy invested in below-ground cannot be invested in above-ground and hence, mycorrhizal interactions may change from positive to negative as nutrients increase (Hart *et al*. 2003; Olsson & Tyler 2004).

Our results are consistent with the resource-ratio hypothesis (Tilman 1982) that defines competitively superior species as those capable of growing at the lower resource level (thereafter R*) and predict that competition will change from below- to above-ground as productivity increases (see Wilson & Tillman 1991, 1993). AMF, by increasing nutrient uptake efficiency, can allow plant species to grow at lower nutrient levels (i.e. decrease its R*; Umbanhowar & McCann 2005). In our study, fungicide addition eliminated AMF, thus changing *S. densiflora* R* from lower to higher values, compared to the unchanged *S. alterniflora* R* (see Umbanhowar & McCann 2005). With high nutrient availability, the limiting resource may be light, and the higher above-ground biomass of *S. alterniflora* (see Emery *et al*. 2001) may determine its advantage on fertilized plots. Fungicide application also eliminates AMF, possibly allowing *S. densiflora* to allocate more energy to above-ground structures, decreasing the competitive advantage of *S. alterniflora*.

Until recently, AMF were considered to be unimportant in wetland communities (see Wolfe *et al*. 2006), but our results demonstrate their importance on salt-marsh community structure. How general are these results? Evidence in the literature suggests a positive association between plant competitive dominance and AMF infection in other marsh systems. Low elevation zone species like *S. alterniflora* (North and South America), *S. anglica* (Western Europe) and *S. maritima* (Western and Southern Europe, Mediterranean Sea coast, Western Africa), for example, are reported as non-mycorrhizial, while dominants like *S. pattens* (North America) or *Puccinellia maritima* (Western Europe, North American West Coast (introduced)) are reported as usually infected, and fugitives like *Distichlis spicata* (North and South America) are moderately or weakly infected (Hoefnagels *et al*. 1993; Carvalho *et al*. 2001). Thus, by affecting competitive abilities, AMF may affect plant zonation in other salt marsh systems and may also play a more important role in structuring other terrestrial plant systems where the processes generating spatial structure are not as well understood and are less amenable to study. AMF have been shown to have plant community effects in terrestrial systems largely based on greenhouse experiments (e.g. van der Heijden *et al*. 1998; Klironomos *et al*. 2000; but see Hart *et al*. 2003). Our field experiments demonstrate not only that AMF can affect plant distribution ranges by affecting competitive hierarchies, but also that this interaction can be a component of the eutrophication impacts on natural plant communities.

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References

Bertness, M.D. (1991) Interespecific interactions among high marsh perennials in a New England saltmarsh. *Ecology*, **72**, 125–137.

Bertness, M.D. (1999) *The Ecology of Atlantic Shorelines*. Sinauer Associates, Inc. Sunderland, MA.

Bertness, M.D. & Ellison, A.M. (1987) Determinants of pattern in a New England saltmarsh plant community. *Ecological Monographs*, **57**, 129–147.

Bouma, T.J., Koutstaal, B.P., van Dongen, M. & Nielsen, K.L. (2001) Coping with low nutrient availability and inundation: root growth responses of three halophytic grass species from different elevations along a flooding gradient. *Oecologia*, **126**, 472–481.

Burke, D., Hamerlynck, E. & Hahn, D. (2002) Effect of arbuscular mycorrhizae on soil microbial populations and associated plant performance of the salt marsh grass *Spartina patens*. *Plant and Soil*, **239**, 141–154.

Callaway, R.M., Thelen, G.C., Barth, S., Ransey, P. & Gannon, J.E. (2004) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology*, **85**, 1062–1071.

Carvalho, L.M., Caçador, I. & Martins-Loução, M.A. (2001) Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza*, **11**, 303–309.

Connell, J.H. (1975) Some mechanisms producing structure in natural communities: a model and evidence from field experiments. *Ecology and Evolution of Communities* (eds M. Cody & J.M. Diamond), Harvard University Press, The Belknap.

Daleo, P., Fanjul, E., Mendez Casariego, A., Silliman, B.R., Bertness, M.D. & Iribarne, O. (2007) Ecosystem engineers activate mycorrhizal mutualism in saltmarshes. *Ecology Letters*, **10**, 902–908.

del Moral, R. (1983) Competition as a control mechanism in subalpine meadows. *American Journal of Botany*, **70**, 232–245.

Emery, N.C., Ewanchuk, P.J. & Bertness, M.D. (2001) Competition and salt-marsh plant zonation: stress tolerators may be dominant competitors. *Ecology*, **82**, 2471–2485.

Grime, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, **111**, 1169–1194.

Gurevitch, J. (1986) Competition and the local distribution of the grass *Stipa neomexicana*. *Ecology*, **67**, 46–57.
Hart, M.M., Reader, R.J. & Klironomos, J.N. (2003) Plant coexistence mediated by arbuscular micorrhizal fungi. *Trends in Ecology and Evolution*, **18**, 418–423.
Hartnett, D.C. & Wilson, G.W.T. (1999) Mycorrhizae influence plant community structure and diversity in tall grass prairie. *Ecology*, **80**, 1187–1195.
Helgason, T., Merryweather, J.W., Young, J.P.W. & Fitter, A.H. (2007) Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *Journal of Ecology*, **95**, 623–630.
Hoefnagels, M.H, Broome, S.W. & Shafer, S.R. (1993) Vesicular-arbuscular mycorrhizae in salt marshes in North Carolina. *Estuaries*, **16**, 851–858.
Jansen, C., van de Steeg, H.M. & de Kroon, H. (2005) Investigating a trade-off in root morphological responses to a heterogeneous nutrient supply and to flooding. *Functional Ecology*, **19**, 952–960.
Johnson, N.C., Graham, J.H. & Smith, F.A. (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, **135**, 575–585.
Kahiluoto, H., Ketoja, E., & Vestberg, M. (2000) Creation of a non-mycorrhizal control for a bioassay of AM effectiveness. *Mycorrhiza*, **9**, 241–258.
Klironomos, J.N., McCune, J., Hart, M. & Neville, J. (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters*, **3**, 137–141.
Levine, J.M., Brewer, J.S. & Bertness, M.D. (1998) Nutrients, competition and plant zonation in a New England salt marsh. *Journal of Ecology*, **86**, 285–292.
McHugh, J.M. & Dighton, J. (2004) Influence of mycorrhizal inoculation, inundation period, salinity, and phosphorous availability on the growth of two salt marsh grasses, *Spartina alterniflora* Lois. and *Spartina cynosuroides* (L.) Roth., in nursery systems. *Restoration Ecology*, **12**, 533–545.
Olsson, P.A. & Tyler, G. (2004) Occurrence of non-mycorrhizal plant species in south Swedish rocky habitats is related to exchangeable soil phosphate. *Journal of Ecology*, **92**, 808–815.
Pennings, S. & Callaway, R.M. (1992) Salt marsh plant zonation: the relative importance of competition and physical factors. *Ecology*, **73**, 681–690.
Smith, M.D., Hartnett, D.C. & Wilson, G.W.T. (1999) Interacting influence of mycorrhizal symbiosis and competition on plant diversity in tall grass prairie. *Oecologia*, **121**, 574–582.
Smith, S.E. & Read, D.J. (1997) *Mycorrhizal Symbiosis*. 2nd edn. Academic Press, London, UK.
Tilman, D. (1982) *Resource Competition and Community Structure*. Princeton University Press, Princeton, NJ.
Umbanhowar, J. & McCann, K. (2005) Simple rules for the coexistence and competitive dominance of plants mediated by mycorrhizal fungi. *Ecology Letters*, **8**, 247–252.
van der Heijden, M.G.A., Boller, T., Wiemken, A. & Sanders, I. (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, **79**, 2082–2091.
van der Putten, W.H., Maas, P.W.Th., van Gulik, W.A.M. & Grinkman, H. (1990) Characterization of soil organisms involved in the degradation of *Ammophila arenaria*. *Soil Biology and Biochemistry*, **22**, 845–852.
Wilson, S.D. & Keddy, P.A. (1986) Species competitive ability and position along a natural stress/disturbance gradient. *Ecology*, **67**, 1236–1242.
Wilson, S.D. & Tillman, D. (1991) Components of plant competition along an experimental gradient of nitrogen availability. *Ecology*, **72**, 1050–1065.
Wilson, S.D. & Tillman, D. (1993) Plant competition and resource availability in response to disturbance and fertilization. *Ecology*, **74**, 599–611.
Wolfe, B.E., Weishampel, P.A. & Klironomos, J.H. (2006) Arbuscular mycorrhizal fungi and water table affect wetland plant community composition. *Journal of Ecology*, **94**, 905–914.
Zar, J.H. (1999) *Biostatistical Analysis*. 4th edn. Prentice-Hall, Upper Saddle River, NJ.

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