FEATURE ARTICLE

Mycorrhizal association with native and invasive cordgrass *Spartina* spp. in San Francisco Bay, California

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ABSTRACT: For the first time, hybrids of *Spartina alterniflora* × *foliosa* are reported to form mycorrhizal associations. This is important in light of the invasion dynamics within San Francisco Bay—where *Spartina* hybrids are invading tidal habitats and causing functional changes in the ecosystem. Mycorrhizal associations can positively influence biomass production in invasive *Spartina* and may contribute to increased invasion success. Of the *Spartina* hybrids investigated, 83% were mycorrhizal. During hybridization, the ability to be mycorrhizal may be contributed by the native *S. foliosa*, also found to be mycorrhizal, whereas the introduced *S. alterniflora* is non-mycorrhizal in its native habitat. Seedlings of *Spartina* hybrids inoculated with a commercial mycorrhizal mix showed greater above-ground growth and total biomass compared to control plants in the greenhouse. Mycorrhizal associations have the potential to influence the invasion trajectory of hybrid *Spartina* in San Francisco Bay, but additional research is needed.

KEY WORDS: Mycorrhizae · *Spartina* spp. · Hybrid · Salt marsh · Mycorrhizal inoculation · Invasion

INTRODUCTION

Facilitative interactions between species can lead to increased invasion success (Richardson et al. 2000, Ricciardi 2001, Grosholz 2005), with symbiotic microorganisms often playing important roles in shaping invasion dynamics (Clay 2001, Pringle et al. 2009). Fungi are among the most important symbionts of animal and plant hosts and have been associated with plants in mycorrhizal associations since the first colonization of land (Cairney 2000, Heckman et al. 2001). Mycorrhizal symbioses are present in 80% of surveyed plant species (Cairney 2000, Wang & Qiu 2006). Mycorrhizae enhance nutrition and increase the fitness of individual plants, and they can shape the structure and dynamics of plant populations and communities in a variety of environments (Isaac 1992, van der Heijden & Sanders 2002, Smith & Read 2008).

The mutualistic basis for mycorrhizal associations is largely due to a bidirectional transfer of nutrients: carbon from plant to fungus, and mineral nutrients (mostly phosphorus and nitrogen) from fungus to host plant (van der Heijden & Sanders 2002). The
location of the fungal symbiont in the plant's root, and its hyphal connections with the soil, ensure that it influences the plant's absorption of nutrients from the soil while obtaining organic carbon from the host plant (Smith & Read 2008). In addition to the survival and productivity of the host plant (Morton et al. 2004, Deacon 2006), mycorrhizal colonization can also influence plant traits such as clonal morphology, foliar quality, and fitness (Gehring & Whitman 2002, Kernaghan 2005). Moreover, mycorrhizal associations can improve water absorption or provide defense against pathogenic microorganisms and herbivores (Smith & Read 2008). Mycorrhizal fungi also play important roles in the succession and maintenance of plant communities (Brundrett 1991, Allen et al. 1995).

The fungi that form arbuscular mycorrhizal (AM) associations are members of the Glomeromycota, which are considered to be obligate mycorrhizal symbionts and which form hyphae that lack cross walls. AM fungi form specialized structures, called arbuscules, in plant root cells, and these structures are the sites of the transfer of nutrients between fungus and plant (Smith & Read 2008). A global analysis of AM fungal communities suggests high variability in taxon richness and species composition between different ecosystems (Opik et al. 2006). While it is thought that water-logged soils reduce mycorrhizal growth (Khan & Belik 1995), some aquatic plants have been reported to show AM infection (Cooke & Lefor 1998, Hildebrandt et al. 2001, Burke et al. 2003, Daleo et al. 2008). Evidence exists that mycorrhizae may be important components of salt marsh ecosystems (Rozema et al. 1986, Vanduin et al. 1990, Hoefnagels et al. 1993, Cooke & Lefor 1998). For example, the presence of AM fungi in salt marsh plants may increase competitive ability through increased phosphorus uptake, and through increased nitrogen acquisition as a result of reduced phosphorus limitation of root-associated diazotrophs (Hildebrandt et al. 2001).

Salt marshes are among the most productive and frequently invaded ecosystems on earth (Grosholz 2002). The halophyte cordgrass *Spartina* spp. is a highly productive primary producer in salt marshes. *Spartina foliosa* is the only native *Spartina* species on the North American Pacific coast (Moerby 1956), *S. alterniflora*, *S. patens* and *S. densiflora* have been introduced there accidentally or deliberately by humans (Ayres et al. 2004). The introduced *S. alterniflora* hybridized with the native *S. foliosa* in San Francisco Bay, and these *S. alterniflora × S. foliosa* hybrids (henceforward ‘*Spartina* hybrids’ or ‘hybrid *Spartina*’) have spread rapidly across the Bay (Daehler & Strong 1997, Ayres et al. 2004). *Spartina foliosa* is restricted to elevations above mean sea level (MSL), whereas hybrids invade mud flats below MSL as well as *Sarcocornia* meadows (Ayres et al. 2004). *Spartina* hybrids have greater above-ground biomass compared to native *S. foliosa* but differences among hybrids have been observed and have been partially explained by their percentage of hybridity (Ayres et al. 2004). I posit that some of these differences in biomass and height may be due to microbial associations, particularly associations with AM fungi.

Previous studies have found no (Hoefnagels et al. 1993) or extremely low (McHugh & Dighton 2004) mycorrhizal infections of *Spartina alterniflora* in its native habitat, but several other species of *Spartina* have been reported as mycorrhizal (Brown 1994, Burke et al. 2002, 2003). The occurrence and importance of AM fungi in native *S. foliosa*, and in introduced *S. alterniflora* and *S. densiflora*, as well as in invasive hybrids, has not been investigated. I present a survey of colonization by AM fungi of salt marsh plants in San Francisco Bay, California, with emphasis on different species of the cordgrass *Spartina* spp. (the native *S. foliosa*, the introduced species *S. alterniflora* and *S. densiflora*) as well as *Spartina* hybrids. Field sampling that spanned the range of tidal extent of each collected species at a site was conducted at Cogswell Marsh and Richardson Bay Park. In a greenhouse experiment seedlings were inoculated with a commercial mycorrhizal mix to determine the effect of mycorrhizal inoculation on plant biomass. The goals of this study were (1) to determine the prevalence and extent of mycorrhizal infection in both native and introduced *Spartina* spp. in San Francisco Bay, (2) to compare mycorrhizal infection rates in *Spartina* spp. with those in other estuarine plants, and (3) to determine, via a greenhouse experiment, whether inoculation with AM fungi influences *Spartina* growth and potential competitive ability.

**MATERIALS AND METHODS**

**Mycorrhizal colonization of *Spartina* spp. in San Francisco Bay**

Two sites were chosen in San Francisco Bay, California, where native, introduced, and hybrid *Spartina* spp. grow close together: Cogswell Marsh (37° 33’ 20” N, 122° 07’ 27” W) and Richardson Bay Park near Blackie’s Pasture (37° 53’ 24” N, 122° 27’ 59” W). Plants were identified on the basis of previous molecular genetic studies of hybridization in *Spartina* spp.
(Ayres et al. 2004, Ayres unpubl.) and on morphological characteristics for field identification (Invasive Spartina Project http://www.spartina.org/species.htm). In addition to cordgrasses, the following native estuarine plants were sampled as a comparison of mycorrhizal infection rates: *Sarcocornia pacifica*, *Jaumea carnosa*, *Distichlis spicata* and *Grindelia* sp. With the exception of *S. pacifica*, these species grow in tidal elevation above the *Spartina* spp. invasion zone (Grewell et al. 2007). Root samples spanning the range of elevation of each species at that site were collected in the summer of 2006. A total of 5 or 6 replicate root samples of each species was collected in a small area, care was taken to eliminate roots of non-target species within the sample. Samples were digested with 1% artificial seawater at 4°C for 4 wk. Seeds were checked every 2 to 3 d to detect the start of germination, and water was changed when cloudy. The germination rate was ~50% for hybrids, but none of the *S. foliosa* seeds germinated. Once the hybrid seedlings had reached a length of ~2 cm, individual seedlings (n = 50) were transplanted to 600 ml pots and kept in the greenhouse with overhead watering for 5 min every 6 h. After 3 wk, seedlings that had grown well (showed no yellow or brown on tiller) were paired according to similarity in plant height and randomly assigned to treatments (mycorrhizal (+M) or no mycorrhizal (−M) inoculate, n = 20 per treatment). All seedlings were replanted in individual pots in a sterile potting mix of 7 parts Yolo County loam and 3 parts potting mix. The bottom one-third of each 600 ml pot was filled with soil mixture. The middle third was filled with either a 1:1 mixture of sterile soil and mycorrhizal inoculate (+M) or soil mix (−M); the top third of all pots was filled with sterile soil mix. The mycorrhizal fungal inoculum used—MycoApply Endo (Mycorrhizal Applications Inc.)—contained a mixture of the following 4 AM fungal species: *Glomus intraradices*, *G. aggregatum*, *G. mosseae* and *G. etunicatum*. Plants were kept in the greenhouse at the University of California Bodega Marine Laboratory (Bodega Bay, CA) under ambient conditions for 12 wk. Plants were measured for height of the tallest tiller and the number of tillers every 2 to 4 wk. At the end of the experiment, soil was carefully rinsed off all roots, and the plants were dried at 60°C for 48 h; dry weight was determined for root and shoot biomass. Twenty treatment pairs were analyzed with paired 2-tailed t-tests to test differences among treatments (+M vs. −M).

**Greenhouse experiment**

Seeds were collected in November 2006, from multiple clones of hybrid *Spartina* at Richardson Bay Park and multiple clones of native *S. foliosa* at China Camp State Park (38° 00' 12” N, 122° 28' 03”). Seeds were separated from stems, sorted in the laboratory for similarity in plant height and randomly assigned to treatments (+M or −M). All seedlings were transplanted to 600 ml pots and kept in the greenhouse with overhead watering for 5 min every 6 h. After 3 wk, seedlings that had grown well (showed no yellow or brown on tiller) were paired according to similarity in plant height and randomly assigned to treatments (+M vs. −M).

**RESULTS**

**Mycorrhizal colonization of Spartina spp. in San Francisco Bay**

Native *Spartina foliosa*, introduced *S. alterniflora* and *S. densiflora*, and hybrid *Spartina* were all colonized by AM fungi (Table 1). *S. alterniflora*, which previous studies have shown to be non-mycorrhizal, contained mycorrhizae in only 30% of the samples in-
Table 1. Mycorrhizal association of native (N) and introduced (I) Spartina spp. and other saltmarsh plants reported
as the percentage of samples with mycorrhizal hyphae (%+Myc) and the average percentage of root length with
mycorrhizal infection (%RLMyc ±SE) per sample. n = number of replicates per sample

<table>
<thead>
<tr>
<th>Species</th>
<th>Native/ introduced</th>
<th>%+Myc</th>
<th>%RLMyc ± SE</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spartina foliosa</td>
<td>N</td>
<td>70</td>
<td>8.9 ± 3.6</td>
<td>10</td>
</tr>
<tr>
<td>Spartina alterniflora</td>
<td>I</td>
<td>30</td>
<td>1.0 ± 0.5</td>
<td>10</td>
</tr>
<tr>
<td>Spartina densiflora</td>
<td>I</td>
<td>100</td>
<td>5.9 ± 1.7</td>
<td>3</td>
</tr>
<tr>
<td>Spartina hybrida</td>
<td>I</td>
<td>83</td>
<td>4.7 ± 1.1</td>
<td>12</td>
</tr>
<tr>
<td>Sarcocornia pacifica</td>
<td>N</td>
<td>100</td>
<td>17.4 ± 3.4</td>
<td>10</td>
</tr>
<tr>
<td>Distichlis spicata</td>
<td>N</td>
<td>100</td>
<td>14.2 ± 3.0</td>
<td>10</td>
</tr>
<tr>
<td>Jaumea carnosa</td>
<td>N</td>
<td>100</td>
<td>19.4 ± 5.5</td>
<td>10</td>
</tr>
<tr>
<td>Grindelia sp.</td>
<td>N</td>
<td>100</td>
<td>14.3 ± 5.0</td>
<td>10</td>
</tr>
</tbody>
</table>

vestigated, whereas 70% of S. foliosa and 83% of hybrids showed mycorrhizal infections. The 3 samples of
S. densiflora were all mycorrhizal, as were all samples of Sarcocornia pacifica, Jaumea carnosa, Grindelia sp.,
and Distichlis spicata. The average percentage of root length with mycorrhizal infection (%RLMyc) per sample
was not significantly different between the 2 sites (Wilcoxon Rank Sums p > 0.05 for all species comparisons)
data from different sites were combined. Mycorrhizal infection rates in all species of Spartina were 9.10% RLMy
and 10-fold lower than infection rates in S. pacifica, J. carnosa, Grindelia sp. and D. spicata (Table 1) which tend to grow at higher elevations than Spartina spp., with the exception of S. pacifica.

Greenhouse experiment

Total and above-ground biomass were greater in hybrids grown with mycorrhizal inoculate in the greenhouse. Plants with this treatment also grew taller (Fig. 1). The mean ± SE total dry weight biomass for Spartina spp. grown in the greenhouse with myc-orrhizal inoculate (+M) was 2802 ± 221 mg as com-
pared to a total biomass of 2292 ± 204 mg in the treat-
ment without inoculate (−M), with a highly significant
difference between groups (paired t = 3.01, p < 0.01, df = 19). Spartina spp. +M showed higher above-ground but not below-ground biomass as compared to
Spartina spp. −M (paired t-tests: shoot biomass t = 3.86 p < 0.01, root biomass t = 1.16, p = 0.26) (Fig. 1). Plants grown with mycorrhizas grew on average taller (mean ± SE; 518.1 ± 19.3 mm) than those without myc-orrhizae (457.7 ± 15.4 mm) as measured by the height of the tallest tiller (paired t = 2.45, p < 0.05), but no difference in the number of tillers per plant could be de-
tected (paired t = 0.64, p = 0.42). Plants grown with mycorrhizal inoculant had a significantly lower rootto-shoot ratio (mean ± SE; +M 0.661 ± 0.12, −M 0.806 ± 0.24, t = −2.43, p < 0.05) (Fig. 1).

DISCUSSION

Swarms of Spartina alterniflora × foliosa hybrids are invading both high and low marshes, are displace
native S. foliosa, and are causing changes in the functioning of the ecosystem by altering water flow, light penetr
ation and sediment deposition within marshes (Levin et al. 2006, Neira et al. 2006). For the first time, Spartina
hybrids are shown to form mycorrhizal associations in this highly invaded habitat. Mycorrhizae have previously been reported only
from S. cynosuroides, S. densiflora, S. gracilis, S. patens and S. pectinata. This is the first report of mycorrhizal
infection of S. foliosa and of Spartina hybrids. In introduced Spartina spp., hybridization may cause an increased
ability to associate with AM fungi. Both native S. foliosa and hybrid
Spartina showed a greater percentage of mycorrhizal infections than did introduced S. alterniflora.

Previous reports indicate that S. alterniflora lacks AM fungi in its native habitat on the Atlantic coast (reviewed in Brown 1994) and failed to become infected after 2 mo of growth with AM fungal inocula in the greenhouse (McHugh & Dighton 2004). I identified plants in the field as pure S. alterniflora based on morphological characteristics and previous molecular work with random amplified polymorphic DNA (RAPD) markers (Ayres et al. 2004). Differences in the degree of hybridity between samples of Spartina hybrids from San Francisco Bay have been shown with molecular markers (RAPD: Ayres et al. 2004, and microsatellite: Sloop et al. 2011). It is possible that some of the samples identified as pure S. alterniflora in this study may be cryptic hybrids with a low percentage of hybridity that could not be detected with RAPD markers.

It is generally difficult to compare infection rates directly among existing studies as different methods are often used (Gange et al. 1999). In the present study multiple species of Spartina are compared, which allows for a relative comparison of infection rates within the study. It appears that the ability to form mycorrhizae may have been conferred on the invasive hybrid by the native S. foliosa.

This study found greater colonization by mycorrhizae of plant species growing in higher elevation zones of marshes as compared to the lower zone occupied primarily by Spartina spp. Pugh (1961) also reported a higher number of fungal isolations from upland zones compared to bare mud flats on the Atlantic coast. He suggested that fungi were probably limited by the reducing conditions present in S. alterniflora sites. Cooke & Lefor (1990) hypothesized that the absence of mycorrhizae in S. alterniflora was due to harsh edaphic conditions common to its flooded environment. This notion was challenged by Hoefnagels et al. (1993) who investigated the mycorrhizal status of 5 salt marsh species—including S. alterniflora, S. patens, S. cynosuroides, Distichlis spicata and Juncus roemerianus—collected from 8 different North Carolina salt marshes. All species except S. alterniflora were mycorrhizal. These authors concluded that S. alterniflora is probably resistant to infection because S. patens grown in the same soil mixtures became mycorrhizal. Except for the presence of AM hyphae in S. alterniflora, the results of the present study match Hoefnagels et al. (1993) wherever equivalent species were investigated.

Glomus spp. appear to be the most commonly encountered AM fungi in salt marsh soils (Sengupta & Chaudhuri 1990, Carvalho et al. 2004, Wilde et al. 2009). A large portion of mycorrhizal hyphae in our samples belonged to this genus, but we also observed mycorrhizal hyphae with much smaller diameters that most likely belonged to the genus Scutellospora (A. Bennet, Scottish Crop Research institute, pers. comm.), in both S. foliosa and hybrids. Detailed analysis of AM diversity was beyond the scope of this study, but future work should make use of morphological characteristics of fungi and available molecular markers (Raab et al. 2005) to determine differences in mycorrhizae associated with native and hybrid Spartina.

Hybrid Spartina grown with mycorrhizal inoculant had a significantly larger total as well as above-ground biomass compared to non-inoculated plants, whereas no significant difference in root biomass was apparent. Mycorrhizal plants also grew taller. The increase in above-ground biomass and the increased height of tillers could represent a fitness advantage to the plant, as it allows it to keep more shoots above tidal inundation and allows for the colonization of lower elevation sites, while maintaining sufficient above-ground shoots for photosynthesis at all times. A reduced root-to-shoot ratio was observed in plants grown with AM fungi. Increased nutrient absorption due to the symbiosis could have reduced the requirement for large root biomass, freeing resources that can be invested in above-ground production (Smith & Reed 2008). Plants may invest fewer resources in root growth as AM fungi perform the work of additional roots (McHugh & Dighton 2004).

In a previous field experiment, the outcome of competition between Spartina alterniflora and S. densiflora was affected by AM infection and depended on nutrient conditions (Daleo et al. 2008). In low nutrient conditions, mycorrhizae conferred a competitive advantage on S. densiflora. Different nutrient conditions were not explicitly investigated in the present study, but should be investigated in future research. Phosphorus levels in the present study were relatively high due to the fertilization regime used. For both S. alterniflora and S. cynosuroides, McHugh & Dighton (2004) showed a greater effect of nutrients on plant biomass in inoculated plants, compared with uninoculated plants, when growth occurs in low-phosphorus conditions but not in high-phosphorus conditions.

The results of this study are a first step in demonstrating the potential effect of mycorrhizae on hybrid Spartina invasion. The results from the greenhouse study should be repeated by comparative inoculation
experiments under more natural conditions (e.g. inoculation with local mycorrhizae, use of local soils and frequent inundation). Future work should also include field manipulation of mycorrhizal associations in *Spartina* spp. via the application of fungicides. This work could be combined with eradication efforts currently under way with the Invasive Spartina Project. This study suggests that a cautious attitude be adopted in restoration efforts targeting native species and using mycorrhizal fungal inoculation to help in the establishment of plants, because, in salt marsh ecosystems, inoculation with AM fungi will not necessarily provide an advantage to native species over invaders. While this study does not conclusively demonstrate that mycorrhizae are facilitating the invasion of tidal lands by *Spartina* hybrids, and further research is required, mycorrhizal associations have the potential to influence the invasion trajectory of *Spartina* hybrids in San Francisco Bay.

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LITERATURE CITED


Grosholz ED (2005) Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. Proc Natl Acad Sci USA 102:1088–1091


McHugh JM, Dighton J (2004) Influence of mycorrhizal
inoculation, inundation period, salinity, and phosphorus availability on the growth of two salt marsh grasses, *Spartina alterniflora* Lois. and *Spartina cynosuroides* (L.) Roth., in nursery systems. Restor Ecol 12:533–545


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