



How Abundant are Root-Colonizing Fungi in Southeastern Louisiana's Degraded Marshes?

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Abstract Despite earlier notions that fungi are not important in wetlands, it is becoming clear that root endophytes are abundant in wetlands and potentially can influence plant community dynamics. Little is known about the effects of wetland degradation on these fungi. We assessed two groups of root endophytes in a degrading marsh in southeast Louisiana that historically was a swamp forest dominated by *Taxodium distichum* (baldcypress) and *Nyssa aquatica* (water tupelo). We determined percent root colonization by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) in each of 18 vascular plant species. Fungi were present in all species that were assessed. In general, monocots were primarily colonized by DSE, whereas dicots were primarily colonized by AMF. *Taxodium distichum* was heavily colonized by AMF, as was the non-native, invasive Chinese tallow (*Triadica sebifera*). This study is the first to show that wetland plants in a degraded marsh harbor abundant and diverse root endophytes. These fungi and their interactions with stressed plants may be important in effective management of degrading wetlands.

Keywords Arbuscular mycorrhizas · Dark septate endophytes · Manchac land bridge · Restoration · Wetlands

Introduction

Fungi form symbiotic associations with plants, inhabiting above- and below-ground tissue. Two types of root-inhabiting fungi are arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE). Both types have been reported in wetland plants (Turner and Friese 1998; Cornwell et al. 2001; Stevens et al. 2002; Muthukumar et al. 2004). These root-inhabiting fungi may affect plants by reducing negative effects of flood and salt stress, enhancing nutrient uptake, and providing protection from pathogens and herbivores (Jumpponen 2001).

Until recently, arbuscular mycorrhizal fungi were considered unimportant in wetlands. Because AMF are aerobic, anoxic conditions associated with waterlogged soils were considered limiting (Malloch et al. 1980; Peat and Fitter 1993), and AMF had rarely been found on plant roots in waterlogged soils. Several studies, however, indicate that AMF are present and widespread in some wetlands, and also may influence wetland plant community structure (Brown and Bledsoe 1996; Stevens and Peterson 1996; Carvalho et al. 2001; Hildebrandt et al. 2001; Landwehr et al. 2002; Bauer et al. 2003; Wang et al. 2004). Several wetland plant species (Cyperaceae, Chenopodiaceae, and Plumbaginaceae) that were thought to be non-mycorrhizal (Hirsch and Kapulnik 1998) have been shown to have high levels of AMF colonization (Hildebrandt et al. 2001; Muthukumar et al. 2004). The presence of AMF has been shown to influence plant zonation in salt marshes (Daleo et al. 2008), and they may play a role in diversifying plant communities by allowing mycorrhizal plants to compete

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with sympatric, non-mycorrhizal plants (Weishampel and Bedford 2006).

Dark septate endophytes in marshes have received less attention than AMF. Evidence suggests, however, that these fungi form associations with some wetland plants and may occur over a wide range of conditions (Addy et al. 2000; Fuchs and Haselwandter 2004). Dark septate endophyte colonization has been shown to occur in both bog and fen species, although bog plant species are typically colonized mostly by DSE, and fen species are colonized mostly by AMF. Some studies suggest that community structure may be influenced as much by DSE as they are by AMF (Fuchs and Haselwandter 2004; Weishampel and Bedford 2006).

Because root endophytes may have important functional roles in wetlands, they may be important in restoration of marshes (Bauer et al. 2003). However, we know little about plant-fungal interactions and their effects on community dynamics in degrading wetlands. In this study, we assessed AMF and DSE colonization of wetland plant species in the Manchac land bridge, a degrading marsh in southeast Louisiana. For several decades, there have been unsuccessful efforts to restore this marsh back to a swamp forest dominated by *Taxodium distichum* and *Nyssa aquatica*. However, only some stunted, remnant *T. distichum* survived from these restoration efforts, and *Triadica sebifera*, a non-native, invasive tree, became established. We had three objectives: 1) to determine if root endophytes colonized plants in this marsh, 2) to determine if a relationship exists between AMF and DSE colonization, and 3) to compare patterns of colonization between two broad categories of

vegetation—monocotyledonous (monocots) and dicotyledonous (dicots) plants.

We collected roots from a total of 18 species, 14 herbaceous plants, two shrubs (*Iva frutescens* and *Baccharis halimifolia*), and two trees (*T. distichum* and *T. sebifera*). We determined the extent of root colonization for each species and distinguished between AMF and DSE colonization. This study is an important first step in determining the role of root endophytes in degrading coastal marshes of southern Louisiana.

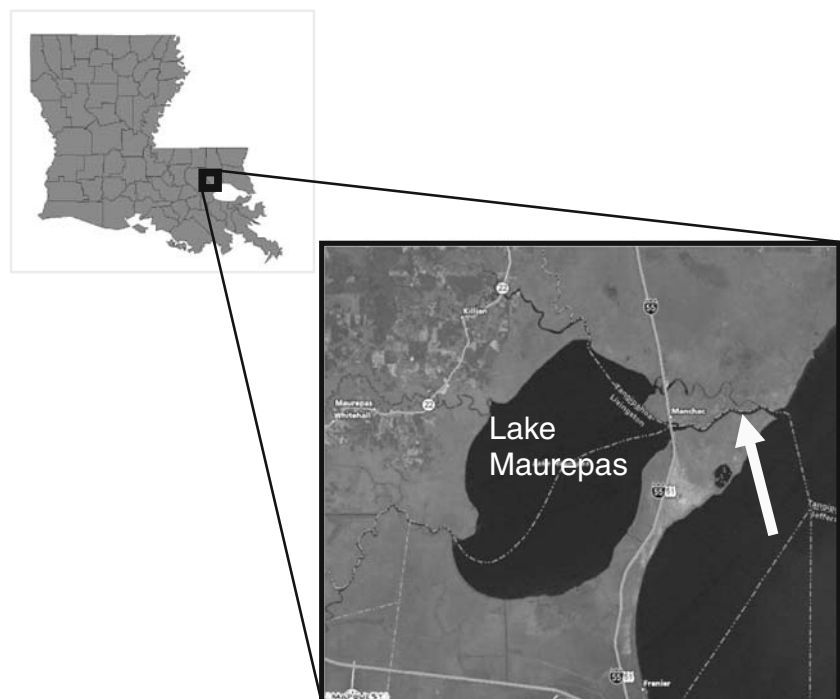
Methods

Study Site

The Turtle Cove Environmental Research Station (N 30° 17'59", W 90° 20'10") fronts a 3,200 ha experimental marsh located on the Manchac land bridge between Lakes Maurepas and Pontchartrain in southeastern Louisiana (Fig. 1). Wetlands on this narrow land bridge have been undergoing rapid degradation over the past century, primarily from hydrologic alteration by canal and levee construction. As a result, the Manchac land bridge *Taxodium distichum* and *Nyssa aquatica* swamps have converted to marsh vegetation, and parts of these marshes are now converting to open water (Barras et al. 2003; Shaffer et al. 2009b).

Wetlands of the Manchac land bridge are currently nutrient limited, with nitrogen (nitrate plus nitrite) rarely

Fig. 1 Location of Turtle Cove Environmental Research Station in southeast Louisiana. Turtle Cove (arrow) is located in the Manchac Wildlife Management Area, on the Manchac Land Bridge between Lakes Pontchartrain and Maurepas (Tangipahoa and St. John the Baptist Parishes)



exceeding 0.05 mg/L (Lane et al. 2003; USGS, National Water Information System 2007). Phosphorus levels may reach 0.2 mg/L (Kandalepas 2004), but concentrations typically are low, averaging 0.055 mg/L (Lane et al. 2003). In contrast, the adjacent Mississippi River, which historically was the source of nutrients and fresh water for wetlands, contains much higher concentrations of nitrogen and phosphorus (Lane et al. 2003).

Salinity in the Manchac land bridge wetlands is usually low, ranging from 0.8 ppt to 1.1 ppt (Kandalepas 2004). However, pulses of salt water from storms frequently inundate the wetlands, and post-hurricane salt concentrations may exceed 5 ppt (USGS, National Water Information System 2005; Shaffer et al. 2009b). Following 2 years (1999, 2000) of drought, Lane et al. (2003) found the salinity on the Manchac land bridge wetlands reached 12 ppt.

Sampling

We collected randomly selected plants along a 1,160-m transect from spring 2005 through fall 2006. We chose plants that broadly represented species of Louisiana marshes. Additionally, we collected roots from *T. distichum* that were planted in an effort to restore the habitat to cypress swamp, as well as from invasive species typical of degraded swamp/marsh habitat (e.g., *Triadica sebifera* and

Alternanthera philoxeroides). With the exception of trees and shrubs, we collected entire plants to ensure roots originated from a given species. For large woody plants we physically followed the roots to their tips with our hands to find the new growth.

We collected three to five individuals (true reps) of 18 of the most common wetland plant species in the area. We assessed these for AMF and DSE colonization (see Table 1). Plants were uprooted, cleaned of debris, bagged, and transported to the laboratory. Plants were kept moist and stored in a refrigerator until further processing. Nomenclature and authorities of plant species were confirmed by the USDA Natural Resources Conservation Service plants database (2008). While grasses (e.g., *Echinochloa walteri*, *Phalaris* sp.) were present, we did not sample them because inflorescences were lacking and thus conclusive identification was difficult.

Processing and Assessment

We initiated processing of roots within 24 h of collecting. Roots waiting to be processed were kept at 4°C. Sediment and debris were washed from the roots, and a sub-sample of non-woody roots large enough to fill a 50 ml centrifuge tube was obtained from each plant and fixed in 50% ethanol. All healthy roots collected were cleared by autoclaving in 10% potassium hydroxide (KOH) for 15–

Table 1 Vascular plant species examined for arbuscular mycorrhizal fungi and dark septate endophytes at Turtle Cove Environmental Research Station, Louisiana

	Species
MAGNOLIOPHYTA (ANGIOSPERMS)	
Liliopsida (Monocotyledons)	
Alismataceae	<i>Sagittaria lancifolia</i> L.
Cyperaceae	<i>Eleocharis cellulosa</i> Torr.
	<i>Eleocharis montevidensis</i> Kunth
	<i>Schoenoplectus tabernaemontani</i> (K.C. Gmel.) Palla
	<i>Schoenoplectus americanus</i> (Pers.) Volk. Exs Schinz & R. Keller.
	<i>Schoenoplectus robustus</i> (Pursh) M.T. Stong
Typhaceae	<i>Typha domingensis</i> Pers.
Magnoliopsida (Dicotyledons)	
Amaranthaceae	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.
	<i>Amaranthus australis</i> (Gray) Sauer
Asteraceae	<i>Symphytotrichum subulatum</i> (Michx.) G.L. Nesom
	<i>Baccharis halimifolia</i> L.
	<i>Iva frutescens</i> L.
	<i>Ipomoea sagittata</i> Poir. In Lam.
	<i>Triadica sebifera</i> (L.) Small.
	<i>Vigna luteola</i> (Jacq.) Benth.
	<i>Sesbania herbacea</i> (Mill.) McVaugh
Polygonaceae	<i>Polygonum punctatum</i> Ell.
PINOPHYTA (CONIFERS)	
Cupressaceae	<i>Taxodium distichum</i> (L.) Rich.

20 min, depending on pigmentation, then stained by autoclaving in 3% Trypan Blue for 15 min (Brundrett et al. 1996) to make structures associated with AMF and DSE colonization visible. A subset of the species were processed as above, but stained with 3% Chlorazol Black E (Brundrett et al. 1984) to achieve higher contrasts between fungal structures and plant tissues. Tryphan Blue and Chlorazol Black E were dissolved in a 1:1:1 lactic acid:glycerine:deionized water solution (Brundrett et al. 1996). Roots were de-stained and stored in a 50% glycerol solution for up to 1 week before mounting on slides in 50% glycerol (Phillips and Hayman 1970). All stained roots were cut into 1-cm segments and mounted on multiple slides.

Root colonization was assessed by viewing stained roots and estimating the proportion of each root that was colonized by AMF, DSE, or both. We did not identify specific fungal species, as this was beyond the scope of this study. We used a Zeiss Axioimage microscope at 200x magnification and images were obtained with a Zeiss AxioCam MRC-5 camera. Colonization levels were quantified using a modified grid line intersect procedure (McGonigle et al. 1990), with 100 fields of view assessed for each slide. We calculated total colonization as the percentage of root length in the 100 different fields of view containing any AMF or DSE fungal structures, including hyphae, arbuscules, or vesicles. Plants were considered to form AMF associations, however, only if arbuscules, the only uniquely distinguishable feature in AMF, were detected in the roots (McGonigle et al. 1990). If characteristic AMF hyphae and/or vesicles were found without arbuscules, the AMF status was deemed unverified, but colonization by hyphae and vesicles was included in the total. For assessment of DSE colonization levels, only hyphae were quantified, because DSE hyphae are distinctive. As a result, total colonization was often greater than the sum of AMF and DSE colonization. Means and standard errors for all estimated percentages of roots colonized by AMF and DSE or both were computed using SAS software, Version 9.1.3, of the SAS System for Windows (SAS Institute Inc. 2000–2004). To determine the relationship between AMF and DSE colonization, Spearman Rank correlations were conducted using GraphPad InStat (ver 3.06, GraphPad Software, Inc.). To determine if levels of colonization differed among monocots and dicots, arbuscular, hyphal, vesicular and DSE colonization levels were compared using Mann-Whitney tests in GraphPad InStat.

We used nonmetric multidimensional scaling (NMS) (Kruskal 1964) in Primer v6 (Clarke and Warwick 2006) to illustrate the pattern of colonization by AMF and DSE in monocots and dicots. We used ANOSIM (Clarke and Warwick 2006) to determine if the observed pattern was different from random. To perform the NMS, we used the

Bray Curtis Similarity Index with 999 permutations. Square root transformation was used to minimize the influence of large values.

Results

Both AMF and DSE were visible in stained cells of roots of wetland plants at the Turtle Cove Biological Research Station. Figure 2 illustrates a cleared, unstained root (Fig. 2a), stained roots with AMF (Fig. 2b–e), and stained roots with DSE (Fig. 2f–g). In Fig. 2b–d, arbuscules are clearly evident. Figure 2(d) and (e) display vesicles. Dark septate endophytes in Fig. 2(f) and (g) are recognized by their thickened walls and septate hyphae. In addition, microsclerotia, segmented structures visible within the plant cells, also are characteristic of DSE. The physical presence of these different structures indicated the presence of AMF, DSE, or both in at least some plants of all species examined (Table 2).

Most species that we sampled had indications of colonization by AMF, and every species, monocot or dicot, had indications of DSE colonization, usually in all individuals (Table 2). Typically, every perennial dicotyledonous plant was colonized by AMF. In contrast, the presence of AMF was only conclusively confirmed for 1 of the 7 monocots sampled—*Schoenoplectus robustus*. The presence of vesicles and hyphae suggested AMF might be colonizing many of the monocots, although there were higher levels of DSE in this group. Three species, *Taxodium distichum*, *Alternanthera philoxeroides*, and *Vigna luteola* contained AMF within all individuals examined, but each contained DSE in only one individual sampled.

Total colonization of roots by AMF and DSE was substantial for most species. Among monocots, proportions of roots colonized by both fungal types ranged from 27% (*Sagittaria lancifolia*, *Typha domingensis*) to > 80% (*Schoenoplectus americanus*, *S. robustus*). Among dicots, root colonization by AMF and DSE ranged from 29–44% (*Amaranthus australis*, *Sesbania herbacea*) to > 80% (*Alternanthera philoxeroides*, *Triadica sebifera*).

Overall, there was a negative correlation between AMF and DSE for, both, monocots ($r=-0.65$; $p=0.0002$; $n=27$) and dicots ($r=-0.44$; $p=0.0073$; $n=36$). All measures of AMF colonization were significantly higher in dicots compared to monocots (mean arbuscular colonization in dicots was $10.2\% \pm 1.8\%$ (SE) vs $0.1\% \pm 0.1\%$ for monocots, $p<0.0001$; mean hyphal colonization was $47.0\% \pm 3.3\%$ for dicots vs $28.8\% \pm 6.2\%$ for monocots, $p<0.05$; mean vesicular colonization was $6.8\% \pm 1.5\%$ for dicots vs $0.7\% \pm 0.3\%$ for monocots, $p<0.0001$). DSE colonization showed the opposite trend and was significantly higher in monocots ($25.0\% \pm 4.5\%$) compared to the dicots ($14.2\% \pm$

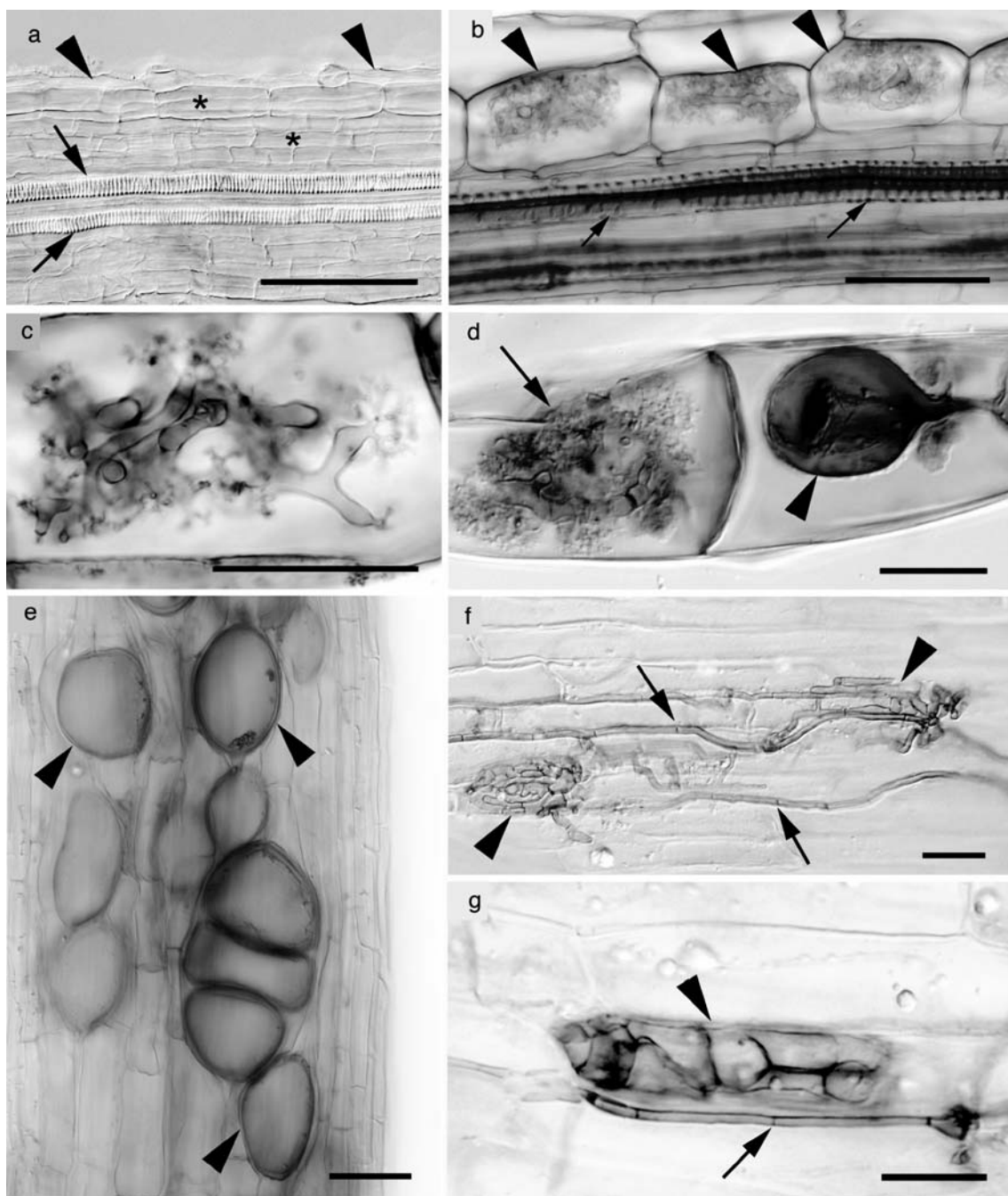


Fig. 2 Cleared and stained roots of seven wetland plant species collected from the Turtle Cove Environmental Research Station in southeast Louisiana. Figure 2a is a non-colonized root; Figures 2b–e illustrate colonization by arbuscular mycorrhizal fungi; Figure 2f–g illustrates colonization by dark septate endophytes. **a** Non-colonized area of a root of *Schoenoplectus robustus*. Cell contents have been removed by clearing in KOH. Cell walls are visible in epidermal cells (*arrowheads*) and cortical cells (*) otherwise cells appear translucent. Helical secondary cell wall thickenings are evident in the xylem tracheary elements (*arrows*). Roots are stained with Chlorazol Black E. Scale bar=50 μ m. **b** Arbuscules (*arrowheads*) within cortical cells of *Vigna luteola*. Non-colonized cells are visible above the colonized cells. Helical secondary cell wall thickenings (*arrows*) are visible in

xylem tracheary elements. Roots are stained with Chlorazol Black E. Scale bar=100 μ m. **c** Arbusculate coils of the Paris-type AMF within a cortical cell of *Triadica sebifera*. Roots are stained with Chlorazol Black E. Scale bar=50 μ m. **d** Arbusculate coils of the Paris-type AMF (*arrow*) and vesicle (*arrowhead*) within isolated cortical cells of *Taxodium distichum*. Roots are stained with Chlorazol Black E. Scale bar=100 μ m. **e** Vesicles (*arrowheads*) in the cortex of *Aster subulatus*. Roots are stained with Trypan Blue. Scale bar=100 μ m. **f** Microsclerotia (*arrowheads*) and septate hyphae (*arrows*) in epidermal cells of *Eleocharis cellulosa*. Roots are stained with Trypan Blue. Scale bar=50 μ m. **g** Septate hyphae (*arrow*) and melanized microsclerotium (*arrowhead*) within an epidermal cell of *Iva frutescens*. Roots are stained with Trypan Blue. Scale bar=20 μ m

Table 2 Arbuscular mycorrhizal fungi (AMF), dark septate endophytes (DSE), are indicated as present (+) absent (–) or indeterminable (?). AMF colonization could not be determined if arbuscules were not detected, even though characteristic AMF hyphae and/or vesicles were present. Numbers of plants of each species positively identified as

harboring AMF and/or DSE are indicated in parentheses (number/total sampled). The proportions of roots containing arbuscules, vesicles, AMF hyphae, DSE, and total colonization by fungi are presented mean percentages \pm 1 standard error

Taxon	Species	AMF (+/-)	DSE (+/-)	Arbuscules	Vesicles	AMF Hyphae	DSE	Total Colonization (all AMF and DSE structures)
MAGNOLIOPHYTA (ANGIOSPERMS)								
Liliopsida (Monocotyledons)								
Alismataceae	<i>Sagittaria lancifolia</i>	? (0/5)	+ (4/5)	0	0.40 \pm 0.40	20.20 \pm 8.62	6.80 \pm 2.48	27.40 \pm 10.39
Cyperaceae	<i>Eleocharis cellulosa</i>	? (0/3)	+ (3/3)	0	0.67 \pm 0.67	1.67 \pm 1.67	43.33 \pm 8.11	44.00 \pm 8.72
	<i>Eleocharis montevidensis</i>	? (0/3)	+ (3/3)	0	0.33 \pm 0.33	7.33 \pm 1.45	46.00 \pm 6.81	50.67 \pm 5.17
	<i>Schoenoplectus tabernaemontani</i>	–(0/3)	+ (3/3)	0	0	0	43.33 \pm 13.74	43.33 \pm 13.74
	<i>Schoenoplectus americanus</i>	? (0/5)	+ (4/5)	0	0	62.20 \pm 11.80	19.20 \pm 15.77	83.20 \pm 7.06
	<i>Schoenoplectus robustus</i>	+ (3/5)	+ (5/5)	0.60 \pm 0.25	2.60 \pm 1.60	67.80 \pm 4.81	9.20 \pm 2.82	80.20 \pm 5.67
Typhaceae	<i>Typha domingensis</i>	? (0/3)	+ (3/3)	0	0.33 \pm 0.33	0.33 \pm 0.33	32.33 \pm 7.69	33.00 \pm 7.57
Magnoliopsida (Dicotyledons)								
Amaranthaceae	<i>Alternanthera philoxeroides</i>	+ (5/5)	+ (1/5)	6.60 \pm 3.26	8.60 \pm 2.44	65.40 \pm 4.07	0.80 \pm 0.80	81.4 \pm 7.94
	<i>Amaranthus australis</i>	? (0/3)	+ (3/3)	0	2.67 \pm 1.45	18.67 \pm 4.26	13.33 \pm 3.93	29.00 \pm 5.03
Asteraceae	<i>Symphotrichum subulatum</i>	+ (3/3)	+ (3/3)	10.00 \pm 1.53	26.67 \pm 5.84	49.33 \pm 2.33	28.00 \pm 6.66	67.00 \pm 6.24
	<i>Baccharis halimifolia</i>	+ (3/3)	+ (3/3)	12.33 \pm 6.44	8.33 \pm 3.29	32.67 \pm 1.45	40.33 \pm 8.57	61.67 \pm 4.48
	<i>Iva frutescens</i>	+ (3/3)	+ (3/3)	8.67 \pm 1.20	9.67 \pm 6.17	28.67 \pm 10.2	42.00 \pm 14.93	65.00 \pm 14.52
Convolvulaceae	<i>Ipomoea sagittata</i>	+ (2/3)	+ (2/3)	2.33 \pm 1.20	0.33 \pm 0.33	62.33 \pm 13.28	17.67 \pm 8.88	82.33 \pm 5.70
Euphorbiaceae	<i>Triadica sebifera</i>	+ (3/3)	+ (2/3)	21.33 \pm 3.18	2.33 \pm 1.86	69.67 \pm 6.69	2.00 \pm 1.15	96.33 \pm 1.20
Fabaceae	<i>Vigna luteola</i>	+ (5/5)	+ (1/5)	27.20 \pm 4.66	4.80 \pm 2.40	45.60 \pm 5.12	0.60 \pm 0.60	76.60 \pm 8.94
	<i>Sesbania herbacea</i>	+ (2/3)	+ (3/3)	9.67 \pm 6.49	8.33 \pm 6.35	35.33 \pm 14.97	12.00 \pm 1.00	44.67 \pm 12.73
Polygonaceae	<i>Polygonum punctatum</i>	+ (3/5)	+ (5/5)	1.00 \pm 0.55	0.80 \pm 0.58	49.40 \pm 7.81	7.80 \pm 3.20	58.80 \pm 10.29
PINOPHYTA (CONIFERS)								
Cupressaceae	<i>Taxodium distichum</i>	+ (3/3)	+ (1/3)	29.33 \pm 1.67	10.67 \pm 0.88	55.67 \pm 0.33	0.33 \pm 0.33	94.33 \pm 0.88

2.9%; $p < 0.05$). Finally, NMS revealed a significant difference in colonization between AMF and DSE in the plant groups examined (ANOSIM, Global $R = 0.391$; $p = 0.003$, Fig. 3).

Discussion

Our study demonstrated that plants inhabiting degrading marshes of coastal Louisiana are colonized by AMF and DSE, and contributes to a growing body of literature documenting the occurrence of AMF and DSE fungi in wetland plants. Only four plant species in this study appear to have been examined previously for mycorrhizal colonization (see Appendix); to our knowledge this is the first record of AMF and/or DSE being present in 13 of the species. All plant species examined contained DSE, and AMF were indicated in all but one plant species, suggesting that mycorrhizal fungi can be expected to be ubiquitous components of southern Louisiana marshes. Moreover,

colonization levels were comparable to those in other U.S. wetlands (e.g., Cornwell et al. 2001; Bauer et al. 2003; Weishampel and Bedford 2006), and higher than those recorded in wetlands of Asia (Kai and Zhiwei 2006) or Europe (Sraj-Krzic et al. 2006).

Plant species at Turtle Cove Environmental Research Station are typical of fresh to intermediate brackish marsh, but often experience salt and flood stress due to natural and anthropogenic influences. Storm surges from hurricanes and periodic droughts occasionally concentrate salts in the soil, thereby bringing about major shifts in vegetation (Visser et al. 1999, 2002). In addition, levees constructed throughout southeast Louisiana have resulted in loss of nutrient input that, historically, occurred with the flooding of the Mississippi River. These hydrologic alterations have contributed to relative sea-level rise, due to subsidence that occurs naturally in south Louisiana. As many marshes in coastal Louisiana, the Manchac land bridge has been converting to open water (Barras et al. 2003) due to saltwater intrusion, increased flooding, nutrient starvation,

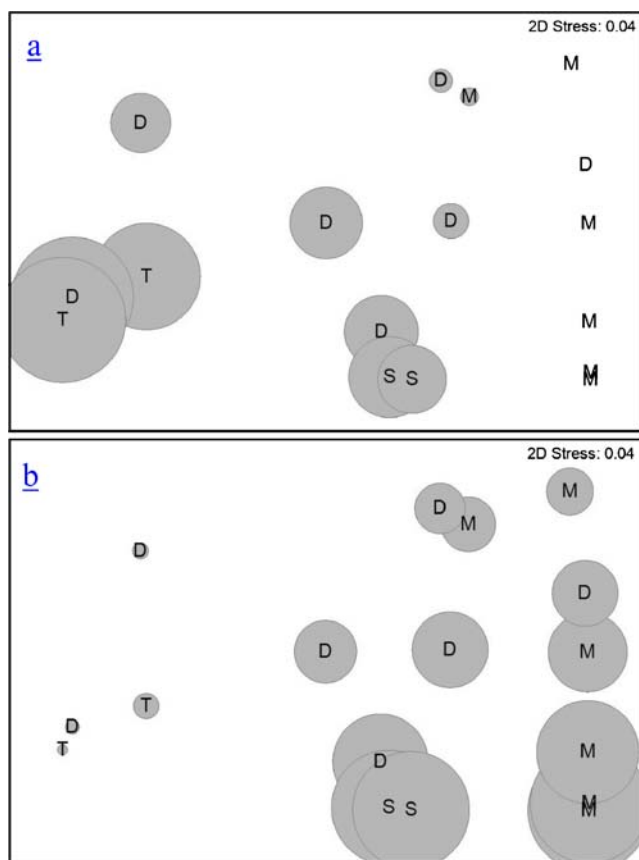


Fig. 3 Nonmetric multidimensional scaling ordination showing pattern of colonization among plant groups by AMF and DSE. Graphs A and B are the same NMS analysis, but each has been overlaid with circles to indicate either AMF (a) or DSE (b) colonization. The x-axis represents a gradient from more AMF colonized on the left to more DSE colonized on the right. 3A illustrates colonization only by AMF and 3B illustrates colonization only by DSE. The size of the circle indicates the level of colonization by AMF or DSE within a particular plant group. The larger the circle, the higher the level of colonization by a particular fungal type. T=tree, S=shrub, D=dicot, and M=monocot

and herbivory by nutria (*Myocastor coypus*) (Myers et al. 1995; Shaffer et al. 2009b). Together, these factors have contributed to wetland loss that is greater than anywhere in the country.

The high density of root-inhabiting fungi found in this study suggests that fungi may play an important role in this stressed ecosystem. Mycorrhizae are known to provide protection against “physiological drought” (Schimper 1898) caused by salt stress in coastal wetlands. Therefore, plants at Turtle Cove may rely on their fungal symbionts’ ability to rehydrate them (Hildebrandt et al. 2001). In addition, AMF can improve tolerance of plants to flooding (Neto et al. 2006) and influence resource allocation (Stevens and Peterson 2007), which is important when nutrients are limiting.

Benefits provided by AMF and DSE to their individual plant hosts may extend to the entire community. Wolfe et al. (2006) showed that presence of AMF influenced wetland

plant community structure. They suggested that wetlands are affected not only by abiotic factors that change soil properties, but also by an interaction of abiotic and biotic factors where AMF provide amelioration for some negative abiotic effects. In addition, Fuchs and Haselwandter (2004) showed that monocots, which dominate fen communities, contain higher levels of DSE than less abundant, sympatric dicots, which are heavily colonized by AMF. This suggests that DSE may confer a competitive advantage for their plant hosts.

One important outcome of this study is that DSE not only were abundant in this degrading habitat but were negatively correlated to AMF in both monocots and dicots. Dark septate endophytes have not been studied for their potential role in wetland plants, but there is a growing body of evidence that suggests these fungi may play an important ecological role in wetland communities similar to that of AMF (Jumpponen 2001). The negative correlation may either suggest competition among fungal types, or that the two fungal types perform optimally under different ecological conditions. For instance, it has been shown that DSE are commonly found in low nutrient environments (Peterson et al. 2004), suggesting that DSE may interact with their plant hosts, the environment, and other fungi in wetlands to influence community structure. The high density of DSE found in the marsh at the Turtle Cove Environmental Research Station warrants further study of these endophytes’ roles. Such studies might examine: 1) effects of changing environmental conditions (e.g., increases in flooding and salinity) on DSEs ability to colonize wetlands plants; 2) the potential effects of DSE on plant hosts’ competitive ability under changing environmental conditions; 3) the role of plant host on DSE colonization; 4) identification of species of DSE in wetland plant hosts and under varying ecological conditions; and 5) possible interactions between DSE and AMF under varying ecological conditions.

Another important outcome of this study is the documentation of AMF in *T. distichum*. Baldcypress is an important component of southern U.S. swamps and has been shown to resist wind-throw during intense hurricanes (Noel et al. 1995; Shaffer and Day 2007; Shaffer et al. 2009a, b). A consequence of the loss of forested wetlands dominated by *T. distichum* is, therefore, diminished hurricane protection (van Heerden et al. 2006; Day et al. 2007; Shaffer et al. 2009a). The Pontchartrain Basin was once old-growth *Taxodium distichum*–*Nyssa aquatica* swamp, but today it is rapidly converting to marsh and open water (Barras et al. 2003). Although efforts are currently being made toward the restoration of this ecosystem throughout Louisiana, improving this ecosystem’s health and increasing its productivity have yet to be attained (Shaffer and Day 2007). Mycorrhizal fungi may be important in attempts to restore degrading wetlands

containing baldcypress. Introducing appropriate AMF and DSE to the soils may facilitate regeneration of baldcypress. The importance of AMF in restoration/forest strategies in terrestrial ecosystems is well documented (Smith and Read 1997), but this has not been examined in coastal systems. This may be a particularly relevant area to explore given the relatively high level of AMF colonization in desirable wetland plant species, especially *T. distichum*.

Introduction of root endophytes to an ecosystem, however, should be done with caution. Undesirable consequences, such as increased likelihood of invasion by non-native plants, might result from adding root endophytic fungi to soils in which they have not evolved, or to plants with which they have not evolved (Collins Johnson et al. 2006). Generalist AMF species often form associations with exotic plants. These associations, compounded by the absence of enemies of exotic plants (Richardson et al. 2000; Keane and Crawley 2002), could facilitate invasion. Some studies suggest that invasive plants suppress growth of native plant species by preventing natives from being colonized by beneficial fungi (Callaway et al. 2008; Nijjer et al. 2008). Louisiana wetlands are threatened by invasive species such as *Triadica sebifera* and *Alternanthera philoxeroides*. Both these plant species showed high levels of AMF colonization in this study. Along with other stressors, these invasive species might accelerate the current trajectory toward open water if they reduce or prevent native plants from forming mycorrhizal associations.

Arbuscular mycorrhizal fungi also exhibit parasitic characteristics under certain environmental conditions, such as when nutrients are abundant. For example, Johnson

(1993) found that inoculating plants with AMF isolated from fertile soils resulted in a net cost in biomass and fitness for the plant. In contrast, plants grown with AMF isolated from soils low in nutrients were taller and had more inflorescences. Therefore, it is crucial that more studies be conducted to demonstrate the role of root endophytes in wetlands, as well as environmental effects on plant-fungal interactions. Determining which fungal species are native to a particular soil and which species of fungi are associated with which plant species will allow managers to make informed decisions when including fungi in conservation/restoration plans. Studying these fungi and their role in plant dynamics, however, is subject to the presence of the plant hosts. Obviously, once a wetland converts to open water, it will be difficult to determine which fungi were present, as well as relationships with indigenous plants. Because wetlands in Louisiana are currently being lost at rates anticipated to occur soon in other deltaic regions around the world, research in Louisiana can be used as a model for studies elsewhere that soon will be affected by sea-level rise. Thus, identification of the AMF and DSE in Louisiana coastal wetlands is of some urgency.

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Appendix

Table 3 Four plant species that were examined in this study were also examined in previous studies for their mycorrhizal status. We compared mycorrhizal levels reported in other studies to levels found in this study. Dark septate endophytes were not examined for any of these species in any previous studies. *Schoenoplectus tabernaemontani* was found to be colonized by AMF in three studies (Wetzel and van der Valk 1996; Cooke and Lefor 1998; Bauer et al. 2003),

whereas we were not able to find any evidence of AMF in our specimens. We did, however, find that *S. tabernaemontani* was heavily colonized by DSE. In addition, many studies do not report arbuscular colonization separately from total colonization, which makes it difficult to know what proportion of the root was, indeed, colonized by AMF. Standard error is reported if it was reported in a particular study

Plant Species	Reference	AMF (arbuscules only)	AMF (Total colonization)	DSE	Notes
<i>Schoenoplectus tabernaemontani</i>	This study	0%	0%	43.33%±13.74%	-field collections
As <i>Schoenoplectus maritimus</i>	1. Gouraud et al. 2008	0%	0%	N/A	-field collections -examined roots for all structures characteristic to AM fungi
as <i>Scirpus validus</i>	2. Bauer et al. 2003		3–85%	N/A	-field collections -total colonization includes arbuscules, as well as vesicles and hyphae

Table 3 (continued)

Plant Species	Reference	AMF (arbuscules only)	AMF (Total colonization)	DSE	Notes
as <i>Scirpus maritimus</i>	3. Wetzel and Van der Valk 1996		37–26%	N/A	-field collections -hyphal coils and arbuscules <i>rarely</i> observed
	4. Cooke and Lefor 1998		70%	N/A	-field collections -only reported colonization by vesicles
<i>Triadica sebifera</i>	This study	21.33%±3.18%	93.33%	2.00%±1.15%	-field collections -total colonization includes arbuscules, vesicles, and hyphae
as <i>Sapium sebiferum</i>	Nijjer et al. 2008		> 8%	N/A	-both field and greenhouse collections -totals include arbuscules, as well as hyphae, vesicles, and hyphal coils -mycorrhizal levels increased to >42% with fertilizer
<i>Vigna luteola</i>	This study	27.20%±4.66%	77.60%	0.60%±0.60%	-field collections -total colonization includes arbuscules, vesicles, and hyphae
	Hernández et al. 2000	N/A	18.6–35.3%	N/A	-experimental pots, inoculated with 0–2 species AMF, with or without associated non-mycorrhizal soil organisms -highest level of colonization observed with all microorganisms present, suggesting synergistic association between mycorrhizas and associated soil organisms -total mycorrhizal colonization includes arbuscules, as well as vesicles and hyphae
<i>Iva frutescens</i>	This study	8.67%±1.20%	47.01%	42.00%±14.93%	-field collection -total mycorrhizal colonization includes arbuscules, as well as vesicles and hyphae
	Cooke and Lefor 1990	only indicated presence of mycorrhizae	N/A	N/A	-field collections -indicated that infection for all plants was usually ≤5%

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