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Seasonal dynamics of arbuscular mycorrhizal fungi in differing wetland habitats

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Abstract The dynamics and role of arbuscular mycorrhizal fungi (AMF) have been well described in terrestrial ecosystems; however, little is known about how the dynamics of AMF are related to the ecology of wetland ecosystems. The seasonal dynamics of arbuscular mycorrhizal (AM) colonization within different wetland habitats were examined in this study to determine the factors that influence AM associations and to further assess the ecological role of AMF in wetlands. Fen and marsh habitats of four wetlands in west central Ohio were sampled monthly from March to September. AMF were found at all four sites for each month sampled and were present in all of the dominant plant species. A significant effect of month ($P<0.001$) on AM colonization did occur and was attributable to maximum colonization levels in the spring and minimum levels in late summer. This trend existed in all four wetlands in both fen and marsh habitats, regardless of variation in water levels, percent soil moisture, or available phosphorus levels. Because abiotic factors had minimal influence on AM colonization variation and the level of AM colonization paralleled plant growth patterns, we conclude that the AM seasonal dynamic was in response to plant phenology. Our data suggest that AM associations in temperate fen and marsh habitats are prevalent in the spring during new root and vegetative growth, even for plants experiencing flooded conditions. Evidence of an overriding AM seasonal trend indicates that future studies should include a seasonal component to better assess the role and distribution of AMF in wetland ecosystems.

Keywords Arbuscular mycorrhizal fungi · Phosphorus · Seasonal dynamics · Temperate wetlands · Soil moisture

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Introduction

Living organisms in wetland habitats are exposed to anaerobic soils, extremely high or low concentrations of soil minerals and available nutrients, and flooding. Since arbuscular mycorrhizal fungi (AMF) require oxygen to thrive and since many wetland plants have been described as non-mycorrhizal (Khan 1974; Mosse et al. 1981; Anderson et al. 1984; Mejstrik 1984), it has been assumed that AMF have little significance in wetland ecosystems. However, recent field studies show that AMF exist in wetlands and colonize many hydrophytic plants (Brown and Bledsoe 1996; Cantelmo and Ehrenfeld 1999; Turner et al. 2000). Indeed, 50–90% of plants in some wetlands were found to be mycorrhizal (Ragupathy et al. 1990; Turner and Friese 1998). Furthermore, in salt marshes, AM colonization levels exceeded 20% in regions where soil moisture levels were above 120% (Brown and Bledsoe 1996), and AM roots were found 42 cm below the soil surface where oxygen was not detectable (Cooke et al. 1993). These studies indicate that AMF tolerate wetland conditions and suggest that AMF are an important component of wetland ecosystems. To determine the extent of their importance and to further elucidate the role of AMF in wetlands, knowledge of significant factors influencing the distribution of AMF and seasonal patterns of AM colonization is necessary.

Several wetland field studies show that AM colonization is generally higher in the drier areas as compared to the wetter, more anaerobic areas of a wetland (Rickerl et al. 1994; Stevens and Peterson 1996; Turner and Friese 1998). For example, Miller (2000) found a negative correlation between water depth and AM colonization of two semi-aquatic grasses in the Carolina Bays. Results from that study also showed that AM colonization rose in some areas that experienced seasonal drying, thus supporting speculation that a fluctuating water table could increase AM colonization by providing periodic aeration of the soil (Van Hoewyk et al. 2001). Studies carried out in salt marshes also have shown that periodic aeration can potentially provide a better environment for AMF (Brown

and Bledsoe 1996). Although these results are expected because of the aerobic nature of AMF, other wetland studies do not demonstrate such a clear relationship between water regime and AM colonization. Instead, these studies indicate that AMF may respond to a complex mix of abiotic and biotic factors rather than to just water regime (Brown and Bledsoe 1996; Wetzel and van der Valk 1996; Miller and Bever 1999).

One of the main functions and ecological roles of AMF is providing enhanced phosphorus (P) nutrition to plants; thus, the effect of soil available P is often assessed in wetland ecosystems (Smith and Read 1997). In upland ecosystems, a negative correlation between soil P concentration and AM colonization is commonly reported, indicating the importance of AMF in nutrient-deficient habitats (Dhillion and Anderson 1993; Anderson et al. 1994). In wetlands, the effect of different soil P concentrations on AM colonization dynamics remains unclear. Greenhouse studies of wetland plants indicate that P does negatively affect AM colonization levels (Wigand and Stevenson 1994, 1997; White and Charvat 1999); however, a correlation between AM dynamics and P seldom occurs in wetland field studies. In studies where a correlation does exist, it is not ubiquitous between and within wetlands (Wetzel and Van der Valk 1996; Cornwell et al. 2001). For example, Rickerl et al. (1994) found that AM colonization of dogbane and reed canary grass in the dry zone of wetlands was highly correlated to P concentration, while in the wet regime there was no correlation. Anoxic soils and fluctuating water tables likely complicate the relationship between AM colonization and P because of changes in P mobility and solubility in flooded soils. The relationship could also be affected by temporal variation of soil P and plant need for P, especially if a wetland experiences very low or high levels of P (Carvalho et al. 2001; Van Hoewyk et al. 2001).

In many terrestrial ecosystems, temporal changes in AM colonization show a seasonal pattern correlated with plant phenology and phosphorus availability changes (Rabatin 1979; Brundrett 1991). Although limited in number, wetland studies on the dynamics of AM colonization show that the extent of AM colonization appears to follow seasonal trends; however, the explanation for the seasonal trend differs from study to study. On the one hand, in freshwater wetlands, Wetzel and van der Valk (1996) and Turner and Friese (1998) show that AM seasonal trends are influenced mainly by flooding and/or soil moisture levels. On the other hand, Stenlund and Charvat (1994) and Miller (2000) show that AM seasonal trends reflect plant phenological changes. Similarly, three salt marsh studies (van Duin et al. 1989; Brown and Bledsoe 1996; Carvalho et al. 2001) had mixed results, showing that the dynamics of AM colonization reflect plant phenology at the drier ends of marshes while AM colonization trends are influenced by flooding at the wetter ends. In short, the literature suggests that AMF are advantageous for wetland plants during certain times of the plant phenological cycle, while at the same time suggesting that flooding and soil moisture will often outweigh plant phenological effects on the dynamics of AM colonization.

This study was designed to further investigate the seasonal dynamics of AM associations in wetlands and to determine the main factors regulating AM colonization by assessing two different types of Ohio wetland habitats: fen and marsh. Ohio fen habitats usually have very low nutrient levels (available P is the main nutrient limiting plant growth and productivity), have high soil moisture and organic content levels, and experience little to no flooding. Also, some fens experience significant mineral deposits on the surface due to groundwater seeps (Amon et al. 2002). On the other hand, marsh habitats have high nutrient levels, experience significant flooding that may or may not fluctuate, and have anoxic soil conditions (Mitsch and Gosselink 1993). The differences between these wetland habitats include the extremes of major factors identified as potentially influencing AM dynamics in wetlands (soil moisture, flooding, and P). Furthermore, the differences include the extremes of factors correlated with AM seasonal dynamics (P availability and plant P need). Encompassing these factors allowed for a thorough study that assessed the factors' significance in relations to AM dynamics in freshwater wetlands. The primary questions addressed in this study were: (1) Does percent AM colonization change over the growing season and does this change differ between fen and marsh habitats? (2) If there are changes and differences in percent AM colonization, are they related to P availability, soil moisture, water levels, and/or plant phenology? (3) How widespread is AM colonization in fen and marsh habitats?

Materials and methods

Study sites

The four wetland sites selected for this study were Spring Valley Marsh (SV), Gingell Parcel Marsh (GP), Travertine Fen (TF), and Siebenthaler Fen (SF). These wetlands are located in Greene and Warren counties in west central Ohio, and are at least partially fed by groundwater (Bohrer 2001). The wetlands are preserved areas that are linked to residential and/or agricultural areas. The climate for this area is temperate and consists of humid, hot summers and cold winters. The highest level of precipitation occurs from April to June and a mild seasonal drought frequently occurs in August. The growing season is from late March to September.

Spring Valley Marsh is a topographically flat, groundwater driven marsh dominated by flood-tolerant plant species such as *Typha latifolia* L and *Sparganium eurycarpum* Engelm. ex Gray. This marsh remains 85% flooded for most of the year, has high nutrient concentrations, and has Linwood Muck soils (organic soil that is poorly drained; Soil Survey of Warren County, Ohio 1973). Gingell Parcel is a hillside fen that grades into marsh habitat. The marsh habitat is inundated most of the year, has Sloan soils (silty clay loam), and is dominated by emergent plant species such as *T. latifolia* and *Acorus calamus* L. The fen habitat of GP has peat and muck soils similar to the Linwood Muck soils of SV (Garner et al. 1978) and is dominated by typical Ohio fen plant species such as *Carex stricta* Lam. and *Carex hystericina* Muhl. ex Willd. Travertine Fen is a hillside fen having rapid discharge of groundwater at the surface and inundated soils at the slope's end. The diverse and unique plant community of this fen results from the

microtopography developed by groundwater seeps and vegetative mounds. Travertine Fen has calcium-laden surface soils and peat subsoils that remain saturated year round (no flooding or inundation occurs). The fourth site, SF, is a mound fen with very patchy and highly diverse plant communities. The surface of SF has a thin muck layer that is underlain by peat soils. The soils are classified as highly organic and remain saturated most of the year. Additional site description data, including water depth, vegetative composition, and soil chemistry, are documented in Bohrer (2001).

Sampling system

In each wetland, eight sampling areas were established along a transect that spanned an existing water table gradient. The gradient began in predominantly "near upland areas" (water table always below soil surface and soil moisture levels below 150%) and ended in predominantly saturated or flooded areas that had soil moisture levels above 250%. The length of the transect in each wetland depended on the size of the gradient and ranged from 35 m to 117 m. The eight sampling areas were designated based on the plant communities along the transect so that at least one sampling area was in each individual plant community (Bohrer 2001). For each month of the study, beginning in March and ending in September, the eight sampling areas were sampled by randomly placing a 1-m^2 quadrat within a 2 m radius circle. Within each quadrat, plants were identified, percent plant cover was recorded, the major period of plant growth (based on plant height and/or width) and flowering was recorded, and water table levels were measured (distance from soil surface) via water table wells (Bohrer 2001).

To sample the soil for analyzing abiotic factors and AM colonization of the overall plant community, four soil cores (2.5 cm in diameter and 15 cm deep) were collected from each quadrat after removing the top layer of debris. The soil was homogenized in marked Ziploc® bags and kept cold until processed in the lab. Within 24 h of collection, portions of these soil samples were analyzed for percent moisture by placing soil in a drying oven for 24 h at 100°C [grams water (grams oven dried weight of soil)$^{-1}$], and organic matter was determined by loss on ignition in a muffle furnace (Brower and Zar 1984). Balance Labs (Marion, Ohio) analyzed air-dried soil samples for measurements of available P, pH, potassium, calcium, magnesium, and cation exchange capacity.

Plant samples were also collected in each quadrat for each month to determine the mycorrhizal status of the dominant plant species for each sampling area. Intact roots of one plant specimen were removed from each quadrat and kept cold until the roots were analyzed for percent AM colonization in the laboratory.

AM colonization assessment

Roots were randomly removed from the soil core samples (representative roots from plant community) and the plant specimens, cut into 1 cm segments, rinsed, cleared in 10% KOH, and then stained with Trypan Blue (Phillips and Hayman 1970). Stained root segments were examined at ×100 magnification and were assayed to determine the total percent root length colonized using the gridline intersection method (Giovannetti and Mosse 1980; Brundrett et al. 1994). Percent AM colonization by arbuscules, vesicles, spores, and hyphae was measured. To accurately identify the presence of arbuscules, root segments were observed at ×400 magnification.

Statistical analysis

All statistical analyses were performed using SPSS Base 10.0 (SPSS, Cary, N.C.). Site and month effects on total AM colonization (percent colonization via all AM structures combined) from the soil core samples were analyzed using one-way and two-way analysis of variance (ANOVA), and pairwise differences among samples were determined by Bonferroni's test (P<0.05). Levene's equal variance test was performed at a 5% significance level and residual plots were examined to verify that the equal variance and normality assumptions were met. Percent AM colonization data were arcsine square root transformed to meet the ANOVA assumptions. The environmental variables (soil available P concentration, percent soil moisture, and water table level) and individual AM fungal structures (arbuscules, vesicles, spores, and hyphae) statistically analyzed were non-normal and the variances were not homogeneous across the treatments; therefore, these variables were converted into ranks, which greatly improved the normality and homogeneity of variance. A Kruskal-Wallis test was used to determine the significance of site and month on the environmental factors. The relationships between percent AM colonization and each environmental variable were analyzed for each site via Spearman's rank correlation coefficient. Spearman's rank correlation coefficient was also used to analyze these relationships for each month within each site. Statistical analysis of AM colonization of individual plant species was not performed because of low sample number.

Results

AM colonization in mixed-root soil samples

Analysis of the soil core samples representing a mixture of plant species at each wetland site and sampling area revealed that AMF were present at all four sites and in all months. AM colonization was indicated by the presence of aseptate hyphae, arbuscules, vesicles, and/or endospores. Unless otherwise specified, "AM colonization levels" refers to total percent AM colonization. Root segments had AM colonization levels ranging from 0–76%. The highest colonization level for any one sampling area within one wetland was 51% for SF in April, 76% for TF in April, 33% for GP in April, and 50% for SV in March. Arbuscules, as well as the other fungal structures, were found along all parts of the water table gradient in all wetlands. Intraradical spores, vesicles, and hyphae were the most dominant mycorrhizal structures found.

Statistical analysis of AM colonization levels indicated a significant effect of wetland site (F=4.961, 3 df, P=0.002) and of month (F=16.419, 6 df, P<0.001); however, there was no significant interaction between site and month. Combining all months together, GP had significantly lower (P<0.05; $\bar{x}$=10.96%) AM colonization levels than either SF ($\bar{x}$=18.41%) or TF ($\bar{x}$=19.96%). Although not significant, the marsh at SV ($\bar{x}$=12.54%) also had lower AM colonization levels than the two fens. Time of sampling (month) was significantly correlated with AM colonization levels (P<0.05), and the seasonal trend of AM colonization was similar for all four wetland locations (Fig. 1). Highest levels of AM colonization were observed in March or April and the lowest levels were observed in July or August for the majority of the sampling areas in all wetland habitats. Since mixed-root soil samples at each transect point were used for the monthly trend analysis of AM colonization, the seasonal trends (Fig. 1) represent the general wetland plant communities located in each wetland. AM colonization levels for vesicles, hyphae, and spores were also affected by month, expressing seasonal trends similar to total AM

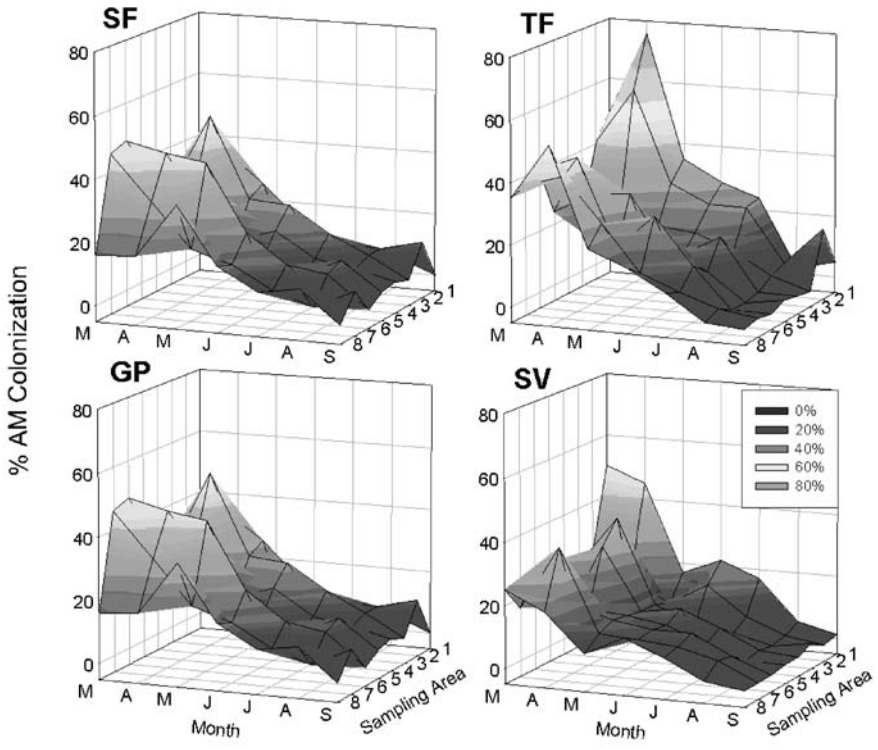

Fig. 1 Seasonal variation of percent arbuscular mycorrhizal (AM) colonization of bulk soil samples for SF (Siebenthaler Fen), TF (Travertine Fen), GP (Gingell Parcel), and SV (Spring Valley Marsh). Sampling area 1 is the driest end of the wetland site and area 8 is the wettest. Each data point represents four soil cores (n=4) obtained within the sampling area

Table 1 Edaphic characteristics for Siebenthaler Fen (*SF*), Travertine Fen (*TF*), Gingell Parcel (*GP*), and Spring Valley Marsh (*SV*). Sample size for each site is 56 (8 samples collected along the transect for each month). Values are means ±SE

	Site			
Soil variable	SF	TF	GP	SV
Phosphorus (P) (μg g^{-1})	4.0±0.2	0.6±0.0	3.1±0.2	9.0±0.7
Soil moisture (%)	236±10	172±11	215±13	187±18
Organic matter (%)	41.0±1.2	17.3±1.0	27.2±2.0	23.1±1.6
Calcium (kg ha^{-1})	11,106±40	10,993±84	10,485±175	9,383±243
Magnesium (kg ha^{-1})	1,784±7	871±46	1,555±39	1,413±40
pH	6.67±0.04	7.98±0.02	7.32±0.05	6.74±0.11
Potassium (kg ha^{-1})	146±10	58±4	123±6	131±9
Cation exchange capacity	344±4	268±3	291±4	300±7

colonization trends (highest levels in March and April and lowest levels in August and September). AM colonization via arbuscules was highest in April (4–10% colonization) and sharply decreased through June (0–4%), after which arbuscules were no longer noticed.

Soil and water analyses

All soil variables were significantly (P<0.001) different between wetland sites. Values of soil variables for each wetland are summarized in Table 1. Siebenthaler Fen had the highest values for all soil variables except P and pH. On the other hand, TF had the lowest values for all soil variables except calcium and pH. Soil P and moisture data by site and by month are shown in Fig. 2. Soil P trends were different between sites: TF consistently had the lowest values and SV consistently had the highest values (Table 1; Fig. 2a). No one site consistently had the highest or lowest values for soil moisture (Fig. 2b). Soil P availability was significantly influenced by month (χ^2=13.377, 6 *df*, P=0.037) for each site, but the trends were site dependent (Fig. 2a). The effect of time of sampling (month) on moisture was also largely dependent on the site (Fig. 2b).

Water table levels were recorded as distance below soil surface. The average transect water table levels are illustrated in Fig. 2c. There was a significant difference in water table levels between wetland sites (χ=19.202, 3 *df*, P<0.001). SF consistently had the lowest water table levels for all months and SV had the highest water table levels for all months except May. At SF, water levels significantly decreased throughout the study (χ=16.845 6 *df*, P<0.01); however, the change in water level for the other sites was not significant. At GP, TF, and SV the water table levels were always at or above the soil surface at the "wet" end of the sampling transect, while at SF the level fell to 18 cm below soil surface in August and September. At the "near upland" end of the sampling transects in the four wetlands, the water table level varied

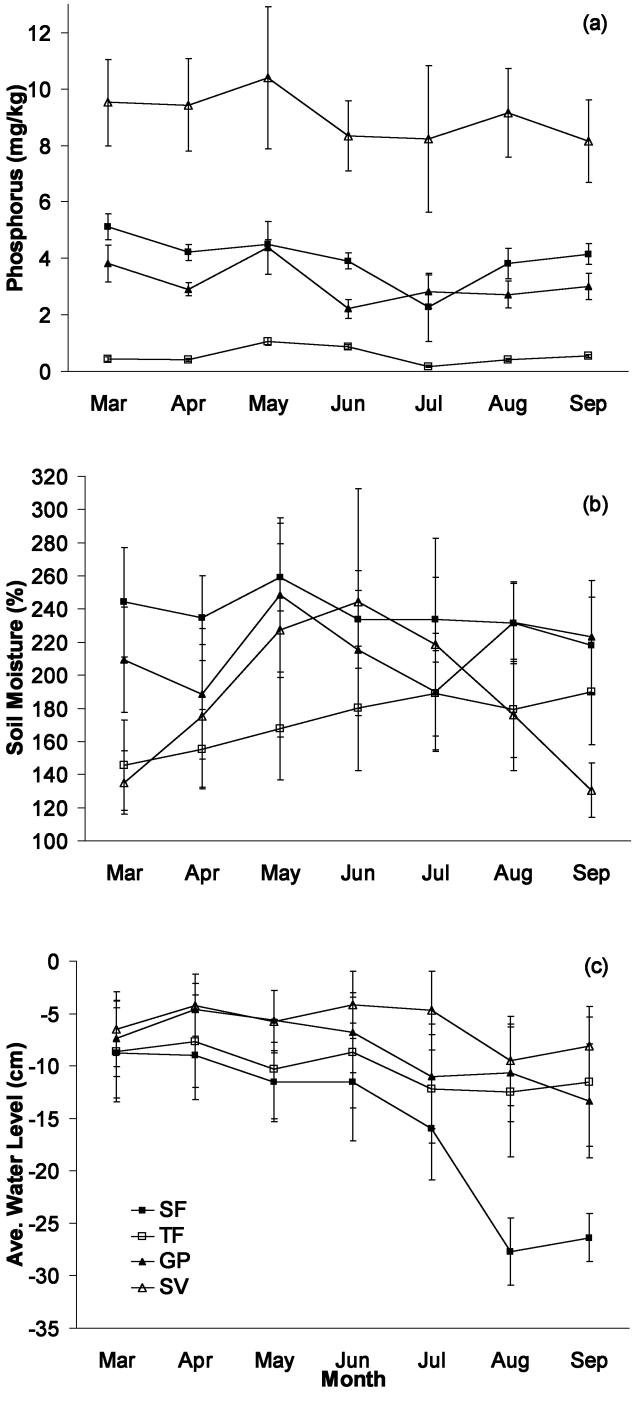

Fig. 2a–c Seasonal variation of **a** soil available phosphorus concentration, **b** percent soil moisture, and **c** average water table levels at each site. Values plotted are means of the eight sampling areas for each month ±SE. A water table level at the soil surface is indicated by 0 cm

from 21 cm to 45 cm below the soil surface, depending on the time of sampling. Average water table level at the "near upland" end for TF and SF was 40 cm below soil surface, while the average level was 30 cm below soil surface for SV and GP.

Table 2 Spearman's rank correlation coefficients (r_s) showing relationships between arbuscular mycorrhizal (AM) colonization (%) data and soil available P concentrations and soil moisture (SM; %) for each site. Correlation coefficients were determined from means of all sampling areas and dates combined (n=56)

Wetland site	Soil variable	% AM colonization
SF	P	0.194
	SM	0.335*
TF	P	0.351**
	SM	–0.354**
GP	P	0.468*
	SM	–0.134
SV	P	0.068
	SM	–0.028

**P<0.01, *P<0.05

AM colonization levels (total and individual fungal structures) at SV and GP were not significantly correlated with soil moisture (SM) (Table 2). Total AM colonization and colonization by arbuscules, vesicules, and hyphae were positively correlated with SM levels at SF; however, all colonization levels were negatively correlated with SM at TF (P<0.05). Total AM colonization was positively correlated with soil P at both TF and GP (Table 2). When each site was analyzed by month, a positive correlation resulted between AM colonization and soil P at TF for the months of March and April (P<0.05). The seasonal trend of AM colonization levels did not appear to be related to the trends of soil available P, SM, or water table level in a significant way (Figs. 1, 2). However, there appeared to be patterns associated with the extent of AM colonization based on transect location (Fig. 1).

AM status of individual plant species

Seventeen different plant species within the four wetland sites were analyzed for the presence of AMF (Bohrer 2001). Each site had between five and eight species selected for analysis depending on the number and size of the plant communities along the transect. All 17 species assessed were colonized by AMF for at least 1 month and arbuscules were observed in 7 of these species (Table 3). Of the plant species examined, 85% had highest levels of AM colonization in March and April (wetland site averages =20–50%) and lowest levels in July through September (0–10%).

Field observations (Table 3) revealed that maximum vegetative growth periods occurred in early- to late-spring for nearly all plant species sampled. Blooming time varied from April through September. Estimates of percent cover and plant height indicated that most of the sampled plant species started growing in April and completed growth by June. Two plant species common in the flooded areas, *T. latifolia* and *A. calamus*, started growing in March and had completed most of their growth by the end of April. Both of these flood-tolerant plant species were colonized by AM fungi during these months. Plants at TF, where P is lowest among all the

Table 3 AM status of the plant species assessed from the four sites. Percent AM colonization value is the highest value found during the study. AM colonization sampling took place the last week of the month indicated. Wetland indicator status is based on the United States Fish and Wildlife Guidelines for Region 1 (Reed 1997): *FACU* facultative upland, *FACW* facultative wetland, *OBL* obligate wetland, *UPL* obligate upland

Plant species	Indicator status	% AM colonization	Arbuscules (March–May)	Major growth period	Bloom time
Alliaria officinalis Andrz ex Bieb	UPL	9% (April)	Not found	May (biennial)	June (2nd year)
Solidago canadensis L.	FACU	18% (May)	Not found	May–July	Aug–Sep
Caltha palustris L.	OBL	9% (July)	Not found	March–April	April
Typha latifolia L.[a]	OBL	79% (March)	Present	March–April	May–June
Carex hystericina Muhl. ex Willd.[a]	OBL	36% (March)	Not found	April–May	June
Phalaris arundinacea L.	FACW	12% (April)	Not found	May and Sep	June
Poa palustris L.	FACW	26% (March)	Not found	April–June	May
Carex stricta Lam.[a]	FACW	33% (March)	Not found	April–May	Late May
Sorghastrum nutans (L.) Nash	UPL	40% (March)	Not found	May–Aug	Sep
Schizachyrium scoparium (Michx.) Nash	FACU	41% (March)	Present	May–Aug	Aug–Sep
Potentilla fructicosa auct. non L.[a]	FACW	68% (April)	Present	April–June	June
Carex sterilis Willd.	OBL	55% (March)	Present	April–May	Late May
Acorus calamus L.	OBL	19% (March)	Not found	March–April	Late May
Carex comosa Boott[a]	OBL	15% (April)	Present	May	Late June
Impatiens capensis Meerb.	FACW	21% (April)	Present	May–Aug	Aug
Sparganium eurycarpum Engelm. ex Gray	OBL	21% (April)	Present	April–May	July
Eleocharis palustris Roemer & J. A. Schultes	OBL	20% (April)	Not found	May	Late May–June

[a] Plant species was assessed at more than one site

wetland sites, grew to approximately one-third the height seen at the other wetland sites or that would be expected based on plant natural history data.

Discussion

The results of this study demonstrate that AM colonization of wetland plants occurs to a great extent in wetland ecosystems. Because the four wetlands had significantly different soil environments and water regimes, the presence of AMF did not seem affected by wetland specific environmental factors or by certain positions within a wetland, in agreement with the results of Carvalho et al. (2001) and Van Hoewyk et al. (2001). This study revealed a similar and significant seasonal trend of AM colonization that existed among all wetlands and all sampling areas within the wetlands. The soils of the sampling areas had water conditions that ranged from mostly dry to consistently flooded, therefore demonstrating, contrary to expectation, that AMF are not limited, and AM seasonal trends are not diluted, by flooding in these wetlands. Turner and Friese (1998) and Miller (2000) similarly indicate that a significant seasonal trend for AM colonization exists regardless of gradient. These studies suggest that AMF are tolerant of a wide range of soil moisture and flooded conditions. Since AMF were present and had similar colonization trends in all parts of the gradient in our study, we speculate that AMF in water-stressed soils have functional roles similar to the roles of AMF in other areas of a wetland gradient.

Both Wetzel and van der Valk (1996) and Rickerl et al. (1994) found that AM colonization levels were lower in soil with elevated P concentrations in wetland habitats, as is common in upland habitats (Koide 1993). In contrast, at TF and GP, AM colonization levels appeared to be positively impacted by increasing soil P concentrations, potentially due to the very low levels of soil available P at TF (<1 ppm) and GP (<4 ppm). Bolan et al. (1984) and Koide and Li (1990) indicate that extremely low levels of P suppress the extent of AM colonization; hence, initial increases in P concentration should cause AM colonization levels also to increase. For this increase to happen, the plants would also have to be P limited, which is quite apparent at TF where the P-deficient environment causes the plants to be very small.

From the amount of P that suppresses AM colonization up to the level of P no longer limiting to plants, it is likely that a correlation between AM colonization and soil P would not occur since the plants are still benefiting from the AM associations. This may explain why a correlation between AM colonization and soil P did not occur at the other two wetlands (SV and SF), both of which had more moderate levels of soil P (5–10 ppm). At these two sites levels of P evidently were above the P concentrations that limit AM colonization and within the P concentrations that limit plants. Miller (2000) and Van Hoewyk et al. (2001) speculate that the narrow range of P (0–35.8 ppm and 2.5–15.7 ppm, respectively) in their study sites caused the lack of significant correlation between AM colonization and soil P. Because the soil P levels are so low, and the range in soil P is so small in our study (and theirs), it is not possible to speculate on whether or not large fluxes of P in other wetland habitats would significantly influence the extent of AM colonization at any given time. Future wetland research assessing the variation of AM colonization along a larger available P spectrum would be valuable for further understanding of the dynamics of AMF in different wetland ecosystems.

SM was not correlated with AM colonization at GP and SV; however, as previous wetland studies have found (Anderson et al. 1984; Brown and Bledsoe 1996; Turner and Friese 1998), AM colonization levels at TF appeared to be negatively impacted by SM along the water table gradient. In contrast, higher AM colonization levels were correlated with increased SM at SF. This was unexpected because SF had the highest average soil moisture (236.42%) of all the wetland sites. It is possible that this positive correlation was due to the unique nature of the peat soil and/or the plant species growing in the highly saturated areas of the fen. Peat soil that is rich in organic matter and has a broad capillary zone has a greater water-holding capacity as compared to other wetland soil types. Peat soil, therefore, allows for more oxygen as compared to other wetland soils with the same soil moisture content (Mitsch and Gosselink 1993). Research is ongoing to better understand the relationship between AM colonization and soil moisture in soils with high peat content.

Our graphical results revealed some differences in the extent of AM colonization based on transect location. Because our transects were based on a water gradient at each wetland site and AM colonization levels appeared to vary between sampling areas, the possibility exists that the water gradient affected the extent of colonization, but not the presence of AMF. This is consistent with other research (Stevens and Peterson 1996; Wetzel and van der Valk 1996; Miller 2000); however, sorting out these differences has not yet been pursued because the multivariate problem requires an analysis geared specifically towards gradient effect. Other differences along the transect, including plant species, fungal species, and mineral deposit differences, could also contribute to the variability in AM colonization levels (Miller and Bever 1999; Miller 2000). Although we did not analyze fungal species differences, we minimized the plant species effect by analyzing roots that were representative of the whole plant community along the transects. Also, we incorporated mineral deposit differences by measuring soil mineral concentrations and comparing them between wetlands.

Wetland studies have found that seasonal variation of AM colonization is linked to seasonal variation in water table levels and/or soil moisture levels. Seasonal variation of water regimes in wetlands likely provides the soil with opportunities to aerate, thus providing the AMF with periods of increased oxygenation for survival (Anderson et al. 1984, 1986; Cooke et al. 1993; Miller and Bever 1999). Results from this study do not support this finding since, in general, all four wetlands had the highest AM colonization levels during periods of higher water tables and vice versa. This indicates that the extent of AM colonization was not controlled by seasonal dynamics related to variations in water table. As for the effects of SM seasonal trends, only TF had a SM trend that appeared to be associated with the AM colonization trend. We speculate that the negative association between the two trends at TF was coincidental since the seasonal trend of AM colonization was similar to the AM colonization trends at the other sites and since TF was the only site where SM continuously increased during the course of the study. Water table and SM levels did not vary greatly at our wetland sites; hence, our results could be different from results of studies performed in wetlands where water levels greatly vary.

Finding the same seasonal trend of AM colonization in wetland habitats ranging from a nutrient-rich flooded marsh to a nutrient-deficient calcerous fen provides evidence that AM seasonal variation is likely influenced by plants rather than by abiotic factors. In terrestrial ecosystems, seasonal variation of colonization is often evident and is correlated with plant phenology. Typically, a higher degree of AM colonization coincides with the stages of the plant life cycle that demand additional P (Rabatin 1979; Dhillion et al. 1988; Sanders and Fitter 1992; Anderson et al. 1994). At our wetland sites, plant demand for P is probably high relative to soil available P in the spring when rapid vegetative growth and new root production occurs; therefore, we predicted AM colonization also to be high in the spring (assuming that AMF would not be restrained by the wetland soil environment). Indeed, AM colonization was highest in the spring (March through May) and lowest in late summer regardless of the level of P during these months and regardless of position along the transect. Furthermore, arbuscules—the site of P exchange between the fungus and the plant—were present in the springtime (March through June) indicating that the AM associations were functional at that time (Smith and Read 1997). These findings suggest that the seasonal trend in AM colonization was influenced by plant phenological events, specifically new root production and vegetative growth. Other wetland mycorrhizal studies also indicate that seasonal variation in AM colonization levels is linked to plant phenological events, including new root growth (van Duin 1989), flowering and fruiting (Stenlund and Charvat 1994; Carvalho et al. 2001), active plant growth (Miller 2000), and seedling establishment (Carvalho et al. 2001).

Given that anoxic, flooded soils are not amenable for aerobic organisms, AMF might be maintaining themselves in certain plants during the summer just to survive. Brown and Bledsoe (1996) observed AM fungi in the aerenchymatous tissue of salt marsh plants, suggesting that AMF tap into this aerenchymatous tissue to survive in low oxygenated soils. Because AMF existed in all parts of the wetland gradient and AM colonization levels showed a similar seasonal trend in all sampling areas, we also suggest that AMF may receive sufficient oxygen from plants that are adapted to life in oxygen-deficient soils. Although it was not noted whether or not AMF were specifically present in the aerenchyma of plant roots in this investigation, AMF were always found in *T. latifolia*, which has extensive aerenchymatous tissue. In August and September, *T. latifolia* roots had the highest AM colonization levels of all plants sampled in all four wetlands suggesting that this plant host may act as a survival mechanism for AMF.

All plant species assessed in this study were colonized for at least 1 month in each site, supporting other wetland research that shows AMF can be abundant in wetland plants (Cooke and Lefor 1990; Wetzel and van der Valk 1996; Miller and Bever 1999; Turner et al. 2000). Nevertheless, some research has shown plant species, such as *Carex* species (Miller and Bever 1999; Thormann et al. 1999) and *Typha* species (Anderson et al. 1984; Rickerl et al. 1994; Thormann et al. 1999), to be non-mycorrhizal. In this study, *Carex* spp. were colonized by AMF, and *T. latifolia* was heavily colonized by AMF at all four sites for at least 1 month. Discrepancies on the mycorrhizal status of certain wetland plants could be the result of sampling regime. If AM colonization varies significantly in wetland plants as it did in this study, previous studies might have missed observing the presence of AMF in certain wetland plants because of sampling time. Therefore, at the very least, wetland studies should sample during major vegetative growth periods to observe active root colonization.

In summary, environmental factors seem to influence only the extent of AM colonization in some instances, while plant species and plant phenological events seem to influence the seasonal variation of AM colonization, a finding consistent with the results of Miller (2000) in a semi-aquatic grass in the Carolina Bays. Because the results of our study indicate a strong AM seasonal dynamic, we suggest that future wetland AM studies be long term and that past studies be re-examined to more fully understand the dynamics and functional roles of AMF in wetlands. Although the roles of AMF are still not fully understood in wetlands, the results from this study imply that AM associations are functional in, and are a significant component of, wetland plant communities, especially during new root production and major vegetative growth. By enhancing plant nutrition at certain stages of the plant life cycle, and possibly helping plant establishment and development, AM associations have significant implications for plant competition, succession, and diversity in fens and marshes (Newman and Reddell 1988; van der Heijden et al. 1998; Carvalho et al. 2001; Facelli and Facelli 2002). Because of these ecological implications, AM associations and the seasonal dynamics of AM colonization should be considered in plans for the ecological restoration of functional wetlands and should be a significant component of studies assessing wetland ecosystem dynamics.

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