

Arbuscular mycorrhizal fungi and water table affect wetland plant community composition

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Summary

1 Most studies of the community-level effects of arbuscular mycorrhizal fungi (AMF) have been conducted in upland grassland plant communities where a majority of the plant species are colonized by AMF. Here, we examine the effects of AMF on plant community composition in experimental wetland plant communities, where the dominant plant species are non-mycorrhizal and subordinate plant species are colonized by AMF. We also assess how an important abiotic soil variable, depth to water table (soil saturation), might mediate the community-level effects of AMF.

2 In the low water table (un-saturated) treatment, above-ground plant biomass increased in the presence of AMF relative to the controls, while in the high water table treatment, biomass decreased with the presence of AMF. Contrary to predictions, plant diversity was unaffected by the presence of AMF in the low water table treatment, but significantly decreased in the presence of AMF in the high water table treatment. Changes in biomass and composition were driven by the interactions between the dominant non-mycorrhizal species *Carex hystercina*, and the remaining mycorrhizal plant species.

3 Our results indicate that AMF have the potential to influence plant community composition in calcareous fens and that these effects can be mediated by soil saturation.

4 This study has implications for understanding how established principles of above-ground/below-ground interactions from upland communities translate to wetland plant communities and for understanding how AMF function can be mediated by abiotic soil properties. Contrary to previous thought, AMF may be important drivers of plant community composition in wetland plant communities.

Key-words: abiotic, above-ground/below-ground interactions, arbuscular mycorrhizal fungi, calcareous fen, diversity, mesocosm, mutualism, plant community, spatial heterogeneity, wetland

Journal of Ecology (2006) **94**, 905–914
doi: 10.1111/j.1365-2745.2006.01160.x

Introduction

Arbuscular mycorrhizal fungi (AMF) have been shown to have idiosyncratic effects on plant community composition in a variety of terrestrial ecosystems. In herbaceous plant communities at small spatial scales,

experimental studies have demonstrated positive (Grime *et al.* 1987; Gange *et al.* 1993; van der Heijden *et al.* 1998), negative (Hartnett & Wilson 1999; O'Connor *et al.* 2002) and negligible (Landis *et al.* 2005) effects on plant community diversity.

Freshwater wetland plant communities are one type of ecosystem where the community-level effects of AMF are poorly understood. In the past, AMF have been considered to be of limited importance in wetland soils because they are thought to be obligately aerobic while many wetland soils are frequently anoxic (Keddy 2000). However, recent work has demonstrated that AMF are present in the roots of many wetland plants

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across many wetland types (Rickerl *et al.* 1994; Turner *et al.* 2000; Carvalho *et al.* 2001; Cornwell *et al.* 2001) and AMF communities in wetlands can be as diverse as in upland systems (Wirsal 2004). Although there has been some work showing that AMF can affect the growth of individual wetland plants (Miller & Sharitz 2000; Stevens *et al.* 2002; Dunham *et al.* 2003), to our knowledge, the community-level effects of AMF in a wetland plant community have not been examined.

In this study, we used experimental calcareous fen plant communities similar to natural fens of central New York State (USA), to test the community-level effects of AMF in wetlands. These species-rich, groundwater-fed peatlands are important conservation priorities in many regions because of the number of rare flora and fauna that occur in calcareous fens and because they are among the most floristically diverse ecosystems in North America (Bedford & Godwin 2003). Undisturbed calcareous fens soils receive high inputs of calcium-rich groundwater and are low in plant-available phosphorus (Godwin *et al.* 2002), suggesting that mechanisms to acquire phosphorus, such as associations with AMF, might be important for the nutrition of fen plant species. Although some of the plants that occur in fens are also found in other freshwater wetlands, many plant species are unique to North American calcareous fens and include many calcicole species that have an affinity with calcium-rich soils (Bedford & Godwin 2003). As with many other freshwater wetland plant communities, the most abundant plant species in calcareous fens are non-mycorrhizal plants, such as sedges (mostly *Carex* spp.) and *Typha* spp., that often have no or low levels of AMF colonization and generally do not respond to the presence of AMF (Miller *et al.*

1999; Muthukumar *et al.* 2004). Within this matrix of dominant non-mycorrhizal sedges are subordinate dicots that are colonized by and can respond to AMF (Fig. 1; Cornwell *et al.* 2001; Weishampel 2005). In this type of plant community, a recent conceptual model proposed by Urcelay & Diaz (2003) predicts that AMF will increase plant diversity as the growth and competitive ability of subordinate species increases in the presence of AMF.

In addition to examining the effects of AMF on fen plant community composition, we also assessed how a local abiotic soil variable, depth to water table, could mediate the community-level effects of AMF. At the level of individual plants, plant responses to AMF are mediated by abiotic characteristics of the local soil environment (Jones & Smith 2004), but it is unclear if community-level effects of AMF are also mediated by abiotic soil factors. In calcareous fens, depth to water table (soil saturation) is spatially heterogeneous. Although the water table in calcareous fens is generally at or slightly below the surface of the soil (Bedford & Godwin 2003), there can be considerable variation in depth to water table due to the presence of hummocks created by bryophytes (Figs 1 and 2 and Wolfe 2005). Over a growing season there is little fluctuation in the water table because these wetlands are primarily ground-water fed (Cornwell *et al.* 2001), so the heterogeneity in water table is mostly spatial and not temporal. This environmental spatial variability may have the potential to mediate plant–AMF interactions. Subsequently, the two specific research questions of this study were: (i) Do AMF increase calcareous fen plant diversity by increasing the abundance of subordinate mycorrhizal plant species? (ii) Does depth to water table mediate the community-level effects of AMF in calcareous fens?



Fig. 1 Photograph of a calcareous fen plant community. Note that in the right hand side of the photo, the soil is saturated, but depth to water table increases on the top and left hand side of the photo due to the presence of bryophyte hummocks. Photo courtesy of Robert Wesley.

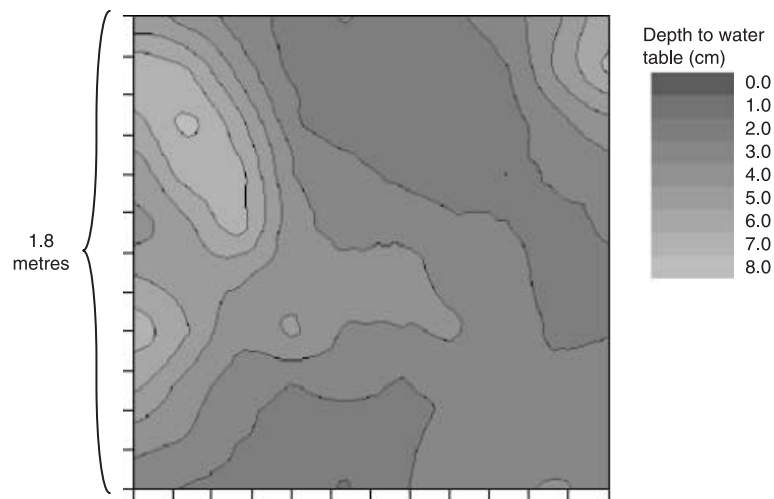


Fig. 2 Krigged map showing spatial variation in depth to water table in an intensively sampled plot at Belle School Fen, a calcareous fen of central New York State. Values represent depth of the water table from the surface of the soil. The krigged map was created based on semivariance analysis of depth to water table using an exponential model with a lag distance of 20 cm using GS+ v. 5.1.1 (Gamma Design Software, Plainwell, MI, USA). Variogram model parameters were: nugget = 3.79, sill = 8.17, range = 60.9, spatial dependence = 0.54, $r^2 = 0.90$. Mean depth to water table in the plot was 2.99 (± 2.67 SD) cm below the surface of the soil.

Methods

MESOCOSM CONSTRUCTION

Plant material

Mesocosms were constructed to simulate small patches of calcareous fen vegetation by collecting seeds of 14 native fen plant species (Table 1) during the 2003 growing season. These seeds originated from populations of plants in North American wetlands and were obtained from a native seed supplier (Prairie Moon Nursery, Winona, MN, USA) or from local fens, when possible. The 14

species that we included in this study are plant species that represent both dominant and subordinate species (in terms of relative abundance across numerous sites) in New York State calcareous fen communities, and have been shown to have differential responses to the presence of AMF and water table (Weishampel 2005). All species included are native to North America and are obligate or facultative wetland plant species (USDA NRCS 2004). Seeds were cold-wet stratified, if necessary for germination, in sterile sand for 1 month and then germinated on Pro-Mix (Premier Tech, Rivière-du-Loup Québec, Canada), which is a peat-based soil free of AMF propagules.

Table 1 Characteristics of species used in this study

	Plant family	AMF colonization*	Number planted†
Graminoids			
<i>Carex hystericina</i>	Cyperaceae	0	9
<i>Bromus ciliatus</i>	Poaceae	57	1
<i>Muhlenbergia glomerata</i>	Poaceae	NA	1
Forbs			
<i>Eupatorium</i> spp.‡	Asteraceae	54	3
<i>Solidago patula</i>	Asteraceae	84	2
<i>Packera aurea</i>	Asteraceae	61	2
<i>Symphotrichum puniceum</i>	Asteraceae	46	1
<i>Doellingeria umbellata</i>	Asteraceae	NA	1
<i>Chelone glabra</i>	Scrophulariaceae	35	1
<i>Lobelia siphilitica</i>	Campanulaceae	74	1
<i>Lycopus americanus</i>	Lamiaceae	78	1
<i>Mentha arvensis</i>	Lamiaceae	NA	1
<i>Parnassia glauca</i>	Saxifragaceae	NA	1

*Percentage root colonization observed in field plants. Data from Weishampel (2005).

†Number of individuals per species planted in each mesocosm.

‡Contained a mix of *Eupatorium maculatum* and *Eupatorium perfoliatum* that were difficult to differentiate as seedlings. Both species are commonly found in calcareous fens.

NA = data not available.

AMF inoculum

The AMF used in this experiment originated from four natural fen communities within the Cayuga Lake Basin in central New York. Inoculum from these fens was generated using trap cultures of sudangrass (*Sorghum sudanese* (Staph.) Piper) grown in homogenized soil and sand collected from the four fens (as described in Weishampel 2005). Homogenized subsamples of the sudangrass inoculum were bulked by growing corn (*Zea mays* L.) in a 1 : 1 mixture of the original inoculum and AMF-free Turface® (Profile Products, Buffalo Grove, IL, USA) for 1.5 months. Control (non-AMF) pot cultures consisting of corn without AMF were also established by growing corn in AMF-free Turface without field soil inoculum. Corn roots were separated from the pot culture soil, rinsed with sterile water, and used within 24 hours to inoculate the mesocosms (see below). No attempt to identify AMF morphospecies was made, but subsamples of this inoculum have been deposited at the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM Accession #s NY304-NY307).

This method of AMF inoculum preparation does not eliminate fungal pathogens, but similar studies that did not use pure cultures of AMF have been used in the past to assess AMF effects (e.g. Kiers *et al.* 2000; Miller & Sharitz 2000; Dunham *et al.* 2003; Moora *et al.* 2004). Pathogens could have been a component of the inoculum used, although, as is described later, there was a low incidence of non-AMF fungal structures in the roots of plants in the +AMF treatments. Moreover, a microbial wash was added to attempt to control for differences in microbial communities between +AMF and –AMF treatments (see below).

Establishment of experimental plant communities

To create experimental mesocosms, 20 × 20 × 10 cm deep plastic containers were filled with a commercially available AMF-free peat soil (Pro-Mix, Premier Tech) to 1 cm below the rim of the containers (c. 2 kg of wet soil). This soil has physico-chemical characteristics similar to peat in North American fens (Godwin *et al.* 2002). Within this soil, 20 g (wet weight) of the inoculum described above was placed 3 cm below the surface of the soil and 3-week-old seedlings of each species were transplanted into the mesocosms. The number of seedlings planted in each mesocosm (Table 1) was based on the relative abundance of each species in calcareous fens of New York State. Seedlings were randomly planted in these mesocosms in a regular grid pattern. The total number of seedlings planted per mesocosm is approximate to densities that occur in the field. Seedlings that died within a week of transplanting were replaced. After the initial 2-week period, there was no further mortality of the seedlings.

To attempt to correct for differences in non-AMF soil microbes between the +AMF and –AMF treatments,

a microbial wash was added to all mesocosms. To prepare the microbial wash, AMF inoculum (roots and soil from trap cultures) was shaken with sterile water for 5 min to create soil slurry. This slurry was then filtered through a 40-µm sieve; 50 mL of this filtered slurry was added to each mesocosm after the seedlings were planted.

Two different water table treatments were established by drilling drainage holes in the sides of the mesocosms. In the high water table (HWT) mesocosms, the water table was maintained at the soil surface, while in the low water table (LWT) mesocosms the water table was maintained 8 cm below the soil surface. Mesocosms were watered twice a day to maintain the water table treatments with local groundwater.

Each of the four treatments, (i) low water table without AMF (LWT/–AMF), (ii) low water table with AMF (LWT/+AMF), (iii) high water table without AMF (HWT/–AMF), and (iv) high water table with AMF (HWT/+AMF), was replicated 10 times for a total of 40 mesocosms. Mesocosms were placed in a completely randomized design in a glasshouse at the University of Guelph during the summer of 2004. No supplemental light was provided during the experiment.

BIOMASS HARVEST

After 12 weeks of growth, all above-ground plant material was harvested, sorted by species, dried to a constant mass at 60 °C for 36 hours, and weighed. Biomass for species with more than one individual per mesocosm was pooled across all individuals of each species. Root biomass for each individual species was not harvested because it was too difficult to separate intertwined roots from the peat substrate, but we were able to sample a small portion of roots of each species from each treatment to check for the presence of AMF structures (see below).

Dried tissue of all plant species, excluding *Carex hystericina*, was combined together and ground with a Wiley mill for tissue nutrient analysis. *C. hystericina* was excluded because its proportionally high biomass and non-mycorrhizal status had the potential to dilute any tissue nutrient effects of the mycorrhizal treatment on species colonized by AMF. We did not measure tissue nutrients of *C. hystericina*, but previous work with this species did not detect an effect of AMF on tissue nutrient concentrations (Weishampel 2005). Tissue nutrient concentrations of N and P were analysed at Agri-Food Laboratories (Guelph, Ontario, Canada). Percentage N was determined using a modified Kjeldahl method and percentage P was determined using plasma-emission spectroscopy.

ASSESSMENT OF AMF COMMUNITIES

We assessed a portion of the roots of each plant species to determine if AMF from the inoculum successfully colonized roots in both +AMF treatments. Subsamples of roots from each treatment from each species were

collected and placed in 70% ethyl alcohol until stained using Chlorazol Black E (Brundrett *et al.* 1984). Roots were examined for the presence of characteristic structures of AMF, including arbuscules, vesicles and hyphae. In addition, we examined roots from the –AMF treatments to ensure the absence of AMF in these treatments.

The abundance of AMF in the roots of plants in the mesocosms was determined by assessing percentage root colonization of one plant species, *Packera aurea*, which is known to be highly colonized by AMF in the field (Cornwell *et al.* 2001). We only chose to assess percentage colonization of one species because of time constraints and because of the difficulties in separating roots as mentioned above. Roots of one individual *P. aurea* from each mesocosm were extracted from the soil, preserved in 70% ethanol, and stained using Chlorazol Black E. Percentage colonization by AMF was determined using the gridline-intersect method (McGonigle *et al.* 1990).

STATISTICAL ANALYSES

Percentage mycorrhizal plant biomass was used to detect shifts in the biomass of all species except for the non-mycorrhizal *C. hystericina*. Percentage mycorrhizal plant biomass = (biomass of mycorrhizal plants)/(biomass of non-mycorrhizal plants) × 100. Plant diversity was calculated using the Shannon diversity index with plant biomass used as abundance. As the number of plants per species did not differ across the mesocosms, Shannon diversity represents community evenness. Total above-ground biomass, plant diversity, tissue nutrient concentrations and percentage mycorrhizal plant biomass were analysed using two-way ANOVAS. Biomass data were square root transformed and percentage mycorrhizal plants biomass and tissue nutrient data (expressed as %N and %P) were arcsine square root transformed to meet assumptions of ANOVA.

A MANOVA was performed with individual species biomass used as the independent variables and water table and AMF as the dependent variables. Protected ANOVAS were performed on the biomass of individual plant species because significant MANOVA effects for the main factors and their interaction were obtained (see Results). All statistical tests were conducted using SAS Version 8 (SAS Institute, Cary, NC, USA).

Results

AMF COLONIZATION OF HOST PLANTS

All plants, except *Carex hystericina*, were colonized by AMF as indicated by the presence of hyphae, vesicles and arbuscules. There were no signs of AMF colonization in the roots of plants in the non-AMF treatments. Across all treatments, there was a small amount (< 3%) of colonization by non-AMF fungi, as indicated by the presence of septate hyphae within the stained roots.

Percentage root colonization of *P. aurea*, including hyphae, vesicles and arbuscules, was significantly higher in the HWT treatment (20.2 ± 2.0%) compared with the LWT treatment (10.0 ± 1.8%) ($P = 0.0046$).

UNIVARIATE ANALYSIS OF COMMUNITY RESPONSES

AMF had a significant effect on total above-ground biomass, with a 21.6% increase in biomass in the presence of AMF relative to the control mesocosms in the LWT treatment and a 33.2% decrease in biomass in the HWT treatment ($F_{1,36} = 4.93$, $P = 0.0328$; Fig. 3a). Water table did not have a significant effect on above-ground biomass ($F_{1,36} = 2.94$, $P = 0.0949$) but there was a significant interaction between AMF and WT ($F_{1,36} = 51.02$, $P < 0.0001$).

Diversity of the experimental plant communities was significantly affected by both main treatment effects (AMF, $F_{1,36} = 2.94$, $P = 0.0949$; WT, $F_{1,36} = 2.94$, $P = 0.0949$) and their interaction (AMF × WT, $F_{1,36} = 2.94$, $P = 0.0949$). Diversity did not change in the presence of AMF in the LWT treatment, but significantly decreased in the HWT treatment (Fig. 3b). Percentage mycorrhizal plant biomass showed the same response as diversity (Fig. 3c), with no change in the LWT environment, but a significant decrease in the high water table environment (AMF, $F_{1,36} = 7.70$, $P = 0.0087$; WT, $F_{1,36} = 70.75$, $P < 0.0001$; AMF × WT, $F_{1,36} = 11.02$, $P = 0.0021$). This change in abundance of the subordinate mycorrhizal species occurred as *C. hystericina* increased from 42.4 (± 3.4)% to 60.4 (± 3.7)% of the community biomass.

Phosphorus content of the combined subordinate species was significantly affected by both main treatment effects (AMF, $F_{1,36} = 10.47$, $P = 0.0026$; WT, $F_{1,36} = 14.29$, $P = 0.0006$) and their interaction (AMF × WT, $F_{1,36} = 7.27$, $P = 0.0106$), with no significant increase of %P in the LWT treatment but a significant increase in %P in HWT treatment (Fig. 4a). There was no significant effect of AMF, WT or AMF × WT on %N concentration (AMF, $F_{1,36} = 2.68$, $P = 0.1103$; WT, $F_{1,36} = 1.28$, $P = 0.2662$; AMF × WT, $F_{1,36} = 2.69$, $P = 0.0610$; Fig. 4b).

MULTIVARIATE ANALYSIS OF SPECIES COMPOSITION

AMF, WT and the interaction between AMF and WT had significant effects on the composition of the plant communities (AMF, Wilk's $\lambda = 0.329$, Wilk's $\lambda F_{13,24} = 2.86$, $P = 0.0125$; WT, Wilk's $\lambda = 0.150$, Wilk's $\lambda F_{13,24} = 10.46$, $P < 0.0001$; AMF × WT, Wilk's $\lambda = 0.232$, Wilks' $\lambda F = 6.12$, d.f. = 13,24, $P < 0.0001$). Subsequent two-way ANOVAS on individual species biomass showed differential responses of the 13 different plant species to the main and interaction effects (Table 2). Two species showed significant AMF main effects, seven species showed significant WT effects, and six species showed significant AMF × WT interaction

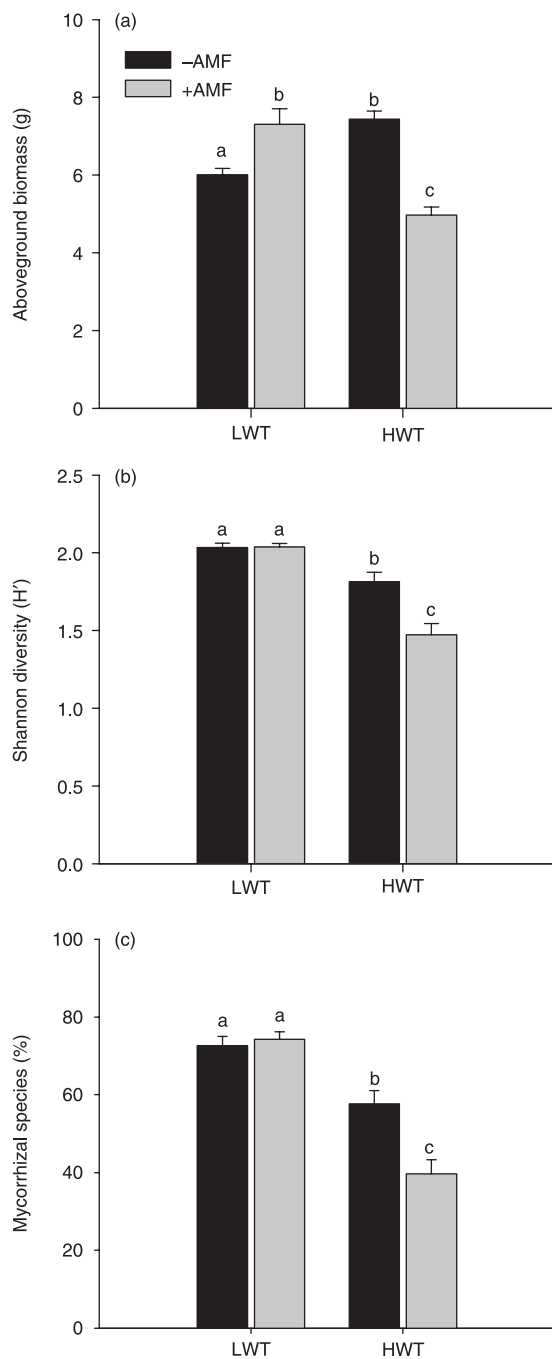


Fig. 3 Effect of water table (WT) and arbuscular mycorrhizal fungi (AMF) on (a) aboveground biomass, (b) diversity and (c) percent mycorrhizal plant biomass. Percent mycorrhizal plant biomass = [(biomass of mycorrhizal plants)/(biomass of nonmycorrhizal plants) × 100]. LWT = low WT, HWT = high WT, -AMF = no AMF inoculum added, +AMF = AMF inoculum added. Error bars are ± 1 SE. Bars with different letters indicate significant differences ($P < 0.05$) based on Ryan-Einot-Gabriel-Welsch (REGW) multiple-range tests.

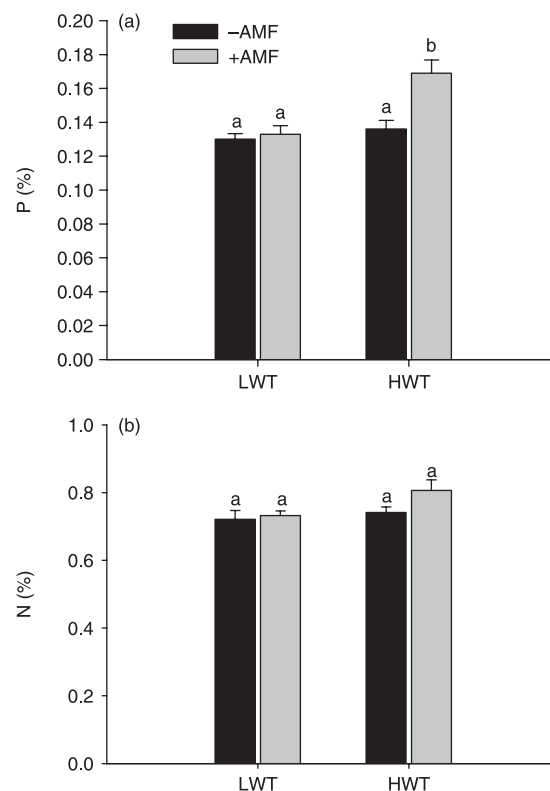


Fig. 4 Effect of water table and arbuscular mycorrhizal fungi on (a) %P and (b) %N in aboveground plant tissues of pooled mycorrhizal species. Data are for composite dry biomass samples of all mycorrhizal plant species within the experimental communities, and exclude the non-mycorrhizal species *Carex hystericina*. Bars represent mean values and error bars are ± 1 SE. Bars with different letters indicate significant differences ($P < 0.05$) based on Ryan-Einot-Gabriel-Welsch (REGW) multiple-range tests. Abbreviations follow Fig. 3.

Discussion

Previous studies have shown that AMF can have positive, negative and neutral effects on the productivity and diversity of upland plant communities (as reviewed in Urcelay & Diaz 2003) and on the growth of individual wetland plant species (Miller & Sharitz 2000; Stevens *et al.* 2002; Dunham *et al.* 2003), but this is the first study indicating that AMF can have community-level effects in a wetland. Plant diversity did not change in the low water table environment and decreased in the high water table environment in the presence of AMF. These findings are contrary to the results predicted by a conceptual model recently proposed by Urcelay & Diaz (2003) and to our hypothesis that AMF would increase plant diversity in experimental calcareous fen communities where non-mycorrhizal plant species dominate. It is important to note that the conceptual model and predictions of Urcelay & Diaz (2003) emphasize absolute positive or neutral effects of AMF. However, we suggest that the model should specifically consider the *relative* dependence of plants on AMF, because AMF can have a range of effects on plant growth from positive to neutral to negative, based on the plant and fungal species and genotypes, and local

effects. The responses of 6 of the 13 species are shown in Fig. 5 to illustrate the different types of individual species responses observed in this study. The final biomass of each species in each treatment is available in Table S1 (see Supplementary Material).

Table 2 *F*-table showing effects of treatments on biomass for individual plant species and the effect of AMF in the two water table environments

	<i>F</i> -values and significance			Effect of AMF	
	AMF	WT	AMF × WT	LWT	HWT
Graminoids					
<i>Carex hystericina</i>	0.75	49.23***	0.34	NE	NE
<i>Bromus ciliatus</i>	1.63	30.49***	1.12	NE	NE
<i>Muhlenbergia glomerata</i> †	9.08**	12.03**	2.53	–	NE
Forbs					
<i>Chelone glabra</i>	0.34	0.35	1.69	NE	NE
<i>Mentha arvensis</i>	0.67	0.41	3.61	NE	NE
<i>Lycopus americanus</i>	0.43	0	4.34*	NE	NE
<i>Lobelia siphilitica</i>	9.63**	0.2	3.1	NE	–
<i>Symphytotrichum puniceum</i>	0.51	1.15	13.47**	NE	–
<i>Parnassia glauca</i> †	1.13	3.94	6.59*	–	NE
<i>Doellingeria umbellata</i>	0.05	23.45***	30.95***	+	–
<i>Eupatorium</i> spp.	1.88	35.68***	23.15***	NE	–
<i>Packeria aurea</i> †	2.54	34.6***	4.23*	NE	NE
<i>Solidago patula</i> †	3.48	32.07***	27.33***	+	NE

LWT = low water table; HWT = high water table; NE = no significant effect on plant biomass; – = significant negative effect on plant biomass; + = significant positive effect on plant biomass. See Table S1 for final plant biomass of each species. *** $P < 0.001$; ** $P < 0.01$, * $P < 0.05$.

†Fen indicator species according to Godwin *et al.* (2002).

environmental conditions (Jones & Smith 2004). This conceptual model could also consider the temporal variation in AMF effects on plant communities given that the effects of AMF on individual plants can vary with time (Hart & Reader 2002). In our short-term assessment of the effects of AMF on fen communities we saw overall negative effects on diversity, but these effects may become neutral or positive as the communities develop over a longer time-scale (see Discussion below). Clearly, additional tests of the predictions of the Urcelay and Diaz model are necessary in other communities where non-mycorrhizal plant species dominate to help clarify the variable role of AMF in plant communities.

Nested within the community-level effects of AMF and water table were the differential responses of individual species to these effects, which are driving the community-level effects. Not surprisingly, the species most likely to drive the community-level effects was the non-mycorrhizal *C. hystericina*. In the low water table treatment, there was no change in the biomass of the non-mycorrhizal *C. hystericina* relative to the biomass of the mycorrhizal plant species, resulting in it having no influence on above-ground plant diversity. However, in the high water table treatment, as AMF caused negative growth responses of the mycorrhizal plants, *C. hystericina* became more dominant and led to a reduction in the evenness of the plant community. All three of the grasses responded to the main effect of water table, with *C. hystericina* and *B. ciliatus* showing no response to AMF. Interestingly, with the exception of *M. glomerata*, all plant species that are considered characteristic of North American fens according to Godwin *et al.* (2002), including *P. glauca*, *P. aurea* and *S. patula*, had significant AMF × WT treatment effects.

While total above-ground biomass was suppressed in the high water table environment when AMF were present, tissue nutrient concentrations increased, although this was only significant for %P (Fig. 4). This increase in tissue nutrient concentrations was probably a result of smaller plants having higher concentrations of nutrients per unit of dried biomass. Although soil saturation can affect the availability of both N and P for wetland plants (Mitsch & Gosselink 2000), there were no differences in %N or %P between low and high water table treatments without AMF present.

Another key finding from this study was that the community-level effects of AMF were mediated by the local soil conditions. Almost all studies examining the effects of AMF on plant communities have only considered the presence/absence of AMF or the composition of the AMF community, and have not considered the interactions between AMF and local abiotic conditions of the soil. The depth to water table in calcareous fens and many other wetlands is highly spatially variable due to the presence of microtopography (Mitsch & Gosselink 2000). Based on the results from these experimental communities, we suggest that variation in depth to water table due to microtopography can interact with AMF to create small-scale functional heterogeneity of AMF within wetland plant communities. As there is considerable small-scale variation in environmental variables in many other plant communities, including nutrient availability, light and soil moisture, this fine-scale spatial variability in AMF function could be widespread and not just novel to wetland plant communities. For example, Johnson *et al.* (2003) showed that atmospheric CO₂ and soil nitrogen mediated the community-level effects of AMF in North American mesic grasslands. Although they did not interpret the

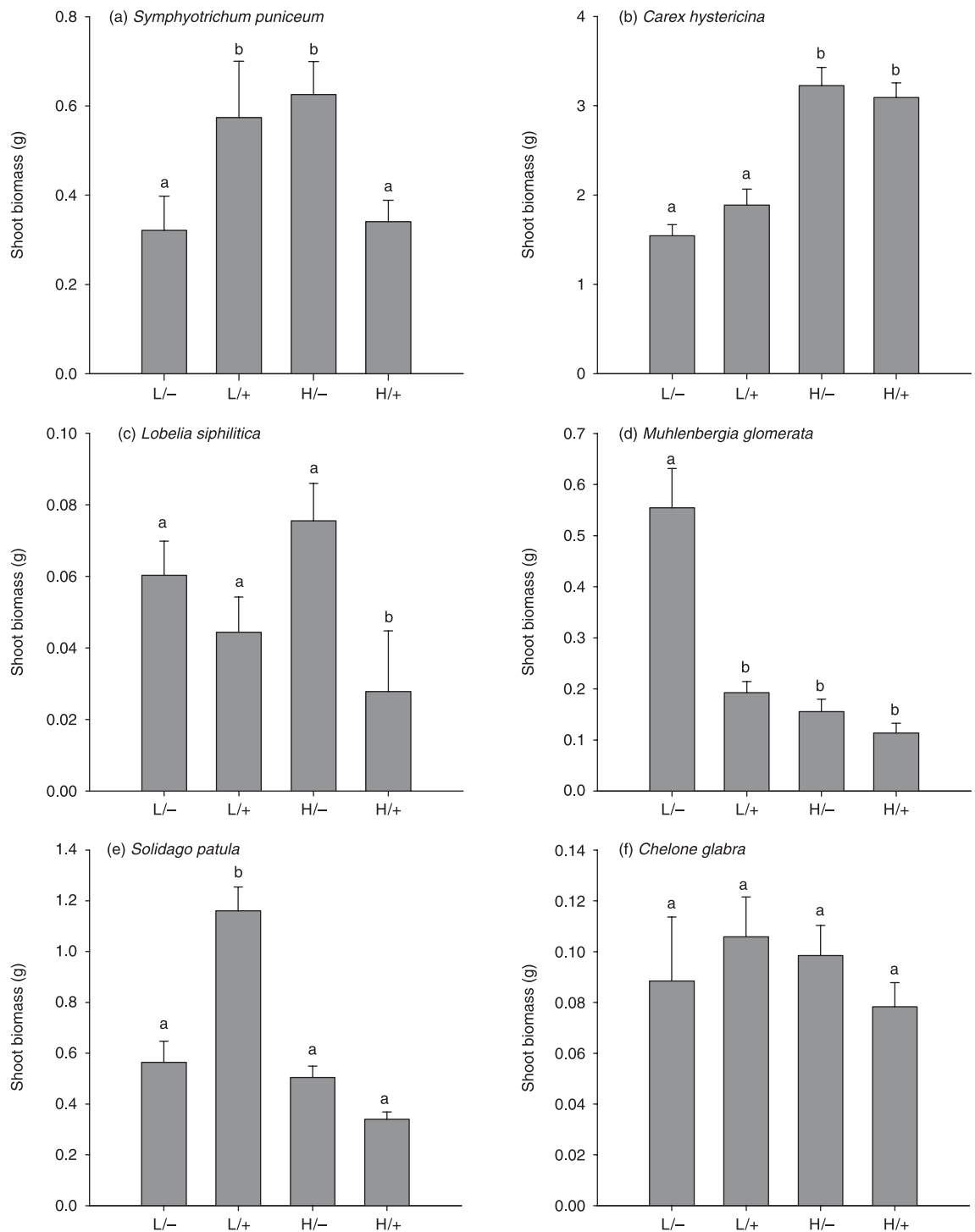


Fig. 5 Differential responses of six of the 13 plant species to water table (WT) and arbuscular mycorrhizal fungi (AMF). The bars represent the mean total biomass for each species within each mesocosm (± 1 SE). Only six species are shown because these species represent the six different types of responses observed (see Table S1 for biomass of each species). L = low water table. H = high water table. '-' = no AMF. '+' = AMF present. *F*-values and significance of main and interaction treatment effects are given in Table 2. Bars with different letters indicate significant differences ($P < 0.05$) based on Ryan-Einot-Gabriel-Welsch (REGW) multiple-range tests.

results of their mesocosm study in a spatial context, we suggest that just as a spatially heterogeneous water table might mediate the effects of AMF in calcareous fens, spatial variation in nitrogen availability within a field may mediate the effects of AMF on the composition of grassland communities.

The potential mechanisms by which water table mediated the effects of AMF in this experiment are not

clear because this experiment was designed to observe potential effects and not to test mechanisms. However, we can speculate as to what may have caused the observed changes in AMF effects in this experiment. Soil saturation can influence the abundance and composition of AMF communities in field and glasshouse experiments (Rickerl *et al.* 1994; Miller & Bever 1999; Miller & Sharitz 2000). The change in percentage colonization of the

one species assessed (*P. aurea*) is indicative of changes in the AMF communities in the mesocosm. However, it is unclear whether the small changes in colonization observed are functionally significant. The composition of the AMF communities may also have changed with alterations of water table depth. Future work using molecular techniques to relate changes in AMF community composition to AMF function may lead to a mechanistic understanding of our mesocosm results.

It is not possible to completely rule out the effect of soil pathogens in driving the effects seen in this experiment because with any study of the function of AMF in plant communities, non-mycorrhizal fungi are always present in experimental units. However, we are confident that the effects were caused by AMF and not soil pathogens. There was a low incidence of non-AMF colonization observed within roots and high levels of AMF colonization in the inoculum when it was added at the beginning of the experiment. Moreover, we did not observe typical plant growth symptoms associated with the presence of pathogenic fungi such as necrotic leaves or roots. Finally, a low level of root colonization by non-mycorrhizal fungi was observed across all treatments and not just the treatments where AMF inoculum was added.

The duration of AMF microcosm/mesocosm studies with experimental plant communities ranges from a few months (e.g. Landis *et al.* 2005) to several years (e.g. Johnson *et al.* 2004; van der Heijden 2004). This study ran for only 3 months because of various constraints. Ideally, to ensure that plant-AMF and plant-plant interactions are similar to what might occur in the field, this experiment would have run much longer. For example, the mean level of colonization of *P. aurea* in this experiment in the low water table treatment ($20.2 \pm 2.0\%$) was much lower than colonization levels observed in the field for this species (61%, Table 1). The negative effects of AMF observed in this experiment may have become neutral or positive over a longer time-scale as the outcome of plant-AMF and plant-plant interactions would probably have changed. Despite this limitation, we have shown that AMF can have community-level effects on the development of wetland plant communities. Future studies over longer time-scales will be needed to better understand the magnitude and temporal dynamics of these effects.

Previous experiments with calcareous fen plant species growing individually in pots have shown neutral and positive responses of plants to AMF (Weishampel 2005), while in this study in experimental communities there were some positive, but mostly neutral or negative, responses of plants to AMF. Studies in other plant communities have shown discrepancies in the responses of plant species to AMF at the individual and community levels (e.g. O'Connor *et al.* 2002). The discrepancy in our study could have been caused by differences in experimental conditions (soil, glasshouse environment, etc.). An alternative explanation may be that competition with neighbouring plant species decreases the benefits

of AMF observed when plants are grown in individual pots. Moreover, a larger number of plant species with more variable life histories and growth forms were included in this study, which may have allowed us to capture a larger amount of the variation in the outcomes of AMF-plant interactions.

In summary, AMF had significant effects on the composition of the experimental wetland plant communities in this study and these effects were mediated by depth to water table. As the depth to water table is highly heterogeneous in calcareous fens over small spatial scales, this environmental variability may lead to small-scale spatial variation in the community-level effects of AMF. Given the widespread efforts to create and restore wetlands without specific consideration of soil biota, further research should be conducted to assess the potential community-level significance of AMF in other types of wetlands. Additionally, further work in wetlands will add to our broader understanding of the role of AMF in plant communities, which has been traditionally focused on terrestrial herbaceous plant communities.

Acknowledgements

Rosie Dell, Eva Kuczynski, Jeff Powell, Stuart Campbell and Jessica Wells provided assistance or insight in the laboratory and/or field. Doug Larson, Hafiz Maherali, Matthias Rillig, Anne Pringle, Rasmus Kj  ller, the editors of this journal and two anonymous reviewers offered thoughtful comments on earlier drafts of this manuscript. Barbara Bedford and members of the Bedford Laboratory provided logistical support and helpful ideas for this work. This work was funded by the Natural Sciences and Engineering Research Council of Canada and the National Science Foundation of the United States.

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Received 31 January 2006

revision accepted 22 May 2006

Handling Editor: Marcel van der Heijden

Supplementary material

The following supplementary material is available online from www.blackwell-synergy.com

Table S1 Mean final biomass of each plant species in each treatment