Arbuscular mycorrhizal fungi alter phosphorus relations of broomsedge (*Andropogon virginicus* L.) plants

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Abstract

Broomsedge (*Andropogon virginicus* L.) is a dominant grass revegetating many abandoned coal-mined lands in West Virginia, USA. Residual soils on such sites are often characterized by low pH, low nutrients, and high aluminium. Experiments were conducted to assess the resistance of broomsedge to limited phosphorus (Pi) availability and to investigate the role that arbuscular mycorrhizal (AM) fungi play in aiding plant growth under low Pi conditions. Pregerminated mycorrhizal and non-mycorrhizal seedlings were grown in a sand-culture system with nutrient solutions containing Pi concentrations ranging from 10 to 100 mM for 8 weeks. Non-mycorrhizal plants exhibited severe inhibition of growth under Pi limitation (<60 mM). Colonization by AM fungi (combined *Glomus clarum* Nicolson & Schenck and *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe) greatly enhanced host plant growth at low Pi concentrations, but did not benefit growth when Pi was readily available (100 mM). In comparison to non-mycorrhizal plants, mycorrhizal plants had higher phosphorus use efficiency at low Pi concentrations and maintained nearly constant tissue nutrient concentrations across the gradient of Pi concentrations investigated. Manganese (Mn) and sodium (Na) accumulated in shoots of non-mycorrhizal plants under Pi limitation. Mycorrhizal plants exhibited lower instantaneous Pi uptake rates and significantly lower $C_{\text{min}}$ values compared to non-mycorrhizal plants. These patterns suggest that the symbiotic association between broomsedge roots and AM fungi effectively maintains nutrient homeostasis through changes in physiological properties, including nutrient uptake, allocation and use. The mycorrhizal association is thus a major adaptation that allows broomsedge to become established on infertile mined lands.

Key words: Nutrient use efficiency, nutrient homeostasis, phosphate uptake.

Introduction

The benefits accrued to plants by their mycorrhizal symbionts are often ascribed to enhanced phosphorus (Pi) nutrition (Smith and Read, 1997). Increased phosphorus acquisition by plants as a result of arbuscular mycorrhizal (AM) fungal colonization may result from (1) the exploration of a larger soil volume by extraradical hyphae; (2) a greater movement of Pi into mycorrhizal fungal hyphae due to high efficiency fungal Pi absorption systems, more effective competition for Pi with soil microorganisms, and formation of polyphosphate inside hyphae; and (3) the solubilization of poorly available phosphorus sources by modification of the rhizosphere (Hayman, 1983; Bolan, 1991; Smith and Read, 1997). However, AM fungal enhancement of plant growth is not always explained by the facilitation of Pi acquisition (Ross, 1971; Plenchette *et al*., 1983; Pacovsky, 1986). Mycorrhizal fungi also enhance the uptake of other nutrient elements, including copper (Cu) and zinc (Zn) (Ross, 1971; Pacovsky, 1986; Leake and Read, 1989). Thus, the maintenance of nutrient balance in the host plants by AM fungi may be more critical than the acquisition of any one nutrient element.

The regulation of nutrient uptake by roots is one process affecting the internal nutrient balance of the plant (Clarkson, 1985; Marschner, 1995). This balance in nutrient acquisition by the plant under edaphic stress, as modulated by mycorrhizal fungi, may be critical to plant growth and survival. For example, the amelioration of Al toxicity by mycorrhizal fungi in *Liriodendron tulipifera* L. was associated not only with the maintenance of Pi acquisition, but also with higher and more stable concentrations of Ca, Cu and Zn in
the roots or shoots (Lux and Cumming, 2001). In addition, AM fungi may suppress the uptake of other elements, such as Al, Fe and Mn, that may be present at toxic levels in some soils (Pacovsky, 1986; Kothari et al., 1990; Ning, 2000). Thus, mycorrhizal fungi play a role in whole-plant nutrient balance by aiding in the uptake of limiting nutrients, maintaining nutrient balance or limiting toxic element accumulation under edaphic stress.

Broomsedge (Andropogon virginicus L.) is a dominant bunch-grass colonizing extreme edaphic environments in the eastern United States (Chapman and Jones, 1975; Gibson and Risser, 1982; Campbell, 1982, 1983; Morton, 1986; Nellessen and Ungar, 1993). Indeed, the improvement of soil conditions, such as through the application of nitrogen fertilizers, can exclude this species from a plant community (Nellessen and Ungar, 1993). Preliminary studies demonstrated that broomsedge is a highly mycorrhizal species (Ning, 2000). Together, these patterns suggest that broomsedge is adapted to infertile habitats and may rely on AM fungi to maintain nutrient balance under both nutrient-limiting and stressful edaphic conditions.

The goals of this study were to assess the capacity of broomsedge to grow under Pi limitation and to investigate the role AM fungi play in aiding growth and nutrient acquisition of this plant species. It was hypothesized that broomsedge plants rely on AM fungi to overcome Pi limitation through changes in the Pi acquisition processes. Further, the enhancement of Pi acquisition would enable broomsedge plants to maintain a more balanced accumulation of other nutrients under variable Pi environments.

Source of AM fungal inoculum
Arbuscular mycorrhizal fungal inoculum was generated from broomsedge plants collected from an abandoned coal mine in Field Crest, near Morgantown, West Virginia, USA. Although the site has been abandoned for about 50 years, much of the surface soil remains devoid of vegetation. The vegetated surface consists of a broomsedge sward surrounding stunted red maple (Acer rubrum L.) and large-toothed aspen (Populus grandidentata Michaux) trees. Soil pH ranged from 3.0 to 3.3 and extractable Pi by Melich III was 3.76 ± 0.54 mg kg⁻¹.

To produce the AM fungal inoculum, broomsedge plants with intact mycorrhizal roots and adhering soil from the mine site were transplanted into 15 cm diameter pots containing a mixture of autoclaved mine-soil and sand (1:3 v/v). Following growth for 1 month, the pot contents became the source of infective inoculum for the experiment, and these pots are termed ‘nursery pots’ hereafter. The dominant fungus present in nursery pots was Glomus claroideicum Nicolson & Schenck, with Gigaspora gigantea (Nicol. & Gerd.) Ger. & Trappe also present.

Preparation of plants
Broomsedge seeds were sown around the transplants in the nursery pots. Broomsedge seeds also were sown in pots containing axenically germinated sudan-grass and the mixture of autoclaved mine-soil and sand (1:3 v/v), which included bacteria extracted from the mine-soil. Seedlings from these pots served as non-mycorrhizal controls. After 4 weeks growth, roots of a subset of seedlings (c. 10) were examined to determine mycorrhizal status (see below). All mycorrhizal seedlings were infected by AM fungi (71.5 ± 6.8%). Tissue P status was analysed after wet digestion (Parkinson and Allen, 1975) by the molybdate blue method (Olsen and Sommers, 1982). Tissue phosphorus concentrations (0.268 mg g⁻¹ in mycorrhizal shoots, 0.207 mg g⁻¹ in mycorrhizal roots, and 0.227 mg g⁻¹ in non-mycorrhizal shoots, and 0.011 g g⁻¹ in non-mycorrhizal roots) and tissue dry weights (0.018 g and 0.011 g for mycorrhizal shoots and roots, and 0.016 g and 0.011 g for non-mycorrhizal shoots and roots, respectively) did not differ significantly between mycorrhizal and non-mycorrhizal plants.

Nutrient solutions and growth conditions
Mycorrhizal and non-mycorrhizal broomsedge seedlings were transplanted and grown in 5 cm diameter × 18 cm height plastic pots (D16 Deepots, Stuewe and Sons, Inc., Corvallis, Oregon, USA) prefilled with 220 cm³ of a 3:1 (v/v) mixture of coarse: fine acid-washed sand. Plants were placed into a growth chamber with 14 h of light at 28 °C, 60% RH, and 10 h of darkness at 21 °C, 50% RH. Average light intensity at plant height in the chamber was 260 μmol m⁻² s⁻¹ from mixed fluorescent and incandescent sources. Plants received a baseline nutrient solution containing Ca (0.675 mM), K (0.79 mM), Mg (0.25 mM), NO₃ (1.5 mM), NH₄ (0.5 mM), SO₄ (0.25 mM), B (23.14 μM), Fe (25 μM as FeEDTA), Mn (4.57 μM), Zn (0.38 μM), Cu (0.16 μM), and Mo (0.06 μM). Phosphate as the treatment was added separately as NaH₂PO₄. All solutions were adjusted to pH 4.0 before application. Solutions (approximately 15 ml) were automatically delivered to each plant three times each day for 8 weeks.

Growth response experiment
Treatments consisted of five Pi concentrations (10, 20, 40, 60 or 100 μM), which were delivered in the nutrient solution outlined above. After 8 weeks, sand was removed from roots under running deionized water, and shoots were rinsed in deionized water. Roots were excised, and shoots and roots were dried at 60 °C and weighed. Percentage mycorrhizal colonization of roots was assessed on a 5% (by mass) root subsample. These root samples were cleared in boiling 10% KOH for 5 min, neutralized by 2% HCl for 2 min, and stained with 0.05% trypan blue for 5 min. Roots were stored in cold H₂O while they were assessed for mycorrhizal colonization by the gridline intersect method (Giovannetti and Mosse, 1980).

Roots and shoots from the same treatments within blocks (see below) were pooled, ground and digested (Parkinson and Allen, 1975). Phosphorus concentrations of digest solutions were determined spectrophotometrically (Olsen and Sommers, 1982). All other mineral elements (Fe, Mn, Mg, Ca, Zn, Cu, Na, and K) of the digests were analysed by ICP by the National Research Center for Coal and Energy Analytical Laboratory at West Virginia University.
Pi depletion experiment

Plants were grown for 8 weeks as noted above and treated with 20, 40, 60 or 100 μM Pi. Plants were removed from sand, roots were gently washed in running deionized water, and plants were transferred into 200 ml glass tubes containing the appropriate treatment solution for each plant. Solutions were aerated and changed every 12 h for 48 h. Following the last solution change, each tube was sampled at 5, 10, 15, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min. Phosphate concentrations of these samples were determined by the molybdate blue method (Olsen and Sommers, 1982) when the Pi concentration was higher than 5 μM and the malachite green method (Motomizu et al., 1983) when the Pi concentration was lower than 5 μM. Roots and shoots were dried and weighed.

Experiment design and data analysis

Within the growth chamber, treatments were arranged in a randomized complete block factorial (Pi-by-mycorrhizal fungal treatment) design, with three blocks accounting for environmental variation within the chamber. Each treatment had 3 or 4 replicates within each block.

Phosphorus use efficiency (PUE) (Chapin, 1980; Baon et al., 1993) was calculated as: (plant dry weight)/(plant P content).

For the Pi depletion experiment, the Pi instantaneous uptake rate and the minimum solution Pi concentration (C\text{min}) at which net Pi influx was zero for each treatment were determined by fitting a 2nd-order polynomial equation (Y = aX^2 + bX + c) to the experimental data (Claassen and Barber, 1974), where Y is Pi concentration in solution and X is time (min). Taking the derivative yields:

\[ Y' = 2aX + b \]

where \( Y' \) is the change in solution Pi concentration with time \( X \). According to the derivative, the instantaneous Pi uptake rate at time \( X = 0 \) is equal to the coefficient \( b \).

To evaluate \( C\text{min} \), the above derivative was solved to obtain the time at which \( C\text{min} \) was attained, the value of time was placed back into the 2nd-order polynomial equation and, after rearranging, the following equation was obtained:

\[ C\text{min} = c - b^2/(4a) \]

Normality of data was tested with the conservative Shapiro-Wilk W Test using the statistical package JMP (SAS Institute, Cary, North Carolina, USA). The distribution of biomass data appeared to be slightly leptokurtic. Standard transformations did not improve this distribution and analysis was undertaken on the original data. Elemental analysis and uptake data were normally distributed. Data were analysed in JMP using blocked two-way analysis of variance (biomass, nutrition and uptake parameters).

Results

At the termination of the 8 week experiment, mean percentage mycorrhizal colonization in broomsedge plants was approximately 70% across all Pi treatments (data not presented). Phosphate treatment had no significant effect on percentage colonization (\( P = 0.637 \)). None of the non-mycorrhizal plants were contaminated by the mycorrhizal fungi.

Mycorrhizal and non-mycorrhizal plants differed significantly in their patterns of growth in response to Pi availability (Fig. 1). At 100 μM Pi, all growth traits (shoot and root mass, cumulative height, and number of tillers) were similar between mycorrhizal and non-mycorrhizal plants. In comparison to non-mycorrhizal plants, however, mycorrhizal fungal colonization sustained broomsedge plant growth between 100 and 60 μM Pi and allowed plants to accumulate significant biomass at 40 μM Pi and below, where non-mycorrhizal plants exhibited extremely limited growth (Fig. 1). Below 60 μM Pi, non-mycorrhizal plants exhibited Pi-deficiency symptoms such as purple leaf margins not evident in mycorrhizal plants.

Differential effects of AM fungal colonization were observed for tissue P concentrations (Table 1). The
concentrations of P in shoots of mycorrhizal plants varied widely over the range of Pi treatments applied (Table 1). At low Pi availability, shoot P concentrations of mycorrhizal plants were actually less than those of non-mycorrhizal plants. For root P concentrations, mycorrhizal and non-mycorrhizal plants had similar response patterns to Pi treatment, although non-mycorrhizal plants had significantly higher root P concentrations than mycorrhizal plants (Table 1). However, mycorrhizal plants additionally retained more P in roots than non-mycorrhizal plants (Table 1), reflecting a change in P allocation resulting from mycorrhizal fungal colonization.

Phosphorus use efficiencies (PUE) of mycorrhizal and non-mycorrhizal broomsedge plants depended on Pi availability (P = 0.006 for the Pi-by-mycorrhizal treatment interaction) (Fig. 2). Mycorrhizal plants exhibited greater flexibility than non-mycorrhizal plants in PUE. Phosphorus use efficiency in mycorrhizal plants was higher than non-mycorrhizal plants at low Pi concentrations, whereas mycorrhizal and non-mycorrhizal plants had similar PUE at the high Pi treatment (Fig. 2).

The different patterns of growth, shoot P concentrations and PUE between mycorrhizal and non-mycorrhizal plants suggested that AM fungal colonization may have altered some physiological process other than Pi acquisition alone. Assessment of other nutrients indicated that mycorrhizal colonization altered the acquisition of several other nutrient elements (Table 2). Across all Pi treatments, shoots of mycorrhizal plants contained significantly higher concentrations of Mg and Cu, but lower concentrations of K, Mn and Na (Table 3). Roots of mycorrhizal plants contained significantly greater Ca and Cu concentrations, but lower K, Mg and Mn concentrations, in comparison to non-mycorrhizal plants. Foliar elemental concentrations were also influenced by Pi treatments (Table 2). In most cases, except for Ca and Mg, tissue elemental concentrations tended to increase with increasing Pi limitation (data not presented), although these effects were modified by mycorrhizal colonization (Table 2). These Pi-by-mycorrhizal treatment interactions were noted for shoot K, Fe, Mn, and Na concentrations and root Mn concentrations (Fig. 3). Tissue elemental concentrations varied less in mycorrhizal plants than in non-mycorrhizal plants in response to Pi treatments, reflecting greater stability in shoot and root nutrient relations of mycorrhizal plants (Fig. 3).

In addition to measuring plant tissue P concentrations and within-plant P allocation, the Pi depletion technique was used to measure Pi uptake rates by root systems of mycorrhizal and non-mycorrhizal broomsedge plants. Mycorrhizal plants had significantly lower Pi uptake rates than their non-mycorrhizal counterparts at the same Pi concentrations (Table 4). Phosphate uptake rates of

### Table 1. Shoot and root P concentrations and P translocation to shoots of broomsedge (A. virginicus) plants as influenced by Pi concentration and mycorrhizal (Myc) colonization

<table>
<thead>
<tr>
<th>Pi (µM)</th>
<th>Myc</th>
<th>Shoot P concentration (mg g⁻¹)</th>
<th>Root P concentration (mg g⁻¹)</th>
<th>P translocation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>+</td>
<td>0.87 (0.08)</td>
<td>0.44 (0.01)</td>
<td>73.8 (0.1)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>1.30 (0.20)</td>
<td>0.54 (0.10)</td>
<td>79.3 (2.3)</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>0.69 (0.02)</td>
<td>0.51 (0.10)</td>
<td>69.6 (4.0)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>1.11 (0.14)</td>
<td>0.66 (0.06)</td>
<td>75.6 (3.8)</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
<td>1.05 (0.24)</td>
<td>0.60 (0.10)</td>
<td>75.8 (2.0)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>1.84 (0.31)</td>
<td>0.92 (0.05)</td>
<td>79.3 (1.3)</td>
</tr>
<tr>
<td>60</td>
<td>+</td>
<td>1.30 (0.21)</td>
<td>0.70 (0.11)</td>
<td>75.0 (4.1)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>1.37 (0.20)</td>
<td>0.81 (0.11)</td>
<td>79.2 (1.3)</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>2.16 (0.28)</td>
<td>1.08 (0.07)</td>
<td>79.7 (2.4)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>1.76 (0.29)</td>
<td>0.89 (0.18)</td>
<td>76.2 (3.0)</td>
</tr>
</tbody>
</table>

P<sub>Pi</sub> 0.001 0.001 ns
P<sub>Myc</sub> 0.007 0.019 0.013
P<sub>Pi + Myc</sub> 0.028 ns ns

Fig. 2. Influence of solution Pi concentration on P use efficiency (PUE) of mycorrhizal (solid) and non-mycorrhizal (open) broomsedge (A. virginicus) plants.
mycorrhizal roots averaged 0.075 μmol g⁻¹ min⁻¹ and were independent of Pi treatment. In contrast, Pi uptake rates of non-mycorrhizal roots increased when Pi became limiting (20 μM Pi) (Table 4). In addition, the uptake data indicated a significant divergence in the minimum Pi concentration (C_{min}) to which mycorrhizal and non-mycorrhizal plants could draw down Pi in the nutrient solution (Table 4). Mycorrhizal root systems drew Pi down to lower concentrations than non-mycorrhizal plants in the 20, 40 and 60 μM Pi treatments.

**Discussion**

Arbuscular mycorrhizal fungi play important roles in enhancing host plant growth under adverse soil conditions (Daft and Nicolson, 1974; Daft and Haekskayo, 1976; Lindsey et al., 1977; Allen and Allen, 1980; Lambert and Cole, 1980; Danielson, 1985; Clark, 1997). The results of the present study are consistent with these previous reports. When Pi availability was low, mycorrhizal fungal colonization significantly increased the number of tillers and their heights, consequently leading to greater biomass accumulation in mycorrhizal plants (Fig. 1). When Pi was readily available, AM mycorrhizal fungi no longer exerted a beneficial effect on host plant growth. Thus, the AM fungi may switch from mutualistic symbiosis under Pi-limited conditions to a commensal or mildly parasitic association when Pi is readily available (Johnson et al., 1997; Smith and Read, 1997). One possible factor leading to the observed convergence in biomass of mycorrhizal and non-mycorrhizal plants at 100 μM Pi is the possible constraint resulting from limited root zone volume (Thomas and Strain, 1991). If mycorrhizal plants at high Pi no longer added biomass due to such limitation, non-mycorrhizal plant biomass would approach that of mycorrhizal plants.

Several physiological changes in plants occur in response to variable Pi conditions, with changes in Pi uptake and translocation being integral aspects of acclimation to Pi limitation (Clarkson and Scattergood, 1982; Mimura, 1995, 1999). In addition, mycorrhizal fungal colonization may additionally alter these responses (Cress et al., 1979). For example, it has been noted that colonization of Medicago truncatula Gaertn. by AM fungi altered the expression of phosphate starvation-inducible genes in this species (Burleigh and Harrison, 1999). Thus, the basic physiology of the host plants may be altered by Pi availability, mycorrhizal fungal colonization and interactions between these factors, as noted in the present study. In non-mycorrhizal broomsedge shoots and roots, biomass production was modulated to maintain tissue P concentrations within narrow bounds (Table 1), which may reflect the general regulation of P homeostasis typically reported for higher plants (Mimura, 1995, 1999). In contrast, mycorrhizal plants had lower shoot Pi concentrations when Pi was limiting and accumulated P to high levels when Pi was readily available, which appears to indicate that the P homeostasis of broomsedge plants was altered by AM fungal colonization.

Phosphorus translocation to the shoot of mycorrhizal plants was less than that of non-mycorrhizal plants under limited Pi availability, but increased as Pi levels increased.

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**Table 2.** P values for elemental concentrations of shoots and roots of broomsedge (A. virginicus) plants as influenced by Pi concentration and mycorrhizal (Myc) colonization

<table>
<thead>
<tr>
<th>Element</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pi Myc</td>
<td>Pi×Myc</td>
</tr>
<tr>
<td>K</td>
<td>0.014</td>
<td>0.005</td>
</tr>
<tr>
<td>Ca</td>
<td>&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Mg</td>
<td>0.008</td>
<td>0.007</td>
</tr>
<tr>
<td>Fe</td>
<td>&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Mn</td>
<td>0.006</td>
<td>0.003</td>
</tr>
<tr>
<td>Cu</td>
<td>ns</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zn</td>
<td>&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Na</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

---

**Table 3.** Influence of AM fungal colonization on foliar and root elemental concentrations of broomsedge (A. virginicus) plants

<table>
<thead>
<tr>
<th>Element</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myc Non-myc</td>
<td>Myc Non-myc</td>
</tr>
<tr>
<td>K (mg g⁻¹)</td>
<td>19.3</td>
<td>21.6**</td>
</tr>
<tr>
<td>Ca (mg g⁻¹)</td>
<td>4.64</td>
<td>4.45</td>
</tr>
<tr>
<td>Mg (mg g⁻¹)</td>
<td>2.17</td>
<td>1.92**</td>
</tr>
<tr>
<td>Fe (μg g⁻¹)</td>
<td>92.6</td>
<td>106.6</td>
</tr>
<tr>
<td>Mn (μg g⁻¹)</td>
<td>183.3</td>
<td>258.9**</td>
</tr>
<tr>
<td>Cu (μg g⁻¹)</td>
<td>14.2</td>
<td>9.07***</td>
</tr>
<tr>
<td>Zn (μg g⁻¹)</td>
<td>21.3</td>
<td>21.5</td>
</tr>
<tr>
<td>Na (μg g⁻¹)</td>
<td>168.0</td>
<td>306.6***</td>
</tr>
</tbody>
</table>
This tendency of mycorrhizal plants to limit P translocation from roots under low Pi availability reflects an increased investment of a limiting resource (P) into below-ground biomass production (roots/hyphae), which would serve to increase the acquisition of this limiting resource and promote growth (Chapin, 1980, 1991). When Pi was readily available, more P was transported to shoots, which leads to P luxury consumption (Chapin, 1980) or Pi storage that could be used in the future to support long-term growth (Aerts and Chapin, 2000). In support of this observation, the change observed in PUE as available Pi changed (Fig. 2) and the differences in this response between mycorrhizal and non-mycorrhizal plants suggest that AM fungal colonization alters within-plant P allocation to overcome Pi limitation under low Pi availability and to support sustainable growth under variable Pi environments (Chapin, 1980; Aerts and Chapin, 2000).

The rates of Pi uptake measured by the Pi depletion technique in the present study reflect the difference in the overall P status and P demand of the mycorrhizal versus non-mycorrhizal plants. The Pi uptake rates were less in mycorrhizal than non-mycorrhizal plants for any given Pi availability.
Table 4. Instantaneous Pi uptake rates and minimum solution Pi concentrations (C_{\text{min}}) for Pi influx in broomsedge (A. virginicus) plants as influenced by Pi concentration and mycorrhizal (Myc) colonization

Mean values (SE) of \( n = 10 \) plants with probabilities (P) of treatment effects for the ANOVAs; ns = P > 0.05.

<table>
<thead>
<tr>
<th>Pi (( \mu \text{M} ))</th>
<th>Myc</th>
<th>Pi uptake rate (( \mu \text{mol g}^{-1} \text{ h}^{-1} ))</th>
<th>( C_{\text{min}} ) (( \mu \text{mol l}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>+</td>
<td>4.89 (0.66)</td>
<td>1.17 (1.27)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>9.49 (1.28)</td>
<td>5.02 (1.68)</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
<td>4.42 (0.55)</td>
<td>1.78 (0.59)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>7.09 (2.33)</td>
<td>6.98 (1.99)</td>
</tr>
<tr>
<td>60</td>
<td>+</td>
<td>4.14 (0.66)</td>
<td>11.92 (2.79)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>7.28 (1.53)</td>
<td>22.53 (4.05)</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>4.58 (0.78)</td>
<td>50.08 (1.48)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>7.16 (0.82)</td>
<td>48.64 (2.38)</td>
</tr>
<tr>
<td>( P_{\text{Pi}} )</td>
<td>ns</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>( P_{\text{Myc}} )</td>
<td>ns</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>( P_{\text{Pi} \times \text{Myc}} )</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

Treatment. Since Pi uptake rates increase in plants under Pi limitation (Clarkson and Scattergood, 1982; Clarkson and Lüttge, 1991), it would appear that mycorrhizal broomsedge plants were under less Pi stress than their non-mycorrhizal counterparts. In the sand-culture system used in the present study, mycorrhizal plants dominated the root zone to a greater extent than non-mycorrhizal plants. Greater root biomass (Fig. 1) and associated mycorrhizal hyphae would more effectively exploit the pool of available Pi in the root zone. In addition, mycorrhizal plants could draw down solution Pi concentrations to lower levels (Table 4), further increasing the total Pi pool exploited and increasing the diffusive gradients to mycorrhizal plant roots. Thus, mycorrhizal plants, with higher PUE (Fig. 2), greater below-ground exploitation (Fig. 1), and lower \( C_{\text{min}} \) (Table 4), more effectively acquired Pi and were under less Pi stress at the lower Pi treatments. As a consequence, mycorrhizal broomsedge plants, overall, did not exhibit the elevated root Pi uptake rates noted for non-mycorrhizal plants (Table 4). These findings are contrary to those of Cress et al., where mycorrhizal tomato plants were shown to have greater Pi uptake rates at low Pi concentrations (Cress et al., 1979). However, the pattern of reduced Pi uptake in mycorrhizal broomsedge roots is supported by other authors (Liu et al., 1998; Burleigh and Harrison, 1999) who noted the down-regulation of phosphate starvation-inducible genes, including those encoding high-affinity Pi transporters, in \( M. \text{ truncatula } \) plants colonized by mycorrhizal fungi. Taken together with the patterns of growth and P utilization within broomsedge plants, this study’s findings suggest that mycorrhizal plants adapt to Pi limitation primarily by allocating more effort to root zone exploitation, which is consistent with model predictions for effective nutrient resource acquisition (Clarkson, 1985). This allocation would be, in the long term, an effective strategy for overcoming Pi limitation (Chapin, 1980; Lambers et al., 1998).

Other nutrient responses suggest that AM fungi effectively alter the acquisition of elements by the host plant (Table 2; Fig. 3). Notable changes included significant reductions in shoot and root Mn and increases in shoot and root Cu concentrations. The critical toxicity concentration for Mn varies widely between plant species, but may be as low as 200 \( \mu \text{g} \text{ g}^{-1} \) (Marschner, 1995). While the concentrations noted here for broomsedge (c. 150 \( \mu \text{g} \text{ g}^{-1} \)) grown at 100 \( \mu \text{M} \) Pi are well below this level, mycorrhizal and non-mycorrhizal plants exhibited divergent responses to Pi limitation (Fig. 3). The accumulation of Mn by non-mycorrhizal plants to near 300 \( \mu \text{g} \text{ g}^{-1} \) may reflect an interaction between Pi nutrition and Mn homeostasis, leading to the uncontrolled accumulation and toxicity of Mn (James et al., 1995).

Copper concentrations of both shoots and roots of mycorrhizal plants were significantly elevated by AM fungi, although the Cu concentrations of plants in all treatments are well above critical deficiency concentrations typically reported (1–5 \( \mu \text{ g} \text{ g}^{-1} \)) (Marschner, 1995). However, enhanced Cu uptake by the mycorrhizal symbiosis in broomsedge may contribute to the observed increases in the plant growth and may be involved in altered PUE exhibited in mycorrhizal plants. For example, if mycorrhizal plants were more effective at energy capture and carbohydrate transformations as a result of enhanced Cu nutrition (Marschner, 1995; Cook et al., 1997), then these plants would be able to capture more carbon given a restricted supply of Pi. Furthermore, Cu deficiency often decreases the activities of anti-oxidative enzymes (Yu et al., 1998), which may play a role in overcoming Mn toxicity (Wissenmeier and Horst, 1992).

Sodium concentrations of shoots in non-mycorrhizal plants were elevated when Pi became limited, and decreased as P status was improved with increased Pi availability (Table 2; Fig. 3). This may reflect a loss of membrane selectivity under Pi limitation and the subsequent loss of Na exclusion. Furthermore, mycorrhizal broomsedge plants maintained relatively constant foliar K, Fe, Mn, and Na concentrations across Pi treatments, which would be important to support the normal metabolism and optimal growth of broomsedge plants in varying Pi environments (Mooney and Winner, 1991). These patterns all indicate that AM fungi are important in balancing the nutritional status of plants under Pi stress.

The association of AM fungi with the roots of broomsedge plants influenced the nutrition of this species in several ways, all of which increased plant growth. Enhanced capture of Pi through greater biomass allocation to root systems and effective Pi drawdown led to better plant growth under Pi-limiting conditions. Higher
PUE of mycorrhizal plants under low Pi conditions increased biomass production when Pi limited growth of non-mycorrhizal plants. The strategies of resource allocation, lower $C_{\text{min}}$ and higher PUE in mycorrhizal plants under low Pi availability undoubtedly facilitated nutrient homeostasis in mycorrhizal broomsedge plants. The loss of uptake selectivity in non-mycorrhizal plants under Pi limitation, as evidenced by Mn and Na accumulation, may additionally contribute to reductions in plant growth. Enhanced acquisition of Cu may play a role in the observed growth dilutions by increasing PUE when Pi is not optimal. These patterns reflect a well-regulated nutrient homeostasis in mycorrhizal broomsedge under varying Pi availability that is not evident in non-mycorrhizal plants. The highly variable tissue P concentrations of mycorrhizal plants suggest that mycorrhizal fungal colonization alters fundamental regulation of Pi relations in this plant species, providing a greater ability to function under variable Pi environments.

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