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Mycorrhiza

Plant life span and response to inoculation with vesicular-arbuscular mycorrhizal fungi

III. Responsiveness and residual soil P levels

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Summary. This study sought to investigate the relationships among residual soil phosphorus (P) pools, plant life span, successional persistence and responsiveness to vesicular-arbuscular mycorrhizal (VAM) infection. Plants of five species which varied in life span, persistence, and VAM responsiveness were grown in nutrient-poor soils in a glasshouse for 8 weeks and given weekly feedings with either high- or low-P solutions. There was little effect of plant life span or VAM status on changes in residual available (1 M KCl extractable) P. In contrast, there were strong correlations between VAM responsiveness and changes in the exchangeable P pool (1 M $NH_4C_2H_3O_2$ extractable). Plants with greater VAM responsiveness and greater persistence through succession were able to reduce this potentially available P pool by as much as 50% in 8 weeks. In contrast, plants with poor or negative responsiveness to mycorrhizal infection and little successional persistence exhibited little control over potentially available P pools. These data confirm other studies which demonstrate that VA mycorrhizae access insoluble forms of P, thereby controlling potential supply rate over the longer term. Such control over soil P pools may contribute to successional persistence via either inhibition or tolerance mechanisms, and should be considered in any comprehensive theory of the mechanisms underlying succession.

Key words: Vesicular-arbuscular mycorrhizae – Succession – Soil P pools – Persistence

Introduction

Although patterns of change in species composition through succession have been documented frequently over the last century (e.g. Vankat and Snyder 1991), experimental studies which clearly demonstrate the mechanisms responsible for such changes are uncommon. As a framework for designing such experiments, Connell and Slatyer (1977) erected three mechanistic models by which successional replacement might occur, two of which (facilitation and inhibition) have been established as functional under both field and laboratory conditions; the third (tolerance) still awaits widespread verification.

Explicit in both the inhibition and tolerance models is the ability of the current occupant of a volume of space to reduce or alter resource levels to the degree that subsequent colonists cannot grow sufficiently well to displace that current occupant. Both interception of light and allelopathy have been suggested as examples of this inhibition (Connell and Slatyer 1977). Less attention has been given to the ability of plants (and their symbionts) to reduce soil nutrient or water availability to levels insufficient for the growth of seedlings which subsequently attempt to invade that volume of space.

Infection of roots of a strongly mycotrophic plant by vesicular-arbuscular mycorrhizal (VAM) fungi can result in the ability of that plant to maintain physiological function at water potentials lower than plants without such infections (Hardie and Leyton 1981; Allen and Boosalis 1983), to take up soluble phosphorus (P) from a greater soil volume (Hayman 1983), and to make use of fixed soil P fractions not available to plants without mycorrhizae (Bolan et al. 1984). For mycorrhizal infection to be important in influencing successional replacement or persistence, however, it must first be demonstrated that: (1) mycorrhizal plants can reduce soil P or moisture levels significantly more than plants without mycorrhizal infections, and (2) reductions in resource levels are greater for species which persist through successional time than for species which are quickly displaced. As mean plant life span (Janos 1980; Vankat and Snyder 1991) and mycorrhizal responsiveness (Boerner 1992a, b) both increase with successional time in mesic ecosystems, this implies that later successional, longer-lived perennials should, with the aid of a mycorrhizal infection, reduce resource levels more than early successional species, without exhibiting a concomitant reduction in their own growth rate.

To determine if these assertions are sound, a set of experiments was designed to answer the following questions: (1) Do plants with a mycorrhizal infection reduce resource levels (i.e. available and unavailable/exchangeable P) below those typical around uninfected plants? (2) Can a perennial species reduce resource levels more than a congeneric annual without a significant change in growth rate? (3) Are species which are more dependent and responsive to mycorrhizal infection and more persistent through succession able to reduce resource levels to a greater extent than species with lesser responsiveness and successional persistence?

Materials and methods

Three of the five species chosen for this study were grasses: *Panicum virgatum* L (switchgrass), *Bromus inermus* Leyss (smooth brome), and *B. secalinus* L. (cheat). The first two are perennials and persist in both grasslands and at forest edges; the latter is a weedy annual of grain fields and waste places. The remaining two species were members of the Caryophyllaceae: *Silene nivea* (Nutt) Otth (snowy campion) and *Silene noctiflora* L (night-flowering catchfly), both of which are common in abandoned agricultural fields. The responsiveness of these fiva taxa to infection was determined at low and high P levels for five to seven growth and survivorship parameters (Boerner 1992a, b). The overall responsiveness ranking, from greatest to least, among these five taxa is: *P. virgatum*, *B. inermus*, *B. secalinus*, *S. nivea*, and *S. noctiflora*. Seed sources and distribution patterns for these species are given by Boerner (1992a, b).

Glomus intraradices Schenck and Smith is among the most common *Glomus* species in the southeastern United States and will form VAM with a wide range of hosts (Schenck and Smith 1982). The culture was obtained from J. H. Gerdemann at the University of Illinois and had been isolated from agricultural field crops. The *G. intraradices* culture had originally been identified as *G. fasciculatum*, but was subsequently re-identified (S. B. Rabatin, personal communication).

Seeds were planted in flats of acid-washed sand and transplanted to 10-cm-diameter pots of 4:1 sand:perlite (v:v) when the first true leaves appeared. Each plant to be VAM inoculated was given 30 ml of an inoculum slurry which contained at least 300 *Glomus* spores. Plants which were to remain free of VAM infection were given an equal amount of a slurry which had been passed throughy a 15-μm filter to remove VAM spores (Jensen 1982).

The plants were grown for 8 weeks during summer in a glasshouse at ambient temperature and light intensity. They were fed weekly with a nutrient solution (Ruakura Solution) designed for sand culture (Smith et al. 1983) modified so as to supply P at rates similar to that found in relatively fertile and infertile forest sites in Ohio (Boerner 1990); high P: 5.0 mg $P \cdot l^{-1}$ in solution and 11.0 mg P total over the course of the experiment versus low P: 2.0 $mg \cdot l^{-1}$ in solution and 4.4 mg P total addition. In addition, for each experiment, six pots of soil (control soils) which received neither plants nor fungi were watered and fed in the same manner. Each species/VAM inoculum/P level combination was replicated 16 (*Panicum* and *Bromus*) – 20 (*Silene*) times in a completely randomized design.

To determine the nature and magnitude of the soil P pools remaining after harvest, we extracted the experimental and control soils with 1 M KCl for available P and 1 M $NH_4C_2H_3O_2$ for exchangeable (potentially available) P. The $NH_4C_2H_3O_2$ extractant is designed to remove exchangeable cations (e.g. Ca, Fe, Al) from mineral complexes; in doing so, it also brings into solution phosphates which were bound by such cations. Soil extracts were assayed for P using the stannous chloride method (American Public Health Association 1976).

All response variables were tested for normality, then analyzed by analysis of variance and the Ryan-Einot-Gabriel-Welch Modified F test (Statistical Analysis System 1985). The total mass of each plant in each pot was used as a covariate in the analyses. All significant differences noted are at $P<0.05$, except where otherwise noted.

Results

Change in P pool sizes

At the beginning of the experiment, the available P pools in these nutrient-poor experimental soils, as measured by weak salt extraction, were on average 14.3±2.0 mg $P \cdot kg^{-1}$ soil, only 1.3% of the available plus exchangeable P total (1093.7±371.5 mg $P \cdot kg^{-1}$ soil). In the absence of plants, the available P pool in the soils increased by a factor of 18.8 in the high P treatment and threefold in the low P treatment. Exchangeable P pool sizes increased less: times 2.3 at high P and times 1.7 at low P. At the end of the experiment, the available P pool in the soil controls had increased to an average of 14.5% and 3.3% of the total available plus exchangeable P pools at high P and low P, respectively.

P. virgatum

Available P pools remaining in *P. virgatum* pots after 8 weeks were four- to fivefold greater than the initial conditions, but only approximately 22% of the soil controls (Fig. 1). VAM infection did not significantly affect the residual available P pool in *P. virgatum* pots. However, available P pools in soils from pots with plants, whether VAM-inoculated or not, were significantly lower than those in soil control pots.

In contrast, exchangeable (potentially available) soil P pools were significantly greater in soils from non-VAM plants than from VAM plants. The exchangeable P pools in soils from VAM plants were not significantly different from the initial soil conditions, whereas exchangeable P pools in soils from non-VAM plants were more similar to those from the soil controls.

B. inermus and B. secalinus

In both *Bromus* species, the residual available P pools in soils from pots given the high P supply rate were intermediate between those from the initial soils and the soil controls (Fig. 1); there were no significant differences between species or VAM treatments. At low P, soils from pots with VAM-inoculated *B. inermus* plants had the lowest remaining available P soil pools, whereas those remaining in soils from non-VAM *B. secalinus* plants were as large as those of the soil controls.

At high P, the exchangeable P pools of VAM-inoculated plants of both *Bromus* species were not significantly different from those in the initial soils. In contrast, at high P, non-VAM of both *Bromus* species had exchangeable P pools not significantly different from those in the soil controls. The same was true for *B. iner-*

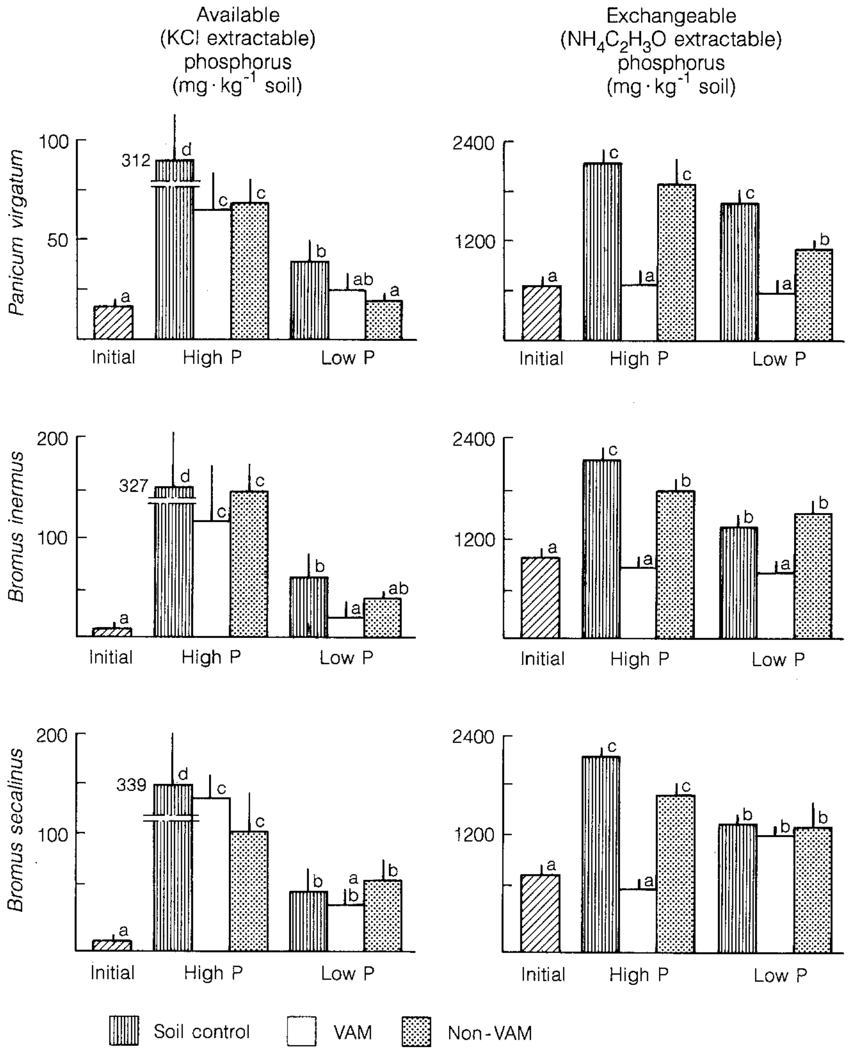

Fig. 1. Soil P pool responses to growth of *Panicum virgatum*, *Bromus inermus*, and *B. secalinus* in relation to inoculation with *Glomus intraradices* and P supply rate. Initial soil conditions, and residual pools in soils from soil controls (no plant), VAM-inoculated plants, and non-VAM plants are given. *Histogram bars* indicate means of six (initials, soil controls) or 16 (VAM-inoculated and non-VAM plants) replicates; standard errors of the means are indicated by the *vertical line*. Within each species/pool type combination, the means labelled with the same lower case letter were not significantly different at $P<0.05$

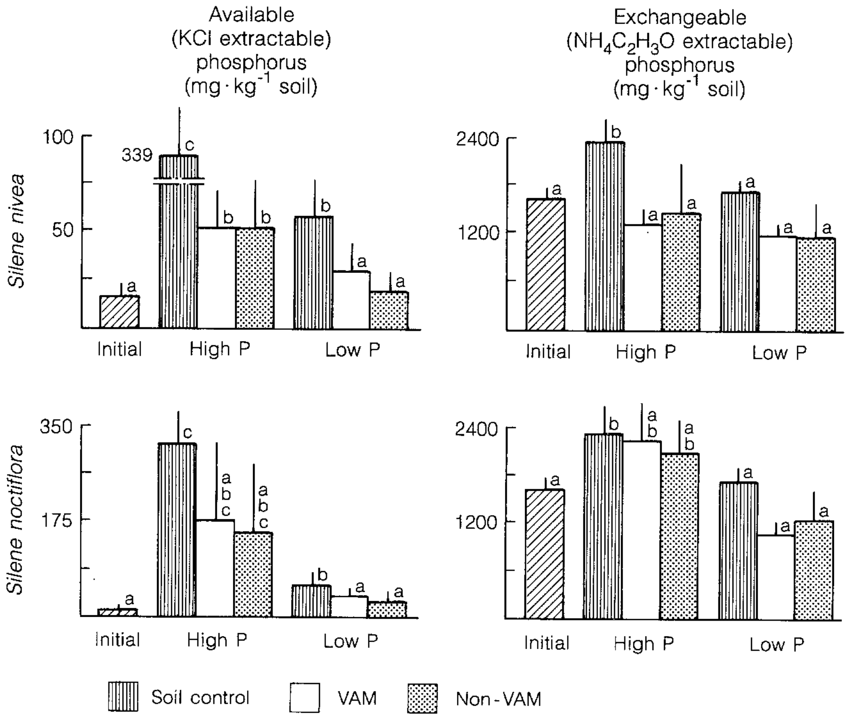

Fig. 2. Soil P pool responses to growth of *Silene nivea*, and *S. noctiflora* in relation to inoculation with *Glomus intraradices* and P supply rate. Initial soil conditions, and residual pools in soils from soil controls (no plant), VAM-inoculated plants, and non-VAM plants are given. *Histogram bars* indicate means of six (initials, soil controls) or 20 (VAM-inoculated and non-VAM plants) replicates; standard errors of the means are indicated by the *vertical line*. Within each species/pool type combination, the means labelled with the same lower case letter were not significantly different at $P<0.05$

mus grown at low P. Soils from low-P *B. secalinus* plants had residual exchangeable soil P pools similar to those of the soil controls, regardless of VAM status.

S. nivea and S. noctiflora

None of the mean residual available P pool sizes from *S. nivea* or *S. noctiflora* pots differed significantly from the initial conditions (Fig. 2). However, at high P the means and variances of *S. noctiflora* pots were three- and fivefold larger, respectively, than those associated with the initials and the *S. nivea* pots. The same trend occurred for the exchangeable P pools from high-P pots, with the means and variances of *S. noctiflora* pots exceeding the others by twofold.

Correlations between soil pools and plant responsiveness

To determine if the residual P pools remaining after plant growth were correlated with the responsiveness of the various plant species to mycorrhizal infection, I assembled a correlation matrix of plant responsiveness (data from Boerner 1992a, b) versus changes in soil P pools (Table 1). There were strong and significant positive correlations between overall plant mycorrhizal responsiveness (biomass plus nutrient uptake responses) and the degree of reduction of the exchangeable soil pool, both overall and for plants grown at high P. There was also a significant positive correlation between the effect of infection on total P uptake (nutrient uptake responsiveness) and the ability of the plant to reduce or minimize overall P pool sizes at high P. In contrast, there were no significant correlations between biomass, nutrient uptake or overall plant mycorrhizal responsiveness and residual available P pool size.

Table 1. Pearson product-moment correlations between plant responsiveness and changes in soil P pools. Correlation coefficients followed by *, **, and *** were significant at the $P<0.10$, $P<0.05$, and $P<0.01$ levels, respectively. For single growth or nutrient parameters, response was calculated as the ratio of VAM:non-VAM performance. For growth/nutrient uptake parameters, ratios >1 indicated positive VAM effects; for soil responses, ratios <1 indicated positive VAM effects. Plant P uptake and biomass responsiveness were each calculated as the sum of positive and negative responses to two to five individual response parameters using data from Boerner (1992a, b); overall plant responsiveness rating was the sum of P uptake and biomass responsiveness. The overall soil responsiveness rating was calculated as the sum of the exchangeable P and available P responses. $n=10$ for each correlation coefficient

	Overall plant responsiveness	Plant P uptake response	Plant biomass response
Pooled treatments			
Available p pool	0.189	−0.130	0.210
Exchangeable P pool	0.744***	0.504	0.481
Soil responsiveness rating	0.768***	0.523	0.559*
High P treatment			
Available P pool	0.561	0.734	0.761
Exchangeable P pool	0.848**	0.676	0.591
Soil responsiveness rating	0.936**	0.900**	0.693
Low P treatment			
Available P pool	0.162	−0.379	0.037
Exchangeable P pool	0.670	0.675	0.921**
Soil responsiveness rating	0.623	0.262	0.643

Discussion

The five species utilized in this study represent a range of life span and responsiveness to mycorrhizal infection. *P. virgatum* and *B. inermus* are both perennial grasses with high mycorrhizal responsiveness and dependency. *B. secalinus* is an annual grass with intermediate responsiveness. *S. nivea* is a herbaceous perennial which shows little response to VAM inoculation, and *S. noctiflora* is an annual which exhibits a negative response to inoculation (Boerner 1992a, b). The first two species are common in mature grasslands in North America and may persist for decades in successions toward forest cover (Sims 1988); in contrast, the latter three generally disappear relatively early in old-field succession.

There were no significant effects of VAM inoculation on the available P pool size for any of the species tested, despite differences in biomass response of the species ranging from >2.85 for *P. virgatum* (Boerner 1992a) to <0.85 for the two *Silene* species (Boerner 1992b). These data confirm the observation of Bolan et al. (1984) that responses of plants to VAM inoculation are not correlated with or explained by changes in the available P pool. This small soil-nutrient pool turns over too rapidly for any instantaneous measurement of its magnitude to be useful for predicting the response of species to mycorrhizae.

In contrast, there were significant differences in the portion of the soil P referred to as the exchangeable P pool (defined as that extractable with $NH_4C_2H_3O_2$ less the available P pool). This larger, less available P pool was of the order of 50-fold larger than the available P pool at the beginning of the experiment, and 7- to 30-fold larger at the end, depending on the amount of soluble P supplied during the experiment. There were significant positive correlations between the growth and nutrient uptake responsiveness of plants to VAM infection and the difference in the degree of reduction of the exchangeable P pool, both overall and in the high P treatment. For the two species with the greatest responsiveness and successional persistence (*P. virgatum* and *B. inermus*), the exchangeable P pools in pots with VAM-inoculated plants were an average only 48% of those in paired non-VAM pots, and only 42% of those in the soil controls. At the opposite end of these gradients of responsiveness and persistence were *S. nivea* and *S. noctiflora*, neither of which showed any significant response to VAM inoculation in either growth

(Boerner 1992b) or changes in exchangeable P pool sizes.

Bolan et al. (1984) have shown that VAM are capable of accessing metal-bound forms of P typically unavailable to non-mycorrhizal plants. It was these forms of P for which we designed our exchangeable P extraction. Duce (1987) demonstrated that the P pools which changed most during the growth of the mycorrhizal perennial grass *Agropyron smithii* were the unavailable mineral P and organic P. Taken together, these results and ours demonstrate that plants with strong mycorrhizal responsiveness are capable of accessing potentially available P pools in the soil and of reducing them by a large amount. The amount of P available in the soil solution at any one time is insufficient to support significant long-term plant growth. Assuming a mean tissue P concentration of 0.2%, the initial available P pool in our pots was sufficient to support plant growth of only 10–30% of that attained by the more responsive, VAM-inoculated plants. To support the observed level of growth, these plants had to be dependent on a continuous supply of available P being generated from the mineral- and organic-bound P pools, both by normal physiochemical soil processes and by VAM-fungus mobilization of mineral-bound P.

The inhibition mechanism of Connell and Slatyer (1977) requires that the original colonist of a volume of space actively inhibit the growth of seedlings of subsequent colonists, either by adding allelopathic compounds to that volume or by reducing resource levels below that necessary for growth of the subsequent early- or late-successional colonist. Through the more common examples of such reductions in the resource mix have involved light interception, the ability of a mycorrhizal plant to reduce the bound P fractions in a volume of soil also has the potential to disable subsequent colonists. This may be particularly important when the subsequent colonists are non-mycotrophic species (see also Allen and Allen 1984) or weakly responsive mycotrophs, as an induced P deficiency may prevent these colonists from growing to a size where competition for water or light becomes important.

According to the tolerance model of Connell and Slatyer (1977), the initial colonists change the environment's resource mix in such a way as to make it unsuitable for successful colonization of early successional species but suitable for late successional species. In the case of mycorrhiza-induced changes in soil P pools, this model may operate as a transitional situation, in which the soil P pools have been drawn down too much to allow successful establishment of weakly responsive or non-mycotrophic species but which still allows colonization by strongly responsive mycotrophs or by plants that can share the established VAM network already in place.

It is clear that the benefits of a mycorrhizal infection increase as the availabilities of mycorrhizally transportable resources decrease, be they water, P, or in the case of ectomycorrhizae, nitrogen. This pattern can be seen in spatial patterns of increasing mycorrhizal infection intensity along a gradient of decreasing soil P availability (e.g. Boerner 1986). Finer-scale studies which demonstrate increased benefits of mycorrhizal infection with increasing competitive pressure (e.g. Allen and Allen 1984; Crowell and Boerner 1988) also verify this relationship. The impact of mycorrhizae on competition between established plants and subsequent colonists in a successional setting is likely influenced by fine-scale mechanisms, such as the size hierarchy of the competing plants (Boerner and Harris 1991), the spatial pattern and overall density of propagules (Janos 1980) and the degree of mycorrhizal responsiveness of the colonists. Though a knowledge of plant-mycorrhizal fungus interactions alone is unlikely to produce a comprehensive, mechanistic view of succession, these studies demonstrate that such a comprehensive theory is equally unlikely without such an understanding.

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