Optimizing microbial associations to enhance N and P soil nutrient availability

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Optimizing microbial associations to enhance N and P soil nutrient availability

Abstract
Sustainable farming systems rely in part on recycling nutrients through materials such as crop residues, manure, or biological inputs. Three nutrients commonly added as fertilizers in agricultural production systems are nitrogen (N), phosphorus (P), and potassium (K). Past research has shown that fungal and bacterial microbial systems may be an important way to add nutrients to the soil or to enhance availability of those nutrients already present. But the interaction of beneficial fungi and bacteria in a synergistic relationship with higher plants is still poorly understood. Moreover, very little is known about the ecology of these beneficial soil fungi.

Keywords
Agronomy, Plant Pathology and Microbiology, Forestry, Soils and agronomy

Disciplines
Agricultural Science | Agriculture | Agronomy and Crop Sciences | Plant Pathology | Soil Science

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Background and goals
Sustainable farming systems rely in part on recycling nutrients through materials such as crop residues, manure, or biological inputs. Three nutrients commonly added as fertilizers in agricultural production systems are nitrogen (N), phosphorus (P), and potassium (K). Past research has shown that fungal and bacterial microbial systems may be an important way to add nutrients to the soil or to enhance availability of those nutrients already present. But the interaction of beneficial fungi and bacteria in a synergistic relationship with higher plants is still poorly understood. Moreover, very little is known about the ecology of these beneficial soil fungi.

A group of beneficial fungi known as vesicular arbuscular mycorrhizae (VAM) symbiotically exchange photosynthate and nutrients with plant roots. Important genera include *Glomus*, *Sclerocystis*, *Gigaspora*, and *Acaulospora*. The vesicles, or storage sacs, and abscules, or branched structures formed by hyphae (threads), form distinct structures inside host root cells. These fungi also grow in the soil surrounding the roots, effectively increasing the absorbing surface for nutrient uptake. This is especially important for P because it is otherwise less available to plants. In addition, the bacterium *Bradyrhizobium*, another rhizosphere organism, infects legumes, causing the nodulation that fixes dinitrogen gas for crop use. The interactions of VAM and bradyrhizobia may result in a synergistic relationship that enhances the supply of both N and P and thus reduces fertilizer inputs for legume production.

The goal of this project was to determine whether dual inoculation and/or colonization with mycorrhizal fungi and *Bradyrhizobium* may provide such a synergistic relationship. Specific objectives were to evaluate (1) the effect of mycorrhizal fungal colonization and/or nodulation of soybean by bradyrhizobia in the greenhouse, (2) the extent of colonization of soybeans in Iowa fields along with the VAM spore levels found, and (3) the dependency of soybean and corn wild-type, or improved, and improved cultivars on mycorrhizal colonization.

Approach and methods
Investigators conducted two greenhouse studies as well as a field survey. In the greenhouse experiments, they used a low-P, uncultivated soil collected from a railroad site. They mixed the soil with silica sand and steam-sterilized it prior to the studies. This mixture was then placed in plastic greenhouse pots and leached twice daily for one week to remove undesirable salts and toxic products.

In the first greenhouse study, which evaluated mycorrhizae-bradyrhizobium-soybean symbiosis, soybean seeds were sterilized, germinated in a crisper, and then incubated to yield seedlings for planting. Three pregerminated seedlings were transferred to each pot and thinned one week later to one seedling per pot.

Three strains of bradyrhizobia—including one that was marked with an antibiotic marker to aid identification—were grown to the desired density. The soybean seedlings were then inoculated with single-strain or mixed-strain cultures. Mycorrhizal fungal inoculum was mixed in the top 10 centimeters (4 inches) of soil in each pot just before seedling transfer. Plants were alternately watered with distilled
water and N-free nutrient solution having three phosphate levels: low, recommended, and excessive. Twice weekly, five pots at random were weighed, and the desired amount of solution/water was added to all plots to bring the moisture contents to field capacity.

Plants were harvested at day 77, and roots were gently washed to remove soil/sand particles. Investigators recorded fresh weight of roots, shoots, and nodules; length of shoots and roots; and number of nodules. Roots were then cut from the shoot at ground level, placed into plastic bags, and stored at 4° C. Shoots were dried at 40° C for 4 days, after which dry weights were recorded.

To determine nodule occupancy of the bradyrhizobia, 80 nodules were typed per treatment from 16 nodules per plant chosen at random to represent tap- and lateral-root nodules. The contents from these nodules were transferred to plates containing antibiotic medium. Following incubation, the colonies growing from the point of inoculation were counted as positive for the marked strain. Mycorrhizal colonization was determined by staining and observing 10 root segments per plant and 50 root segments per treatment. The extent of colonization was rated on a 1 to 10 scale.

The second greenhouse study evaluated soybean and corn cultivar dependency on mycorrhizae. Mycorrhizal inocula were multiplied on soybeans in greenhouse pot cultures. Seeds of five soybean and six corn cultivars were pregerminated after surface sterilization, and three pregerminated seeds were planted per pot; later, they were thinned to one per pot. Two VAM fungi and a non-inoculated control were used with three replicates. Again, plants were randomly arranged and watered with distilled water and N-free nutrient solution.

After ten weeks of growth, investigators harvested plants and measured dry weight, P and N contents of shoots, leaf area, and VAM colonization levels. They then determined the proportion of roots colonized by VAM fungi and the P content in plant samples. Mycorrhizal dependency is expressed on a percentage basis as the difference between the total dry matter yield of inoculated and non-inoculated plants.

In the field study of VAM spores and soybean colonization, soybean plants and rhizosphere soil were collected from 15 locations representing five soil series in nine counties. In mid-July, when most of the crop was in late vegetative to early reproductive growth stages, workers took two subsamples for each soil. For each subsample they decapitated five soybean plants, noted their physiological growth stages, and removed the soil and plants by using a soil-core extractor centered over the tap root. Each soil and plant sample was placed individually into plastic bags and stored until transport to the laboratory.

The plant tap root and attached lateral roots were carefully extricated from adhering soil and washed gently to remove soil particles. Fine root segments were preserved until they could be mounted for study under the microscope. Slight pressure on the cover slip of the glass slides flattened the glass segments so the vesicles, arbuscules, longitudinally running internal hyphae, and entry points of the fungi stood out clearly against the outlines of the root cells. Investigators noted the presence or absence of certain of these features per segment and tallied the number of root segments infected per plant. For each plant, 50 root segments were used to determine the extent of colonization, or the percentage of roots showing either mycelia (fungal filaments), vesicles, or arbuscules.

In addition, five soil subsamples were thoroughly mixed to form a composite subsample from which spores were counted and moisture determined. VAM fungal spores from the field soils were propagated in the greenhouse to get fresh spores with complete diagnostic characteristics such as spore walls, colors, and hyphal attachments for identification by genera. A combination of soil and roots from each field site was mixed thoroughly with sand and seeded with soybean seeds. Plants were harvested after 14 weeks, and the soil from each pot was sieved to collect VAM spores for identification. The first 20 spores from each
millipore filter were identified under the microscope at 100 to 400x magnification.

Findings
The first greenhouse study revealed that VAM fungal colonization and phosphate fertilization significantly increased shoot dry weights when P was limiting. Compared with non-inoculated controls, mycorrhizal inoculation increased the shoot dry weights, total N, and total P in soybeans grown at low P levels. Inoculation with *Bradyrhizobium* by itself did not improve shoot dry weights, but dual inoculation of the two resulted in significantly higher shoot dry weights, total N, and total P. When P was supplied at the recommended rate, the mycorrhizal inoculum significantly decreased soybean shoot dry weight. Significant reduction in total N per shoot also suggested that the VAM endophyte had an excessive drain on the host carbohydrates. In addition, VAM likely competed with the host plant for N, thus limiting its availability; in fact, the VAM-inoculated plants appeared chlorotic (see Fig. 1).

With the excessive P application, shoot dry weights were not increased significantly with either single inoculation of *Bradyrhizobium*, mycorrhizal fungus, or with dual inoculation. In the former treatment, however, total N significantly increased from an average of 263 milligrams (mg) per shoot in the control treatments to 351 mg in the inoculated treatments. Total P significantly increased from 58.50 mg per shoot in the control treatments to 61.52 mg in the inoculated treatments. Similar significant increases were present with dual inoculation.

Only at the low P level did inoculation with mycorrhizal fungi significantly affect nodule number. With dual inoculation, nodules per plant averaged 35.6 compared with 13.8 nodules per plant without mycorrhizal fungal inoculation. Mycorrhizae apparently improved the nutrition and growth of the host, which aided in nodule development. At the recommended and excessive P levels, when P was not limiting plant growth, mycorrhizal fungal inoculation did not affect nodule number, nor was percentage nodule occupancy by the marked inoculum increased significantly. VAM colonization did not affect strain competition for nodulation sites, as investigators had expected on the basis of earlier data.

Mycorrhizal fungal colonization decreased significantly with an increase in P fertilization from 100% in the low P treatment to 3% in the excessive P treatment. No significant differences were found in VAM colonization between treatments inoculated by bradyrhizobia and those not inoculated.

In the second greenhouse study, shoot dry weights were significantly different among soybean cultivars and treatments within cultivars. Inoculation of soil with mycorrhizal fungi improved plant dry weights in all soybean cultivars. Response of corn cultivars was also similar except for one, in which growth was not significantly enhanced. Most improved cultivars had uniform response; unimproved cultivars showed more variation. For example, growth of one soybean cultivar increased almost 16 times; in another, growth increased 2 to 3 times. One corn cultivar's growth was enhanced 4 to 5 times, whereas another showed a decrease in dry weight. Growth of most improved cultivars increased about twice as much as that of non-inoculated controls.

![Fig. 1. The effect of VAM is apparent in the soybean plant above (left).](image)
Total and percentage P were improved with VAM fungal colonization in both soybean and corn cultivars, and differences were highly significant among cultivars and treatments within cultivars. Mycorrhizal and non-mycorrhizal plants also differed significantly.

Total N was higher in soybean mycorrhizal plants, but the percentage N was higher in the tissue of non-mycorrhizal plants. Total N was significantly different among soybean and corn cultivars; differences among treatments were significant within treatments in soybean cultivars but not in corn cultivars. Inoculation with the genus *Glomus* improved shoot dry weight, total P, and total N in soybean cultivars, whereas *Gigaspora* was more effective with corn cultivars. VAM colonization ranged from 49 to 68% with *Gigaspora* and from 46 to 67% with *Glomus* in corn cultivars; percentage colonization did not differ significantly among improved and unimproved cultivars. In soybeans, VAM fungal colonization ranged from 62 to 83% with *Gigaspora* and from 71 to 87% with *Glomus*. Colonization by each VAM species was significantly different in all cultivars except one.

Mycorrhizal dependency of soybean cultivars was higher than for corn cultivars. Two unimproved cultivars showed higher relative mycorrhizal dependency (RMD) than the improved cultivars. Additional results indicate that the RMD of a plant species may be related to the proportion of root colonized by VAM fungi.

The relationship between root morphology and RMD observed in this investigation supports the hypothesis that plant species having coarse roots with few or no root hairs are more dependent on VAM fungi than those having fine roots with numerous root hairs. Further investigations in progress, regarding differences in such root characteristics as diameter, hair length, hair incidence, length, and density, will help to explain variation in RMD of cultivars within plant species.

Results from the field study indicate that most plants from most soil samples contained VAM fungal colonization regardless of soil test P levels. Investigators found that VAM fungal colonization was not restricted to poorer soils, as is commonly assumed, but was also characteristic of soils with relatively high levels of available P. VAM spores were quite common in all the soils sampled; total spore numbers were significantly different among the soil series. Additionally, total spore numbers varied significantly from one soil to another, indicating a dominant role of soil factors in VAM sporulation and colonization. A negative correlation was found between soil organic matter, P, and VAM colonization. *Glomus* was the most abundant VAM fungal genus associated with the soybean rhizosphere soils surveyed. *Gigaspora* was the second most common genus found, but only one species was dominant.

**Implications**

One of the goals of sustainable agriculture research is to maintain productivity while reducing the level of chemical inputs, including nutrients, that farmers must purchase. Because this goal depends in part on renewing resources within the soil, a better understanding of these natural microbial partnerships is crucial to maximizing and manipulating their contributions.

The results of this study revealed that Iowa soils contain a high level of mycorrhizal colonization in spite of high soil P levels. This unexpected finding suggests that fungi have adapted to high P fertilization over the years. The VAM fungi present in our soils now may not be as efficient as those present naturally before heavy P fertilization. Soybeans clearly benefit by microbial associations when soil P levels are low. Plant cultivars also prefer specific mycorrhizal fungi.

This work represents only the beginning of a long-term effort to understand the reasons for these plant preferences as well as how to manipulate these factors for improved plant growth in the field. Achieving a better understanding of the VAM organisms in their natural habitat will also require additional study.