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# Belowground Microbial Processes Underpin Forest Productivity

By

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## Summary

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Nitrogen-fixing bacteria associated with mycorrhizal fungi and mycorrhizas can be demonstrated with microaerophilic procedures. The chemical substrates in mycorrhizal fungi or mycorrhizas often stimulate the growth and nitrogenase activity of the associated N<sub>2</sub> fixers. In addition, the associated N<sub>2</sub> fixers are producers of plant-growth-promoting substances and B-group vitamins. Combined inoculation of mycorrhizal fungi with N<sub>2</sub> fixers enhances mycorrhiza formation. Other microbes in the mycorrhizosphere have capacities to breakdown primary minerals, thereby releasing nutrients available for uptake by plants. Thus, land restoration can be achieved by planting trees with nitrogen-fixing and rock weathering capacities, such as alders and some pines. The treatment can enhance nutrient availability and increase soil organic matter that provides organic substrate for nutrient release, maintain soil structure and enhance water-holding capacity. Also changes in tree species compositions on the site are likely to alter belowground processes through changes in functional processes of organisms that constitute ecosystems.

## Introduction

Soil microbial populations are metabolically active in the mycorrhizosphere, where a continuous supply of energy-rich carbon substrates from plants, selective absorption of nutrients by roots, and active respiration by roots and microbes creates a microaerophilic environment. Together, these processes often selectively stimulate microaerophilic associative nitrogen-fixing microbes and other microor-

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ganisms that, for example, produce plant-growth-promoting substances or change the soil's chemical composition in ways that enhance nutrient availability and absorption by plants. Thus, dynamic microbial processes in the mycorrhizosphere regulate mobility and cycling of limiting nutrients in forest ecosystems, thereby improving ecosystem development and protection. Microbially mediated ecosystem processes change as plant communities develop, however, and they can be severely disrupted by natural and human caused disturbances. Understanding how plant species and environmental conditions affect these processes is essential in evaluating belowground biological contributions to ecosystem resilience and ecosystem productivity.

This paper presents key biogeochemical processes mediated by microbe-plant interactions occurring in mycorrhizospheres and their contributions to ecosystem functioning in forests.

## Materials and Methods

### Nitrogen-fixing bacteria associated with fungal sporocarps

Three to five pieces of interior tissue of each fresh sporocarp of ectomycorrhizal fungi were aseptically removed and placed in a N-free liquid medium in a serum bottle (LI & CASTELLANO 1987). The bottles were capped and flushed for 5 min with nitrogen gas. Oxygen was injected into the bottles to obtain an atmospheric mixture of N<sub>2</sub>-O<sub>2</sub> (99:1). The bottles were incubated for 3 days at 30 °C under these microaerophilic conditions. Bottles showing bacterial growth were injected into each bottle to 10 % of the total gas volume. After 18 h a 0.1 ml gaseous sample from each bottle was removed and analyzed for ethylene and acetylene with a gas chromatograph fitted with a 2 m x 2.1 mm, 80-100 mesh, Porapak R column with oven temperature at 70 °C. Injection temperature and flame-ionization detector temperature was each adjusted to 100 °C, and flow rate of the nitrogen carrier gas was adjusted to 40 ml per min.

### Nitrogen-fixing bacteria associated with mycorrhizas

Mycorrhizas were thoroughly washed under tap water to remove soil particles. They were then immersed in 1 % Chloramine-T (J. T. Baker Chemical Co., Phillipsburg, NJ) for 1 h, shaken frequently, then washed in five changes of sterile distilled water. Mycorrhiza was afterwards aseptically cut into 0.2-0.5 cm segments and inoculated into a N-free liquid medium in a serum bottle (LI & HUNG 1987). The bottles were incubated as described under the microaerophilic conditions. Bottles showing bacterial growth were tested for acetylene reduction as described with a gas chromatograph.

### Weathering of rocks by mycorrhizal fungi and associated microbes

Limestone, marble, and tricalcium phosphate in less than 90 um particle size were sterilized by anhydrous ethyl ether and each was amended at 0.25 % to a medium developed by HENDERSON & DUFF 1963. The microbes were each inoculated onto the center of the medium and incubated at room temperature (21-24 °C). Halo zones produced around the microbial colonies indicated solubilization of rock minerals.

## Results and Discussion

With the microaerophilic techniques N<sub>2</sub> fixers were isolated from the interior tissues of sporocarps of a number of mycorrhizal fungi (Table 1). It was only with the sporocarp extract, the bacteria were able to grow and produce nitrogenase

activity under only microaerophilic conditions (LI & CASTELANNO 1984, LI & al. 1992).  $N_2$  fixers associated with mycorrhizae were also isolated and their  $N_2$  fixation capacities demonstrated (LI & HUNG 1987, TILAK & al. 1991, Table 2).

Table 1.  $N_2$  fixers isolated from sporocarps of ectomycorrhizal fungi.

Sporocarp	Geographic location	$N_2$ fixer
<i>Barssia oregonensis</i>	Apiary, Oregon	<i>Azospirillum</i>
<i>Hymenogaster parksii</i>	Blodgett, Oregon	<i>Azospirillum</i>
<i>Hysterangium setchellii</i>	Mary's Peak, Oregon	<i>Azospirillum</i>
<i>Hebeloma crustuliniforme</i>	Grants Pass, Oregon	<i>Azospirillum</i>
<i>Laccaria laccata</i>	Corvallis, Oregon	<i>Azospirillum</i>
<i>Leccinum scabrum</i>	Corvallis, Oregon	<i>Azospirillum</i>
<i>Rhizopogon parksii</i>	5 miles south of Yaquina Bay Bridge, Lincoln County, Oregon	Not determined
<i>Rhizopogon vinicolor</i>	Peavy Arboretum, Benton County, Oregon	<i>Azospirillum</i>
<i>Suillus ponderosus</i>	Corvallis, Oregon	<i>Pseudomonas</i>
<i>Tuber melanosporum</i>	France	<i>Klebsiella pneumoniae</i>
<i>Tuber aestivum</i>	Italy	<i>Azospirillum</i>
<i>Tuber brumale</i>	Italy	<i>Azospirillum</i>

Table 2.  $N_2$  fixers isolated from Douglas-fir ectomycorrhizas

Mycorrhiza	$N_2$ fixer
<i>Gautieria monticola</i>	<i>Azospirillum</i>
<i>Hebeloma crustuliniforme</i>	<i>Clostridium</i>
<i>Hysterangium setchellii</i>	<i>Azospirillum</i>
<i>Laccaria laccata</i>	<i>Clostridium</i>
<i>Rhizopogon vinicolor</i>	<i>Azospirillum</i>
<i>Thelephora</i> sp.	<i>Azospirillum</i>
Tuberculate by <i>R. vinicolor</i>	<i>Bacillus</i>

Of the ectomycorrhizal fungi tested, *Rhizopogon vinicolor*, *Suillus bovinus*, and *Hysterangium setchellii* degraded limestone, marble, and tricalcium phosphate (CHANG & LI 1998, Table 3). *Penicillium* sp and yeast associated with the sporocarp of *R. vinicolor* were also able to solubilize these rock minerals. All associated fluorescent *Pseudomonads* and  $N_2$ -fixing *Azospirillum* spp. showed positive degradation activity (Table 3).

*Azospirillum* was the most frequent  $N_2$  fixer associated with mycorrhizas or sporocarps of mycorrhizal fungi. It was also the producer of B-group vitamins (DAHME & al. 1993) and growth-promoting substances (STRZELCZYK & al. 1994). Inoculation of *Frankia* with *Azospirillum* resulted in enhanced growth and nodule formation of actinorhizal red alder (*Alnus rubra*) (LI 1987). The associative  $N_2$  fixers also enhanced germination of Fungal spores (TILAK & al. 1989, TILAK & al. 1990) and mycorrhiza formation (GRYNDLER & HRSELOVA 1998). GARBAYE & al. 1990, FILIPPI & al. 1995, 1998 and GAZZANELLI & al. 1999 isolated bacteria from within sporocarps of mycorrhizal fungi and mycorrhizas. Some of the bacteria, especially fluorescent pseudomonads, enhance mycorrhiza formation (DUPONNOIS & GARBAYE 1991, GARBAYE 1994, FREY-KLETT & al. 1997).

Table 3. Solubilization of limestone, marble, and tricalcium phosphate as indicated by a halo formation around microbial colonies. - = halo formation not detected, + = halo formation positive.

Species and isolate number	Limestone	Marble	Calcium Phosphate
<i>Cenococcum geophilum</i> , A145	-	-	-
<i>Hebeloma crustuliniforme</i> , 8166	-	-	-
<i>Hysterangium setchellii</i> , HS 206	+	+	+
<i>Laccaria laccata</i> , 10440	-	-	-
<i>Piloderma croceum</i> , 85.009	-	-	-
<i>Rhizopogon vinicolor</i> , 11970	+	+	+
<i>Suillus bovinus</i> , SB 24.6	+	+	+
<i>Azospirillum</i> sp., Az-1	-	-	-
<i>Azospirillum</i> sp., Az-2	+	+	+
<i>Azospirillum</i> sp., Az-4	+	+	+
<i>Penicillium</i> sp.	+	+	+
<i>Pseudomonas fluorescens</i> , Ps-1	+	+	+
<i>P. fluorescens</i> , Ps-2	+	+	+
<i>P. fluorescens</i> , Ps-3	+	+	+
<i>P. putida</i> , R20	+	+	+

Mycorrhizas removed from the mycorrhizosphere in order to measure rates of nitrogen fixation, therefore, need a microaerophilic environment, relatively similar to that of mycorrhizosphere. Excised mycorrhizal roots of red pine (*Pinus rigida*) and pitch pine (*P. rigida*) growing in sandbox ecosystems had rapid rates of nitrogen fixation, measured under low oxygen environment (BORMANN & al. 1993). *Equisetum* sp. and *Carex* sp. from Alaska had high rates of fixation, measured under low oxygen conditions (MARSH 1996).

Solubilization of rock minerals by roots and microbes in rhizospheres can accelerate release of inorganic nutrients, thereby enhancing nutrient availability for uptake by plants (PARIS & al. 1996, KIM & al. 1997). The combined effect of plant roots and rhizosphere microbes on solubilization of primary minerals can enhance plant growth, ecosystem development, and maintain ecosystem structure. Most researchers attribute solubilization of rock mineral to the release of organic acids by plants or microbes (HINSINGER & GILKES 1995, VASSILEV & al. 1995, VASSILEV & al. 1997). Mycorrhizal inoculation increased mineral weathering (WATTEAU & BERTHELIN 1990, 1994, PARIS & al. 1996).

Studies in progress showed poor growth of Douglas-fir (*Pseudotsuga menziesii*), Manchurian fir (*Abies nephrolepis*), sitka spruce (*Picea sitchensis*), but very good growth of red alder (*Alnus rubra*), in a mixed basalt rock-perlite mixture. Lodgepole pine (*Pinus contorta*) and red pine (*P. densiora*) showed positive growth in the mixture, compared to their poor growth in only perlite.

Land restoration and increasing sustainability of soil ecosystem productivity in forests, therefore, can be achieved by planting different tree species. Forest ecosystems with a genetic diversity of tree species can reduce impacts of insects and pathogens on plant productivity. Belowground terrestrial processes such as nutrient cycling, ecological functions and other properties are mediated by interactions among different functional groups of organisms. Changes in tree species are

likely to significantly alter these processes through creation of new functional processes of other organisms. Alders and tree species with associative or symbiotic nitrogen fixation increase soil organic matter, which can provide organic substrate for nutrient release, maintain soil structure and enhance water-holding capacity. A tree species can alter resource supply by tapping an otherwise unavailable source. Examples are trees with mycorrhizal associates, with associative, or symbiotic N<sub>2</sub> fixers, or with rock-weathering capacities. Thus, trees with different types of microbial association can have significant impacts on biogeochemical processes, affecting recovery of degraded ecosystems and forest sustainability.

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