Microbe-driven fertility of boreal forests: insoluble phosphorus made available by ectomycorrhizal fungi and associated bacteria

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Introduction

In boreal forests, the nutrition of trees is made possible mostly by mycorrhizal symbioses. Mycorrhizas are associations in plant roots where sugar is provided to the fungal partner in exchange for water and minerals. The ectomycorrhizal [ECM] symbiosis is dominant in conifer stands.

Fig. 1 Ectomycorrhizal symbiosis between *Pinus strobus* and *Laccaria bicolor* (J.A. Fortin 2012).

Several species of ECM fungi are able to weather P, K, Mg and Ca-bearing minerals by releasing protons, organic acids and siderophores (Courty et al. 2010). Organic acid production is also displayed by soil bacteria (Uroz et al. 2009) which can be found within bacterial communities associated with ECM fungal hyphae (Nazir et al. 2012).

The province of Québec hosts rich deposits of igneous apatite, a calcium phosphate mineral, that could be used to fertilize P-deficient stands. However, igneous minerals are reported to be recalcitrant to weathering by most soil microorganisms (Bashan et al. 2012). We hypothesized that igneous apatite is a P source readily available to common ECM fungi and that *Picea glauca* (Moench) Voss mycorrhizae would host apatite-weathering bacteria.

Objectives

- To investigate igneous apatite solubilization by *ECM* fungi commonly associated to *Pinaceae*.
- To characterize ECM fungi-associated apatite-weathering bacteria.
- To demonstrate the value of igneous apatite as a fertilizer in *P. glauca* stands.

Materials and Methods

-Apatite weathering by ECM fungi

Five ECM fungal species (*Hebeloma crustuliniforme*, *Suillus granulatus*, *Paxillus involutus*, *Tricholoma scalpturatum* and *Cadophora finlandia*) were grown in liquid media containing either tricalcium phosphate [TCP] or igneous apatite. Controls were soluble P and without P. Incubation lasted 8 weeks after which the biomass was harvested and then freeze dried. Growth was measured by weighing dried biomass. Clarification of the media was also used as direct confirmation of mineral amendment solubilization.

-Apatite weathering by ECM-associated bacteria

White spruce specimens were collected at the Montmorency experimental forest and grown in greenhouses for a year. *Wilcoxina sp*. mycorrhizas were harvested from their root systems, then washed and ground into a slurry to be used as inoculum. Efficient weathering strains were obtained through liquid culture while solid media provided a diversity of strains. Identification of strains displaying stable weathering abilities was performed with partial 16S gene sequencing.

Forest fertilization

White spruce stands located at Forêt Montmorency were selected for a forest fertilization trial, using apatite and orthoclase as insoluble P and K sources. Forest floors were amended with apatite and orthoclase at a rate of 650 and 900 g/m^2 either separately or in combination. Changes in tree growth and nutrition were assessed with dendrometric measurements and foliar analyses. The data was collected one year prior to forest floor amendment and one year after.

Results

-Apatite weathering by ECM fungi

Contrast		P
-P vs +P	*H. crustuliniforme*	0.0128*
	S. granulatus	0.0315*
	T. scalpturatum	<.0001***
	C. finlandia	<.0001***
-P vs Apatite	*H. crustuliniforme*	0.0242*
	S. granulatus	0.0022**
	T. scalpturatum	<.0001***
	C. finlandia	<.0001***
TCP vs Apatite	*H. crustuliniforme*	0.6766
	S. granulatus	0.9179
	T. scalpturatum	0.1554
	C. finlandia	0.2801

Fig. 2 Differences in biomass in relation to P sources for media-clarifying fungal species. Asterisks indicate significant difference.

- Apatite is a P source available to ECM fungi.
- TCP and apatite are equivalent sources of P for ECM fungi.

-Apatite weathering by ECM-associated bacteria

Amendment	Subculture	Nb. strains
TCP	1	455
TCP	2	428
TCP	3	203
Hydroxyapatite	1	140
Igneous apatite	1	98
Igneous apatite		27

Fig. 3 Number of ECM-associated bacterial strains able to dissolve increasingly difficult to weather insoluble P sources.

- *Wilcoxina* sp. hosts insoluble phosphate-weathering bacteria.
- Igneous apatite is more resistant to bacterial weathering than TCP and hydroxyapatite.

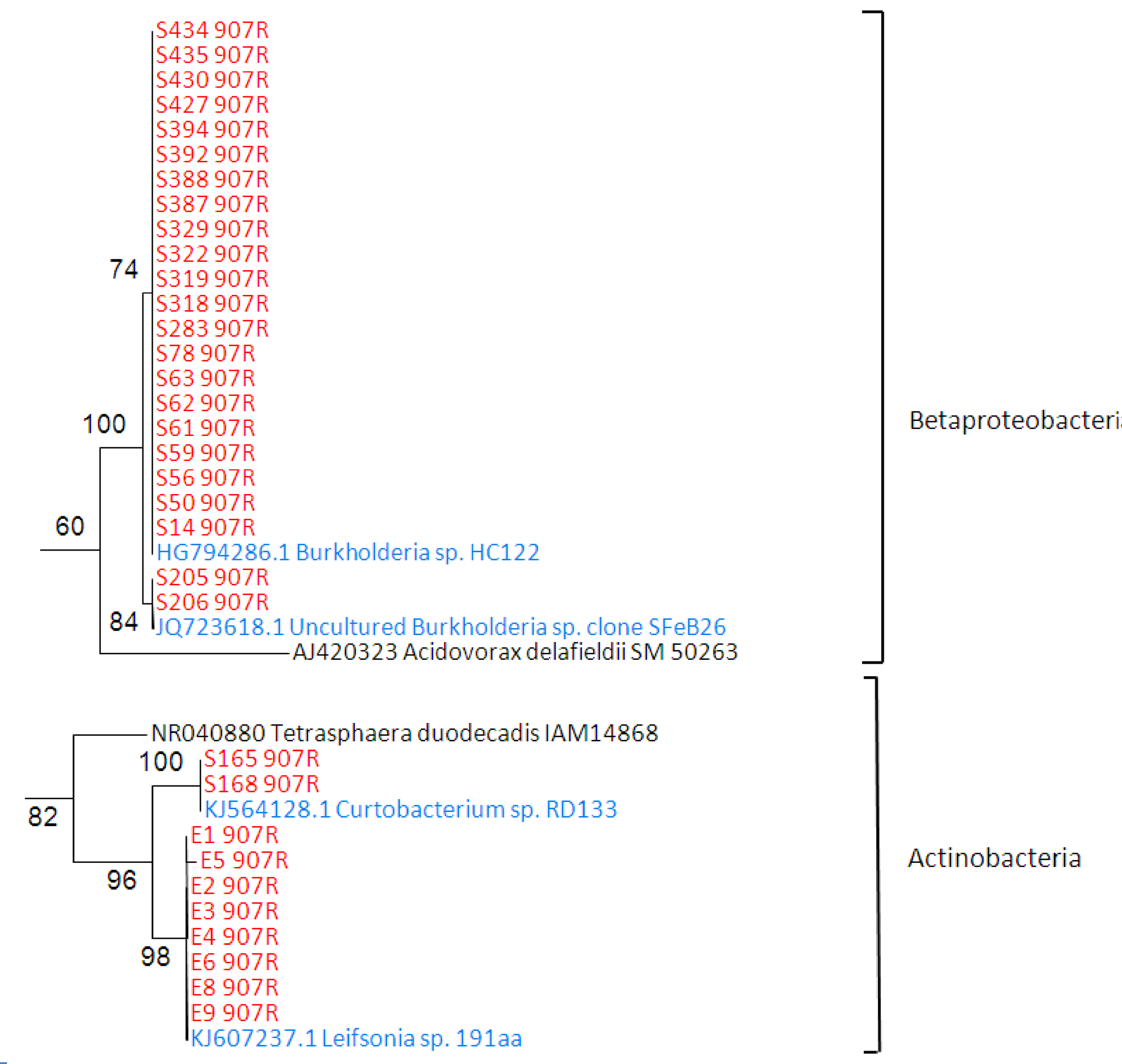

Fig. 5 Apatite weathering bacteria identified with partial 16S gene sequencing.

- Forest fertilization

t Grouping		Mean	N	Treatment
	A	4.8000	9	Apatite
	A			
B	A	3.6833	9	Mixed
B	A			
B	A	3.3167	9	Orthoclase
B				
B		2.8444	9	Control

Fig. 6 LSD grouping of means for tree girth increases one year after fertilization.

- The fertilized treatments make one group.
- Apatite fertilization differs significantly from the other treatments.

Discussion and Conclusion

Igneous apatite is readily used by common ECM fungi and associated bacteria hosted by white spruce. It suggests this mineral is valuable as an amendment for conifer stands in order to increase P availability to the ecosystem.

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