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Enhanced revegetation and reclamation of oil sands disturbed sites using actinorhizal and mycorrhizal biotechnology

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Abstract

*A functional soil microbial community is crucial for plant growth and survival and a key component for successful land reclamation. Mycorrhizal fungi, a component of the microbial community, play an essential role in plant nutrient uptake, water relations, and buffer plants against biotic and abiotic stresses to enhance ecosystem establishment. Actinorhizal bacteria, such as Frankia spp., another component of the soil microbial community, help plants establish in nutrient poor soils by supplying plants with atmospherically fixed nitrogen. Newly reconstructed landscapes after oil sands exploitation may be characterised by low soil organic matter, high salinity and alkalinity, low nutrient status, and limited microbial activity. Several organic amending materials are used to cap reconstructed soils in order to support plant community establishment. However, landscape disturbance may result in destruction of mycorrhizal fungal networks and affect actinorhizal bacterial numbers in soil. Therefore, ensuring the development of functional symbioses in trees and shrubs during greenhouse production before outplanting, is an important biotechnological approach to the reclamation of oil sands disturbed lands. In the present study, actinorhizal alders (*Alnus viridis *ssp crispa and* A. incana *ssp. rugosa) and ectomycorrhizal fungi (ECM) inoculated Jack pine (*Pinus banksina *Lamb.) and White spruce (*Picea glauca *(Moench) Voss) seedlings were outplanted on the Suncor MD5 reclaimed overburden site. The study was designed to evaluate the impact on survival and growth of inoculating plants prior to outplanting. The seedling volume index (SVI) or plot volume index (PVI) were used to evaluate field performance and compare inoculated verses uninoculated plants. In all cases inoculated plants outperformed uninoculated plants. Inoculated alders had a SVI that was 3- to 4-fold greater than uninoculated plants. In addition, alder rhizosphere bacterial populations showed increases in diversity and catabolic activity. There were significant increases in the PVI of ECM inoculated white spruce and jack pine seedlings when compared with uninoculated controls. These preliminary results, after two growth seasons, show promise in the use of pre-inoculated seedlings in enhancing growth and establishment of alders and conifers on oil sands reclamation sites. Knowledge gained from this research will increase our understanding of actinorhizal and ECM symbioses with forest seedlings on reclamation sites and their ability to accelerate successful revegetation and reclamation.*

1 Introduction

Canada has one of the largest oil sands reserves in the world, located mainly in central Canada. It is estimated that 1.7 trillion barrels of bitumen are contained in 4 deposits (Fung and Macyk, 2000). The largest deposit (700 billion barrels), in the Athabasca region of Alberta, is a near-surface deposit that allows recovery through surface mining. This involves removal of the existing vegetation and overburden to reach the oil sands. The current area that has been disturbed by mining activity is approximately 600 km^2 (CAPP, 2009).

Seedling establishment is a critical phase in the reclamation process and depends on the capacity of the seedlings to efficiently capture available resources (Duñabeitia et al., 2004) to withstand pest and pathogen attacks or survive certain climatic and nutritional stress factors (Perry et al., 1987). Nursery inoculation of plants with actinorhizal bacteria (Roy et al., 2007; Lefrançois et al., 2010) and mycorrhizal fungi (Ortega et al., 2004; Parlade et al., 2004; Ringe and Graves, 1990) has demonstrated the ability to improve plant hardiness and survival rate when outplanted on disturbed sites. Increased litter and plant cover reduces erosion and increases soil water retention (Cerdà, 1997). Moreover, continuous addition of organic matter, through living plants can contribute to soil stability (Huang et al., 2005).

The objectives of this study were to evaluate the ability of Frankia-inoculated alders and mycorrhizal fungus inoculated conifers (Jack pine and White spruce) to grow on reclaimed overburden material capped with mineral soil and peat according to current practices (Fung and Macyk, 2000), and to characterise how plant establishment would impact soil quality and microbial communities. This study presents the results of plant survival and growth after two growing seasons and preliminary results on the impact of plant growth on soil microbial community activity.

2 Methodology

2.1 Study site and seedling establishment

The site is located at Suncor Energy Inc., Fort McMurray, Alberta, Canada. Overburden material was capped in 2008, with a mixture of mineral soil and peat. Green alder (*Alnus crispa* (Ait.) Pursh, seedlot 8360546) and Red alder (*A. rugosa*, seedlot 8431680) seedlings were started in an industrial greenhouse in March 2009 (Bonnyville Forest Nursery, Bonnyville, AB), and a portion of the seedlings were inoculated with *Frankia* sp. strain AvcI1 (Quoreshi et al., 2007), subsequently referred to as F9. The seeds were obtained through the National Tree Seed Centre (Fredericton, NB, seedlot 8360545.0). Alders were out-planted on the research site in June 2009: two plots, each with eight random subplots were planted with F9-inoculated alders or non-inoculated alders (control) (four subplots for each alder species in each plot), with each plot having 100 plants planted at 1 m intervals. An adjacent zone was kept unplanted.

Jack pine (*Pinus banksiana*) seed lot no: syn-95-10-4-90 and White spruce (*Picea glauca*) seed lot no: syn-16-92-11-4-05 were inoculated with three different ectomycorrhizal (ECM) species and a consortium of the three-ECM fungi and grown at a commercial nursery (Bonnyville Forest Nursery). The seedlings were grown for approximately six months and hardened prior to out planting in the spring, 2009. To inoculate seedlings, a stock mycelial slurry solution (1 L) was diluted with water to produce 3.9 L of liquid inoculum with a minimum final concentration of 5 × 103 viable propagules/mL or 0.46 mg dry mycelia/mL. The diluted mycelial slurry was mixed into the growing medium (peat:vermiculite) and the mixture was used to fill the Styroblock cavities. Each container cavity (98 mL) received 10 mL of diluted liquid mycelial slurry. The seeds were sown in each cavity containing the media.

2.2 Study design and treatments

The study was carried out in a semi-randomised (alders) or complete randomised block design (conifers) (56 × 30 m) with four replicates of each treatment, which includes the control and an unplanted plot. For alder plots, they were separated to maintain inoculated alders separate from uninoculated alders. Within each plot there were four subplots of each alder species, with 100 plants per subplot. For conifers, this study tested three different ectomycorrhizal species and a consortium of the three-ectomycorrhizal fungi. The ECM fungi tested were *Hebeloma crustuliniforme* (HC) UAMH 5247, *Laccaria bicolor* (LB) UAMH 8232, and *Suillus tomentosus* (ST) CEFGB5. Eight inoculation treatments were established for both Jack pine and White spruce plots. The treatments were HC, LB, ST, HC+LB, HC+ST, ST+LB, HC+LB+ST, and Control (CTRL). Each experimental unit was planted with 30 seedlings each with a distance of 1 m and block distance of 2 m. Nine hundred and sixty seedlings per plant species were planted on each experimental plot.

2.3 Data collection and analyses

All seedlings (alder, Jack pine and White spruce) were measured for shoot height and root collar diameter at the base. Twenty-five randomly selected alders per subplot, or five randomly selected Jack pine of white

spruce seedlings from each block were assessed for growth measurements. Data were used to calculate the seedling volume index [(root collar diameter)^2 × total shoot height] or plot volume index (PVI). The PVI was calculated by multiplying the seedling volume index by the number of surviving plants per treatment (Marx et al., 1991). Due to plot flooding in the alder plots, survival data was not assessed. All growth and survival data were subjected to the analysis of variance (ANOVA) using SAS software. To minimise the effect of early differences in height, relative growth was used to determine yearly gains of stem length computed as height (Pera et al., 1999). This was calculated using the formula $(h_f - h_i)/h_i$, where h_f is the height at end of one growing season and h_i is the height at the beginning of the growing season. Seedling survival was used as a response variable to evaluate the performance to mycorrhizal inoculation (Maestre et al., 2002) and expressed as a percentage.

2.4 Bulk soil sampling

During sampling after two growing seasons (September 2010) bulk soil was randomly collected from each alder plot. Three composite soil samples (ca. 500 mL) from each subplot were taken using a sterile spatula, thoroughly mixed, transferred into sterile plastic bags and immediately placed on ice for transport back to the laboratory. In the laboratory the soils were re-mixed and 20 g aliquots were transferred into sterile 120 mL serum bottles for the mineralisation assays.

2.5 Mineralisation assays

Mineralisation assays were performed for two representative petroleum hydrocarbon substrates (hexadecane and naphthalene) in microcosms. Bulk soil microcosms (20 g of soil) microcosms were set up and sampled as described by Greer et al. (2003). Samples were spiked with 100,000 dpm hexadecane-1-C^{14} (specific activity: 12 mCi/mmol) in 100 mg kg^{-1} hexadecane, or 100,000 dpm naphthalene-1-C^{14} (specific activity: 2.3 mCi/mmol) in 10 mg kg^{-1} naphthalene. Microcosms were incubated at room temperature and $^{14}CO_2$ evolution was regularly measured using a KOH trap and analysed by liquid scintillation spectrometry to determine the cumulative amount of substrate mineralised.

3 Results

3.1 Alders

3.1.1 Alder growth and seedling volume index

Alder growth on the MD5 plots was extensive after only two growing seasons. There was a highly significant difference between F9-inoculated and non-inoculated alders (Figure 1). The seedling volume index was four times greater for the F9-inoculated *A. crispa* than for the non-inoculated plants and more than three times greater for F9-inoculated *A. rugosa* than for the non-inoculated *A. rugosa*. These results are strongly suggestive that greenhouse inoculation of the alders had a positive impact on their ability to establish and grow on the oil sands reclamation site.

3.1.2 Alder survival

Survival of both inoculated and non-inoculated alders was generally very good, but it could not be accurately assessed due to surface water inundation in the plots. There was high precipitation shortly after outplanting in June 2009, which resulted in surface water accumulation in both plot areas and caused total mortality of plants in completely flooded subplots. However, in adjacent plots, plant survival was essentially 100% in both the inoculated and non-inoculated plots.

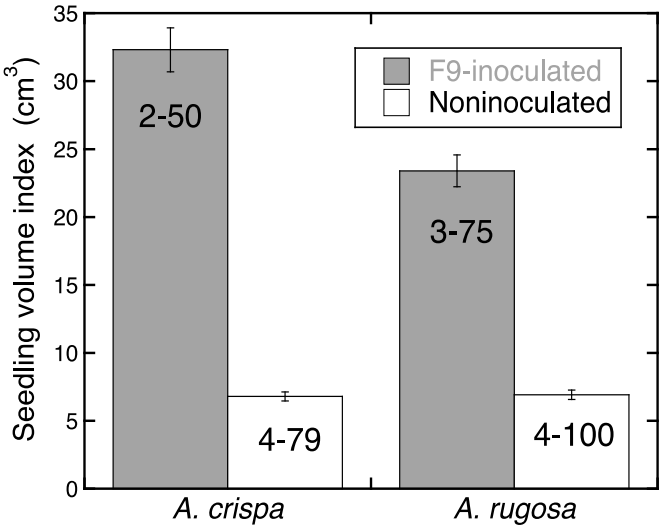

Figure 1 Seedling volume index of *Frankia*-inoculated (F9) and noninoculated alders (*Alnus crispa* and *A. rugosa*) outplanted on site MD5 after two growing seasons. Numbers in bars indicate the number of subplots-number of plants per subplot counted

3.1.3 Hydrocarbon mineralisation potential in bulk soil

Analysis of soil changes during the first two growing seasons was not highly significant in terms of soil physico-chemical parameters (data not shown). However, as an indicator of change in the indigenous soil microbial population activity, we examined hydrocarbon mineralisation potential using two substrates, one representative of the alkane fraction (hexadecane) and one representative of the aromatic fraction (naphthalene) of hydrocarbons. Since this region is rich in natural hydrocarbons, it is expected that soil microorganisms would have the capacity to use these substrates, so monitoring their metabolism would be a good method for detecting change in the local soil environment. Bulk soil samples taken at the start of the experiment before alder outplanting had highly variable and generally lower hydrocarbon mineralisation potential than the bulk soils analysed after two growing seasons (Figures 2 and 3). Hexadecane mineralisation activity was generally lower and more variable initially than after two years of growth (Figure 2). After two years of growth the indigenous microbial population in the soil demonstrated a more consistent and higher degradation potential for hexadecane mineralisation (Figure 2). Similarly, the degradation of naphthalene by the indigenous microbial population was generally more variable than the degradation activity after to growing seasons (Figure 3). These results indicate that the indigenous soil microbial populations have shifted in response to alder growth. The differences observed could not be correlated with inoculated verses non-inoculated alders, but do show that after two growing seasons there are clearly shifts in microbial activity in the soils being evaluated.

3.2 Jack pine and White spruce

3.2.1 Height, seedling volume and plot volume indices

There was no significant difference in height between treatments at the time of outplanting in the first growing season for both conifer species (data not shown). However, in the second year of growth significant differences were observed for which most of the data shown was obtained. In Jack pine, there was a significant overall effect of treatment (inoculation) on plant height. Differences were observed between treatments and the control, of which treatments HCLB, HC, HCST, LBST, HCLBST and LB showed at least a 7% increase in height over the controls. The treatment with the highest and lowest percentage increase in height was HCLB at 18% and LB at 8%, respectively (Figure 4A). In White spruce, significant differences in height were observed between treatments HC, HCLB and HCLBST compared to the controls. However, there was no overall effect of treatment on plant height (Figure 4B).

The PVI of Jack pine increased with all inoculated seedlings particularly with treatments HC, LB, ST and HCST but the differences were not statistically significant (Figure 5). The seedling volume on the other hand of Jack pine seedlings inoculated with HC, LB and HCST increased by about 63, 40 and 41%, respectively, relative to the controls. In White spruce, the PVI of seedlings inoculated with HCLB showed greater significant increases of up to 124% when compared to the control (Figure 6). There was minimal increase in the seedling volume of treatments, which were not significantly different from the controls. In treatment groups, the PVI of single treatments increased by about 57% compared to the control for Jack pine and the volume by 45%. In White spruce, the double treatment increased the PVI by about 83% and the volume by 10% (data not shown).

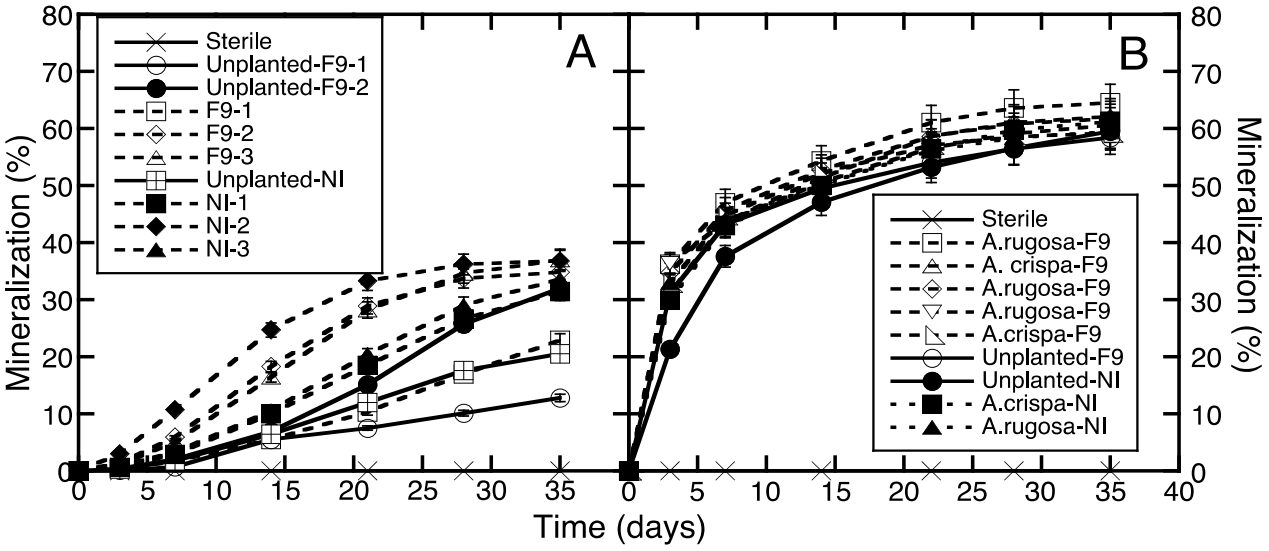

Figure 2 Mineralisation of hexadecane in bulk soil from MD5 site. Samples were from the initial plot site prior to planting (A) or from the plots after two growing seasons (B). Composite soils were collected from subplots as indicated (unplanted, F9 refers to inoculated subplots, NI refers to non-inoculated subplots, alder species (*A. crispa* or *A. rugosa* are indicated)

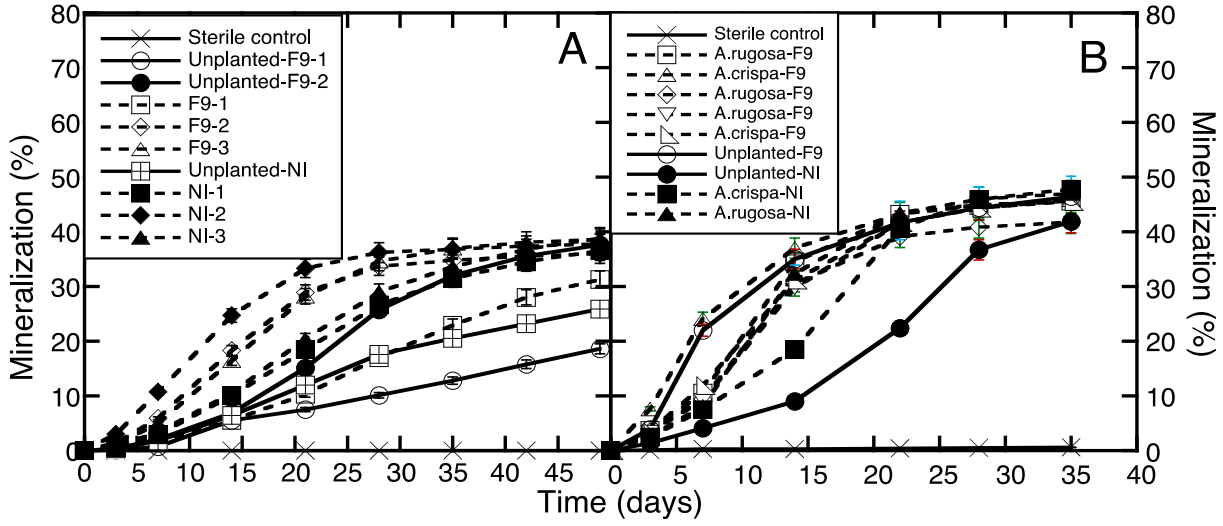

Figure 3 Mineralisation of naphthalene in bulk soil from MD5 site. Samples were from the initial plot site prior to planting (A) or from the plots after two growing seasons (B). Composite soils were collected from subplots as indicated (unplanted, F9 refers to inoculated subplots, NI refers to non-inoculated subplots, alder species (*A. crispa* or *A. rugosa* are indicated)

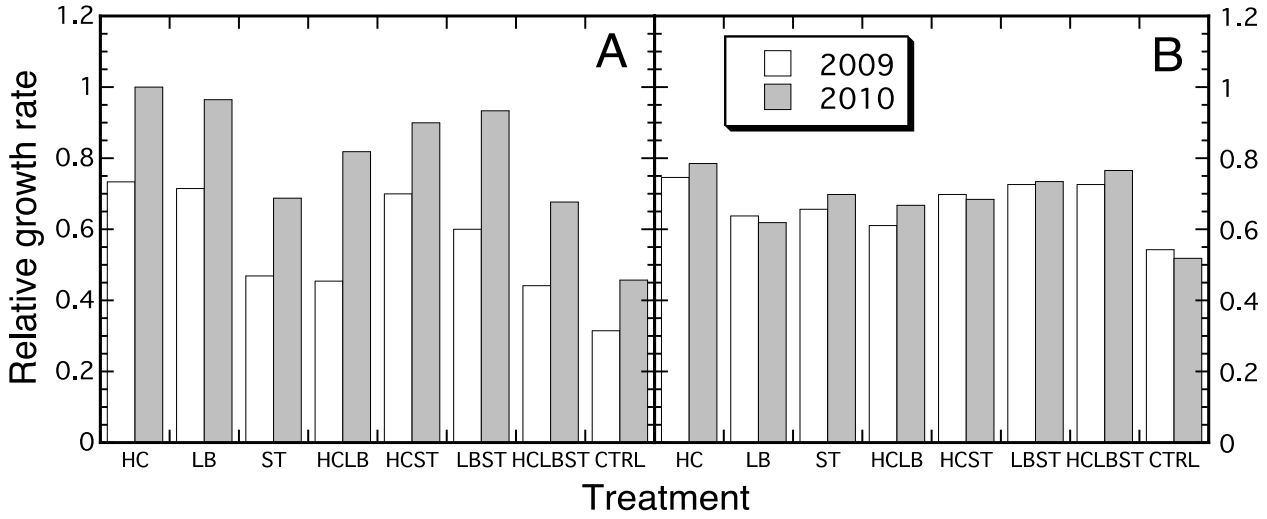

Figure 4 **Relative annual growth rate of inoculated and non-inoculated Jack pine (A) and White spruce (B) seedlings for two growing seasons at the Suncor MD5 site. Treatments are *Hebeloma crustuliniforme* (HC), *Laccaria bicolor* (LB), *Suillus tomentosus* (ST), *H. crustuliniforme* + *L. bicolor* (HCLB), H. *crustuliniforme* + S. *tomentosus* (HCST), L. *bicolor* + *S. tomentosus* (LBST), H. *crustuliniforme* + L. *bicolor* + S. *tomentosus* (HCLBST) and non-inoculated (CTRL)**

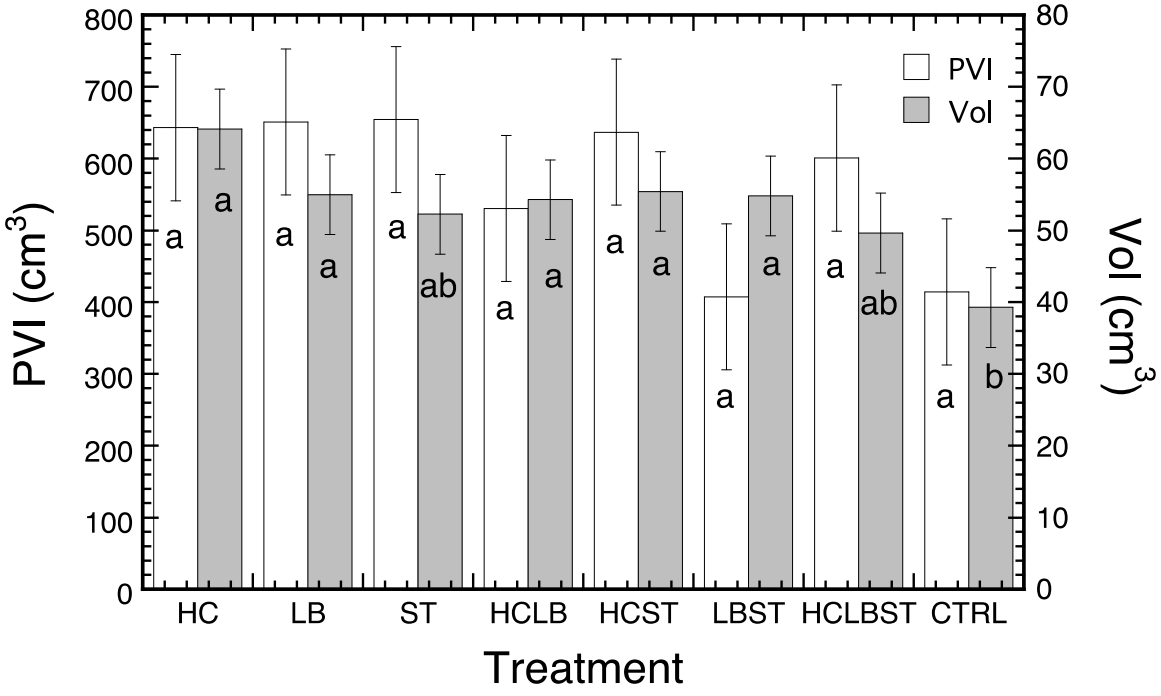

Figure 5 **Effect of nursery mycorrhizal inoculation on mean seedling volume and plot volume index (PVI) of outplanted Jack pine seedlings after two growing seasons at the Suncor MD5 site. Vertical bars represent ± standard error of the mean. The same letters are not significantly different from each other at $p < 0.05$ (Fisher LSD). Treatments are as in Figure 4**

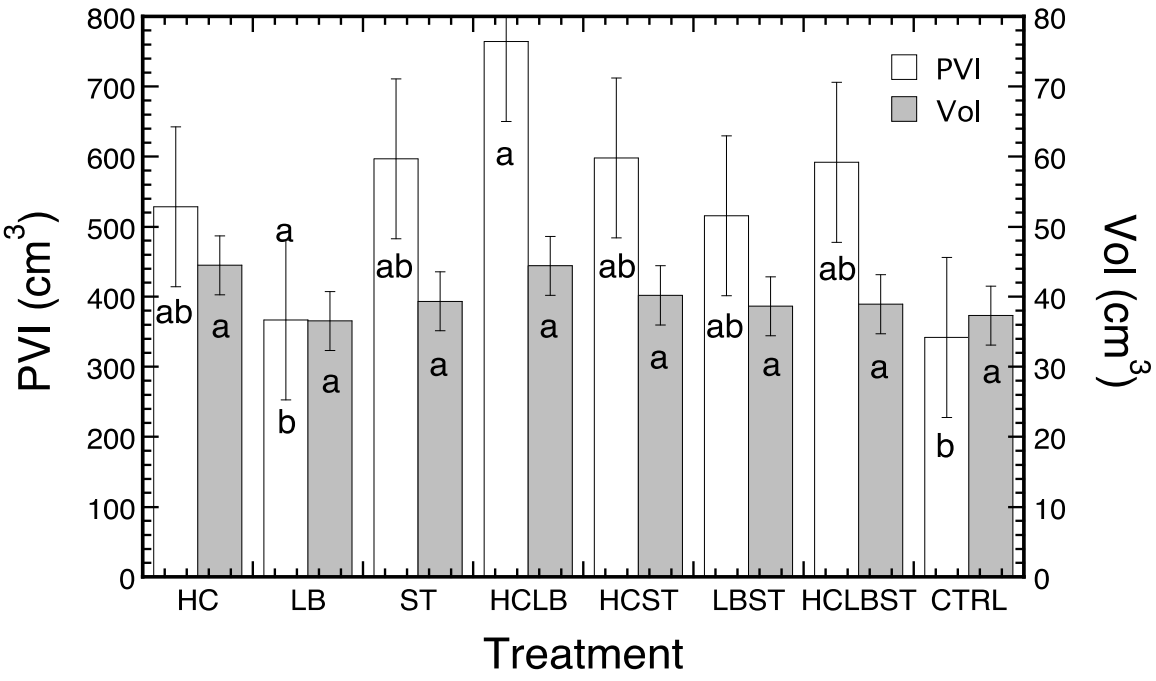

Figure 6 Effect of nursery mycorrhizal inoculation on mean seedling volume (open bar) and plot volume index (PVI) (shaded bar) of outplanted White spruce seedlings after two growing seasons at the Suncor MD5 site. Vertical bars represent ± standard error of the mean. The same letters are not significantly different from each other at p < 0.05 (Fisher LSD). Treatments are as in Figure 4

3.2.2 Survival rate

Generally, the survival rate of seedlings differed in the two conifer species. In Jack pine, there was no overall significant effect of inoculation on seedling survival. However, significant survival differences between the treatments LBST, ST and LB were observed (data not shown). In White spruce, survival of seedlings was significantly influenced ($p < 0.05$) by inoculation with ECM fungi. There was also an average survival rate between 36% and 56% for White spruce seedlings inoculated with different strains of ECM, as opposed to the controls that had minimum and maximum survival rates of 22 and 41%, respectively.

4 Conclusions

The present study demonstrated that greenhouse inoculation of alder and conifer seedlings with beneficial symbiotic bacteria or fungi gave these plants a clear competitive advantage when outplanted on oil sands sites. Greenhouse alders inoculated with the nitrogen-fixing actinomycete, *Frankia*, demonstrated far superior growth on oil sands sites after only two growing seasons. Similarly, ectomycorrhizal fungus inoculated Jack pine and White spruce outperformed non-inoculated plants in terms of survival, relative growth and PVI. These results demonstrate the potential of this approach to contribute to successful reclamation of oil sands sites and the future restoration of the local ecosystem. The successful establishment and growth of pioneer plants will lead to succession by other types of vegetation, a dynamic process that will gradually spread into adjacent areas.

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