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BRi Biotechnology
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***Alder-Frankia* Symbionts Enhance the Remediation and Revegetation of Oil Sands Tailings**

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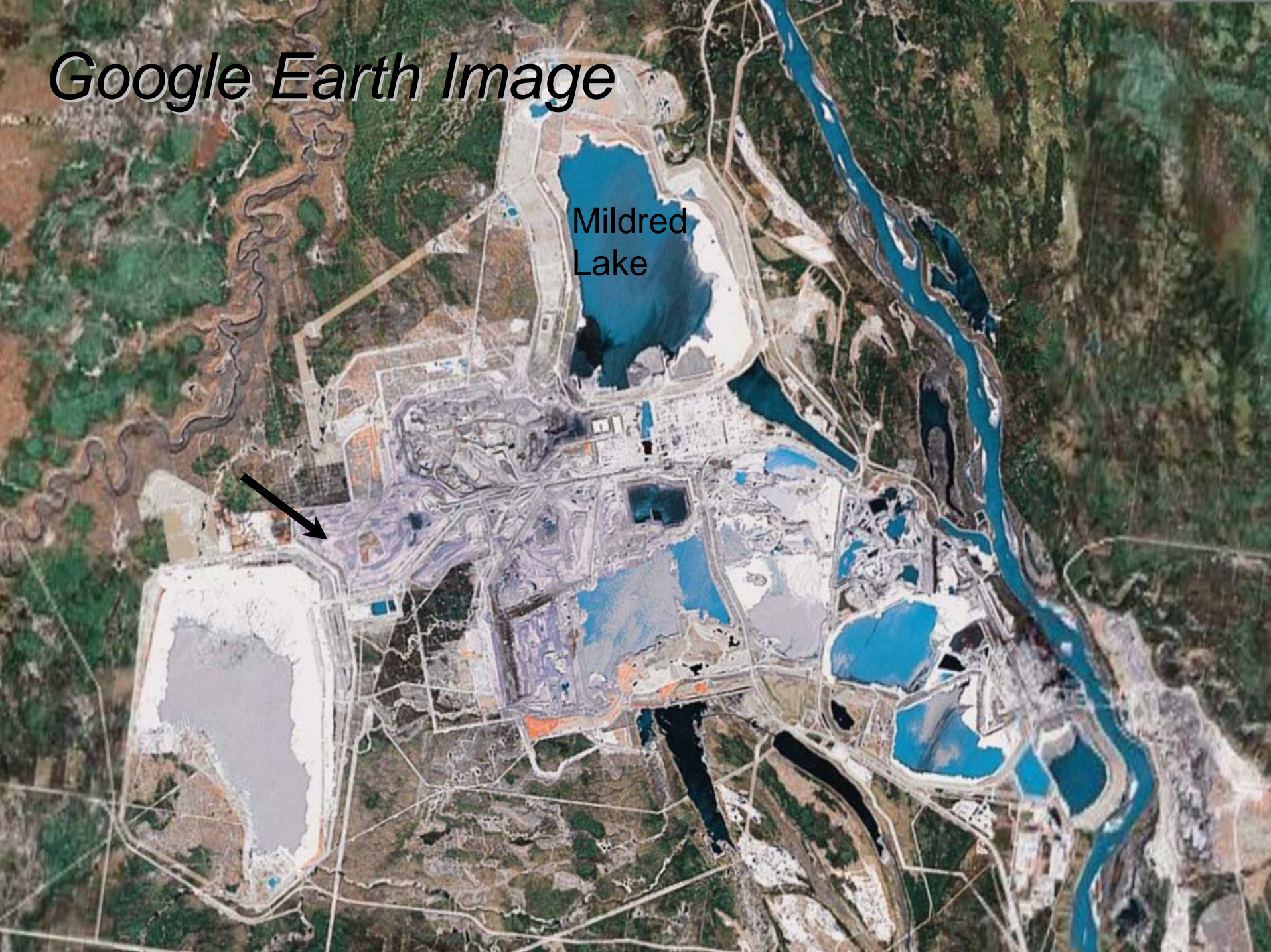
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Oil Sands Operations, Fort McMurray



Tailings sands and tailings water



Objectives

- Develop efficient greenhouse production procedures for alder-*Frankia* symbionts
- Develop a protocol for studying rhizosphere microflora inside and outside the root system
- Screen alders and symbionts in greenhouse trials for use on tailings sands (TS) and composite tailings (CT)
- Evaluate the performance of symbiont alders in a field trial on tailings sands
- Determine the impact of rhizosphere microflora on the degradation of hydrocarbon contaminants



F9-inoculated, 6.5 months

Alder roots penetrating CT sand



alkB PCR

M CSAg CSAr TS TSnp TSAr TSAg CT CTnp CTAr CTAg + - M

500 bp →



CT: Composite Tailings

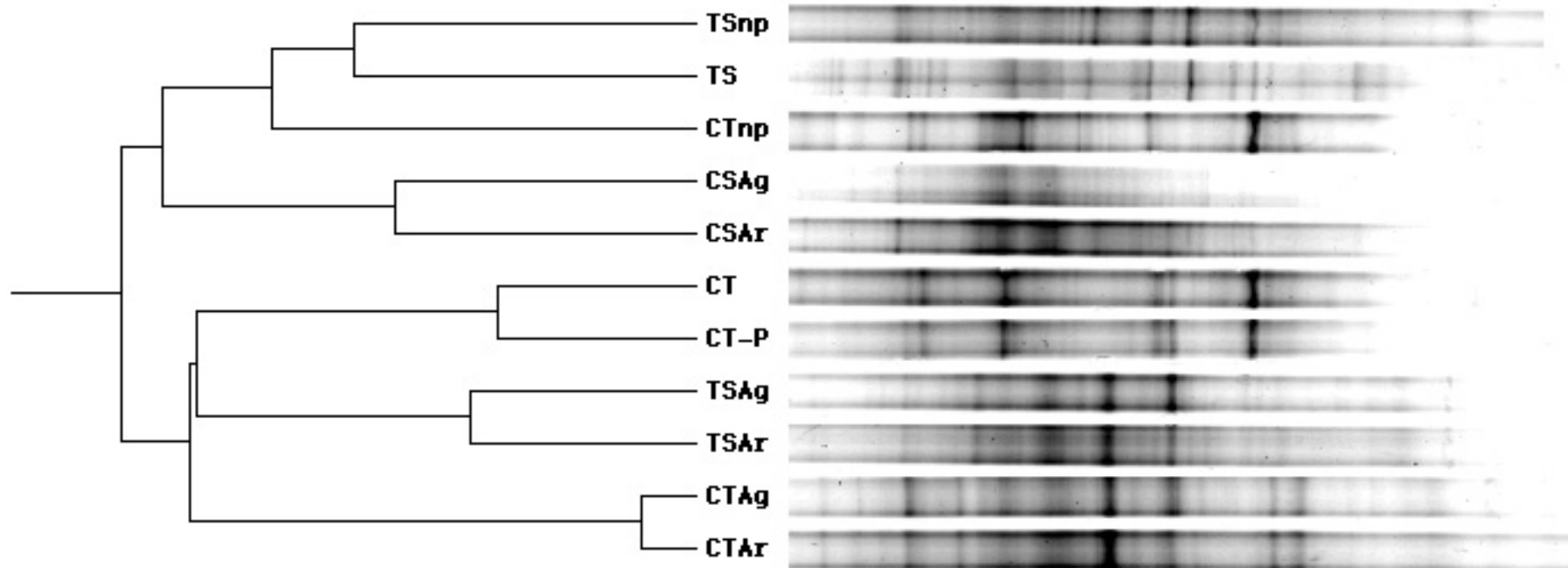
TS: Tailings

AR: *A. rugosa*

AG: *A. glutinosa*

Denaturing Gradient Gel Electrophoresis (DGGE)

- demonstrates relatedness of different microbial communities



0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1 S_{AB}

CT: Composite Tailings

TS: Tailings

Ar: *A. rugosa*

Ag: *A. glutinosa*

np: not planted

Detection of F9 in endophytic community



- primers designed from IGS between *nifD-nifK*
- inoculated strain can be detected in alder endophytic microbial community
- can determine if 'right' microorganisms present
- can also determine if undesirable microorganisms are present

***High saline-alkaline site
composed of mainly overburden***



2005 6 10



Initial Planting

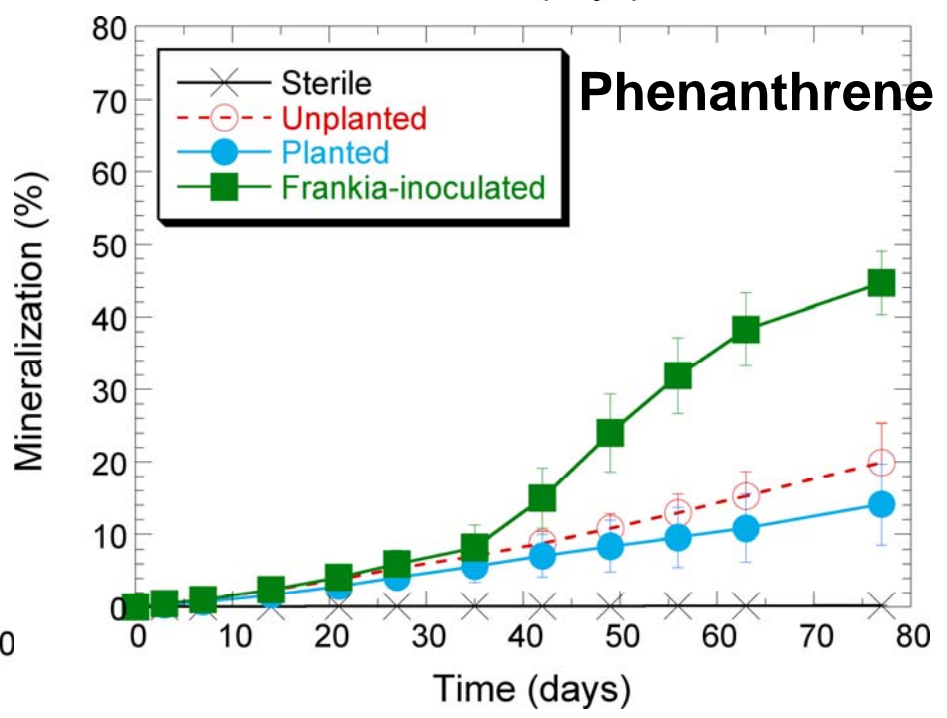
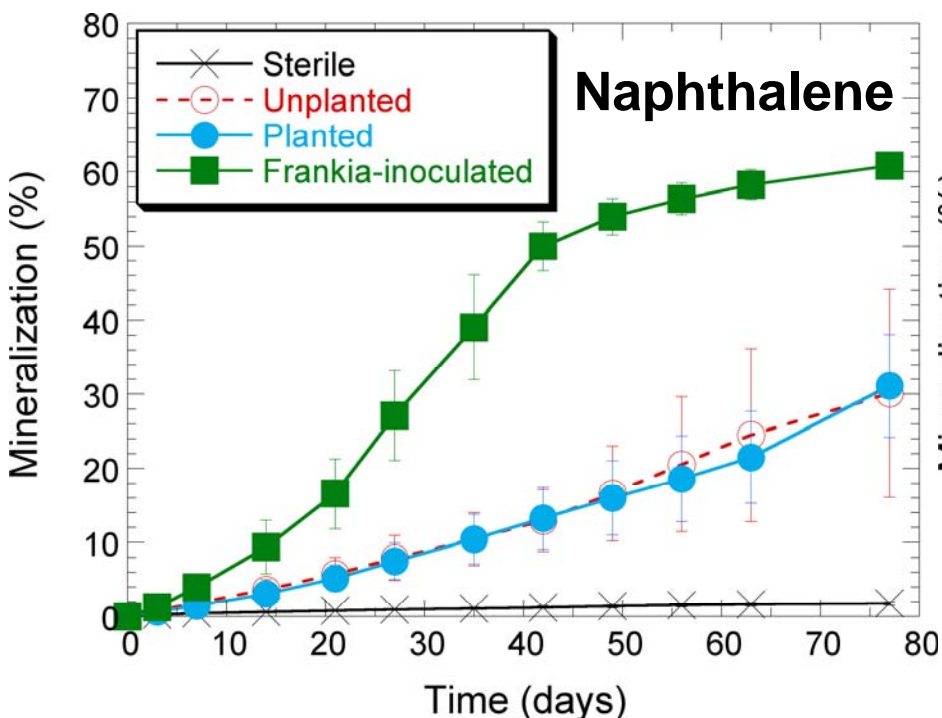
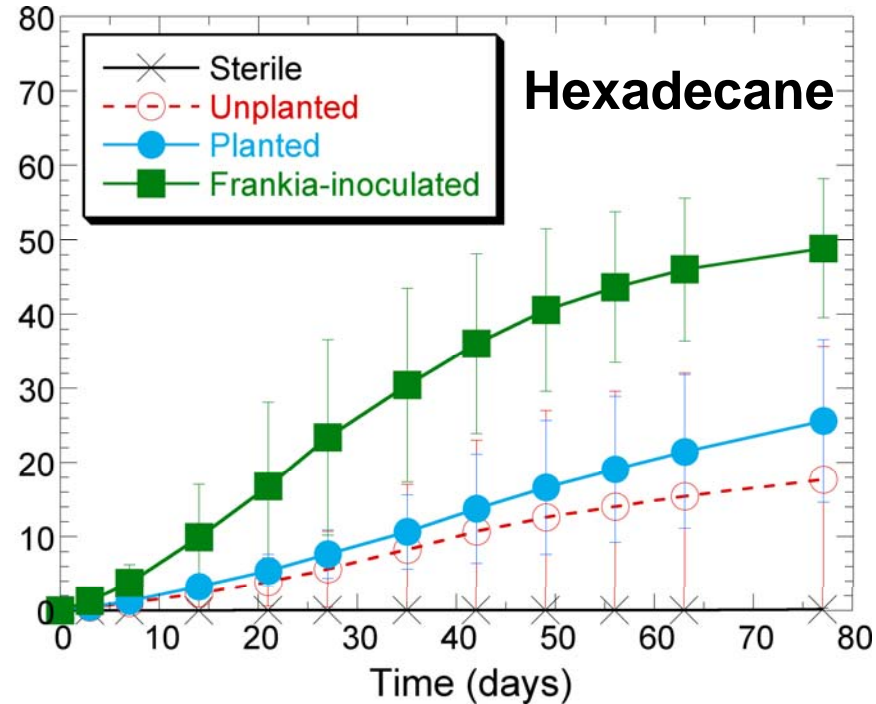
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Alders growing in the field (1.5 yrs)



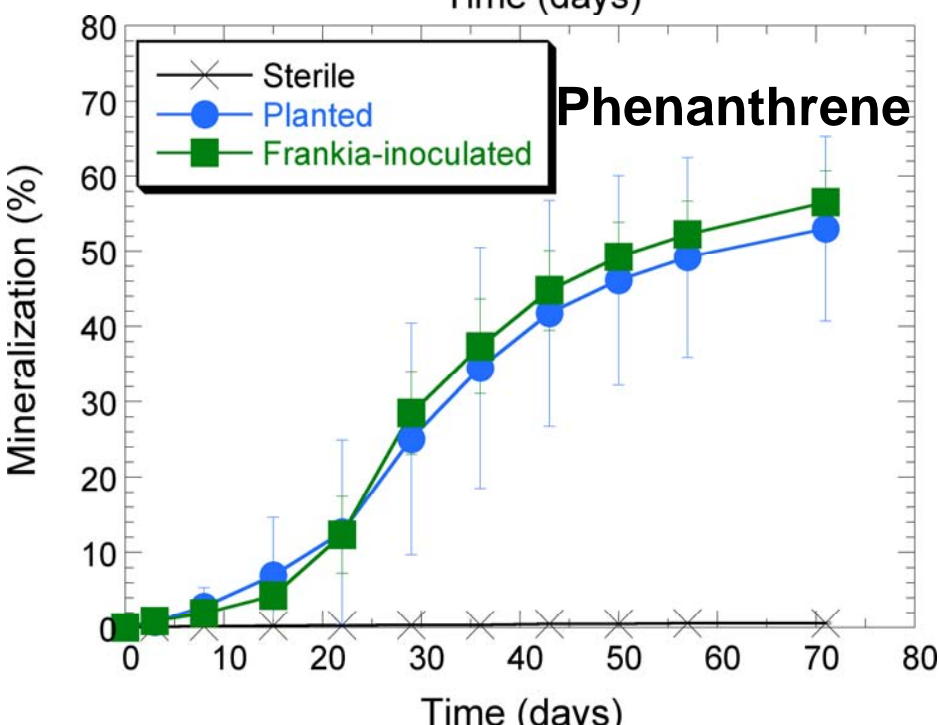
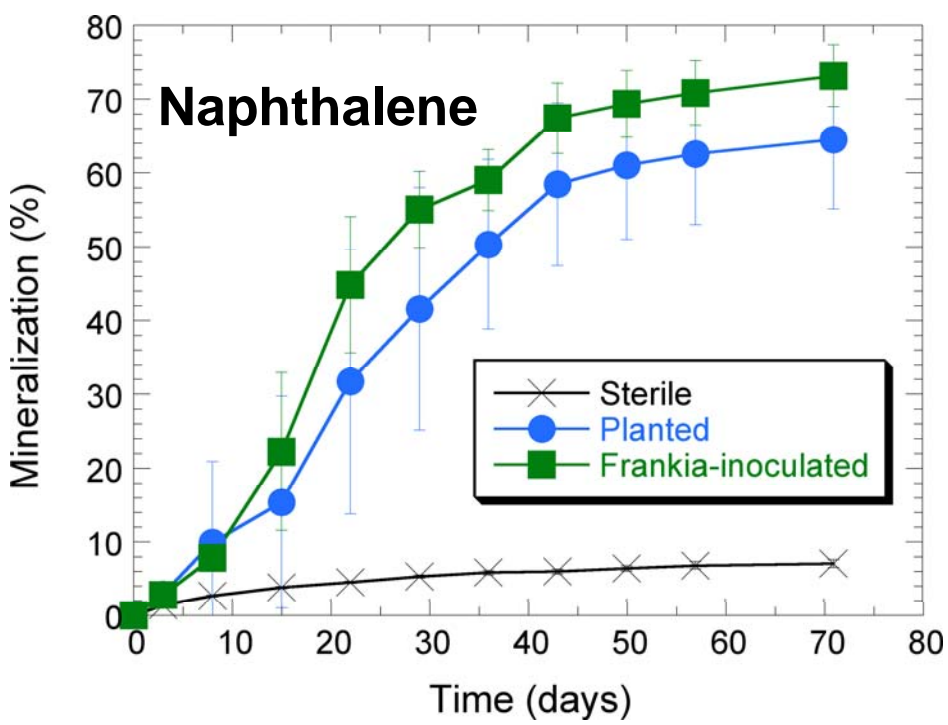
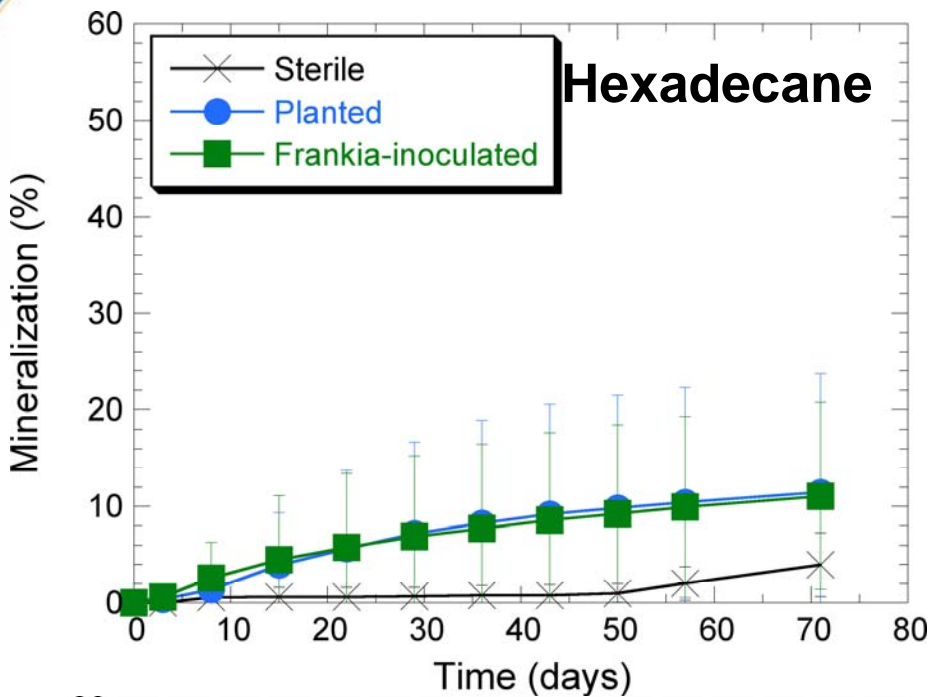
Hydrocarbon mineralization in bulk field soil planted with alders

Mineralization (%)

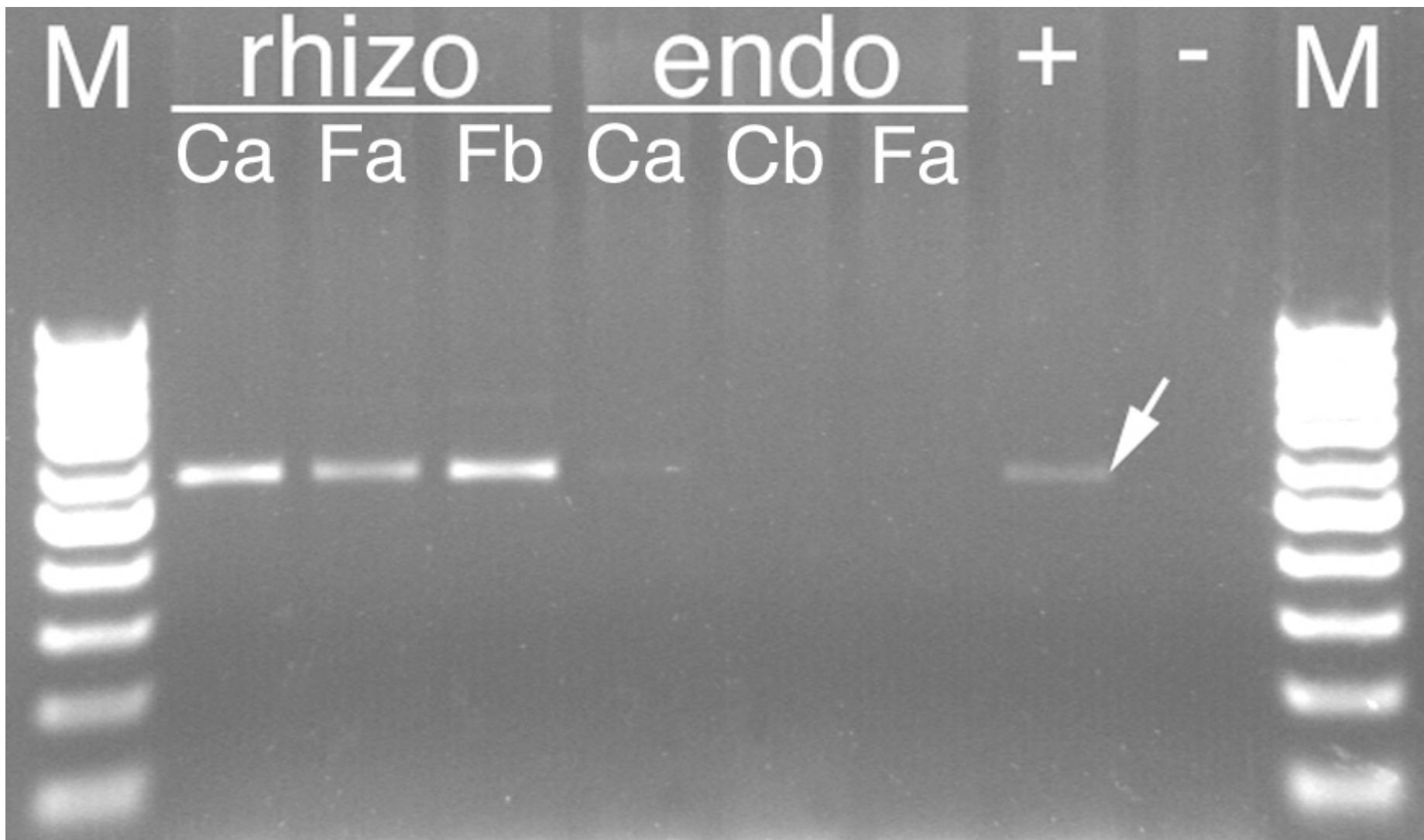




Hydrocarbon mineralization in the rhizosphere of alder planted soil



PCR analysis of *alkB* in field plants



alkB Analysis of Bulk Soil
From Plots of Unplanted, Control
Alder Planted and Frankia-inoculated Alder

Control

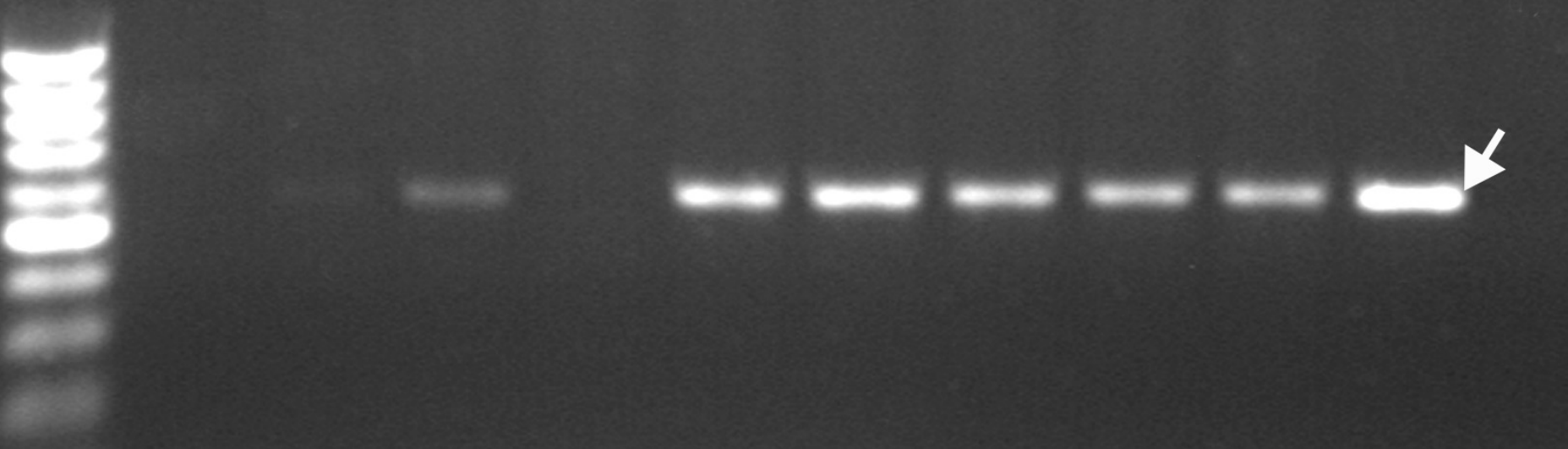
Frankia

unplanted

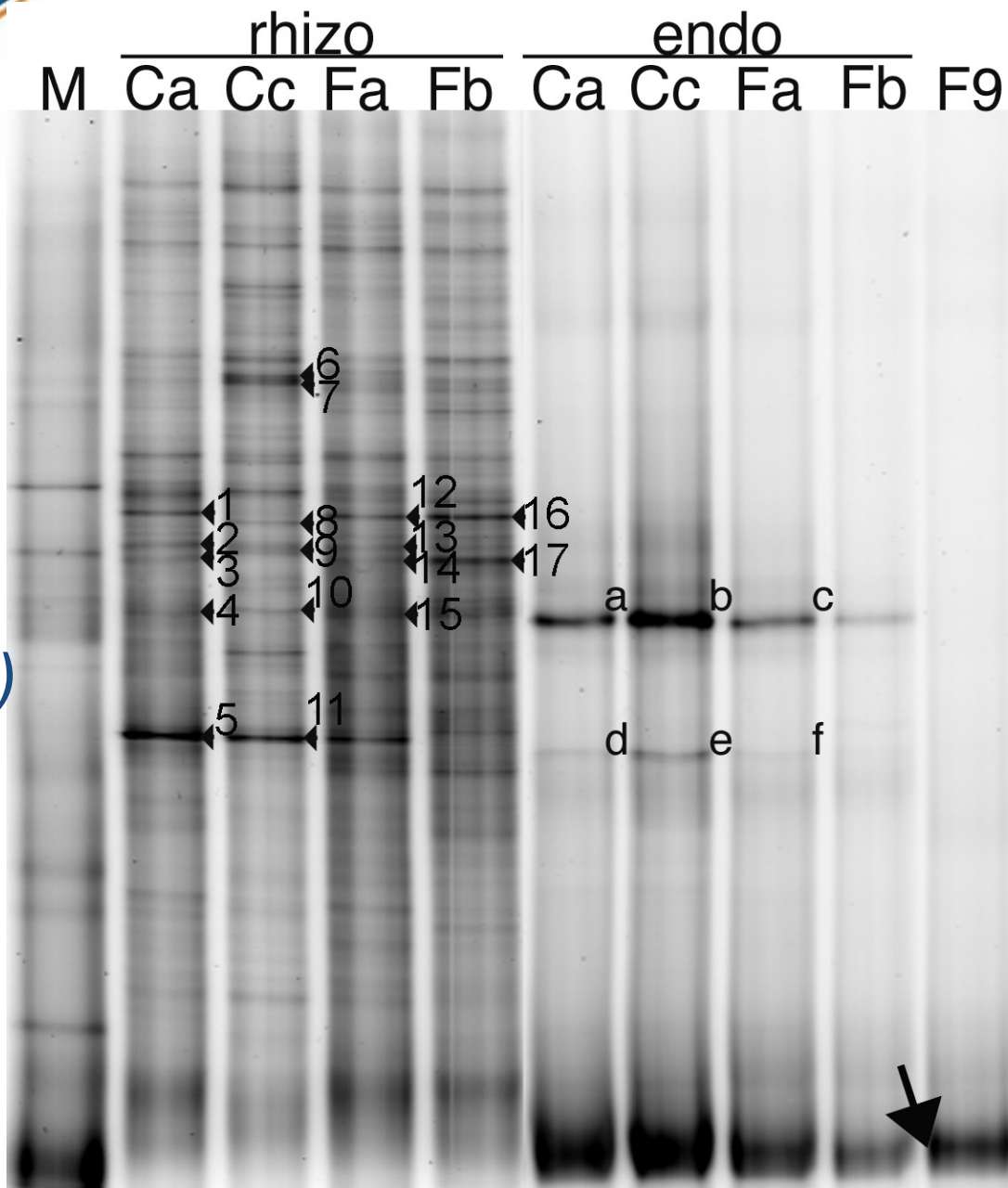
planted

inoculated

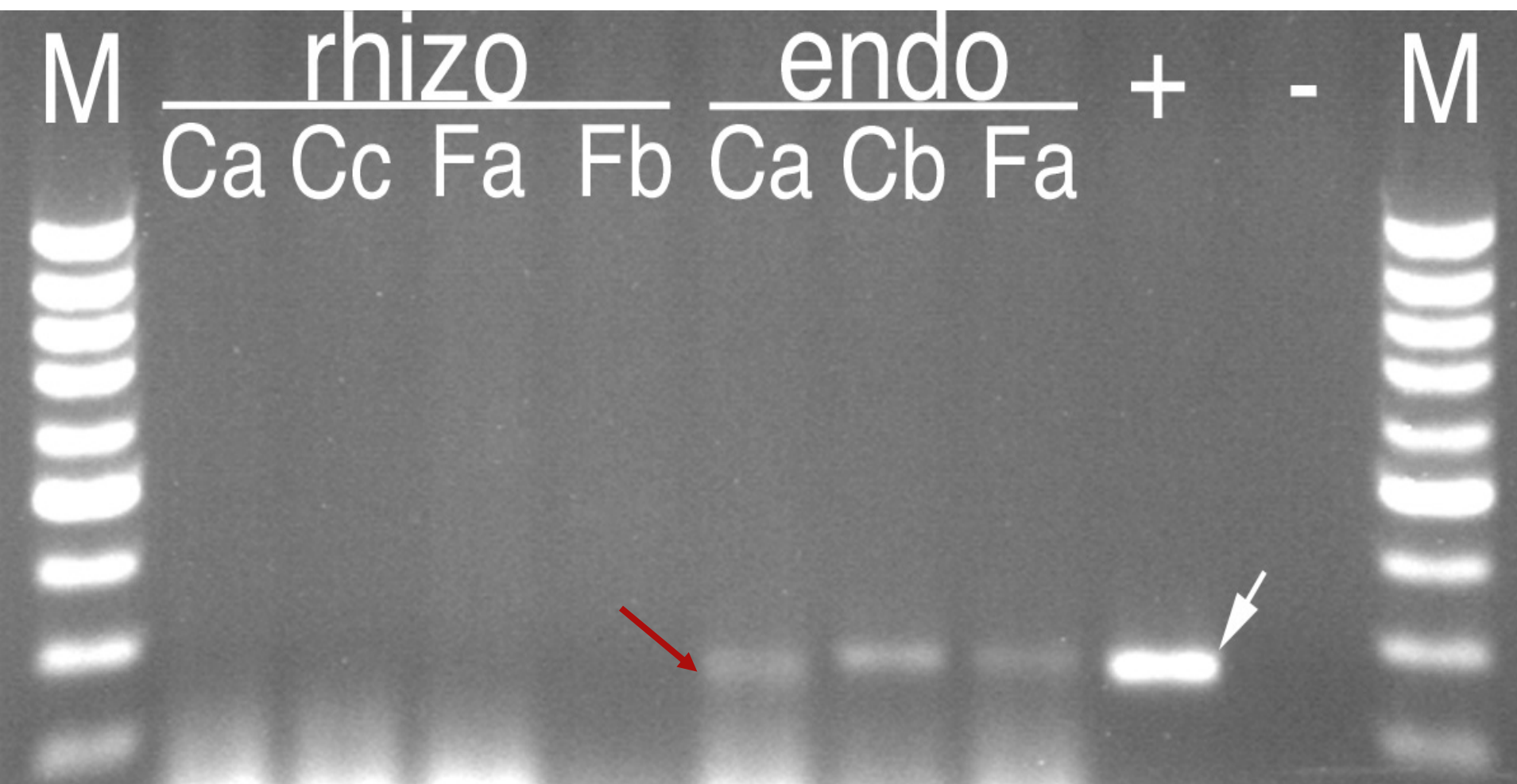
M A B C A B C A B C + -



*DGGE Analysis of
Rhizospheric and
Endophytic Microbial
Communities From
Frankia-inoculated (F)
and Non-Inoculated (C)
Alders*



Detection of *Frankia* in endophytic community



Soil Quality

- pH ↓
- OM ↑
- Electrical conductivity ↓
- CEC ↑
- Bulk Density ↓
- Nutrients and salt content

Field Results: Soil Quality Parameters

	pH	buffer-pH	K (kg/ha)	Mg (kg/ha)	Na (kg/ha)
Unplanted	7.5	7.5	211	1533	498
Planted Control	7.5	7.5	217	1697	359
Planted Frankia	6.6 ↓	7.0 ↓	116 ↓	1637	160 ↓

	Saturation (%)				Estimated CEC
	K	Mg	Ca	K+Mg+Ca	
Unplanted	1.1	25.1	73.8	100.0	23.0
Planted Control	1.0	25.9	73.1	100.0 ↓	24.5 ↑
Planted Frankia	0.5	22.4	62.4	85.3 ↓	27.2 ↑

Summary

- in greenhouse trials, *Frankia*-inoculated alders grew better in tailings sands (biomass, root development), and had a positive impact on indigenous soil microorganisms (higher population densities, greater hydrocarbon degradation activity)
- field trials on tailings sands were initiated in 2005 using *Frankia*-inoculated (actinorhizal) and *Hebeloma*-inoculated (ectomycorrhizal) green alders (*A. crispa*).
- *Frankia*-inoculated alders showed comparable results to greenhouse trials (increase in microbial degradation activity, plant biomass) after more than 2.5 years in the field.
- field trials showed improved soil quality characteristics after only one year, demonstrating the promise of the technology.
- analysis of field plants after 2.5 years currently in progress.

Frankia-inoculated, after 2.5 years



Control plant



Frankia-inoculated alder (A. crispa)



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The Syncrude logo consists of the word "Syncrude" in a bold, red, sans-serif font. A white maple leaf is integrated into the letter 'o'. The logo is set against a light green rectangular background.