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Plant growth and arbuscular mycorrhizae development in oil sands processing by-products

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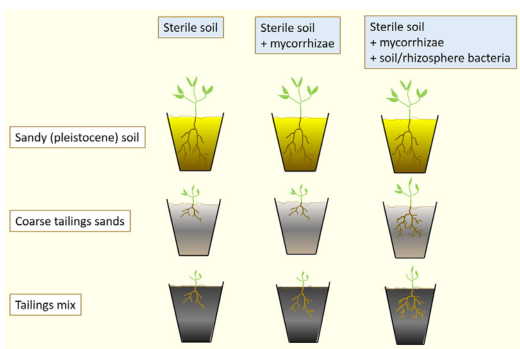
HIGHLIGHTS

- Plant growth was reduced in oil sands processing by-product substrates.
- AMF colonization of *Elymus trachycaulus* was not influenced by soil hydrocarbons.
- AMF colonization of *Lotus corniculatus* without bacteria decreased in coarse tailings.
- Mycorrhizae plus bacteria inoculation improved plant growth in coarse tailings sand.
- Soil bacteria increased root branching in coarse tailings sand and tailings mix.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil pollutants such as hydrocarbons can induce toxic effects in plants and associated arbuscular mycorrhizal fungi (AMF). This study was conducted to evaluate if the legume *Lotus corniculatus* and the grass *Elymus trachycaulus* and arbuscular mycorrhizal fungi could grow in two oil sands processing by-products after bitumen extraction from the oil sands in northern Alberta, Canada. Substrate treatments were coarse tailings sand (CTS), a mix of dry mature fine tailings (MFT) with CTS (1:1) and Pleistocene sandy soil (hydrocarbon free); microbial treatments were without AMF, with AMF and AMF plus soil bacteria isolated from oil sands reclamation sites. Plant biomass, root morphology, leaf water content, shoot tissue phosphorus content and mycorrhizal colonization were evaluated. Both plant species had reduced growth in CTS and tailings mix relative to sandy soil. AMF frequency and intensity in roots of *E. trachycaulus* was not influenced by soil hydrocarbons; however, it decreased significantly over time in roots of *L. corniculatus* without bacteria in CTS. Mycorrhizal inoculation alone did not significantly improve plant growth in CTS and tailings mix; however, inoculation with mycorrhizae plus bacteria led to a significantly positive response of both plant species in CTS. Thus, combined inoculation with selected mycorrhizae and bacteria led to synergistic effects. Such combinations may be used in future to improve plant growth in reclamation of CTS and tailings mix.

1. Introduction

Soil and biotic factors, such as those induced from soil pollutants like heavy metals, hydrocarbons and other industrial chemicals, influence

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plant physiological and ecological responses to environmental stress (Orcutt and Nilsen, 2000). Bitumen extraction from the oil sands in northern Alberta, Canada, results in large areas of disturbed land and large amounts of by-products, known as tailings. Tailings contain a mixture of water, clay, un-recovered bitumen, solvent and dissolved chemicals, including some organic compounds (Natural Resources Canada, 2013). The presence of naphthenic acids in oil sands tailings has led to environmental and industrial concerns, as they are known to be toxic to aquatic organisms, algae and mammals (Mohamed et al., 2008).

Tailings are stored in large ponds similar to water dams. The water released from the ponds can be recycled and reused in oil sands processing, however the majority has historically remained as mud and has to be stored long term. Due to settling processes in tailing ponds, different fractions of tailings including coarse tailings sands (CTS) and mature fine tailings (MFT) can be obtained. Those materials still contain residual hydrocarbons (Lefrancois et al., 2010). These hydrocarbons can increase stress levels for plants (Alkio et al., 2005). Dried MFT (processed MFT treated with a flocculant for dewatering) contains significantly more polycyclic aromatic hydrocarbons than coarse tailings sands due to enrichment during the consolidation process (Noah et al., 2014). Both materials are currently used for land reclamation; however, knowledge of effects of these materials on plant growth, especially herbaceous pioneer plants, is sparse. Environmental challenges associated with oil sands mining continue to require improved reclamation activities to address environmental sustainability and the reconstruction of the disturbed landscapes.

Various studies were carried out on reclamation oil sands sites using organic materials such as peat mineral mix and forest floor as a cover (Brown and Naeth, 2014; Jamro et al., 2014; Kwak et al., 2015). Studies of Adam and Duncan (1999) and Olson and Fletcher (2000) showed that Bermuda grass (*Cynodon dactylon* L. (Pers.)), sunflower (*Helianthus annuus* L.), southern crabgrass (*Digitaria ciliaris* (Retz.) Koeler) and red clover (*Trifolium pratense* L.) are hydrocarbon tolerant plants (Kaimi et al., 2007). Thus grasses and legumes could be useful for reclaiming hydrocarbon polluted soils, although, less is known about the direct interactions of plants and oil sands processing by-products.

Residual hydrocarbons in soil can influence plant development by inducing phytotoxic effects from volatile compounds and hydrophobicity (Kaimi et al., 2007; Alejandro-Cordova et al., 2017). Volatile hydrocarbons can easily move through cell membranes, causing toxic effects (Adam and Duncan, 2002). Hydrophobicity of oil contaminated soils can prevent water infiltration and aeration, which may disrupt plant-water relations and plant metabolism (Racine, 1994; Kaimi et al., 2007), reducing plant and shoot biomass and increasing carbohydrate contents of plants (Rahbar et al., 2012). High concentrations of hydrocarbons in soil can inhibit and limit microbial community biodiversity (Alejandro-Cordova et al., 2017), such as arbuscular mycorrhizal fungi. Over 80% of land plants live in a mutualistic relationship with AMF (Smith and Read, 2010) and many of them are obligate dependents on their symbiotic AMF partner. These fungi are known to improve plant growth and vitality by enhancing nutrient supply and by increasing the tolerance to abiotic and biotic stresses (Clark and Zeto, 2000; Turnau and Haselwandter, 2002). Some oil sands processing by-products may negatively influence AMF development and plant establishment due to their high content and number of hydrocarbons. Gaspar et al. (2002), Rabie (2005) and Wu et al. (2009) found that polycyclic aromatic compounds (PAH) negatively affected AMF spore germination, extraradical hyphae elongation, root colonization and sporulation, and thus may impact successful and sustainable plant development. Mycorrhizal fungi and bacteria in the rhizosphere can synergistically interact, through surface attachment and intimate and obligatory symbiosis (Perotto and Bonfante, 1997). This synergism may be important in promoting plant growth and health in hydrocarbon containing soils, since bacteria such as plant growth promoting rhizobacteria can support plant growth directly by releasing phytohormones and antimicrobial compounds against pathogens (Perotto and Bonfante, 1997) or indirectly by stimulating the relationship between the host plant and mycorrhizal fungi (Hrynkiewicz and Baum, 2011).

A greenhouse study was conducted to determine plant response to two substrates of oil sands processing by-products in combination with different microbial treatments. We hypothesized that AMF would survive in oil sands processing by-products, that microbial additions would stimulate mycorrhization, and that AMF plus bacteria inoculation would have the most positive effect on plant growth. Grasses and legumes with their very different root systems may have different relationships with AMF and bacteria in oil sands processing by-products. Thus, the legume *L. corniculatus* and the grass *E. trachycaulus,* commonly used species for reclamation of other materials in Alberta, were investigated. Such information is important for utilization of substrates and cover materials containing residual hydrocarbons in reclamation.

2. Material and methods

2.1. Substrate materials

Two oil sands processing by-products, coarse tailings sand (CTS) and dry mature fine tailings (MFT), were provided by Shell Canada in Fort McMurray (Alberta, Canada). A Pleistocene, calcareous, sandy overburden substrate (sandy soil) was retrieved from the forefield of the open cast lignite mining area at Welzow-Süd in Germany to serve as a reference material with physical properties similar to those of CTS, but without the influence of oil sands derived hydrocarbons. A detailed substrate characterization for petroleum hydrocarbons and oil sands derived biomarkers was conducted by Noah et al. (2014); oil sands derived hopanes, steranes, monoaromatic and triaromatic steroids, PAHs and dissolved inorganic and organic ions in pore water were analyzed in all MFT and CTS from reclamation sites. In dried MFT mean concentration of hopanes and tricyclic terpanes (aliphatic hydrocarbon fraction) was 1182 ng/g sediment and for CTS 474 ng/g sediment. Aromatic hydrocarbons were significantly higher in dried MFT (365–1646 ng/g sediment) then in CTS (below the detection limit of 97 ng/g sediment). Mean chloride and sulfate concentrations were higher in dried MFT (chloride 10–300 mmol L^{-1}; sulfate 1–100 mmol L^{-1}) than in CTS (chloride 0.01–0.1 mmol L^{-1}; sulfate 1.4–22 mmol L^{-1}). For dried MFT mean sodium concentration (20–300 mmol L^{-1}) was higher than for CTS (0.4–50 mmol L^{-1}).

Sandy soil and CTS were air dried and sieved (<2 mm fraction). MFT was air dried, and ground to powder with a crusher, allowing for thorough mixing with CTS. MFT was applied as a mixture with CTS (1:1) (hereafter tailings mix), to reduce its toxic and hydrophobic properties. Thus there were three substrates: pure CTS, tailings mix (CTS plus MFT) and pure sandy soil. All substrates were sterilized by gamma radiation (50 to 90 kGy for 24 h, Synergy Health GmbH, Radeberg, Germany) to eliminate indigenous soil microorganisms.

2.2. Microbial materials

Three AMF species were used in equal proportions as fungal inoculum for the two plant species. *Funneliformis mosseae* (former *Glomus mosseae*), *Rhizophagus intraradices* (former *Glomus intraradices*) and *Claroideoglomus etunicatum* (former *Glomus etunicatum*). Inocula were purchased as granules including spores and fungal hyphae (200 propagules per gram granules; pathogen and bacteria free) from Aurea Systems GmbH, Neumarkt, Germany.

The bacterial suspension was composed of 18 bacterial strains which were potential hydrocarbon degraders or typical soil and rhizosphere bacteria (Table 1). All strains were isolated from oil sands derived by-products such as processed mature fine tailings (dried mature fine tailings) and from bulk soil of tailings sands from a reclamation site in the Athabasca oil sands mining site, Canada (Noah et al., 2014). Bacteria were grown in tryptone soy medium until late log phase. Cells were