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# Early colonization of root associated fungal communities on reclamation substrates at a diamond mine in the Canadian Sub-Arctic

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ABSTRACT

Mining sites are harsh environments and establishment of root associated symbiotic fungi may be crucial for plant establishment and long term community development. Diamond mining in the Northwest Territories produces large amounts of processed kimberlite, and in some cases lake bed sediment and reclamation is needed for re-establishment of ecosystem function. This study investigated early fungal colonization with arbuscular mycorrhizae and dark septate endophytes on common reclamation substrates of different ages, relative to native tundra. Natural colonization of vegetation free sites with mycorrhizal spores on a trajectory associated with substrate age and type was very low. Fungal spore quantity and diversity was significantly accelerated by establishment of vegetation. Dark septate endophytes dominated native site Cyperaceae whereas reclamation site grasses were dominated by arbuscular mycorrhizae. Topsoil amendment was most effective for fungal colonization on reclamation substrates suggesting that a single application of topsoil can have a long term effect on the soil fungal community.



## 1. Introduction

Plants growing in undisturbed, natural soils are typically associated with a variety of endorhizal fungi (Ormsby et al., 2007; Walker et al., 2010), suggesting an important ecological role of those symbiotic associations for ecosystem development and stability (Soka and Ritchie, 2014). Symbiotic associations between plant roots and soil fungi are complex interactions occurring worldwide. Relatively little is known about these associations in sub-arctic regions and especially about re-establishment of symbiotic communities in regions where mining activities have led to larges disturbances.

Mining produces large amounts of waste materials. Anthropogenic disturbances in the sub-arctic cause long term changes in tundra landscapes (Kwiatkowski, 2007; Truett and Kertell, 1992) and reclamation is needed for re-establishment of ecosystem function. Inherent limiting environmental factors, combined with the inhospitable nature of mining substrates and their exposed surface prone to continued physical disturbance through processes such as erosion, provide challenging conditions for plant establishment. Establishment of a functioning soil biological community including mycorrhizal associations may be a key factor for successful plant growth on reclamation sites.

Arbuscular mycorrhizae are present in approximately 80% of all vascular plant species on earth, making them the most abundant and widespread form of mycorrhizal symbiosis (Smith and Read, 2008). These fungi have a large impact on soil related processes such as weathering, mineralization and soil structure development (Abiala et al., 2014; Jayachandran et al., 1992; Rillig and Mummey, 2006). They improve plant nutrition and plant health (Smith and Read, 2008), especially under poor soil conditions, making them an important component of many terrestrial ecosystems (Dickie et al., 2013).

Two other types of root colonizing fungi, the dark septate endophytes and the fine endophytes, are known as endorhizal symbiotic partners with plant roots, although their role in supporting plant growth and ecosystem function is not well understood (Mandyam and Jumpponen, 2008). Both can occur with arbuscular mycorrhizae in the same plant (e.g. Kauppinen et al., 2014). Dark septate endophytes are primarily a group of ascomycetes all belonging to the fungal endophyte group of Nonclavicipitaceous class 4 endophytes (Rodriguez et al., 2009); they are anamorphic fungi that form symbiotic relationships with

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plant roots and are sometimes capable of forming mutualistic interactions similar to mycorrhizae (Jumpponen, 2001). Fine endophytes are assigned to arbuscular mycorrhizal fungi, although the taxonomic position remains questionable because their colonization structures are different from other genera included in *Glomales* (Thippayarugs et al., 1999). Arbuscular mycorrhizal fungi and dark septate endophytes are ubiquitous in many terrestrial ecosystems (Mandyam and Jumpponen, 2008; Smith and Read, 2008). Dark septate endophytes and fine endophytes have an increased presence in cold, droughty and nutrient poor environments (Frenot et al., 2005; Olsson et al., 2004; Robinson, 2001; Wu, 2009; Walker et al., 2010), suggesting they are significant components of extreme and stressed environments (Barrow and Osuna, 2002; Jumpponen, 2001). Such extreme environments include arctic, subarctic, boreal, alpine, savannas and dunes, which are characterized by extreme temperature ranges, low soil fertility, drought, intensive solar radiation and short growing seasons (arctic zones). Those environmental characteristics, and that natural tundra vegetation consists largely of ericoid shrubs, mean that ericoid mycorrhizae play an essential role in Ericaceae dominated ecosystems (Gardes and Dahlberg, 1996). Seed for reclamation from plant dwarf shrubs and sedges that dominate natural heath tundra is extremely difficult to acquire; thus native grass species have historically been used for northern reclamation.

Research on occurrence, distribution and development of mycorrhizal fungal communities in the Canadian sub-arctic are rare, and to our knowledge there are no publications addressing root colonizing fungal community development on reclamation sites. Assessing the root fungal community of native, heath dominated vegetation relative to that of grass dominated reclamation sites can provide information on whether surrounding native sites could be a natural source of root colonizing fungi for land reclamation. Thus understanding the natural establishment and development of root colonizing fungal communities on reclamation sites is necessary to improve reclamation practices, in that natural invasion of fungal communities may not be sufficient and further reclamation amendments or mycorrhizal inoculants may be necessary for successful reclamation.

The objectives of this study were to investigate temporal development of root associated fungal communities on reclamation sites with lake bed sediment and processed kimberlite as reclamation substrates, to assess the effect of reclamation soil amendments on fungal establishment of septate endophytes and arbuscular mycorrhizal fungi, and to compare their occurrence on reclamation sites with those of a native site. We hypothesized that the number of arbuscular mycorrhizal fungal spores in the reclamation substrate would increase with age of unvegetated sites by natural spore invasion. Reclamation grass species would hypothetically increase occurrence of root associated fungi in both substrates. Topsoil amendment would hypothetically increase the amount and intensity of root colonizing fungi in the substrate and plant roots relative to fertilizer.

## 2. Material and methods

### 2.1. Study site description

Four sampling sites were located at the Diavik Diamond Mine (64°24′46″N 110◦16′24″W) on East Island, Lac de Gras, 320 km northeast of Yellowknife in the Northwest Territories, Canada. Sites are in the continuous permafrost zone, 200 km south of the arctic circle. Three sampling sites were reclamation study areas for other research (Drozdowski et al., 2012; Naeth and Wilkinson, 2014) differing in reclamation substrates, age since reclamation and soil amendment. The fourth sampling site was undisturbed native tundra, approximately 7 km south east of the reclamation research sites, representing native soil and vegetation.

Reclamation sites were one, three and ten years old, with relatively homogeneous substrate material (Drozdowski et al., 2012). Substrates were processed kimberlite, the material from which diamonds were removed; and lake bed sediment, removed during mining. Substrates were divided into areas with and without amendments of inorganic fertilizer (11:52:0, ammonium: phosphorus) or salvaged topsoil added in summer 2005 and 2006. The three and ten year old sites were divided into areas with vegetation (seeded and natural colonization) and without vegetation; the one year old site was without vegetation.

The natural, undisturbed site was dominated by common herbaceous species of the family Cyperaceae, *Carex* (sedge) and *Eriophorum* (cotton grass). They were closely surrounded by low growing shrub species including *Vaccinium vitis idaea* (lingon berry), *Empetrum nigrum* (crow berry), *Loiseleuria procumbens* (alpine azalea), *Arctostaphylos rubra* (red bear berry) and *Ledum decumbens* (dwarf labrador tea).

The reclamation sites lacked common native vegetation, especially shrubs, since the ten year old sites were seeded to grasses (family Poaceae) (Drozdowski et al., 2012). *Poa glauca* (glaucous blue grass), *Festuca saximontana* (rocky mountain fescue), *Calamagrostis canadensis* (blue joint grass) and *Arctagrostis latifolia* (polar grass) naturally colonized the three year old sites. The ten year old sites were densely populated with *Agropyron trachycaulum* (slender wheat grass), *Calamagrostis canadensis* and *Arctagrostis* latifolia.

### 2.2. Soil sampling

Sampling sites consisted of approximately 300 $m^2$ areas for each reclamation treatment. Native, undisturbed sampling sites (natural soil) consisted of grass or shrub dominated sections. Three year old reclamation sites were on two substrates (processed kimberlite, lake bed sediment), with and without two amendments (fertilizer, topsoil), with and without grass vegetation. Ten year old reclamation sites were on two substrates (processed kimberlite, lake bed sediment), with and without two amendments (fertilizer, topsoil), with and without grass vegetation. One year old reclamation sites had no amendment and no vegetation. An adjacent native topsoil stockpile (with soil salvaged from a natural site before mining activity occurred), less than one year old, was included for sampling.

Soil samples were taken in areas with and without plants to assess the influence of plants on fungal soil colonization. Sampling was conducted on June 18, 2014. Samples of 200 $cm^3$ were taken with a dutch auger from the upper 15 cm of the soil; samples with roots were taken directly under the plants. Six samples were taken from each treatment or location. Samples were stored in plastic bags in a cooler, transported to the laboratory and stored at 4 °C until processing. Each sample was run through a 2 mm sieve then a 1 mm sieve to separate roots and soil. Roots were collected from the sieve with forceps. After sieving, the volume of each soil sample was determined using a measuring cup. Soil samples were then air dried in paper bags in a greenhouse for 2 weeks.

### 2.3. Root staining and assessment of fungal colonization

To evaluate fungal root colonization, roots were processed and stained according to the method of Vierheilig et al. (1998). Roots were boiled in 10% potassium hydroxide solution for 5 min, rinsed twice with tap water and placed for 5 min in a 5% solution of ink and acetic acid. From each sample, 40 randomly selected pieces of root were cut to 1 cm lengths and placed side by side on two microscope slides, each slide loaded with 20 of the 1 cm segments