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MICROBIAL COMMUNITIES IN BOREAL PEATLANDS OF THE ATHABASKA REGION, CANADA: BUILDING A REFERENCE FOR FEN CREATION

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SUMMARY

Microbial communities were investigated in fen peat in a greenhouse experiment, and in three different peatlands of the Athabaska region, in the North of Alberta, where oil sand extraction is currently a major disturbance to the landscape. More specifically, the composition and diversity of archaea, fungi and bacteria were investigated using molecular approaches. It was hypothesized that the diversity and composition would vary as plant composition and redox potential would change. The results will serve as a reference database for the monitoring of microbial communities in the peat, following the upcoming large-scale creation of a fen in post-mined areas.

KEYWORDS: Fen, fungi, microbial diversity, oil sands

INTRODUCTION

Open-pit oil sand mining affects large tracks of land in Northern Alberta where peatlands are a major ecosystem type, with wooded fens (20-30%) being the dominant class, and saline fens also being common (Vitt et al. 1996). Peatlands are widely recognized for their capacity to sequester carbon, a consequence of the positive imbalance between primary productivity and decomposition. They can also be large sources of methane, the end product of anaerobic decomposition. However, in the sub-humid boreal climate landscape of this region, they play an instrumental role in the retention and movement of water. Because of the particular properties of the peat, peatlands stay frozen longer, they regulate water storage and discharge (Price et al. 2005), and they remain wet throughout the year and have lower rates of evaporation than other wetlands (Mitsch and Gosselink 2000), consequently increasing water storage across the landscape. There is no doubt that peatlands should thus be targeted by reclamation efforts following mine closure in the Oil Sand area. However, the selection of an appropriate reference system to be targeted by fen reclamation requires an understanding of the range and variability of the hydrological and ecological characteristics typical of the various peatland types of the region. This includes the above- and belowground composition and diversity of organisms and their role in peatland functioning.

Soil microorganisms are involved in many key biogeochemical processes like organic matter decomposition: nutrient cycling and detoxification, and they are determinant in plant productivity (Van Der Heijden, Bardgett and Van Straalen 2008). In fens, many plant species allocate a lot of resources to the creation of below-ground biomass, which eventually forms the majority of the peat (Chimner, Cooper and Parton 2002), as it is decomposed by the microbial communities. On the other hand, plants also impact on the microbial community and can override the influence of site-specific environmental conditions in some cases (Thormann, Currah and Bayley 2004, Trinder, Johnson and Artz 2009).

In the context of Oil Sand reclamation, restoring the C-sequestration functions poses another challenge: the presence of salts and organic acids (naphthenic acids) in the groundwater could affect the recovery of vegetation and microorganisms. The contaminants from oil sand processed water (OSPW) can increase hydrocarbon-degrading bacteria while reducing overall diversity (Hadwin et al. 2006), thereby disrupting the tight interactions among the microbial consortia. Changes in the availability of electron acceptors (e.g. SO_4) could stimulate CH_4 production or foster the co-activity of methanogens or other archaea with anaerobic bacteria (Yavitt 2003) or inhibit it as a consequence of direct competition for electron acceptors between sulphate reducers and methanogens (Abram and Nedwell 1978).

To gain a better understanding of the influence of vegetation and redox potential on microbial communities, we performed a preliminary study in greenhouses using peat from a poor fen and contrasting vegetation types. We also sampled and analysed peat from different peatland types of the Athabaska region. It was hypothesized that the diversity and composition of the microbial community would vary between peatland types in response to the redox conditions, and within peatland types in response to vegetation changes.

METHODS

Fen peat was put into large containers in a greenhouse and planted with propagules from 1) *Campylium stellatum, Aulacomnium palustre, Tomentypnum nitens and Limprichtia cossonii* (*Scorpidium cossonii*) and will be referred hereafter as the "Mosses" community; 2) *Calamagrostis stricta;* or 3) none (control). Three replicate composite peat samples were collected at three different moments in the growing season in 2010. Then, twice during the summer 2011, three replicate peat samples were taken in hummocks (or ridges) and hollows (or pools) of three different peatlands of the Athabaska region covering a large gradient of minerotrophy and salinity (Table 1). Once collected, all samples were immediately sent to the James Hutton Institute where they were kept frozen until they were analysed.

DNA was extracted from 300 mg of peat from each sample using the FastDNA® spin kit for Soil (MP bio) and following the manufacturer's instruction. DNA extraction was verified with electrophoresis on agarose gel. The DNA was then amplified with polymerase chain reaction (PCR) with ITS, 16S rRNA, arch primers. PCR amplicons were verified on agarose gel. PCR products were then purified using the Wizard® SV gel and PCR clean-up system, following manufacturer's instruction. The DNA concentration of the clean PCR products was measured

Table 1. Water chemistry (mean and standard deviation) and dominant vegetation characteristics of the three natural fens in hummocks/hollows (Poor fen, Rich treed fen) or ridges/pools (Saline fen) where samples have been taken during the summer 2011. EC = Electrical conductivity, ppt = parts per thousand. For water chemistry, n= 67 (Poor fen), 29 (Rich Treed fen) and 36 (Saline fen).

Site description	**Poor fen**	**Rich treed fen**	**Saline fen**
pH	5.6 (0.7)	7.0 (0.2)	6.4 (0.3)
EC (µS)	121 (107)	440 (144)	26105 (16335)
Salt (ppt)	0.05 (0.07)	0.2 (0.1)	16.7 (10.4)
Dominant vegetation on hummock (ridges)	*Sphagnum magellanicum* *Andromeda polifolia* *Chamaedaphneae calyculata* *Ledum groendlandicum*	*Tomenthypnum nitens* *Larix laricina* *Betula pumila* *Salix sp.*	*Calamagrostis inexpensa* *Hordeum jubatum* *Hierochloe odorata*
Dominant vegetation in hollow (pools)	*Sphagnum angustifolium* *Carex aquatilis*	*Equisetum palustre* *Maianthemum trifolium* *Campylium stellatum*	*Triglochin maritima* *Juncus balticus* *Atriplex prostata*

using spectrophotometer (NanoDrop system) and digested with restriction enzyme, and 2 µl of each sample was mixed with 0.3 µl of LIZ labeled GS500 (-250) internal size standard and 12 µl of formamide, denaturated at 95°C for 5 minutes and then chilled on ice for 5 min. Fragment size analysis was carried out with ABI PRISM 3130xl genetic analyzer. The TRF profiles were produced using Genemapper (4.0) software. We estimated diversity (Shannon's H) and richness for each taxonomic group (Fungi, Bacteria and Archaea) and for the whole community.

We used permutational analyses of variances (Anderson 2001) using the function *Adonis* in the package vegan in R (R development team 2011) to compare microbial communities between plant or peatland types, and ANOVAs to compare diversity and richness between plant or peatland types. We also used ordinations to further explore the relationships between microbial communities and other variables.

RESULTS

Fungi were the only microbial group that was significantly influenced by the above ground community ($F= 1.10$, $p=0.02$, see also Figure 1). There was no effect of above ground plant community on the TRF profiles of bacteria or archaeas. The diversity and the richness did not vary between treatments or over time for any microbial group, contrarily to what had been hypothesized.

The analyses for the natural samples are not completed at this stage, but we have observed differences in rates of methane production measured between sites (Strack, personal communication) and large differences in water chemistry (table 1) that could be related to differences in archaeal populations, and in particular methanogens. Moreover, we anticipate contrasts in fungal profiles because the dominant vegetation types are functionally different with respect to their mycorrhizal status: one site is dominated by ericaceous shrubs which form association with ericoid fungi, another is dominated by ecto-mycorrhizal trees, and the last one is dominated by grasses known to form arbuscular mycorrhiza.

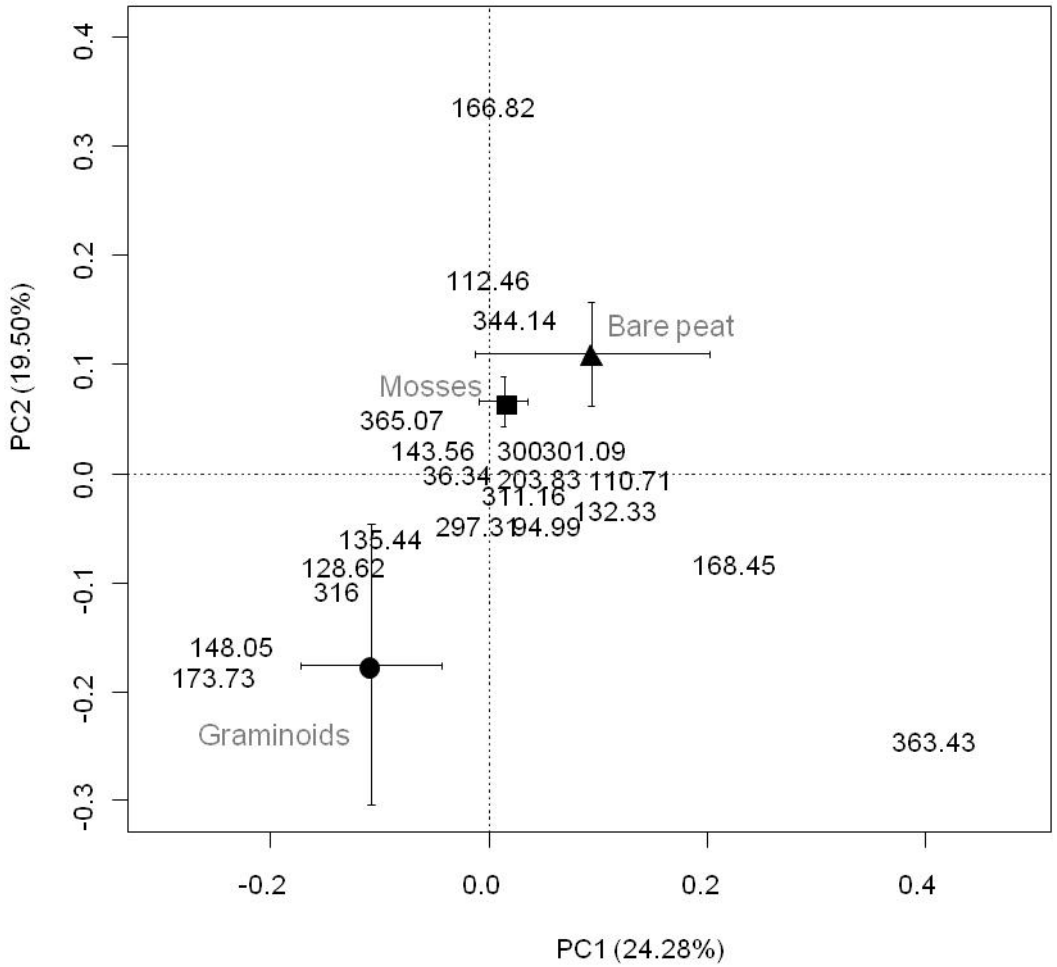

Figure 1. Ordination biplot showing the first two axes of a Principal Component Analysis (PCA) on dominant (present in more than 10% of the sample) fungal Terminal Restriction Fragments (TRF) extracted from fen peat in the greenhouse. Treatments are shown as centroids with standard error.

DISCUSSION AND CONCLUSION

Other studies have previously shown that root exudates can modulate the growth of fungi or bacteria that colonize the rhizosphere by altering the chemistry of soil in the area of the plant roots and by serving as selective growth substrate for soil microorganisms (Orwin et al. 2010) and support this observation. However, even the response of fungi was not very strong, with only a small number of TRF displaying a small preference for a particular vegetation type. Indeed, while a few TRF were dominant across all samples, most TRF occurred in a small number of replicates or accounted for a small (<1%) proportion of the community.

It has been shown that pH (e.g. Fierer and Jackson, 2006) and fluctuations in water table level are important regulators of microbial community structure. Those two variables were controlled in the greenhouses and variations were minimal between treatments, which may have had a homogenizing impact on the microbial community structure. It is possible that the sampling, limited to surface peat was not optimal for archaeas, which are more often found in the anaerobic

horizon. Another important factor structuring microbial communities the redox potential (e.g. Artz 2009): this is likely to be different between graminoids with extensive root systems and mosses, but this difference may not be detected in the upper horizons. Finally, the absence of observed effect does not mean that the microbial communities are not different. Microbial activity is strongly influenced by carbon quality and nutrient availability, which vary between plant and litter types; however DNA-based approaches do not discriminate between active and inactive portion of the community. Therefore, while the same organisms may be present, they may be dormant in one case and active in another: those differences could be examined with physiological approaches, or molecular approaches targeting RNA.

While this remains a largely observational study, the results will serve as a reference database for the monitoring of microbial communities in the peat, following the upcoming large-scale creation of a fen in post-mined areas. By integrating belowground processes and microbial community in monitoring of created peatlands, we will gain a better of capacity to predict how contamination by oil sand processed water, rich in sulphate and salt, could impact the greenhouse gas emissions. A better knowledge of reference peatlands will also help understanding how changes in microbial structure are related to changes in ecosystem function (e.g. production of methane, litter decomposition) and how this could feedback on plant establishment, growth and survival in the created fen.

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