Short-term exposure to Oil Sand Process-Affected Water does not reduce microbial potential activity in three contrasting peatland types

Daté, Vinay; Nwaishi, Felix C.; Price, Jonathan S.; Andersen, Roxane

Published in:
Wetlands

Publication date:
2018

Short-term exposure to Oil Sand Process-Affected Water does not reduce microbial potential activity in three contrasting peatland types

Vinay Date[1], Felix C. Nwaishi[1], Jonathan S. Price[1], Roxane Andersen[1,2]

[1]Department of Geography and Environmental Management, University of Waterloo, 200 University Avenue West, Waterloo, ON, N2L 1R9, Canada

[2] Environmental Research Institute, University of the Highlands and Islands, Castle St., Thurso, KW147JD

Abstract

Reclamation of sites affected by oil sands mining in the Athabasca Oil Sands Region (AOSR) targets the construction of new fen watersheds, which are dominant wetland types in the region. The aquifers of slopes that supply water to the fen watershed are formed with tailings sands containing residual oil sands process-affected water (OSPW) contaminants, whose effects on peat microbial community function are poorly explored. To understand the effect of potential OSPW contamination on microbial communities typical to the range of peatlands in the AOSR, we measured microbial functional characteristics (overall substrate-induced respiration (SIR) and catabolic evenness) and tested the effect of short-term in-vitro exposure to OSPW in peat samples from three representative fen types (treed rich fen, poor fen, and hypersaline fen) within the AOSR at the start (early May) and middle (late June) of the growing season. Overall, our results suggest that short-term exposure to OSPW has negligible impact on peat aerobic microbial activity, and that time of growing season and site physicochemical characteristics are the primary control on microbial potential activity. Further studies are necessary to assess the effects of OSPW contaminants on microbial-driven processes in the medium and long terms, under anaerobic conditions, which dominate in peatlands.

Keywords: Fens, microbial potential respiration, peatland reclamation, Alberta oil sands, MicroResp™

1.0 Introduction

Peatlands store about one-third of the world's terrestrial carbon, a disproportionately large fraction compared to their land area (Tarnocai 1999; Blodau 2002; Limpens et al 2008). This carbon-storage capacity is a result of the imbalance between photosynthetic carbon uptake and net losses, which principally comprises respiration by micro-organisms (Clymo 1984). Any disturbance which changes this imbalance (*e.g*. by inducing greater microbial respiration or by suppressing photosynthesis) could cause the disturbed peatland to change from a net sink to a source of carbon to the atmosphere (Kim et al. 2012; Yavitt et al. 1987). Therefore, understanding the controls and feedback between microbial community structure, activity, and environmental conditions in northern peatlands would be valuable. Aside from direct impacts from industrial activity, the ongoing concerns about rising concentration of atmospheric CO_2 and its effect on global warming makes greater understanding of the controls on carbon release in the world's largest terrestrial carbon sinks more important than ever.

Microbes are responsible for the majority of carbon and nutrient cycling in soil, and are therefore indispensable to the function of any ecosystem, including peatlands (Van Der Heijden et al 2008). Soil microbial communities can also potentially shape the plant community of their ecosystem, by mediating the cycling and availability of nutrients (Lamers et al 2012; Lin et al 2012; Myers et al 2012; Bragazza et al 2015). Microbial activity and dominant microbial processes vary with ecosystem type, land use and other environmental and edaphic factors. The effect of changes in one or more of these factors on microbial community structure and function has been fairly well explored. However, how changes in microbial function feedbacks on the soil system and other ecosystem processes are less well understood, especially in peatlands (Andersen et al. 2013; Classen et al. 2015). In fact, little is known about how microbial re-mineralization activity varies

with peat botanical composition and properties, surface vegetation, physicochemical characteristics, and especially contamination (Bardgett et al. 2008).

The Athabasca Oil Sands region (AOSR) of Alberta, Canada, is pertinent to discussions both of peatland microbial responses to changes and of peatland carbon cycling in general - the area is dominated by wetland environments (>50% of the terrestrial surface area), 95% of which are minerotrophic peatlands, or fens (Vitt et al 1996). The AOSR is also the site of extensive bitumen mining, expected to cover an area of 1400 km^2 by 2023 (Alberta Government 1999). Part of the bitumen deposit is extracted from these sites via open-pit mining, which completely removes the surface vegetation and the underlying peat, and thus severely disrupts the ecosystem functions of these sites, including carbon accumulation functions (Turetsky et al 2002; Johnson and Miyanishi 2008; Rooney and Bayley 2012). Previous studies have indicated that severely disrupted peatlands have limited ability to regenerate either vegetation structure or microbial community function without intervention (Andersen et al 2010; Elliott et al 2015). However, the Alberta government's land use regulations require reclamation of leased sites to a state of 'equivalent land capability' (Alberta Government 2000). Given that this should include the recovery of functional soil activity – which in peatlands includes carbon accumulation functions – one of the goals of ongoing experimental reclamation efforts is to understand the role of microbial community in the carbon cycling processes across the range of natural peatlands in the oil sands region.

Re-habilitation of the vegetation community does not guarantee restoration of microbial function. Studies have shown that microbial community structure and functions lagged behind recovery of vegetation composition in restored cutover peatlands, compared to natural regional reference systems (Andersen et al., 2006). Furthermore, the disruptions to bitumen mining sites are severe enough to require site reconstruction rather than restoration efforts, and even less is known about

72 microbial activity recovery in these constructed systems - at the moment, only two constructed
73 peatlands exist within the Athabasca Oil Sands region (Ketcheson et al 2016). Nevertheless, to be
74 accountable for their land reclamation strategies, the energy industries will require a means of
75 determining whether constructed peatland sites have achieved functions, including microbial
76 activity, equivalent to their natural counterparts. There is evidence that the source of the donor
77 peat used in the creation of a constructed peatland system influences the trajectory of development
78 of peatland functions (including microbial activity) in that system (Nwaishi et al 2015). It is
79 therefore necessary, in order to assess the development of a constructed peatland, to acquire that
80 information from reference sites encompassing the range of peatland types that might serve as peat
81 donors, for use as a reference baseline.

82 The development of microbial activity in constructed peatlands may be further altered by the
83 presence of contaminants; as such sites can contain traces of oil sands process-affected water
84 (OSPW) from tailing materials used in constructing upland landscapes that supply water to
85 constructed fen watersheds. OSPW contains elevated levels of both salt (especially sodium, Na^+)
86 and naphthenic acids (NAs), which have both been shown in mesocosm experiments to affect plant
87 communities (Pouliot et al. 2012) and microbial community activity (Degens et al 2001) alike.
88 One study observed a decrease in microbial catabolic diversity in peat samples exposed to OSPW,
89 although there was a delay between exposure and the onset of deleterious effects (Rezanezhad et
90 al. 2012). It is therefore possible that the microbial activities of these constructed fens, even given
91 full recovery of the peat microbial community, would exhibit some differences relative to their
92 undisturbed state. As contaminants would likely enter the peatland through runoff from the tailings
93 sand-containing upland landscapes, they would enter the peat column through infiltration.
94 Consequently, any influence the contaminants might exert would first be evident in aerobic

microbial community activity. Understanding how microbial communities in reference peatlands respond to OSPW addition will be useful to contextualize observations in constructed sites.

The objectives of this study were therefore twofold – 1) to characterize the aerobic microbial functional diversity of reference fen types representative of the Athabasca oil sands region at the beginning and the middle of the growing season, and 2) to assess the impact of in-vitro addition of OSPW on these functions. We hypothesized that 1) microbial activity would vary between sites as a function of their unique vegetation and biogeochemistry, and 2) that the in-vitro addition of OSPW would generally lead to a reduction in microbial potential activity in all samples.

2 Materials and methods

2.1 Site descriptions

Field sampling for this study was conducted in three natural peatlands located within the Athabasca Oil Sands Region. The region's climate is characterized as a boreal continental climate, which entails long, cold winters and short summers, resulting in a mean annual temperature of 1°C and a mean annual precipitation of 418.6 mm, based on data collected from 1981-2010 (Environment Canada, 2015). These peatlands were chosen as sampling sites, as they encompassed a gradient of vegetation types and physicochemical regimes that represent the range of fen peatlands in northern Alberta. As such, they could be used as reference baselines for the state of microbial activity in pristine peatlands and to gauge possible responses of reclaimed peatlands to contaminant addition. These sites comprised : 1) A treed moderate-rich fen (TRF) located 20 km northwest of Ft. McMurray, characterized by vegetation survey as containing treed poor fen and treed rich fen ecosite phases (Beckingham et al 1996). Sampling was conducted in the latter, whose vegetation

is dominated by *Larix laricina, Betula glandulosa, Equisetum fluvatile, Smilacina trifoliata, Carex prairea, Carex diandra,* and *Stellaria longipe*. The moss layer included *Tomenthypnum nitens, Campyllium stellatum and Hylocomnium* splendens ; 2) a hypersaline fen (Saline), located 10 km south of Ft. McMurray, characterized as containing shrubby rich fen and graminoid rich fen ecosite phases. Sampling was conducted in the marsh grass fen community phase, where the peat and groundwater contain very high concentrations of NaCl, and whose vegetation is dominated by *Calamagrostis inexpansa*, *Carex tenax*, and *Hordeum jubatum*. The sparse moss layer included *Campyllium stellatum*; 3) a poor fen (PF) located 40 km south of Ft. McMurray, characterized as containing treed poor fen and shrubby poor fen ecosite phases. Sampling was conducted in the latter, where vegetation is dominated by *Picea mariana*, *Carex aquatilis*, and *Chamaedaphne calyculata*. The moss layer was dominated by *Sphagnum angustifolium* and *Sphagnum magellanicum*.

2.2 Sampling

Five replicated composite samples were extracted by hand at each site (PF, Saline, TRF). For each composite sample, five 5 cm^2 x 15 cm deep cores were taken around pre-existing randomly distributed collars, used for measuring greenhouse gases in a different study. The five cores thus sampled were homogenized in the field to make a single composite sample, then sealed in a Ziploc bag and stored at 4°C for transport from field to the laboratory. Replicate sampling locations were more than 100 m away from each other and can therefore be considered independent. Two sets of samples were taken for the study: one at the start of the growing season (early May 2014) and one in the middle of the growing season (late June 2014).

2.3 Measurement of substrate-induced respiration with MicroResp™

To evaluate catabolic activity, the MicroResp™ method described in Campbell et al (2003) adapted for peat as per Artz et al (2006) was used, with changes noted below. Briefly, in the MicroResp method, a 96-well deep well (2 mL volume per well) microplate is prepared with 0.3g of peat individually weighed into each well, to which a solution containing a single carbon source is added. Aerobic respiration is measured spectrophotometrically using a detection plate capturing the CO_2 emitted by the incubation plate. The detection plate wells contain a pH indicator (cresol red) in agar gel, which changes colour in response to dissolution of CO_2 into the gel and its subsequent conversion to carbonic acid. Absorbance of the detection microplate is measured at 570 nm (A570), before and after the 6-hour incubation period of the assay, and the change in absorption used to calculate CO_2 production. Briefly, the A570 values are normalised by dividing the A570 data by the A570 data at time 0 and multiplying by the mean of the A570 reading at time 0, before being converted into $\%CO_2$ using calibration curves. The CO_2 rate is then calculated by converting the % CO2 to µg/g/h CO2-C using gas constants and constants for headspace volume (vol) in the well (µl), fresh weight of soil per well (g), incubation time (h) and soil sample % dry weight (Campbell et al. 2003).

The carbon sources used in this experiment fell into three functional groups: amino acids (comprising l-alanine, arginine, l-cysteine-HCl, and l-lysine), carboxylic acids (comprising α-ketoglutaric acid, citric acid, γ-aminobutyric acid, L-malic acid, and oxalic acid,) and saccharides (comprising l-arabinose, d-fructose, d-glucose, N-acetylglucosamine, and trehalose). All these carbon sources were made in solution in two variants – one using Milli-Q water as a solvent, one using OSPW as a solvent.

Each deepwell plate contained peat from a single composite sampling point, and each well received a single carbon source in 25 µL of solvent. Within each deepwell plate each carbon source - treatment combination was applied in triplicate (e.g. three wells per deepwell plate received it). All carbon source solutions were made to 300 mg/mL of the respective carbon source, or to saturation, for those carbon sources whose maximum solubility was below 300 mg/mL.

The OSPW used was taken from a tailings pond on the Suncor lease, near Fort McMurray, Alberta, and had Na^+ concentration of 1331 mg L^{-1}. Due to technical difficulties, the concentration of naphthenic acids in the OSPW solution was not measured (Rubi Simhayov, personal communication). However, a previous studies analyzing the composition of undiluted OSPW from a pond on the Syncrude lease near Fort McMurray found it to have an electrical conductivity of 2370 µS cm^{-1} and a naphthenic acids concentration of 22.7 mg L^{-1} (Kavanagh et al 2009). Another analysis of OSPW content from mining operations north of Fort McMurray found the naphthenic acid concentration of OSPW samples taken within a single date to vary between 24.4 and 35.3 mg L^{-1} (Frank et al 2016). OSPW also contains other contaminants, including assorted dissolved carbon compounds (NAs included) of 50 – 100 mg L^{-1}, and other inorganic contaminants including sulfate (200 – 300 mg L^{-1}) and ammonia (14 mg L^{-1}). Finally, OSPW is known to be basic (pH 8.0 – 8.4) and alkaline (800 – 1000 mg L^{-1} HCO_3^-) (Allen 2008).

2.4 Statistical methods

All data were subjected to a normality test before use in statistical analysis; where data were found to be non-normal they were transformed as appropriate to improve homoscedasticity. All statistical analyses were performed in R (R Development Core Team, 2013) with packages and functions used as noted below.

Site microbial potential activity was quantified as average well colour development (AWCD), or the mean substrate-induced respiration (SIR) response across all substrates in a site. Catabolic evenness, or the uniformity of substrate use, was quantified by the inverse Simpson-Yule Diversity Index. Both microbial potential activity and catabolic evenness were compared between treatments on each reference fen at each sampling date using a nested analysis of variance (ANOVA) using the function 'aov' in the R core package. Significant differences in the overall carbon utilization profiles of these sites within a given sampling date and contaminant treatment were tested using non-parametric permutational analysis of variance (PERMANOVA) under the function 'adonis' in the package 'vegan' (Oksanen et al 2015). Differences in substrate-specific SIR response between sites were analyzed by nested MANOVA, using the function 'manova' in the package 'stats,' and post-hoc difference tests. Significant differences between sites across sampling dates were determined using ANOVA and post-hoc difference tests using the functions 'TukeyHSD' in the 'stats' core package and 'multcompLetters4' in the package 'multcompView' (Graves et al 2015).

3 Results

3.1 Variability in site overall respiration response

Sampling date significantly affected both microbial potential activity and catabolic evenness, as did site within a sampling date (Table 2). The effects of these factors on overall carbon utilization patterns (as determined by permutational multivariate ANOVA) were very similar to the responses of AWCD: sampling date and peatland type within sampling date had a significant effect on pattern of carbon utilization, while treatment (OSPW or control) within peatland type within sampling date did not (Table 2).

The microbial potential activity (Figure 1a) of the PF and Saline microbial communities did not differ significantly from one another, and the microbial potential activity of both communities was significantly greater than that of the TRF site. Additionally, the microbial potential activity of the PF and Saline sites decreased significantly ($p_{PF}<1.0 \times 10^{-7}$, $p_{Saline}<1.0 \times 10^{-7}$) between the start of season and midseason sampling dates, while no such difference was observed at the TRF site ($p_{TRF} = 0.943$).

In contrast, the catabolic evenness (Figure 1b) of all three field sites was similar at the start of the growing season ($p_{TRF-PF}= 0.165$, $p_{TRF-Saline}= 0.997$, $p_{PF-Saline}=0.386$), but was greater in the TRF and Saline sites than in the the PF site at midseason, while not significantly differing between TRF and PF. ($p_{TRF-PF} < 8.0 \times 10^{-7}$, $p_{TRF-Saline}= 0.726$, $p_{PF-Saline}=0.0004$). Differences in catabolic evenness were not consistent between dates: the catabolic evenness decreased significantly over time in PF, increased significantly in Saline, and did not vary significantly between start of season and midseason in TRF.

Substrate-specific SIR (Figure 2) was significantly greater at the start of season in Saline than in PF for four substrates - alanine ($p = 0.0071$), d-fructose ($p < 1.0 \times 10^{-7}$), d-glucose ($p = 1.0 \times 10^{-6}$), n-acetylglucosamine ($p = 0.0014$), and trehalose ($p = 2.4 \times 10^{-6}$). In both PF and Saline, SIR was significantly higher at start of season than at midseason for all carbon sources. In contrast, SIR in TRF samples did not differ significantly between sampling dates in response to any carbon source except for oxalic acid ($p = 0.015$), which caused higher SIR at the start of the growing season. Start of growing season SIR was significantly lower in TRF samples than in PF and Saline samples for all carbon sources except for oxalic acid, where SIR did not significantly differ between ($p_{TRF-PF} = 0.993$, $p_{TRF-Saline} = 0.999$). In contrast, midseason SIR generally did not differ significantly between sites for C sources. Notable exceptions were citric acid, where it was

significantly higher in TRF than Saline samples ($p_{TRF\text{-}Saline} = 0.0044$), and α-ketoglutaric acid ($p_{TRF\text{-}PF} = 0.030$, $p_{TRF\text{-}Saline} = 0.0041$) and oxalic acid ($p_{TRF\text{-}PF} = 0.0037$, $p_{TRF\text{-}Saline} = 7.1 \times 10^{-5}$), for which SIR was significantly higher in TRF than in PF or Saline samples.

3.2 Effect of OSPW contamination on microbial community function

Contrary to our hypothesis, the addition of OSPW did not significantly influence either the community microbial potential activity (AWCD) ($F=0.67$, $p=0.68$) or community catabolic evenness ($F=0.27$, $p=0.95$). Exposure to OSPW caused marginally significantly different SIR responses across sites and dates for four substrates: D-glucose ($F=3.46$, $p=0.003$), arabinose ($F=2.25$, $p=0.040$), D-fructose ($F=2.30$, $p=0.037$), and N-acetylglucosamine ($F=2.3226$, $p=0.03$), but these changes were not significant within a given site and sampling date (Figure 3).

4 Discussion

4.1 Microbial community functional diversity of reference fens

SIR responses to individual substrates generally followed the pattern set by overall site microbial potential activity, *i.e.* Saline and PF were not significantly different from one another, but were much higher than TRF, at start-of-season, while all three were roughly equal at midseason. However, the addition of three carbon sources (citric acid, α-ketoglutaric acid, and oxalic acid) led to significant increases in TRF SIR response for at least one sampling date. If this increase in SIR were a result of increased consumption due to influx of labile carbon in a carbon-limited system, the other substrates used should have provoked a similar response, which was not the case. If the increased SIR were due to a community preference for carboxylic acids, all the carboxylic acids used would have provoked such a reaction, which was likewise not the case. The increased

respiration observed is thus likely not principally due to respiration of of oxalic, citric, or α-ketoglutaric acids themselves. It seems more likely that some property common to the specific carboxylic acids in question allowed for greater respiration of labile carbon already present in the peat sample.

Both oxalic acid and citric acid are among the low molecular mass organic acids (LMMOA) which are thought to play a role in mobilization of soil micronutrients, nitrogen, and phosphorus (Clarholm et al. 2015; Dotaniya et al. 2014; Taghipour and Jalali 2013; Wei et al. 2010). Different LMMOA are most effective at mobilizing these nutrients in different environments, with citric acid being more effective in low-pH forest soils (Clarholm et al. 2015; Wei et al. 2010), while oxalic acid is more effective in higher-pH soils (Clarholm et al. 2015; Dotaniya et al. 2014; Seshadri et al. 2014; Taghipour and Jalali 2013). As OSPW contains ammonia (Allen 2008) but OSPW treatment had no significant effect on TRF SIR, nitrogen is unlikely to be the limiting nutrient in question. This suggests that phosphorus limitation or the limitation of some other organic acid-mobilizable nutrient may be responsible for the low start-of-season TRF microbial community activity. As none of the carboxylic acids used provoked a similar SIR spike in Saline and PF samples, these sites were likely not subject to either the same nutrient limitation.

A response shared across all sampling dates and sites was the low – occasionally negative – SIR response to arginine. Arginine catabolism by soil microbes is known to produce ammonium as a byproduct (Abdelal 1979), which, as a weak base, would likely dissolve and dissociate alongside any CO_2 produced, negating some of the colour change used in the assay and causing the observed unusually-low SIR values.

4.2 Impact of OSPW contamination on aerobic microbial community function

Contrary to the hypothesis, contamination with OSPW did not significantly decrease the overall microbial potential activity or catabolic evenness of any of the three reference sites. One possible explanation for this arises from the chemical properties of the peat soil. Peat contains abundant carboxyl, phenol, and alcohol functional groups because of the high carbon content of the soil and very slow decomposition of organic matter in the anaerobic part of the peat column. These functional groups have been shown to form chelation complexes that immobilize and limit the bioavailability of heavy metal ions (Clemente and Bernal 2006; Kumpiene et al. 2007; Lee et al. 2013). Studies of the movement of OSPW and NaCl through peat have shown that the amount of NaCl and NAs in OSPW adsorbed onto the peat in a contaminant uptake experiment was an order of magnitude higher than the amounts in the liquid phase once the OSPW had traveled through a 40 cm peat column (Rezanezhad et al. 2012). It is thus possible that some attenuation of potential toxic effects of the contaminants occurred through sorption of the contaminants to the peat substrate itself. Furthermore, this experiment only measured substrate-induced respiration, which is necessarily a short-term response – the duration of the assay is only 6 hours. Thus, any long-term toxic effects of OSPW would not have been detectable in the time frame of the experiment. Such effects could be better detected by amending peat samples with OSPW and pre-incubating for some days or weeks prior to the MicroResp assay.

Additionally, as this experiment gave very little insight into the link between microbial community structure and functional potential, integration of 16s rRNA sequencing experiments before and after exposure to OSPW would allow greater understanding of the role of microbial community structure in resistance to OSPW toxicity (Baldwin et al 2006; Sun et al 2014; Chambers et al 2016).

4.3 Seasonal change in carbon utilisation patterns

Almost all midseason PF and Saline samples displayed lower microbial potential activity than start-of-season samples. This was likely not due to peat temperature; while there is conflicting evidence as to the effect of temperature on soil respiration, with some studies showing that increased temperature can either increase (Bonnett et al 2006; Kurbatova et al 2013; Wang et al 2015), decrease (Bradford et al 2008), or not impact (Giardina and Ryan 2000; Luo et al 2001; Kirschbaum 2013) soil microbial respiration, the direct influence of temperature on soil respiration was minimized in our experiments, as all incubations were conducted at 24 - 25 °C in the lab rather than at field soil temperature. Any effect that temperature would have on these results would be indirect ones, e.g. via increased substrate availability (Eliasson et al 2005; Hartley et al 2007) associated with the deposition of fresh plant litter over the growing season. The observed decrease in microbial potential activity in the face of greater substrate availability (both through changes in peat chemical conditions and through the application of substrate as part of the SIR assay) suggests two possible explanations. First, that carbon is not the limiting resource at any of the sites at midseason, or second, that the midseason communities genuinely have diminished microbial activity potential, even without nutrient stress. Our results provide some evidence for the first explanation – start-of-season TRF respiration was more likely limited by nutrient availability rather than carbon availability, as discussed earlier in the section regarding LMMOA. Furthermore, of the three LMMOA that appeared to provoke greater TRF respiration than other substrates, the midseason SIR response to two of them (α-ketoglutaric acid and citric acid) was not significantly less at than at start of season and significantly greater than at least one of the other two sites. For the other, oxalic acid, while midseason TRF SIR was less than at start of season, it was still significantly greater than at the other two sites. In the case of the other two sites the issue of resource limitation is somewhat more unclear – the lack of response to amino acids suggests that

317 nitrogen is not limiting, and the lack of response to LMMOA suggests that phosphorus is likewise
318 not the limiting resource.

319 There is less direct evidence for the second explanation. However, decreases in microbial biomass
320 over the course of the growing season have been observed (Weedon et al 2012; Weedon et al 2013;
321 Wang et al 2015) and decreased microbial biomass would likely lead to similarly diminished
322 carbon cycling potential. This explanation could be tested by a repetition of this experiment
323 coupled with measurement of microbial biomass of each peat sample, and could be lengthened to
324 include a full year rather than only the growing season.
325

326 ## 5. Conclusions and Implications for Fen Reclamation projects in the AOSR

327 Both microbial functional diversity and aerobic microbial carbon cycling potential varied
328 significantly between reference sites, though the respiratory potential profiles of the Saline and PF
329 sites were largely similar. With respect to microbial functional diversity, the TRF site microbial
330 community evidenced a strong respiratory response to certain low molecular weight organic acids.
331 The strong preference for specific LMMOA indicates a potential organic-acid-mobilizable nutrient
332 limitation at the TRF site.

333 Contrary to expectation, addition of OSPW did not significantly reduce overall site carbon cycling
334 potential activity or catabolic evenness in any samples. Thus, the aerobic microbial potential
335 activity of communities from a range of different peatlands appears to be unaffected by OSPW in
336 the very short term. However, it is not certain that OSPW will have no effect on aerobic microbial
337 activity overall, as the duration of the assay period in this study was only six hours, which was
338 likely insufficient time for deleterious effects to make themselves known, given the ability of peat
339 to chemically retard the movement of metal ions and organic contaminants. Future studies on the

matter should include longer-term incubations to determine the detrimental effects of OSPW on microbial community function over timescales that better approximate the duration of contaminant exposure in reclaimed peatlands.

Our findings regarding patterns of microbial community activity response can be of immediate value in already-constructed reclamation sites as a 'baseline' against which the microbial community function of a developing constructed site can be compared. However, given the evident seasonality of carbon utilization patterns, if such a method was used for evaluating constructed against reference sites, all sites should be sampled at the same time for a valid comparison. Alongside monitoring of the edaphic and vegetation-related variables, this may allow managers of such sites to make informed predictions about the site's eventual successional trajectory.

Acknowledgements

Funding for this project was provided through an NSERC Collaborative Research and Development Grant (CRD), # 418557-2011, with support from Suncor Energy Inc., Shell Canada Ltd., Imperial Oil Resources Ltd. This initiative is a part of a Joint Industry Project convened under Canada's Oil Sands Innovation Alliance (COSIA). We would like to thank members of the Wetland Hydrology lab for support in the field and in the lab, in particular Corey Wells and James Sherwood. We thank the anonymous reviewers who have provided constructive comments, which have helped improve our MS.

List of figures

Figure 1 a) Microbial potential activity and b) catabolic evenness by field site and sampling date. Start-of-season samples are indicated by white bars while midseason samples are indicated by grey bars. Groups that share a letter are not significantly different

Figure 2 Subtrate-induced respiration profiles organized by carbon source functional group. White bars indicate start of growing season samples, grey bars indicate midseason. Samples within a carbon source that share a letter are not significantly different, as determined by Tukey post-hoc test

Figure 3 Substrate-induced respiration profiles of carbon sources varying significantly with treatment. White bars indicate start-of-season samples, grey bars indicate midseason. Crosshatched bars indicate treatment with OSPW, while bars without indicate control treatment. Samples within a carbon source that share a letter are not significantly different, as determined by Tukey post-hoc test

References

Abdelal AT (1979) Arginine catabolism by microorganisms. Annual review of microbiology 139–168.

Alberta Government (1999) Guidelines for reclamation to forest vegetation in the Alberta oil sands region. Conservation and reclamation information letter, C&R/IL/99-1.

Alberta Government (2000) Guideline for wetland establishment on reclaimed oil sands leases. i–iv.

Allen EW (2008) Process water treatment in Canada's oil sands industry: I. Target pollutants and

treatment objectives. Journal of Environmental Engineering and Science 7:123–138. doi: 10.1139/S07-038

Andersen R, Chapman SJ, Artz RRE (2013) Microbial communities in natural and disturbed peatlands: A review. Soil Biology and Biochemistry 57:979–994. doi: 10.1016/j.soilbio.2012.10.003

Andersen R, Francez AJ, Rochefort L (2006) The physicochemical and microbiological status of a restored bog in Québec: Identification of relevant criteria to monitor success. Soil Biology and Biochemistry 38:1375–1387. doi: 10.1016/j.soilbio.2005.10.012

Andersen R, Grasset L, Thormann MN, et al (2010) Changes in microbial community structure and function following Sphagnum peatland restoration. Soil Biology and Biochemistry 42:291–301. doi: 10.1016/j.soilbio.2009.11.006

Artz RRE, Chapman SJ, Campbell CD (2006) Substrate utilisation profiles of microbial communities in peat are depth dependent and correlate with whole soil FTIR profiles. Soil Biology and Biochemistry 38:2958–2962. doi: 10.1016/j.soilbio.2006.04.017

Baldwin DS, Rees GN, Mitchell AM, et al (2006) The short-term effects of salinization on anaerobic nutrient cycling and microbial community structure in sediment from a freshwater wetland. Wetlands 26:455–464. doi: 10.1672/0277-5212(2006)26[455:TSEOSO]2.0.CO;2

Bardgett RD, Freeman C, Ostle NJ (2008) Microbial contributions to climate change through carbon cycle feedbacks. 805–814. doi: 10.1038/ismej.2008.58

Beckingham J., Archibald J., Corns IGW (1996) Field guide to ecosites of northern Alberta.

403 Natural Resources Canada, Northern Forestry Center, Edmonton, Alberta. Spec. Rep. 5. doi:
404 18769

405 Blodau C (2002) Carbon cycling in peatlands - A review of processes and controls.
406 Environmental Reviews 10:111–134. doi: 10.1139/a02-004

407 Bonnett SAF, Ostle N, Freeman C (2006) Seasonal variations in decomposition processes in a
408 valley-bottom riparian peatland. Science of the Total Environment 370:561–573. doi:
409 10.1016/j.scitotenv.2006.08.032

410 Bradford MA, Davies CA, Frey SD, et al (2008) Thermal adaptation of soil microbial respiration
411 to elevated temperature. Ecology Letters 11:1316–1327. doi: 10.1111/j.1461-
412 0248.2008.01251.x

413 Bragazza L, Bardgett RD, Mitchell EAD, Buttler A (2015) Linking soil microbial communities
414 to vascular plant abundance along a climate gradient. New Phytologist 205:1175–1182. doi:
415 10.1111/nph.13116

416 Campbell CD, Chapman SJ, Cameron CM, et al (2003) A rapid microtiter plate method to
417 measure carbon dioxide evolved from carbon substrate amendments so as to determine the
418 physiological profiles of soil microbial communities by using whole soil. Applied and
419 Environmental Microbiology 69:3593–3599. doi: 10.1128/AEM.69.6.3593

420 Chambers LG, Guevara R, Boyer JN, et al (2016) Effects of Salinity and Inundation on
421 Microbial Community Structure and Function in a Mangrove Peat Soil. Wetlands 36:361–
422 371. doi: 10.1007/s13157-016-0745-8

423 Clarholm M, Skyllberg U, Rosling A (2015) Organic acid induced release of nutrients from

metal-stabilized soil organic matter - The unbutton model. Soil Biology and Biochemistry 84:168–176. doi: 10.1016/j.soilbio.2015.02.019

Classen AT, Sundqvist MK, Henning JA, et al (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6:art130. doi: 10.1890/ES15-00217.1

Clemente R, Bernal MP (2006) Fractionation of heavy metals and distribution of organic carbon in two contaminated soils amended with humic acids. Chemosphere 64:1264–1273. doi: 10.1016/j.chemosphere.2005.12.058

Clymo RS (1984) The Limits to Peat Bog Growth. Philosophical Transactions of the Royal Society B: Biological Sciences 303:605–654. doi: 10.1098/rstb.1984.0002

Degens BP, Schipper LA, Sparling GP, Duncan LC (2001) Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biology and Biochemistry 33:1143–1153. doi: 10.1016/S0038-0717(01)00018-9

Dotaniya ML, Datta SC, Biswas DR, et al (2014) Production of Oxalic Acid as Influenced by the Application of Organic Residue and Its Effect on Phosphorus Uptake by Wheat (Triticum aestivum L.) in an Inceptisol of North India. National Academy Science Letters 37:401–405. doi: 10.1007/s40009-014-0254-3

Eliasson PE, McMurtrie RE, Pepper DA, et al (2005) The response of heterotrophic CO2 flux to soil warming. Global Change Biology 11:167–181. doi: 10.1111/j.1365-2486.2004.00878.x

Elliott DR, Caporn SJM, Nwaishi F, et al (2015) Bacterial and Fungal Communities in a Degraded Ombrotrophic Peatland Undergoing Natural and Managed Re- Vegetation. 1–20.

445 doi: 10.1371/journal.pone.0124726

446 Frank RA, Milestone CB, Rowland SJ, et al (2016) Assessing spatial and temporal variability of
447 acid-extractable organics in oil sands process-affected waters. Chemosphere 160:303–313.
448 doi: 10.1016/j.chemosphere.2016.06.093

449 Giardina CP, Ryan MG (2000) Evidence that decomposition rates of organic carbon in mineral
450 soil do not vary with temperature. Nature 404:858–861. doi: 10.1038/35009076

451 Graves S, Piepho H-P, Selzer L, Sundar D-R (2015) multcompView: Visualizations of Paired
452 Comparisons.

453 Hartley IP, Heinemeyer A, Ineson P (2007) Effects of three years of soil warming and shading
454 on the rate of soil respiration: Substrate availability and not thermal acclimation mediates
455 observed response. Global Change Biology 13:1761–1770. doi: 10.1111/j.1365-
456 2486.2007.01373.x

457 Johnson EA, Miyanishi K (2008) Creating New Landscapes and Ecosystems. Annals of the New
458 York Academy of Sciences 1134:120–145. doi: 10.1196/annals.1439.007

459 Kavanagh RJ, Burnison BK, Frank RA, et al (2009) Detecting oil sands process-affected waters
460 in the Alberta oil sands region using synchronous fluorescence spectroscopy. Chemosphere
461 76:120–126. doi: 10.1016/j.chemosphere.2009.02.007

462 Ketcheson SJ, Price JS, Carey SK, et al (2016) Constructing fen peatlands in post-mining oil
463 sands landscapes: challenges and opportunities from a hydrological perspective. Earth
464 Science Reviews. doi: 10.1017/CBO9781107415324.004

465 Kim SY, Freeman C, Fenner N, Kang H (2012) Functional and structural responses of bacterial

and methanogen communities to 3-year warming incubation in different depths of peat mire. Applied Soil Ecology 57:23–30. doi: 10.1016/j.apsoil.2012.02.015

Kirschbaum MUF (2013) Seasonal variations in the availability of labile substrate confound the temperature dependence of organic matter decomposition. Soil Biology and Biochemistry 57:568–576. doi: 10.1016/j.soilbio.2012.10.012

Kumpiene J, Lagerkvist A, Maurice C (2007) Stabilization of Pb- and Cu-contaminated soil using coal fly ash and peat. Environmental Pollution 145:365–373. doi: 10.1016/j.envpol.2006.01.037

Kurbatova J, Tatarinov F, Molchanov a, et al (2013) Partitioning of ecosystem respiration in a paludified shallow-peat spruce forest in the southern taiga of European Russia. Environmental Research Letters 8:45028. doi: 10.1088/1748-9326/8/4/045028

Lamers LPM, van Diggelen JMH, Op den Camp HJM, et al (2012) Microbial Transformations of Nitrogen, Sulfur, and Iron Dictate Vegetation Composition in Wetlands: A Review. Frontiers in Microbiology 3:156. doi: 10.3389/fmicb.2012.00156

Lee SJ, Lee ME, Chung JW, et al (2013) Immobilization of lead from Pb-contaminated soil amended with peat moss. Journal of Chemistry. doi: 10.1155/2013/509520

Limpens J, Berendse F, Blodau C, et al (2008) Peatlands and the carbon cycle : from local processes to global implications – a synthesis. 1475–1491.

Lin X, Green S, Tfaily MM, et al (2012) Microbial community structure and activity linked to contrasting biogeochemical gradients in bog and fen environments of the glacial lake agassiz peatland. Applied and Environmental Microbiology 78:7023–7031. doi:

10.1128/AEM.01750-12

Luo Y, Wan S, Hui D, Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prairie. Nature 413:622–625. doi: 10.1038/35098065

Myers B, Webster KL, Mclaughlin JW, Basiliko N (2012) Microbial activity across a boreal peatland nutrient gradient : the role of fungi and bacteria. 77–88. doi: 10.1007/s11273-011-9242-2

Nwaishi F, Petrone RM, Price JS, Andersen R (2015) Towards Developing a Functional-Based Approach for Constructed Peatlands Evaluation in the Alberta Oil Sands Region, Canada. Wetlands 35:211–225. doi: 10.1007/s13157-014-0623-1

Oksanen J, Blanchet FG, Kindt R, et al (2015) vegan: Community Ecology Package.

Pouliot R, Rochefort L, Graf MD (2012) Impacts of oil sands process water on fen plants : Implications for plant selection in required reclamation projects. Environmental Pollution 167:132–137. doi: 10.1016/j.envpol.2012.03.050

Raghoebarsing AA, Smolders AJP, Schmid MC, et al (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436:1153–1156. doi: 10.1038/nature03802

Rezanezhad F, Andersen R, Pouliot R, et al (2012) How fen vegetation structure affects the transport of oil sands process-affected waters. Wetlands 32:557–570. doi: 10.1007/s13157-012-0290-z

Rooney RC, Bayley SE (2012) Development and testing of an index of biotic integrity based on submersed and floating vegetation and its application to assess reclamation wetlands in Alberta’s oil sands area, Canada. Environmental Monitoring and Assessment 184:749–761.

508 doi: 10.1007/s10661-011-1999-5

509 Seshadri B, Kunhikrishnan A, Bolan N, Naidu R (2014) Effect of industrial waste products on
510 phosphorus mobilisation and biomass production in abattoir wastewater irrigated soil.
511 Environmental Science and Pollution Research 21:10013–10021. doi: 10.1007/s11356-014-
512 3030-5

513 Sun R, Xing D, Jia J, et al (2014) Methane production and microbial community structure for
514 alkaline pretreated waste activated sludge. Bioresource Technology 169:496–501. doi:
515 10.1016/j.biortech.2014.07.032

516 Taghipour M, Jalali M (2013) Effect of low-molecular-weight organic acids on kinetics release
517 and fractionation of phosphorus in some calcareous soils of western Iran. Environmental
518 Monitoring and Assessment 185:5471–5482. doi: 10.1007/s10661-012-2960-y

519 Tarnocai C (1999) The effect of climate warming on the carbon balance of Cryosols in Canada.
520 Permafrost and Periglacial Processes 10:251–263. doi: 10.1002/(SICI)1099-
521 1530(199907/09)10:3<251::AID-PPP323>3.0.CO;2-5

522 Turetsky M, Wieder K, Halsey L, Vitt D (2002) Current disturbance and the diminishing
523 peatland carbon sink. Geophysical Research Letters 29:7–10. doi: 10.1029/2001GL014000

524 Van Der Heijden MG a, Bardgett RD, Van Straalen NM (2008) The unseen majority: Soil
525 microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology
526 Letters 11:296–310. doi: 10.1111/j.1461-0248.2007.01139.x

527 Vitt DH, Halsey L, Thormann MN, Martin T (1996) Peatland Inventory of Alberta.

528 Wang Y, Bölter M, Chang Q, et al (2015) Driving factors of temporal variation in agricultural

soil respiration. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 65:589–604. doi: 10.1080/09064710.2015.1036305

Weedon JT, Aerts R, Kowalchuk GA, et al (2013) Temperature sensitivity of peatland C and N cycling: Does substrate supply play a role? Soil Biology and Biochemistry 61:109–120. doi: 10.1016/j.soilbio.2013.02.019

Weedon JT, Kowalchuk GA, Aerts R, et al (2012) Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. Global Change Biology 18:138–150. doi: 10.1111/j.1365-2486.2011.02548.x

Wei L, Chen C, Xu Z (2010) Citric acid enhances the mobilization of organic phosphorus in subtropical and tropical forest soils. Biology and Fertility of Soils 46:765–769. doi: 10.1007/s00374-010-0464-x

Yavitt JB, Lang GE, Wieder RK (1987) Control of carbon mineralization to CH4 and CO2 in anaerobic,Sphagnum-derived peat from Big Run Bog, West Virginia. Biogeochemistry 4:141–157. doi: 10.1007/BF02180152