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ARTICLE

How Fen Vegetation Structure Affects the Transport of Oil Sands Process-affected Waters

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Abstract Oil sands mining in the Athabasca oil sands region disturbs large tracts of peatlands as the vegetation-soil layer must be removed. Processing oil sands produces large volumes of wet material containing oil sand process-affected water (OSPW) that has elevated concentrations of sodium (Na) and naphthenic acids (NAs). Attempts to reclaim mined landscapes to peat-forming systems command knowledge of the transport, fate and impact of OSPW in organic soils. Four mesocosms placed in a greenhouse were randomly assigned with two treatments: 1) a moss carpet (*Bryum pseudotriquetrum*) and 2) graminoids (*Carex aquatilis* and *Calamagrostis stricta*). Transport of Na and NAs through peat was significantly delayed by sorption and diffusion in peat matrix. After two growing seasons of receiving OSPW, the graminoid plants continued to grow without showing stress from OSPW, while mosses showed a considerable decline in health. Microorganisms were more active under sedges than mosses and their activity varied over time either because of seasonal variation or as a consequence of variation in Na concentration. The findings of this study are limited due to the small number of replicates and the lack of a control, but represent a first step towards the creation of peatlands in the post-mined areas.

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Introduction

Vast tracts of land in the Athabasca region, located near Fort McMurray in northeastern Alberta, Canada, are being disturbed by oil sands mining. The total area deemed suitable for surface mining is approximately 2,500 km^2 and active mining is occurring on over 250 km^2 (Woynillowicz et al. 2005) and is expected to cover approximately 1,400 km^2 by 2023 (Alberta Environment 1999). Open pit mining removes the vegetation-soil layer to access the oil sands beneath, thereby completely disturbing the landscape on an unprecedented landscape level. After mining operations have ceased, oil companies are entrusted to reconstruct these landscapes to the best of their abilities. Peatlands are a dominant feature of this region and cover up to half (Vitt and Chee 1989), therefore, are greatly affected by oil sands mining.

The extraction of bitumen from oil sands (using an aqueous process) produces process affected tailings. These tailings contain sand, silts and clays in suspension, soluble organic chemicals (i.e. naphthenic acids and hydrocarbons), ammonia, heavy metals and salts (Bott 2007). Process-affected material that cannot be recycled is stored in settling basins, where further settling produces sand edges and mature fine tailings. These tailings are collectively are termed oil sand process-affected water (OSPW) (Bott 2007). The presence of chemicals in the OSPW (leaching from tailings) has a toxic effect on aquatic life and may adversely affect the ecology of the system being reclaimed, including peatlands. The presence of salts, heavy metals and organic acids (NAs) in the groundwater from OSPW present a potentially

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significant barrier to the establishment of peatland vegetation is. Given the recent impetus to reclaim post-mined landscape to fen peatlands (Price et al. 2010) and the use of peat in the soil layer during reclamation (Shurniak and Barbour 2002), determining the transport and fate of OSPW in peat soils and its impact on peatland plant communities is essential.

Many of the current environmental problems in the Athabasca oil sands region involve the leaching of chemicals associate with OSPW (Alberta Environmental Protection 1996), since they may be at concentrations toxic to aquatic plants and animals (Trites and Bayley 2005; Apostol et al. 2004). OSPW is considered toxic because of elevated concentration of sodium (Na) and naphthenic acids (NAs) (commonly referred to as naphthenic acids but strictly speaking are deprotonated naphthenate anions). Na salts and NAs are transported through porous geological media with little attenuation beyond mechanical dispersion (Gervais and Barker 2005). However, their movement in peat has received very little attention. Janfada et al. (2006) found NAs to be strongly adsorbed by organic matter and Na was also adsorbed by peat soils (Ho and McKay 2000; Montemayor 2006). However, even the transport of non-reactive anions (e.g. Cl^-) though peat can be slowed as a consequence of the dual-porosity structure (with interconnected and dead-end or closed pores) of peat (Hoag and Price 1997). Moreover, in the peat matrix, transport of reactive substances like NAs and Na is substantially retarded (Rezenezhad and Price in review).Currently, it is not known whether and to what extent peatland vegetation and microbial communities can tolerate exposure to OSPW. Both increasing salinity (Munns and Termaat 1986) and NAs (Kamaluddin and Zwiazek 2002) inhibit water uptake and can reduce plant growth. There is a concern that the combined effects of NAs and salinity may intensify plant water stress in reclamation areas (Apostol et al. 2004). Much research has been carried out on the response of boreal forest species (Renault et al. 1998, 1999, 2000, 2001; Apostol et al. 2004), as well as marsh species (Brendell-Young et al. 2000; Crowe et al. 2001; Crowe et al. 2002; Cooper 2004) to OSPW. However, little research has been carried out on peatland species (Trites and Bayley 2005) with none on peatland mosses, even though these plants occupy approximately half of the pre-mined landscape.

In both natural and reclaimed systems, microorganisms are instrumental to nutrient cycling and carbon turnover and feedback on plant productivity and ecosystem functioning (van der Heijden et al. 2008). Studies have shown that NAs can be at least partially degraded by soil microorganisms (Herman et al. 1994). Hadwin et al. (2005) found that exposure to NA levels slightly higher than naturally occurring in wetlands shifted the microbial community structure to one that is capable of metabolizing NAs. Thus, there may be some capacity for wetlands to degrade NAs from process-affected water (Toor et al. 2007). Nevertheless, whether the microbial population can remain functional during progressive contamination by OSPW or to what extent the microbial response is mediated by the vegetation type under which they are found has not been formally investigated.

In this study, greenhouse mesocosm experiments were run to determine how OSPW is transported through peat under a hydraulic regime driven by the evapotranspirative demand associated with moss or vascular plant cover and how these plants and microbial communities reacted to OSPW contamination. The main objectives were to characterize: (1) the movement of process-affected water in peat substrate, (2) survival and growth of common fen species native to the oil-sands region in a realistic greenhouse trials; and (3) the changes in microbial functional diversity in the contaminated peat under two fen vegetation structures.

Materials and Methods

Peat, Plants and OSPW

The peat used in this study was moderately decomposed rich-fen sedge-peat (pH=7.4) obtained from a fen on the Suncor Energy lease in the Athabasca oil sands region in Alberta, Canada and shipped to Université Laval for greenhouse experiments. The peat was highly disturbed by digging operations similar to its anticipated condition in reclaimed peatlands (Price et al. 2010) currently under construction at Suncor Energy Pilot Fen reclamation site. The mosses and graminoid plants used in this study were collected from rich fens approximately 50 km northwest of Fort McMurray, Alberta in October, 2008. The plants were stored at 4°C for approximately 3 months until the experiment began. The OSPW was obtained from the Suncor Energy oil sands operation in Fort McMurray, Alberta, with initial concentration of 525 mg l^{-1} of Na and 53 mg l^{-1} of NAs.

Experimental Design and Instrumentation

Four containers with dimension 120×80×76 cm were used in a randomized block design with two treatments: 1) pioneer invading fen mosses (*Bryum pseudotriquetrum* (Hedw.) G. Gaertn., B. Mey. & Scherb., *Dicranella cerviculata* (Hedw.) Schimp. and *Pohlia nutans* (Hedw.) Lindb.) and 2) graminoid plants (*Carex aquatilis* Wahlenb. and *Calamagrostis stricta* (Timm) Koeler) replicated two times. More replications were not possible due to the sheer volume of peat needed for each container (0.38 m^3). As the rich fen peat was not available locally, all peat was transported over 4,500 km from the oil sands region to the study greenhouse. A lower number of replications in very large containers were chosen because the purpose of the research was to study contaminant transport in a scale that is relative to field conditions.

The system was designed to create groundwater discharge from a 10 cm layer of sand at the base, which would move upward into 40 cm of overlying peat (Fig. 1). Solute was distributed into the sand layer at the base of the peat from a reservoir, through three evenly spaced parallel manifolds buried in the sand. One mesocosm for each treatment (one graminoid and one moss plant cover) was instrumented with TDR (time domain reflectometry), soil moisture sensors, tensiometers, suction-lysimeters, and pressure transducers. Moisture content was measured hourly with a Campbell Scientific TDR100 system with 30 cm CS-605 probes connected to a CR10x data logger. Four probes were installed horizontally into the peat at −5, −10, −15, and −25 cm depths below peat surface. The soil-water pressure (ψ) was measured weekly with tensiometers installed at the same depths as the TDR probes. Water samples (for NAs and Na analysis) were extracted (also at the same depths) with suction lysimeters installed vertically into the peat (Fig. 1). A 2-week sampling period was selected and a 70 ml sample was taken from each depth and stored at 4°C until they were transported to the University of Waterloo for Na and NAs analysis.

On the basis of the dielectric constant (K_a) data reported by the TDR system, the water content (θ) was computed using a calibration equation between water content and dielectric constant developed separately in the laboratory for the OSPW and peat used in this experiment. In the calibration procedure, the first K_a value was recorded at 100% saturation and then the TDR readings were taken at progressively lower water contents, determined gravimetrically, during the drainage process. The correlation $\theta = 0.000017K_a^3 - 0.0019K_a^2 + 0.073K_a - 0.054$ was found between the dielectric constant and volumetric water content with correlation coefficient of 0.96.

The plants were first given 2 months to establish before the contaminated water was introduced. Thereafter, the peat mesocosms were saturated slowly with rain water (collected in a cistern) rising from input manifolds at the base of the containers in several input steps, and then held at saturation for 24 h. The saturated containers were completely drained for 24 h and then OSPW, with initial ~385 mg l^{-1} of Na salts and ~40 mg l^{-1} of NAs, was introduced at the base of the mesocosm until a water table of −15 cm was achieved. Thereafter OSPW was pulled upward by evapotranspiration and the water level in the supply reservoir was reset every 2 days to −15 cm by filling it to the prescribed level with OSPW. Two pressure transducers were installed inside the inflow reservoirs to measure the amount of contaminant introduced to the mesocosms. The water table in the mesocosm was monitored every 15 min using pressure transducers installed into 2.5 cm dia. pvc wells that penetrated the peat. The experiment was carried out over two greenhouse growing seasons from February 2009 until June 2010 (growing conditions are summarized in Table 1). The first growing season was from February 4th to October 30th, 2009 and the second from January 26th to June 6th, 2010. Between the growing seasons, all vascular plants were cut and containers were placed outside the greenhouse to simulate a wintering period. During watering, valves connecting the inflow reservoir to the mesocosms were closed to prevent backflow into the reservoir. When watering regime was changed after 180 days, mosses were misted with a spray-bottle atomizer approximately three times per week to ensure a moist carpet and protect against the potential bias of moss desiccation vs. moss contamination.

All water samples were analyzed for total NAs using Fourier Transform Infrared spectroscopy (FTIR) developed

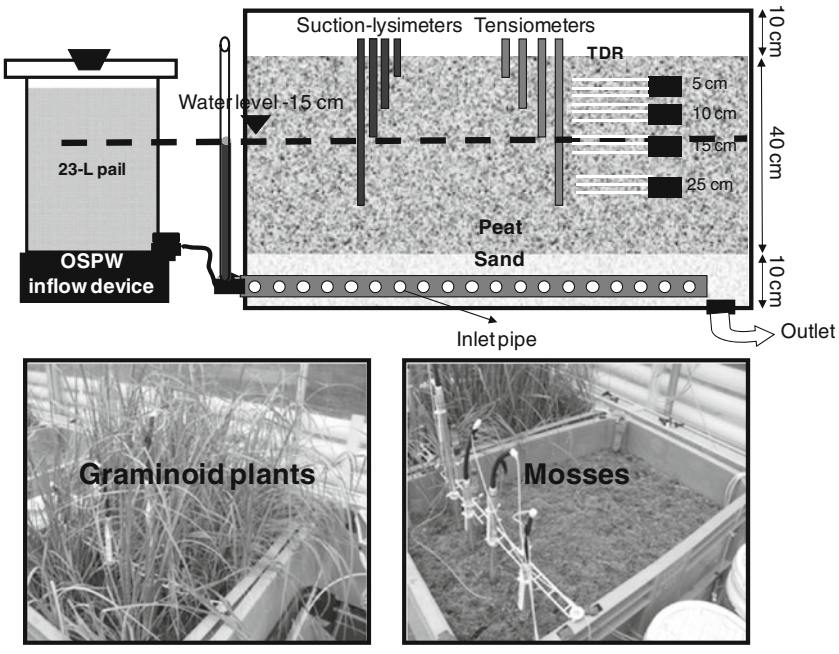

Fig. 1 Experiment design for moss and graminoid plant mesocosms and installed instruments

Table 1 Growing conditions during the experiment

Growing conditions		Duration (days)	Simulated precipitation (mm/months)	Photoperiod (hours)	Day/night temperature (°C)	Day/night humidity (%)
1st growing season	Establishment phase	70	40–70	16	19/10	50/60
	With contaminants	110	40–70	16	19/10	50/60
	With contaminants	90	10	16	19/10	50/60
	Winter period	90	–	0 (under snow)	−8[a]	67/75
2nd growing season	Pre-contamination phase	21	10	16	20/16	60/72
	With contaminants	115	10	16	20/16	60/72

[a] mean temperature for the wintering period (for Québec City, between November 1st 2009 and January 26th 2010)

by Jivraj et al. 1995; and for total Na using Perkin Elmer model 3100 atomic absorption spectroscopy. Naphthenic acids standards (in methylene chloride) were prepared using Merrichem Refined NAs and a Perkin Elmer Spectrum RX 1 FT-IR System was used to obtain the spectrum for NAs standards and samples with the method detection limit (MDL) 2.5 mg l^{-1}. The atomic absorption spectroscopy was calibrated using the standards 25, 50, 100 and 500 mg l^{-1} Na with a detection limit of 1 mg l^{-1}.

The liquid phase Na and NAs masses (M_{liquid}) were obtained based on liquid phase Na and NAs concentrations and the volume of solution available at each depth (volumetric water content × peat volume at each depth). Based on total amount of introduced OSPW, initial water content of the peat, liquid phase Na and NAs concentrations at each depth and the Freundlich adsorption isotherm coefficients obtained for Na and NAs (Rezanezhad et al. 2010), the mass of adsorbed Na and NAs on the peat was determined in the solid phase (M_{solid}) for both moss and graminoid plant mesocosms at the last day of experiment. The percentage of plant uptake (M_{uptake}) was calculated based on solid phase and liquid phase concentrations of Na and NAs at −5, −10, −15, and −25 cm depths by:

$$M_{uptake} = \left(1 - \frac{\sum M_{liquid} + \sum M_{solid}}{M_{input}}\right) \times 100 \quad (1)$$

where M_{input} is the total input concentration (mg) and the total liquid phase (ΣM_{liquid}) and solid phase (ΣM_{solid}) concentrations (mg) from four different sampled depths are defined as:

$$\sum M_{liquid} = M_{liquid}^{d=-5cm} + M_{liquid}^{d=-10cm} + M_{liquid}^{d=-15cm} + M_{liquid}^{d=-25cm}, \quad (2)$$

$$\sum M_{solid} = M_{solid}^{d=-5cm} + M_{solid}^{d=-10cm} + M_{solid}^{d=-15cm} + M_{solid}^{d=-25cm}, \quad (3)$$

At the end of the experiment, all instruments and plants were removed and the peat samples were taken at different depths to determine the peat bulk density, total porosity and chemical properties. Bulk density and total porosity of each peat sample were gravimetrically determined following the method of Boelter (1976) based on the original sample volume, the sample saturated mass and the oven-dry (at 80°C) mass.

Vegetation Assessment

Eight plant assessments were carried out over the two growing seasons. During the first growing season, the first assessment was carried on after 2 months of plant establishment and just before adding the OSPW contaminants. The others were 70, 140 and 200 days after start of introducing the contaminant to the mesocosms. In the second growing season, the first assessment was carried on 3 weeks after winter period and just before adding the contaminants. The others were 35, 80 and 115 days after first assessment.

During these assessments, a plant health index was noted by species. The plant health index ranged from 7 to 1: 7= 100% healthy (100% green), 6=1–10% dead (1–10% with yellow or brown leaves), 5=10–20% dead, 4=20–40% dead, 3=40–70% dead, 2=70–99% dead and 1=100% dead (100% with yellow or brown leaves). Percentage cover of the moss carpet was visually estimated within three quadrats (25×25 cm) placed randomly in each experimental unit during each assessment and results were pooled for each mesocosm for a given vegetation assessment. For the graminoid species, number of leaves per plant as well as length of their longest shoot was measured for each individual plant during the first growing season. Because of the greater number of shoots during the second growing season, these measurements were done only for 10% of individuals systematically chosen. For example, if 100 individuals were counted, measurements were done for ten individuals (the 10th, 20th, 30th, 40th, 50th, 60th, 70th, 80th, 90th and 100th). At each sampling period, 1–2 g of plant tissues of the above-ground phytomass were sampled and the K and

Na concentration in plant tissues were analyzed for moss, *Calamagrostis stricta* and *Carex aquatilis* in a composite sample for each vegetation assessment. Because of the limited number of replications and the lack of a control treatment (treatment where OSPW was applied), only descriptive statistics were used.

Microbial Functional Diversity

Three composite soil samples (total of 300 g) were taken from each tub in conjunction with the first four vegetation assessments (pre-contamination=day 0, days 70, 140 and 200 after contaminations during the first growing season). It is worth mentioning that pre-contamination sampling (day 0) was done after 70 days of vegetation establishment. The results obtained after the first season and the costs of the analyses led us to carry out the microbial evaluation for the second growing season only in a parallel experiment where controls (without vegetation and without contamination) were available.

The samples were sent along with a sample of OSPW to the Macaulay Land Use Research Institute (Aberdeen, UK) where they were analyzed within a week. For each sample, the moisture content was determined using subsamples of 3 g. The changes in microbial functional diversity were investigated by carbon utilisation profiles obtained using Micro-Resp™ (Campbell et al. 2003). The system is set up with a "detection" microplate, containing 150 μl purified agar (1%), cresol red indicator dye (12.5 μg ml^{-1}), potassium chloride (150 mM) and sodium bicarbonate (2.5 mM), attached to a 1.2 ml deepwell plate (96 wells), containing soil(s) and a selection of carbon sources, with a rubber gasket and a seal formed when clamped together. For each sample, one 96-well plate was prepared with 0.30 g of peat individually weighed in each well (Artz et al. 2006). The deep-well plates were covered with parafilm and incubated at 25° for 3 days prior to exposure to C sources. Then two sets of 16 carbon sources were added to the peat in the deep-well plates in triplicates: one set was prepared with de-ionised water according to the manufacturer's instruction, and the other set was prepared in OSPW. The carbon sources used were l-alanine, l-arabinose, arginine, γ amino-butyric acid, l-cysteine–HCl, citric acid, d-fructose, d-galactose, d-glucose, α ketoglutaric acid, l-lysine, l-malic acid, *N*-acetyl glucosamine, oxalic acid, acid and trehalose (all Sigma Aldrich, UK). Those compounds are common rhizoexudates and have been successfully used in other study targeting microbial communities in peatlands (e.g. Artz et al. 2007). In addition, there was a control without any C source (water) and a control with OSPW only. Detection plates were read in a spectrophotometer at absorbance wavelength 570 nm and then placed onto a MicroResp™ seal on top of each 96-well plate containing the peat and C sources. The systems were incubated for 6 h at 25°C, following which the detection plate was removed and read again in the spectrophotometer. The CO_2 production rates were then calculated from the difference between absorbances at times 6 h and 0 h.

While the number of true replicates per treatment (2) was still too low to use conventional statistical approaches, we tested the effect of time and types of plants on carbon utilisation profiles with permutational multivariate analysis of variance (MANOVA, Anderson 2001), a robust approach suited for small number of samples. We used the function "adonis" from the "vegan" package (Oksanen et al. 2006) in R (R core development team, 2010). The CO_2 production rates were log transformed prior to statistical analysis to reduce heteroscedasticity. The variability in carbon utilisation profiles was investigated by principal component analysis (PCA). The effect of time and types of plants on carbon utilisation profiles were then tested with permutational multivariate analysis of variance (MANOVA) using distance matrices with the function "adonis" from the "vegan" package (Oksanen et al. 2006) in R (R core development team, 2010). Catabolic evenness, the variability of substrate use across the range of substrates used, was estimated as $E = 1/\sum p^2{}_i$ where p_i is the respiration response to individual substrates as a proportion of total respiration activity induced by all substrates for a soil (Magurran 1988; Degens et al. 2000) and tested using permutational non-parametric ANOVAs. For both the MANOVA and the ANOVA, the permutations were restricted to account for the design (repeated measures and repetitions within mesocosms).

Results

Hydrology

The water table depth varied over the duration of the experiment according to the evapotranspiration rate, watering regime and OSPW discharge to the mesocosm. The mean depth to water table was −7.4±3.2 cm in the moss mesocosm and −20.8±7.5 cm in the graminoid plant mesocosm for the first growing season and −15.9±2.9 and −22.2± 6 cm, respectively, in the second growing season (Fig. 2). The water table under the moss carpet was closer to the surface than with the graminoid plant cover. The average amount of OSPW introduced to the mesocosms (i.e. to reset the supply reservoirs to the desired −15 cm level as described in the methods section) is a measure of the evapotranspiration loss. This was approximately 3 l per week to the moss mesocosm and 15 l per week to the graminoid plant mesocosm in the first 5 months of first growing season, then increased to approximately 9 and 25 l per week

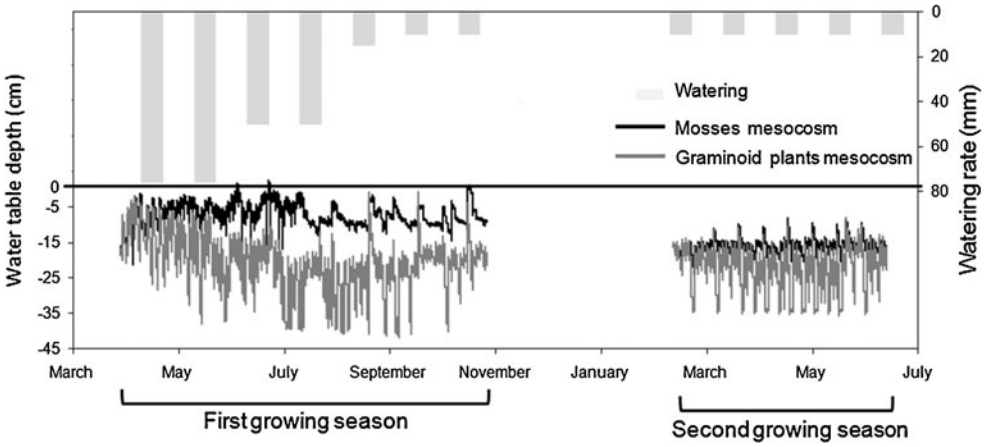

Fig. 2 Water table depth (cm) for mosses and graminoid plant mesocosms with the watering rate (mm) throughout the first (after introducing OSPW) and second growing seasons

for all other months, respectively, corresponding to the reduced watering rate (Table 1 and Fig. 2) (i.e. more OSPW was needed to reset the water table to the desired −15 cm elevation). The corresponding evaporation rates for the moss and vascular plant mesocosms (calculated based on the amount of OSPW needed to reset the supply reservoirs to −15 cm level and the irrigation water rate) were approximately 2.54 and 4.72 mm per day during the initial phase, respectively, and approximately 1.94 and 4.97 mm per day, respectively during the second phase. The total OSPW used in the moss and graminoid treatments was ~415 l and ~1,120 l, respectively.

The measured bulk density of the peat ranged approximately 0.12 to 0.15 gcm^{-3} in both moss and graminoid plant treatments. The average total porosity was 81% to 84% in both treatments. Average water content in the moss and graminoid plant mesocosms at −5 cm was ~0.55 and ~0.50 cm^3 cm^{-3} and increased to 0.75 and 0.76 cm^3 cm^{-3} at −25 cm depth, respectively (Fig. 3). The higher Na concentration in the second growing season (see below) attenuated the TDR signal (McIsaac 2010) resulting in loss of θ data.

In the moss mesocosm, the increase in mass of Na and NAs in the liquid phase (M_{liquid}) was sequentially delayed at higher elevations in the profile (Figs. 4a and 5a). At −25 cm, ~34% of total introduced mass (M_{input}) of Na salts (164.6 g) and ~40% of total NAs (17.7 g) was adsorbed on peat after two growing seasons of receiving OSPW. At −15 cm, −10 cm and −5 cm, the adsorption rate was 20.5%, 19.5% and 17.5% for Na and ~34%, ~11% and ~8% for NAs, respectively (Figs. 4a and 5a). However, in the graminoid plant mesocosm after ~6 months of receiving contaminated water the liquid Na concentration at the −5 cm and −10 cm level matched that of the layer below it, as Na accumulated in the upper layers as evapotranspiration removed the water and left the solute in the soil. After two growing seasons of receiving OSPW with a total input (M_{input}) Na mass of 461 g and NAs mass of 71.3 g, the final Na concentration of the −5 cm and −10 cm layer in the graminoid plant mesocosm exceeded that of the −15 and −25 cm layer (Fig. 4b). The plant uptake (M_{uptake}) was calculated using Eqs. 1, 2 and 3 as 1.45% of Na and 3.06% of NAs in mosses and 0.65% of Na and 2.1% of NAs in graminoid plants (% of total introduced mass of Na and NAs).

Vegetation

After the first growing season, neither mosses not graminoid plants showed signs of poor health beyond normal senescence by the end of the growing season (graminoid plants only). After the second growing period, graminoid plants still were in good health, however, mosses showed a considerable decline in their health towards the end of the experiment (Fig. 6).

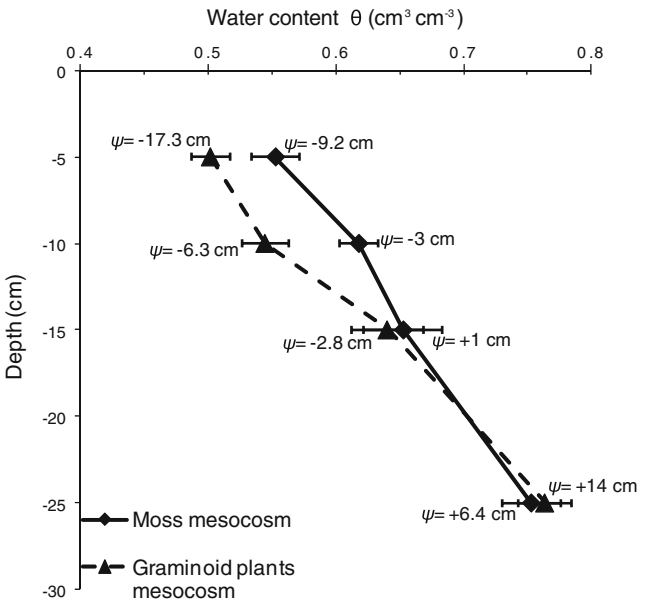

Fig. 3 Volumetric water content (θ) at four depths when the water table was approximately −15 cm in the moss and −18 cm in the graminoid plant mesocosms. ψ represents the water pressure measured using the tensiometers installed at different depths

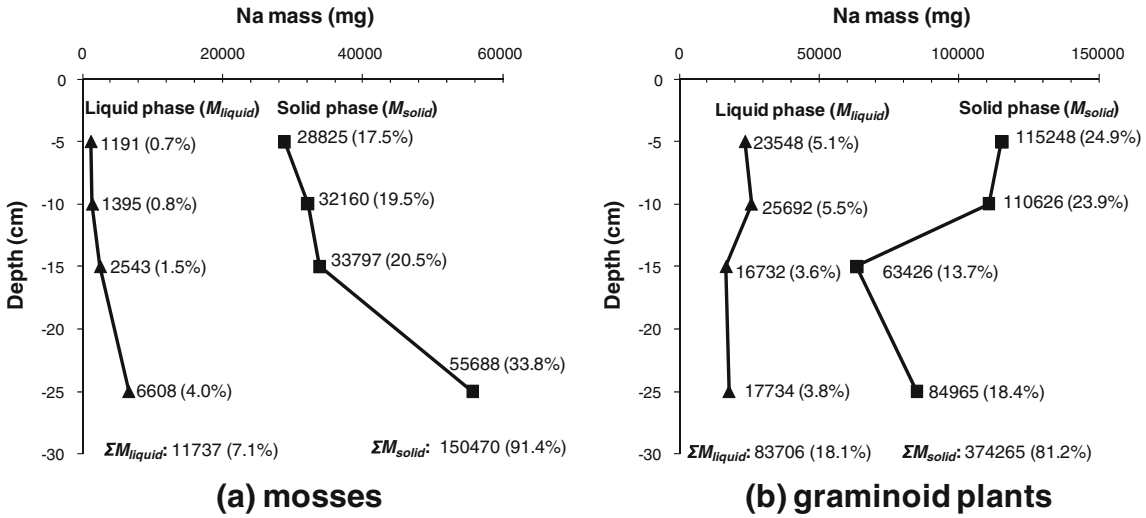

Fig. 4 Na mass profiles with depth for liquid phase (M_{liquid}) and solid phase (M_{solid}) (adsorbed on peat) for mosses and graminoid plants at the last day of experiment, after two growing seasons of receiving OSPW. The total input mass (M_{input}) of Na salt was 164.6 g for the moss mesocosm and 461.0 g for the graminoid mesocosm. The amount of adsorbed Na salts on the peat is approximately an order of magnitude larger than Na in liquid phase and also more in graminoid plants than in mosses. The plant uptake mass (M_{uptake}) was calculated using total liquid phase (ΣM_{liquid}) and solid phase (ΣM_{solid}) masses from four different sampled depths and Eq. 1 as 1.45% and 0.65% in mosses and graminoid plants respectively

Graminoid plants health index (mean ± SE) decreased from 6.3±0.1 to 5.8±0.4 during the first growing season and from 6.4±0.5 to 5.1±0.8 during the second growing season, mainly because of natural senescence. The number of individual plants, number of leaves by experimental unit, number of leaves and maximum shoot length for an individual plant all showed trends to increasing values over the course of the two growing seasons for *Carex aquatilis* (Table 2). Trends for decreasing values for the number of individual plants and number of leaves by experimental unit were visible throughout the second growing period for *Calamagrostis stricta*, probably caused by the competition with *Carex aquatilis*.

Moss health index decreased from 6.2±1.2 to 1.5±0.7 during the second growing season, indicating nearly all

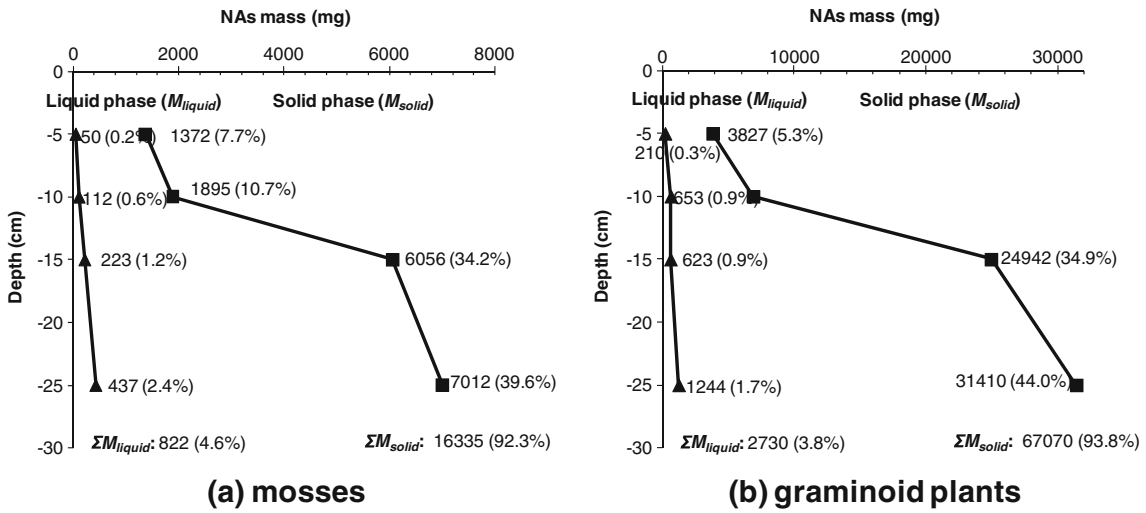

Fig. 5 NAs mass profiles with depth for liquid phase (M_{liquid}) and solid phase (M_{solid}) (adsorbed on peat) for mosses and graminoid plants at the last day of experiment, after two growing seasons of receiving OSPW. The total input mass (M_{input}) of NAs was 17.7 g for the moss mesocosm and 71.3 g for graminoid mesocosm. The amount of adsorbed NAs on the peat is more than an order of magnitude larger than NAs in liquid phase and also more in graminoid plants than in mosses. The plant uptake mass (M_{uptake}) was calculated using total liquid phase (ΣM_{liquid}) and solid phase (ΣM_{solid}) masses from four different sampled depths and Eq. 1 as 3.06% and 2.1% in mosses and graminoid plants respectively

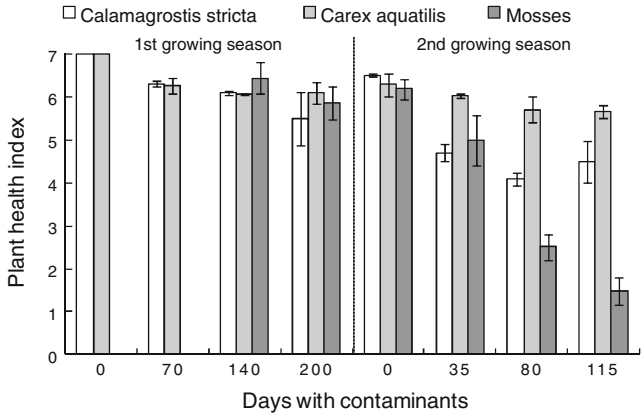

Fig. 6 Graminoid plants and mosses health index (mean ± SE) tested over two growing seasons. For the plant health index, a value of 7 indicates 100% healthy, and a value of 1 indicates 100% dead

mosses were dead at the end of the experiment. Moss cover reached 95±1.9% after 140 days of growth in the first growing season. At the end of the experiment, the moss cover appeared still high (92±4.7%) even though the moss carpet had turned all brown (even though kept moist) with little photosynthetic green or signs of growth to be seen.

Na concentration in plant tissue increased during the experiment (Table 3). At the end of the experiment (end of the second growing season), Na concentration was 2.2 (mosses), 9.2 (*Calamagrostis stricta*) and 12.8 (*Carex aquatilis*) times higher than before the contaminants were added for the first time (beginning of the experiment). K concentration in plant tissue decreased 2.9 times for *C. stricta* leaves and 1.4 times for mosses through the experiment, but was more variable for *C. aquatilis* where no pattern was

Table 2 Variation of growth parameters for vascular plants during the two growing seasons

Variable	Days with contaminants	Species	
		Calamagrostis stricta Mean ± SD	*Carex aquatilis* Mean ± SD
Number of individual plants	1[a]) 0	15±1	18±3
	1) 200	17±4	42±22
	2) 0	123±3	114±12
	2) 115	91±33	194±18
Total number of leaves	1) 0	581±180	660±33
	1) 200	780±332	1,143±64
	2) 0	2,384±179	2,517±151
	2) 115	1,130±176	3,385±108
Maximum shoot length for individual plant (cm)	1) 0	43.8±3.3	54.6±5.9
	1) 200	72.5±0.4	81.8±2.3
	2) 0	50,4±7.5	55.5±0.8
	2) 115	60.8±0.5	70.6±4.6

[a] the growing season

present (Table 3). K concentration was always higher than Na concentration. NAs concentrations in plant tissue are unavailable because there is no established analytical technique at the time of this analysis.

Microbiology

The temporal variation of the functional diversity, evaluated as carbon utilization profiles at the community level, was different under the two types of vegetation structure, (F=3.82, p=0.001). The patterns of variation at the community level are visible on the biplot of the PCA (Fig. 7a). The change in the microbial community over time is expressed as the shift along the horizontal axis, with the left hand side of the graph where all the carbon sources are displayed representing higher activity rates. The diversity was higher in the peat under the graminoid cover than under moss carpet before the start of OSPW inflow (day 0) and until day 70 after inflow of the first growing season. The CO_2 production rates then decreased between day 70 and day 140 after contamination in the first growing season albeit more sharply (greater distance on the graph) under the graminoid cover than under the moss carpet, before increasing slightly again. Catabolic diversity was also affected by time and plant type (F=4.52, p=0.005) (Fig. 7b) and followed the same pattern as functional diversity at the community level. Another pattern visible on Fig. 7 is the change in preferred C source over time, represented by the shift along the vertical axis, with microbial community preferentially using glucose and γ amino-butyric acid at the start, and lysine toward the end of the season.

Respiration rates increased with exposure to OSPW as sole energy source, in comparison with the water control (Fig. 8a). In addition, combining a carbon source with OSPW modified the respiration rates in comparison with the same carbon source in deionised water. However, the effect was variable: in some case, adding OSPW had little or no effect (e.g. glucose, Fig. 8b), in some cases it seemed to stimulate it (e.g. malic acid, Fig. 8c) and in other, it inhibited (e.g. D-galactose).

Discussion

Contaminant Transport, Adsorption, Uptake

The water content (θ) and soil water pressure (ψ) in the peat above the water table was higher for the mosses due to lower evapotranspiration (higher water table; Fig. 3). The moss cover had no underground root system but its cover impacted the moisture and temperature of the underlying peat matrix (Price et al. 2009). However, in the graminoid plant mesocosm, *Carex aquatilis* and *Calamagrostis stricta* had an extensive belowground root systems (~−25 cm) that affected the way NAs and Na salts flowed through the peat

Table 3 Potassium (K) and sodium (Na) concentration (ppm) in plant tissues over the course of the two growing seasons

Growing season	1st	1st	2nd	2nd	2nd	2nd	2nd	2nd
Days with contaminants	0	0	0	0	80	80	115	115
	K	Na	K	Na	K	Na	K	Na
Calamagrostis stricta	15,023	166	14,424	529	8,748	277	5,134	1,524
Carex aquatilis	13,323	217	17,685	1,734	8,852	3,045	12,385	2,770
Mosses	5,270	1,327	6,904	2,914	4,583	4,120	3,654	2,881

matrix. The interaction of the plants with the contaminated substrate is therefore different under a moss vs. graminoid cover. The results showed that OSPW introduced at the base of the containers (with initial ~385 mg l^{-1} of Na salts and ~40 mg l^{-1} of NAs) was pulled upwards by evapotranspiring fen plants. Higher evapotranspiration and extensive belowground roots from the graminoid plants in comparison to the mosses increased the upward migration of water and contaminant compared to the mosses (Figs. 4 and 5). The contamination profiles reflect the rate of upward transport, the sorptivity of the peat and the concentrating effect of evapotranspiration. In the moss mesocosms (Figs. 4a and 5a) the general level of contamination is lower because of the lower evaporation rates (reduced flux and negligible evapo-concentration). The highest concentrations occur at the base where the solute is introduced, and decrease upwards due to: a) the greater distance from the source, and b) the fluid arriving there already had solute stripped from it by sorption in the lower layers. For Na salts in graminoid plant mesocosm (Fig. 4b) there is a notable increase in concentration throughout the upper layers as a consequence of evapo-concentration below the surface.

The liquid-phase concentration profile is dependent on the adsorption of Na and NAs onto the peat in each layer. The amount of adsorbed Na salts and NAs on the peat (at different depths) was determined to be approximately an

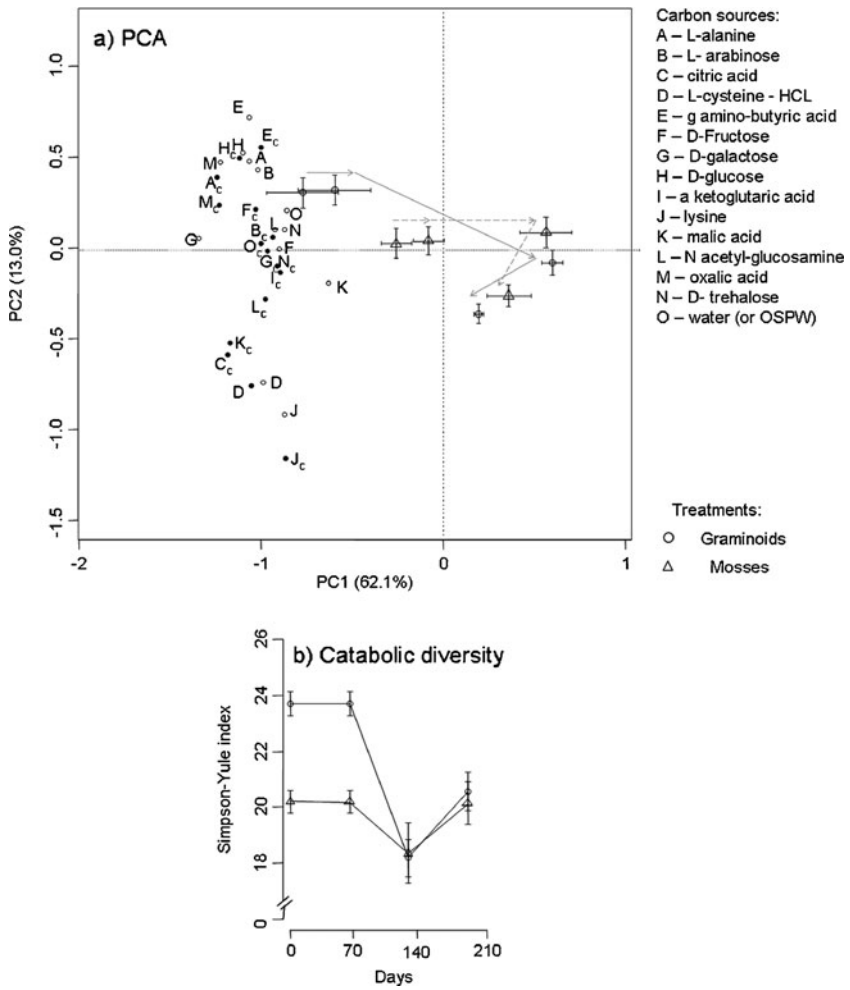

Fig. 7 Temporal evolution of **a**) the functional diversity (average CO_2 respiration rates) at the microbial community level presented on the 1st and 2nd axis of the principal component analysis (PCA) and **b**) catabolic diversity calculated as the Simpson-Yule index using all carbon sources. The days of exposure to OPSW in the first growing season are indicated on the PCA biplot next to treatment symbols. For the carbon sources (identified on the right hand side), closed symbol and the letter "c" following the carbon source on the biplot represent sources prepared in OSPW, whereas open symbols were sources prepared in de-mineralised water. The last measurement was made at Day 200

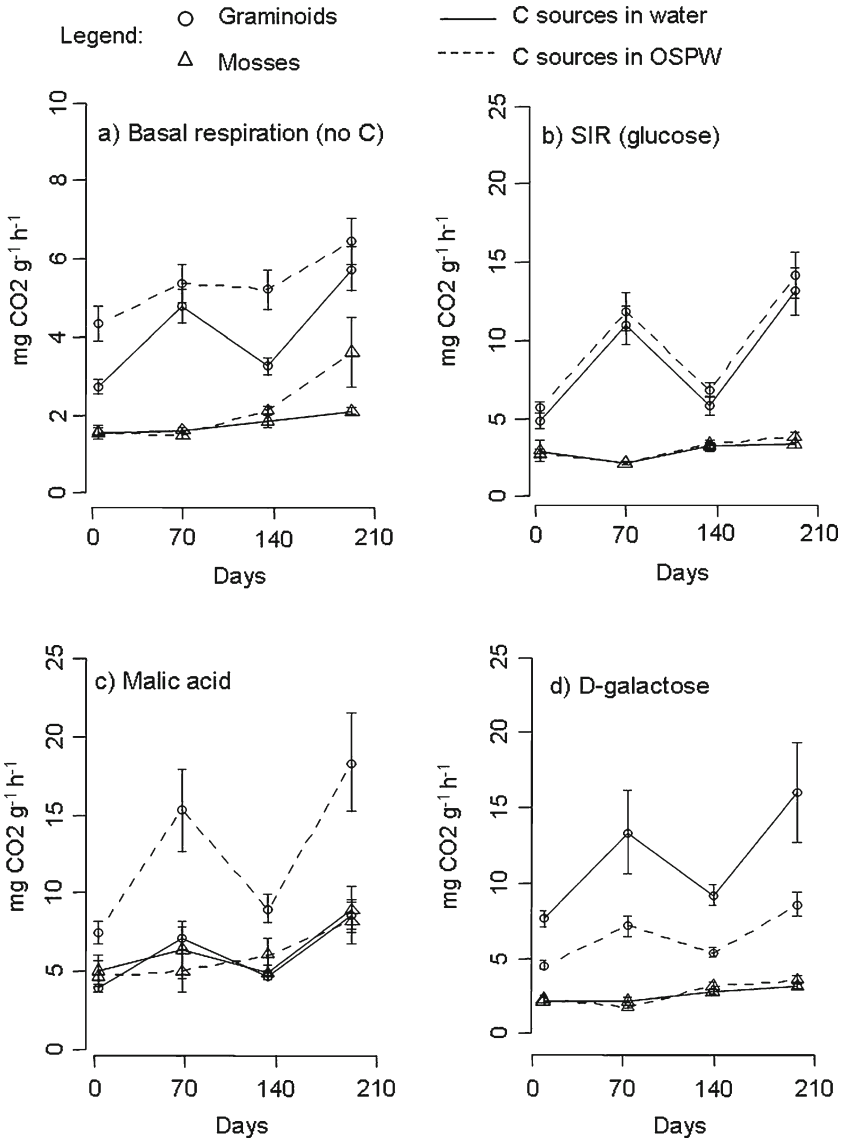

Fig. 8 Evolution of **a**) water (basal respiration), **b**) glucose (Substrate Induced Respiration, SIR), **c**) Malic acid and **d**) D-galactose over time in the first growing season for the two vegetation structure treatments (legend on the graph). Note the scale difference between basal respiration and other carbon sources. The last measurement was made at Day 200

order of magnitude larger than Na and NAs in liquid phase (Figs. 4 and 5). In laboratory experiments reported by (Rezanezhad et al. 2010), for the same peat and OSPW samples, approximately 94% of of NAs at 43.5 mg l^{-1} was sorbed by 1 kg of peat and ~84% of Na was sorbed with 382 mg l^{-1} kg^{-1} of peat, based on measured Freundlich linear isotherms with adsorption coefficients of 6.53 and 5.74 l/kg, respectively. As the NAs in OSPW is molecular weight naphthenate anion dimmers, the vapor pressure will be too low to enter the gas phase meaning there is no volatilization process during the migration of NAs through peat layers (J. Fournier, pers. Comm., 2011).

In addition to sorption, (Rezanezhad et al. 2010) found that diffusion of Na and NAs from the flowing solution (mobile water) into closed and dead-end pores (immobile water) associated with peat soils, delayed the advance of the solute. They offered three hypotheses differentiating pore size distributions as*:* (*I*) open and connected macropores and partially open pores where most solute transport probably occurs and (*II*) hyaline cells and dead-end or isolated pore spaces where solute transport is attenuated and retarded by diffusion into these immobile water spaces (Loxham and Burghardt 1983; Price and Woo 1988; Viraraghavan and Ayyaswami 1989; Hoag and Price 1997). Previous studies also showed that transport of contaminants through the peat may experience ion exchange, ion exclusion, chemical and biological transformations, volatilization, dissolution and precipitation, biodegradation and dispersion (Tindal and Kunkel 1999).

Effects of OSPW on Plants

After receiving contaminated water for two growing seasons, graminoid plants were in good health and continued to

grow. In fact, graminoid plants remained healthy, despite a reduction in irrigation part-way thought the first season which resulted in a higher basal flux of OSPW.

Graminoid plants appear to be resistant to OSPW salt concentration. In salt marshes, plants from the Gramineae and Cyperaceae families block the entry of sodium by having high potassium concentrations in their tissue (Albert and Popp 1977; Gorham et al. 1980; Cooper 1982). We found that K and Na concentrations in leaves of *Carex aquatilis* and *Calamagrostis stricta* followed the same trends (higher K concentration than Na concentration), but were not as high as those found in tissue of salt marsh species (for example: up to 7,955 mg l^{-1} for *Spartina anglica* Hobbard, Gorham et al. 1980; and 66 300 mg l^{-1} for *Juncus gerardii* Loisel., Cooper 1982). Vascular plants might not require this particular adaptation to survive under the contamination tested in the experiment (~385 mg l^{-1} of Na salts and ~40 mg l^{-1} of NAs). Such salt contamination is not significant considering that sea water contains around 35 000 mg l^{-1} of Na (or between 1,000 and 10 000 mg l^{-1} in brackish water). Thus, the resilience of *C. aquatilis* and *C. stricta* to Na, which reached average concentrations (523 mg l^{-1}) in the rooting zone of ~25 cm below the surface, suggests that these species are tolerant to the levels of contamination tested.

We believe the conditions in the field will be less stressful for the plants than those simulated in this greenhouse experiment. The peat depth planned for the Suncor Pilot Fen (2 m, Price et al. 2010) is more than four times greater than in these mesocosms, suggesting that the graminoid plants will have several years without OSPW contamination to establish. Moreover, the average precipitation in the oil sands region is higher than the simulated precipitation applied in the latter part of the first and all of the second growing seasons. Furthermore, during a wet year in the field setting, there is potential for leaching, horizontal transport and surface runoff losses which could provide a solute sink not available during this experiment. Overall, it appears that *Carex aquatilis* and *Calamagrostis stricta* should be able to establish successfully in reclaimed oil sands landscapes.

Mosses were more sensitive to the presence of OSPW than were vascular plants. Under greenhouse conditions that simulated the average precipitation of the oil sands area in the first months of the first growing seasons (until 110 days with added OSPW), moss health was not adversely affected (plant health index of 6.6±0.6). A decrease in plant health (dropping to a plant health index of 1.5±0.7) occurred when watering was reduced after day 180, which was associated with a significant increase in the concentration of OSPW in the upper layers of the mesocosms (Figs. 4 and 5) and in the mosses themselves (Table 3). There is a possibility that moss health was affected by reduced watering. However, during the experiment we regularly (three times per week) misted the moss to prevent moss desiccation (no more than several litres of water over the entire experiment). Furthermore, in a parallel greenhouse experiment with the same watering regime but with additional fen moss species, and no OSPW (unpublished data) plant health was not adversely affected by the same reduced watering regime. In the microcosm experiments, therefore, the decline in moss health was attributable to the presence of OSPW. The single cell thickness of the moss membrane and the high cationic exchange capacity (Clymo 1963) are two features that favor the absorption of contaminants, which may increase the sensitivity of mosses to contaminants. In the context of rehabilitation of oil sands exploitation areas, drier years will result in mosses acquiring more water by capillarity (with higher concentration of OSPW) than during humid years where precipitation is sufficient to keep the moss carpet moist, and where leaching of solutes will be stronger.

Microbes

The temporal variation in CO_2 respiration by microorganisms at the community level (Fig. 7) and for individual carbon sources (Fig. 8) as well as the shift in the preferred carbon sources (Fig. 7) under both vegetation structures could reflect seasonal variation (Schmidt et al. 2007), toxicity by NAs or water stress caused by higher levels of Na. Changes in the relative sizes of carbon pools in the peat surface over the course of the growing season are known to cause shifts in catabolic diversity and carbon utilisation profiles of the microbial community (Degens et al. 2000). Exposure to high NaCl concentrations can also affect microbial diversity (Baldwin et al. 2006). The upward migration of Na was greater in the tubs with graminoid plants, leading to higher concentrations in the surface where samples were taken in comparison with the moss mesocosm. This is consistent with the sharper decrease in respiration and catabolic diversity observed between 70 and 140 days under graminoids. Nevertheless, at the end of the first growing season, most of the NAs were still adsorbed much lower in the profile and that the plants remained healthy; therefore we ruled out the hypothesis that NAs affected the microbial populations of the peat surface during the first growing season. Similarly, since NAs did not reach the surface peat where microbial communities were investigated, we could not test the long-term effects of this contaminant.

We tested the short-term effect of direct exposure to NAs by preparing carbon sources in OPSW. The presence of NAs increases the C/N ratio in the pore water of the peat in the plates (Robertson et al. 2007), which affects some metabolical pathways more than others and can modify microbial activity (Greenwood et al. 2009). This could explain why in some cases microbial respiration was stimulated in presence of OSWP (e.g. Malic acid) and in other cases inhibited (e.g. D-galactose). It has also been suggested that crude oil

extract could at least temporarily stimulate the metabolism of surviving microorganisms (Nyman 1999) and that microorganisms indigenous to wetlands (Hadwin et al. 2005; Del Rio et al. 2006) and oil sands tailings (Herman et al. 1994) were able to at least partially metabolize the NAs. The higher respiration rates measured when OPSW are present without any other carbon source (Fig. 8a) point in the same direction.

In any system, plants and microorganisms feedback on each other. In the mesocosms, microorganisms were more active (higher CO_2 respiration rate for a given C source) and diverse (higher catabolic diversity, see Fig. 8e) under the graminoid plants than under the mosses. This could be due to the presence of a well developed rhizosphere that can increase the diversity of the microbial community (Grayston et al. 1998), hence its capacity to metabolize various substrates. Changes in microbial community structure between vegetation types, over time, or following contamination could also lead to changes in catabolic diversity and preferred carbon sources, and this could be investigated with analyses such as Phospholipid fatty acid (PLFA) (e.g. Andersen et al. 2010) profiles, or DNA-based analyses (e.g. Curlevski et al. 2011).

Conclusion

This study examined the movement of oil sands process-affected water (OSPW) through peat substrate, the establishment and growth of fen plant species contaminated with OSPW, and microbial structure changes in the contaminated peat. The results showed that transport of Na and NAs in peat substrates is highly retarded where the peat absorbed most of the contaminants and affected the concentration of potentially toxic compounds in the rooting zone. This special property of peat soil allows the plants to grow on peat contaminated with OSPW with little or no stress.

Typical fen graminoid plants were able to tolerate a realistic contamination scenario (~385 mg l^{-1} of Na salts and ~40 mg l^{-1} of NAs) as may occur in the oil sands region and probably do not need particular management actions to counteract OSPW additions. However, mosses appeared to have a lower tolerance threshold to OSPW, especially under drier conditions when they acquire water by capillarity from OSPW. Further experiments are needed to clearly identify those thresholds and to test the resistance of mosses to OSPW or salts since they are an important part of fen vegetation and should be included in rehabilitation of oil sands regions.

Microbial communities under graminoid and mosses evolved differently over time, in response to the effects of seasonal variation associated with plant phenology and/or high salinity associated with OSPW contamination. More active and diverse communities were found under graminoids. Higher catabolic diversity could increase the resilience of the microbial community and its capacity to adapt to changing conditions (Naeem and Li 1997). We hypothesize that it can provide access to different nutrient pools and potentially reduce NA concentration or toxicity, thus could give a competitive advantage to graminoids in a created fen.

Finally, the impact of OSPW leaching from tailings sand depends on the transport processes in peat and the resiliency of fen plants and the peat attenuates transport sufficiently that the presence of Na and NAs in the rooting zone may take several years to reach toxic levels, and that some fen plants are relatively tolerant of these substances. This lag time before reaching full contamination potential can give enough time to the reintroduced plant communities to form a good litter layer, further isolating itself from the below-ground contaminants. We believe that despite the limited number of replicates and the absence of different controls, the information gathered from this mesocosm study is an important first step for understanding the transport of contaminants in constructed or natural settings and the response of vegetations on peat contaminated with OSPW. However, unlike in this mesocosm study, the natural fen system outflow is a sink for solutes, and dry followed by wet cycles will redistribute contaminants in the profile.

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