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Fen mosses can tolerate some saline conditions found in oil sands process water

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ABSTRACT

Mosses are keystone species in peatlands and are an important part of the vegetation of the pre-mined peatlands. Therefore, mosses should be included in rehabilitation projects following oil sands exploitation in north-western Canada. However, mosses growing in post-mined landscapes must tolerate elevated salinity levels found in oil sands process water (OSPW). Knowledge of salinity tolerance and thresholds for fen mosses is needed to place these mosses in the newly created landscapes. We tested the effects of NaCl and Na_2SO_4 on four fen moss species growing in Petri dishes in growth chambers. We simulated two scenarios: (1) four immersion times (¼, 1, 3 and 7 days) in NaCl (0%, 20%, 60% or 100% of the concentration found in OSPW) mimicking periodic flooding and (2) a permanent saline influence (NaCl or Na_2SO_4 alone or in combination at 0%, 30%, 50% or 70% of the concentrations found in OSPW) mimicking situations of high water tables with different contamination levels. The effects on moss growth were estimated by counting new innovations of *Bryum pseudotriquetrum*, *Campylium stellatum*, *Sphagnum warnstorfii* and *Tomenthypnum nitens*. All tested mosses tolerated saline levels typically found in post-mined landscapes (up to 500 mg L^{-1} of NaCl and 400 mg L^{-1} of Na_2SO_4) for up to 100 days of exposure. Short periods of immersion (up to 7 days independently of salt concentrations) induced the production of innovation in non-*Sphagnum* species, but *S. warnstorfii* was more rapidly impacted at higher salt concentrations. Short pulses of salt (from 6 h to 7 days) did not influence the formation of new innovations for *C. stellatum* and *T. nitens*. Salt type (NaCl and/or Na_2SO_4) had no effect on moss growth. However, a longer exposure (100 days) with saline water, even at low concentrations, diminished the formation of new innovations for *B. pseudotriquetrum* and *T. nitens*. *C. stellatum* was the least affected by salinity and thus we suggest it is the best species to reintroduce in constructed fens.

1. Introduction

Over the last decades more than 600 km^2 of north-western Canada have been disturbed by open-pit mining activities (Government of Alberta, 2011). These activities change the landscape from a mosaic of peatlands and forest to a landscape dominated by settling basins, tailings ponds, reclaimed forests, and reclaimed wetlands. The process of extracting bitumen from oil sands produces process-affected tailings, which are stored in settling basins. Tailings contain sand, silts and clays in suspension as well as soluble organic chemicals, ammonia, trace metals, and salts (Bott, 2010). Additionally, gypsum is sometimes added to accelerate the settling process. Tailings are contaminated with considerable addition of ions such as SO_4^{2-} from the gypsum and Na^+ from Ca^{2+} exchange in the clays (Bott, 2010).

Due to a zero discharge policy, oil sands process water (OSPW), which remains after solids have settled, must be stored in tailings ponds. The volume of these ponds currently exceeds one billion m^3 (Han et al., 2008). Soil water salinities in reclaimed wetlands in contact with OSPW vary from 335 to 2881 mg L^{-1} (Renault et al., 1998; Purdy et al., 2005; Trites and Bayley, 2009). Oil sands mining companies have to take these salinity levels into account when reclaiming post-mined landscapes.

After mining activities have ceased, laws require that mined landscapes be reclaimed by oil companies to a state comparable to the original ecosystem (OSWWG, 2000). However, current practice favors the reclamation of upland forests and storage lakes (Rooney et al., 2012). As wetlands covered up to half of the original landscape (Vitt et al., 1996), a large part of post-mined sites should be reclaimed back to wetlands. Marshes have been the focus of first reclamation projects as their hydrology is easier to recreated than that of peatlands (Harris, 2007) and some marsh species like *Typha latifolia* L. have proven to be resistant to the salt contamination present in post-mined landscapes (Bendell-Young et al., 2000;

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Crowe et al., 2001; Foote and Hornung, 2007). However, around 90% of wetlands in pre-mined landscapes of the Canadian oil sands region are peatlands, mostly fens (Vitt et al., 1996). Fens, in contrast to marshes, sequester and store carbon over the long-term (Strack, 2008).

The most important factors in restoring the peat-accumulating function of peatlands are hydrology and vegetation (Rochefort, 2000). Recent reclamation research efforts focused on hydrology and engineering of constructed fens in post-mined landscapes (Price et al., 2010). Mosses and sedges form the dominant vegetation in fens (Vitt et al., 1996). Additionally, mosses are important in restoring the peat-accumulating function of fens because of their low decomposition rates (Graf and Rochefort, 2009), but little research has been carried out on moss tolerance to post-mined conditions.

In constructed fens mosses will be in contact with salts, trace metals and naphthenic acids (NAs) from groundwater influenced by OSPW. This contaminated water could hinder the establishment of fen mosses in areas to be reclaimed because the concentrations may be toxic to aquatic plants and animals (Apostol et al., 2004). Vascular plants such as common herbaceous and cyperaceous fen plants have good survival rates in wetlands influenced by OSPW (Trites and Bayley, 2008) and show no sign of stress in greenhouse peatland mesocosms influenced by a groundwater discharge composed up to 70% of OSPW over two growing seasons (Pouliot et al., 2012). Mosses, on the other hand, are considered to be intolerant of saline conditions (Boerner and Forman, 1975).

In natural ecosystems, mosses are usually absent in saline conditions (Shacklette, 1961; Adam, 1976). Naturally saline fens are occasionally found in the Canadian oil sands region or in the boreal zone in general (Trites and Bayley, 2009). These fens are characterized by having a pH above 6.5, unstable seasonal water tables and, in some cases, the presence of sodium deposits (Vitt et al., 1993). They support salt tolerant plant communities that can occasionally include small proportions of mosses such as *Bryum pseudotriquetrum* (Hedw.) G. Gaertn., B. Mey. & Scherb., *Campylium stellatum* (Hedw.) C.E.O. Jensen, and *Drepanocladus aduncus* (Hedw.) Warnst. (Vitt et al., 1993; Trites and Bayley, 2009). Studies about habitat limitations along physical gradient show that these species with *Tomenthypnum nitens* (Hedw.) Loeske and *Sphagnum warnstorfii* Russow are present in species groups which tolerate the highest concentrations of sodium (7.6 ± 15.64 mg L^{-1} and 10.4 ± 2.9 mg L^{-1}; Gignac and Vitt, 1990; Gignac et al., 1991). These concentrations found in nature are more than 30 times lower than the lowest salt concentrations measured in reclaimed wetlands in contact with OSPW.

More information about salinity tolerance and thresholds for fen mosses is thus crucial to guide fen construction in post-mined landscapes following oil sands exploitation. Our objective was to test the effects of two salts abundant in OSPW, NaCl and Na_2SO_4, on the regeneration of fen mosses at salt concentrations corresponding to the levels found in OSPW. As mosses are not expected to tolerate saline conditions, it was hypothesized that the toxic threshold for salt tolerance would be less than the concentration found in OSPW. We also believe that a short, strong pulse of salt should be less harmful for mosses than a long and constant pulse. When mosses experience a short, strong pulse, they will have the possibility to eliminate the surplus salt after the salt stress.

2. Materials and methods

2.1. Moss species

Four common fen moss species were selected for experiments during this study: *B. pseudotriquetrum*, *C. stellatum*, *S. warnstorfii* and *T. nitens*. All these species are abundant in fens within the Canadian oil sands region (Chee and Vitt, 1989; Johnson et al., 1995) and in the northern hemisphere in general. *B. pseudotriquetrum* and *C. stellatum* are occasionally found in saline fens (Trites and Bayley, 2009; Vitt et al., 1993). Mosses were collected in rich fens in the provinces of Alberta and Québec and were then stored in airtight plastic bags at 4 °C before their use.

2.2. Experimental setup

Two scenarios of salt contamination (with NaCl or Na_2SO_4) that could occur in constructed fens were simulated: (1) immersion for different periods of times in saline solutions mimicking short periodic floods (up to a week) as occurs during the snowmelt period or heavy rain events and (2) a longer influence of saline solutions mimicking a situation of high water tables or during a wet summer (over several months). It is expected that plants in constructed fens of post-disturbed landscapes would be in contact with a certain amount of OSPW but not necessarily at full concentrations, accounting for a certain dilution factor from precipitation or filtration through soil. Thus, diluted concentrations were tested along with full concentration of OSPW. OSPW contains around 500 mg L^{-1} of NaCl and 600 mg L^{-1} of Na_2SO_4 (estimated in OSPW samples taken by Suncor Energy in 2009 in their installations close to Fort McMurray, Alberta and shipped to Quebec City in clean oil barrels).

Experiments were carried out in Petri dishes (plastic; diameter = 14.2 cm) set in a growth chamber with a 14-h photoperiod and a stable temperature of 22 °C. Moss growth was assessed by counting the innovations (i.e. new shoots or capitula emerging from a moss fragment) at the beginning and end of experiments. The Petri dishes were initially randomly placed, and thereafter, re-randomized every 2 weeks. No signs of distress (for example: color change or innovations degradation) were observed during any experiments.

2.2.1. Scenario with different immersion times in saline solutions

In this scenario, *C. stellatum*, *S. warnstorfii*, and *T. nitens* were immersed in saline water. For each species, a factorial experiment was conducted with four NaCl concentrations (0%, 20%, 60% or 100% of the concentration found in OSPW) and four immersion times (¼, 1, 3 and 7 days). Each treatment was replicated three times for a total of 48 experimental units per species (see treatments in Table 1). NaCl was dissolved in deionized water. Saline solutions were poured into in glass jars with lids or airtight plastic bags containing mosses. Jars or bags were maintained at 4 °C for a given immersion time. At the end of the immersion period, 10 individual mosses were removed from jars or bags and the first 4 cm in length (from the moss apex) were cut. Ten 4 cm long fragments were put in a Petri dish. One Petri dish corresponded to one experimental unit. All Petri dishes were lined with a thin layer of peat (circa 1 cm). The peat came from a natural fen of the oil sands region and had a pH between 5.0 and 5.5 and other chemical parameters of peat from a moderate-rich fen (Vitt and Chee, 1990). Once the mosses were mounted on a Petri dish, they were watered only with rainwater (see Appendix 1 for chemical information) at least once a week or more if signs of desiccation were observed. The recovery period was set at 65 days (*C. stellatum* and *S. warnstorfii*) or 100 days (for the trial with *T. nitens*, which was run in a different laboratory).

2.2.2. Scenario with permanent water using saline solutions

In this scenario *B. pseudotriquetrum*, *C. stellatum* and *T. nitens* were watered with different saline solutions. For each species factorial experiments with three salt solutions (NaCl, Na_2SO_4 and a combination of both) and four concentrations (0%, 30%, 50% or 70% of the concentrations found in OSPW) were conducted with five replicates, for a total of 60 experimental units per species (see