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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 ($7.6 \pm 15.64\,\mathrm{mg\,L^{-1}}$ and $10.4 \pm 2.9\,\mathrm{mg\,L^{-1}}$; 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 $500\,\mathrm{mg\,L^{-1}}$ of NaCl and $600\,\mathrm{mg\,L^{-1}}$ 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

Table 1
An overview of the treatments tested on *Campylium stellatum*, *Sphagnum warnstorfii* and *Tomenthypnum nitens* in the scenario with different immersion periods in saline solutions (factorial experiments repeated three times). Concentrations in OSPW were measured in samples taken by Suncor Energy in 2009 in their installations close to Fort McMurray, Alberta.

Treatment number	Adsorption time (days)	Concentration NaCl ($mg\ L^{-1}$)	Concentration of Na^+ ($mg\ L^{-1}$)	% of salt in OSPW
1	0.25	0	0	0
2	0.25	100	39	20
3	0.25	300	118	60
4	0.25	500	197	100
5	1	0	0	0
6	1	100	39	20
7	1	300	118	60
8	1	500	197	100
9	3	0	0	0
10	3	100	39	20
11	3	300	118	60
12	3	500	197	100
13	7	0	0	0
14	7	100	39	20
15	7	300	118	60
16	7	500	197	100

treatments in Table 2). As for the other scenario, the first four cm in length of individual mosses were cut and ten fragments were put in a Petri dish (one Petri dish = one experimental unit). For this experiment the Petri dishes were lined filter paper, not peat. Peat was not used for two reasons: (1) to remove the buffering effect of peat (to test more directly the salt effect as peat adsorb efficiently contaminants in OSPW, Rezanezhad et al., 2012a) and (2) to make it easier to count new innovations (more visible). Moss fragments were then watered one time per week or more if signs of desiccation were observed, with the corresponding salt treatment. NaCl or Na_2SO_4 were dissolved in deionized water. Petri dishes receiving the treatment without salt additions (concentration of $0\ mg\ L^{-1}$) were watered with rainwater (see Appendix 1 for chemical information). Each month, all filter papers in Petri dishes were rinsed with deionized water to remove salt incrustation. This scenario was tested for 100 days.

B. pseudotriquetrum spontaneously regenerated in contact with a groundwater discharge of OSPW, and tests with *S. warnstorfii* were not positive in other experiments (Pouliot et al., 2012; Rezanezhad et al., 2012a). We thus decided to replace *S. warnstorfii* by *B. pseudotriquetrum* in that second scenario of the experiment.

2.3. Statistical analyses

The difference between the numbers of innovations counted at the beginning (time = 0 day) and the end of each experiment (time = 65 or 100 days) were used to compare the effects of the treatments on the moss growth. ANOVAs for factorial designs were carried out with the procedure GLM in SAS software (SAS Statistical System software, v. 9.1, SAS Institute Inc., Cary, NC, USA). Polynomial contrasts (linear and quadratic effects) were run when a significant effect was observed for the immersion period and the salt concentration. A constant positive or negative effect of saline solutions on moss growth will be seen by a linear effect. A quadratic effect could indicate a toxicity threshold. If needed, protected LSDs (least significant difference) were run for salt type in the scenario with longer influence of saline solutions. Significant probability levels were set to α of 0.05, all data were tested for homogeneity and normality, and no data transformation was needed.

3. Results

3.1. Scenario with different immersion times in saline solutions

C. stellatum and *T. nitens* tolerated all immersion times in saline conditions ($p = 0.002$ or below for immersion period). The number of new innovations increased linearly with the duration of immersion period (linear effect with $p < 0.001$ for immersion period; Fig. 1A and C). The relative increases in number of innovations from immersion time ½ day to an immersion time of seven days were 42% (19 ± 2 to 27 ± 2 innovations, means ± SE) for *C. stellatum* and 70% more innovations (50 ± 5 to 85 ± 7, means ± SE) for *T. nitens*. In addition, formation of new innovations for *C. stellatum* was influenced by salt concentration ($p = 0.043$ for salt concentration; Fig. 1B), but no linear or quadratic effect was found ($p = 0.197$

Table 2
An overview of the treatments tested on *Bryum pseudotriquetrum*, *Campylium stellatum* and *Tomenthypnum nitens* in the scenario with permanent watering in saline solutions (factorial experiment repeated three times). Concentrations in OSPW were measured in samples taken by Suncor Energy in 2009 in their installations close to Fort McMurray, Alberta.

Treatment number	Salt type	Total concentration of salts ($mg\ L^{-1}$)	Concentration of Na^+ ($mg\ L^{-1}$)	% of salt in OSPW
1	NaCl	0	0	0
2	NaCl	150	59	30
3	NaCl	250	98	50
4	NaCl	350	138	70
5	Na_2SO_4	0	0	0
6	Na_2SO_4	100	32	30
7	Na_2SO_4	300	97	50
8	Na_2SO_4	500	162	70
9	NaCl + Na_2SO_4	0	0	0
10	NaCl + Na_2SO_4	150 NaCl + 100 Na_2SO_4	91	30
11	NaCl + Na_2SO_4	250 NaCl + 300 Na_2SO_4	195	50
12	NaCl + Na_2SO_4	350 NaCl + 500 Na_2SO_4	300	70

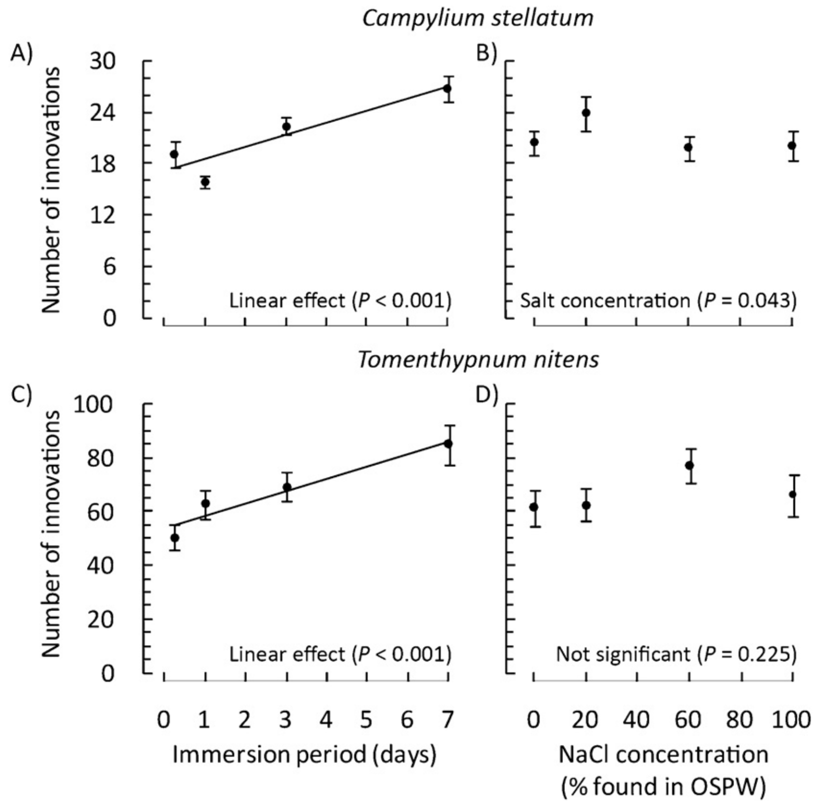

Fig. 1. Scenario with different immersion periods in saline solutions: number of new innovations (mean ± SE) under different immersion periods (A and C) and under different NaCl concentrations (B and D) for *Campylium stellatum* and *Tomenthypnum nitens* ($n = 3$). The recovery period was set up at 65 (*Campylium stellatum*) or 100 days (*Tomenthypnum nitens*). Refer to Table 1 for treatment description. Linear and quadratic contrasts were run for both variables.

or above). *T. nitens* was not affected by salt concentration ($p = 0.225$ for salt concentration; Fig. 1D).

Unlike the other two species, the production of *S. warnstorfii* new innovations was affected by the immersion time (interaction between immersion time and concentration; $p = 0.001$; Fig. 2A). Low concentrations (0 or 100 mg L^{-1}) induced an increase of the formation of new innovations with the increase of immersion time (Fig. 2A, linear effect with $p = 0.013$ or below). However, as the concentration of salts increased (300 or 500 mg L^{-1}), the capacity to produce more innovations became limited and reached a maximum at a shorter immersion time (quadratic effect with $p = 0.006$ for 300 mg L^{-1} and no linear or quadratic effects found for 500 mg L^{-1} but $p = 0.018$ for the immersion time; Fig. 2A).

The increase of salt concentration had no effect on the formation of new innovations of *S. warnstorfii* after an immersion time of 6 h ($p = 0.151$ for the concentration; Fig. 2B). The increase of salt concentration had a positively linear significant effect after an immersion of one day (linear effect with $p = 0.011$). After an immersion time of three days, the production of new innovations reached a maximum at 300 mg L^{-1} (quadratic effect with $p = 0.045$). Finally, after an immersion time of seven days, the number of new innovations decreased with the increase of salt concentration (quadratic effect with $p = 0.004$).

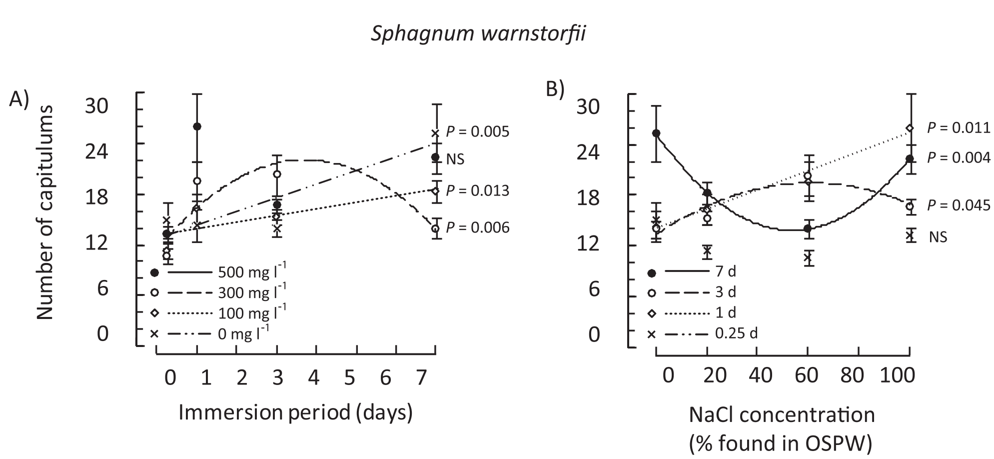

Fig. 2. Scenario with different immersion periods in saline solutions: number of new innovations (mean ± SE) under different immersion periods (A) and under different NaCl concentrations (B) for *Sphagnum warnstorfii* ($n = 3$). The recovery period was set up at 65 days. Refer to Table 1 for treatment description. Linear and quadratic contrasts were run for both variables. NS, not significant.

For *S. warnstorfii*, an extreme value effect was suspected for the concentration of 500 mg L^{-1} with an immersion of seven days. An increase of the new innovations that followed a significant decrease was difficult to explain biologically therefore, we did not include this point in our discussion.

3.2. Scenario with longer influence of saline solutions

All species were not affected by salt type (NaCl, Na_2SO_4 or a combination of both) (no effect of salt type; $p = 0.158$ or above; Fig. 3A, C and E). However, an increase of salt concentration resulted in a linear decrease in the formation of new innovations for two of the three species with no effect on *C. stellatum* ($p = 0.895$; Fig. 3D). The number of innovations for the rainwater treatments declined for the 70% OSPW treatments by 70% for *B. pseudotriquetrum* (38 ± 3 to 11 ± 2, means ± SE; $p < 0.001$) and by 45% for *T. nitens* (70 ± 5 to 38 ± 2, means ± SE; $p < 0.001$) with (Fig. 3B and F).

4. Discussion

B. pseudotriquetrum, *C. stellatum*, *S. warnstorfii* and *T. nitens*, all common fen moss species of north-western Canada, showed some tolerance to the salt concentrations typically found in post-mined landscapes after oil sands exploitation. These species did not show any of the typical signs of physiological distress as color change or a reduction in production of innovations when growing in saline conditions. Contrary to what we expected, these Petri dish trials showed that within the range of salt concentrations tested (up to 750 mg L^{-1} of salt) and time of exposure (up to 100 days), toxic thresholds for salt tolerance of these moss species were not reached. The ability to regenerate decreased for *S. warnstorfii*, but

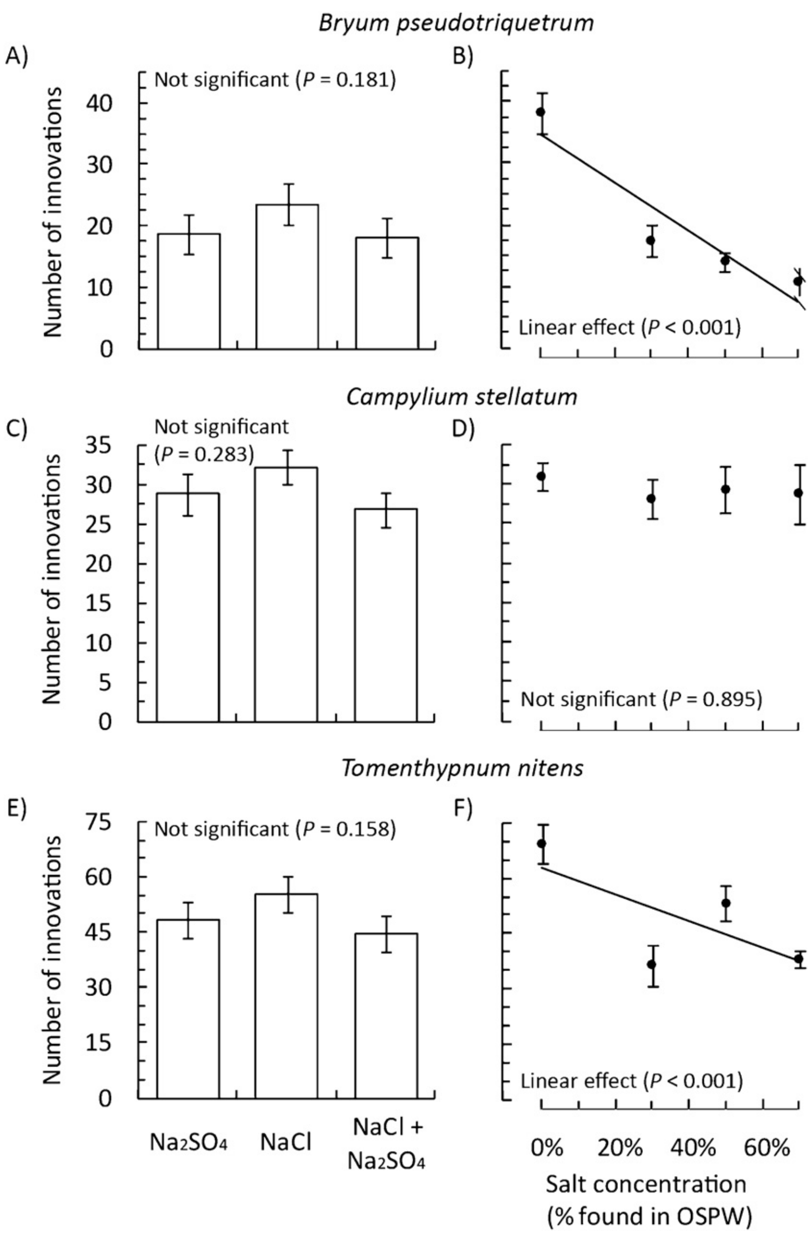

Fig. 3. Scenario with permanent watering in salt solutions during 100 days. Number of new innovations (mean ± SE) under different salt type (A, C and E) and under different salt concentration (B, D and F) for *Bryum pseudotriquetrum*, *Campylium stellatum* and *Tomenthypnum nitens* ($n = 5$). Protected LSDs (for salt type) and polynomial contrasts (for salt concentration) were run. Refer to Table 2 for treatment description.

this species remained healthy. Our hypothesis that a long and constant period of exposure to of salt (in our cases, 100 days of growth in saline conditions) would be more harmful than a short and strong pulse of salt was confirmed even at the lowest concentration of exposure (30% of the concentration found in OSPW). A slowing of the formation rate of new innovations was observed for *B. pseudotriquetrum* and *T. nitens*, even though the mosses appeared to stay in good health throughout the experiments. However, *C. stellatum*, which naturally appears in saline fens, was not affected by salt treatment.

4.1. Scenario with different immersion times in saline solutions

C. stellatum, *S. warnstorfii* and *T. nitens* would likely survive in situations imitating periodic inundations in OSPW as may occur during spring snowmelt or after heavy rains. Regardless of the concentration, increasing immersion time induced a positive effect on the number of innovations of *C. stellatum* and *T. nitens*. Similar effects of flooding creating better regeneration have also been observed for *Sphagnum* sp. in bogs (Rochefort et al., 2002). A leaching of salt accumulating in moss cells during the immersion in saline conditions probably occurred in Petri dishes. That could induce a physiological response forcing mosses to be more productive after an important stress (increase of new innovations).

S. warnstorfii, on the other hand, responded inconsistently to prolonged immersion in saline conditions and high salt concentration. At least, the formation of new innovations also occurred at all treatments for that species. *Sphagnum* mosses have a higher cationic exchange capacity than brown mosses (Rippy and Nelson, 2007), which could promote salt absorption and may make the *Sphagnum* mosses (in our case, *S. warnstorfii*) more sensitive to salt present in OSPW than the other mosses. This sensitivity to salt is consistent with the results by Wilcox (1984), who studied the effects of NaCl on *Sphagnum recurvum* P. Beauv. receiving de-icing salt runoff from a storage area in Indiana, USA. In that case, salt concentrations between 500 and 2472 $mg\,L^{-1}$ of NaCl (up to around five time the highest concentration tested in our experiment) significantly reduced growth of *Sphagnum* mosses in laboratory experiments after 45 days.

4.2. Scenario with longer influence of saline solutions

B. pseudotriquetrum, *C. stellatum* and *T. nitens* would likely tolerate constant exposure to OSPW, mimicking fens with a high water table during a wet summer. Mosses still developed new innovations at a salt concentration corresponding to 70% of salt concentration found in OSPW (350 $mg\,L^{-1}$ of NaCl and 400 $mg\,L^{-1}$ of Na_2SO_4). However, constant watering with salt concentration even at only 30% of concentration in OSPW was sufficient to reduce the generation of new innovations for *B. pseudotriquetrum* and *T. nitens*. However, *C. stellatum* showed a constant number of new innovations for all salt concentrations. Contrary to the scenario with different immersion time, these mosses were grown in saline solutions without peat substrate. That was a worst case scenario because peat has been proven to be very effective in adsorbing contaminants in OSPW (Rezanezhad et al., 2012a). In addition, even if all filter papers in Petri dishes were rinsed with deionized water to remove salt incrustation, the real salt concentration in Petri dishes could be higher than the one we wanted to test. Despite this, mosses still were able to generate new innovations. The salt type is also an important factor influencing plant growth (Franklin and Zwiazek, 2004) and NaCl is indeed more harmful for some terrestrial boreal species than Na_2SO_4 (Nguyen et al., 2006). Wilcox (1984) proposed that chloride is a stronger growth inhibitor than sodium for *S. recurvum*. However, our results did not reflect any salt type effect on the tested species.

Even if our experiments have shown that common fen moss species of the oil sands regions can grow in contact with saline solutions, further experiments are needed to clearly identify the toxic threshold levels of moss tolerance to OSPW or salts in growth chambers, in greenhouses, and in the field. The maximum concentration tested (750 $mg\,L^{-1}$; in the combination of NaCl and Na_2SO_4 at 70% of the concentration found in OSPW) was located in the lower portion of the interval found in soil water salinities in reclaimed wetlands of oil sands region (335–2881 $mg\,L^{-1}$; Renault et al., 1998; Purdy et al., 2005; Trites and Bayley, 2009). In addition, the mean sodium concentration found in natural saline wetlands of that region is around 800 $mg\,L^{-1}$ (Trites and Bayley, 2009), but mosses are rare in these wetlands (Vitt et al., 1993). Thus, a larger range of concentrations and time and frequency of exposure should be tested. The temperature of incubation was not an important factor on *Sphagnum* moss growth in a similar flooding experiment (Rochefort et al., 2002), but differences between the effect of a spring flooding (temperature: 4–10 °C) and a summer flood (temperature: 25–30 °C) should be verified. Finally, moss sensitivity to naphthenic acids at concentrations found in OSPW has never been studied. This information is crucial to understanding which constituents of OSPW are toxic to mosses.

4.3. Implications for constructed fens and conclusions

Mosses are an important part of fen vegetation and should be included in rehabilitation of oil sands regions. Our results indicate that *C. stellatum* is the best moss among the four tested species to reintroduce during fen construction following oil sands exploitation. The formation of new innovations for *C. stellatum* was not slowed down by immersion or by a permanent watering in saline solutions. As no signs of stress were observed for other tested species, they could all be included in the matrix of plants to be reintroduced in constructed fens. The only negative impact of salt concentration at the level found in OSPW could be a slower development for *B. pseudotriquetrum*, *S. warnstorfii* and *T. nitens* compared to *C. stellatum* which could, thus, outcompete them.

Under field conditions, the saline stress on mosses could be higher or mediated by organizational complexity. Higher stress for mosses was observed during greenhouse experiments where the microclimate was less humid than in confined, sealed Petri dishes. The greenhouse experiment showed detrimental impact of OSPW on mosses at salt concentrations similar to the present study (Pouliot et al., 2012; Rezanezhad et al., 2012a). Indeed evapoconcentration caused Na (and other salts) to reach 1.2 and 23.5 g after two growing season in moss and vascular plants, respectively (Rezanezhad et al., 2012a). So, as proposed by Price et al. (2010), it may be important to reintroduce plants on a peat layer to create stable fen conditions. In fact, peat has a great buffering capacity and the transport of contaminant (salts and naphthenic acids) in the peat column will be retarded (Rezanezhad et al., 2012b).

Organizational complexity might modify the response of mosses to elemental concentration. Indeed, *Sphagnum* mosses are more responsive to N application when vascular plants are absent than when they are presence (Limpens et al., 2012). Litter accumulation could with time diminish the flushing of mosses by OSPW. However, as the development of new innovations for *B. pseudotriquetrum* and *T. nitens* (but not for *C. stellatum*) was reduced by a permanent watering with salt concentration of only 30% of concentration in OSPW, persistent inundation with OSPW should be avoided if a moss carpet with several species is planned to be reintroduced. When designing fens in post-mined landscapes, the average seasonal precipitation should be taken into account so that OSPW concentrations stay below toxic levels. Further field experiments are required to test the long term survival of mosses in a constructed fen under the more or less continuous influence of OSPW.

This study identified a moss species that is a good candidate to be reintroduced in constructed fens (*C. stellatum*). Further field investigations should reveal if a greater bryophyte community can be established at the onset of the fen creation. Long-term field experiments (more than one growth season) mimicking a situation where the OSPW will be diluted by rainwater in a created fen with a ground water flow system is needed to better understand the OSPW effect on moss and vascular plant growth. Over a period of years one could anticipate, the development of a new moss cover (dominated by *C. stellatum*) with vascular plants (such as *Carex* sp. and *Triglochin maritima* that grew well in contact with OSPW; Pouliot et al., 2012) could result in the accumulation of new organic matter in constructed fens. This new peat accumulation could create a biological cap, which would isolate plants from OSPW contamination.

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Appendix 1.

Chemical analyses for rainwater used to watering mosses in both scenarios (in mg L^{-1}, mean ± SD for all values, $n = 5$).

Element	Unit	Value	Element	Unit	Value
pH	–	6.04 ± 0.53	Ca	mg L^{-1}	1.78 ± 0.75
E.C.	µS	17.45 ± 11.62	Cu	mg L^{-1}	<0.03 ± 0.00
N-NH_4	mg L^{-1}	0.22 ± 0.07	Fe	mg L^{-1}	<0.05 ± 0.00
N-NO_3	mg L^{-1}	0.89 ± 0.99	Mg	mg L^{-1}	0.18 ± 0.09
P-PO_4	mg L^{-1}	0.26 ± 0.16	Mn	mg L^{-1}	<0.02 ± 0.00
K	mg L^{-1}	1.48 ± 1.53	Na	mg L^{-1}	0.43 ± 0.38
			Zn	mg L^{-1}	0.22 ± 0.12

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