Environmental Pollution 259 (2020) 113945

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Growth and physiological responses of tree seedlings to oil sands non-segregated tailings☆

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ARTICLE INFO

Article history:
Received 26 September 2019
Received in revised form
16 December 2019
Accepted 7 January 2020
Available online 8 January 2020

Keywords:
Soil salinity
Soil pH
Boreal forest
Reclamation
Chlorosis

ABSTRACT

Bitumen recovery from oil sands in northeastern Alberta, Canada produces large volumes of tailings, which are deposited in mining areas that must be reclaimed upon mine closure. A new technology of non-segregated tailings (NST) developed by Canadian Natural Resources Limited (CNRL) was designed to accelerate the process of oil sands fine tailings consolidation. However, effects of these novel tailings on plants used for the reclamation of oil sands mining areas remain to be determined. In the present study, we investigated the effects of NST on seedlings of three species of plants commonly planted in oil sands reclamation sites including paper birch (*Betula papyrifera*), white spruce (*Picea glauca*) and green alder (*Alnus viridis*). In the controlled-environment study, we grew seedlings directly in NST and in the two types of reclamation soils with and without added NST and we measured seedling growth, gas exchange parameters, as well as tissue concentrations of selected elements and foliar chlorophyll. White spruce seedlings suffered from severe mortality when grown directly in NST and their needles contained high concentrations of Na. The growth and physiological processes were also inhibited by NST in green alder and paper birch. However, the addition of top soil and peat mineral soil mix to NST significantly improved the growth of plants, possibly due to a more balanced nutrient uptake. It appears that NST may offer some advantages in terms of site revegetation compared with the traditional oil sands tailings that were used in the past. The results also suggest that, white spruce may be less suitable for planting at reclamation sites containing NST compared with the two studied deciduous tree species.

1. Introduction

Oil sands surface mining in the Athabasca oil sands region (AOSR) in northeastern Alberta, Canada, has disturbed nearly 1000 km^2 of the boreal forest so far (Government of Alberta, 2017). Oil sands operators are obliged to return the disturbed land to self-sustainable aquatic or terrestrial habitats equivalent to that which existed prior to disturbance through land reclamation, revegetation, and closure activities (Alberta Environment, 2010). The process of bitumen recovery involves logging, clearing the remaining vegetation, and striping away the thin layer of boreal soil. The removed soil (peat, muskeg, and LFH - litter, fermented, and humic horizons) is then deposited directly on reclamation sites or stockpiled for future reclamation purposes (Fung and Macyk, 2000). In open-pit oil sands mines, a highly saline overburden lies between the surface soil and the oil sands layer. The oil sands ore is transported from the mining site to a processing plant and extracted with hot water and alkaline chemicals, producing large volumes of tailings. The tailings, which contain clays, water, chemicals, and traces of bitumen as the main by-products of the extraction process, are pumped to the tailings ponds. The water from the top 3 m in the tailings ponds is recycled back to the processing plant and the tailings sand may be used as a substrate in traditional reclamation practices (Fung and Macyk, 2000). Soil reconstruction is one essential procedure in land reclamation and peat-mineral soil mix (PMM) or LFH mineral soil (top soil, TS) are used as capping materials above an underlying substrate of overburden or tailings sand (Naeth et al., 2013).

Natural consolidation and settlement of oil sands tailings in ponds might take more than one hundred years (Mikula et al., 1996). Conventionally, oil sands companies have used gypsum

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☆ This paper has been recommended for acceptance by Jörg Rinklebe.
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https://doi.org/10.1016/j.envpol.2020.113945
0269-7491/

($CaSO_4 \cdot H_2O$) as a coagulant to accelerate the consolidation process and produce composite tailings (CT) (MacKinnon et al., 2000). However, CT contain elevated levels of Na^+, which in addition to high pH pose severe challenges to the revegetation of the oil sands mining sites (Redfield et al., 2003; Renault et al., 2001; Zhang et al., 2015). Consequently, Canadian Natural Resources Limited (CNRL) has invested substantial resources to develop more environmentally-friendly technologies for tailings consolidation. One of the recently-developed new technologies uses thickeners in combination with CO_2 to consolidate fine tailings and produce non-segregated tailings (NST) (CNRL, 2019). NST combines thickener underflow and cyclone underflow to make a non-segregating sand-and-fines mixture. Carbon dioxide (CO_2) is injected into the process as a rheology modifier to increase yield strength of the slurry and decrease the potential for mixture segregation (CNRL, 2019). The primary advantage of NST is the increased fine particle capture by the tailings sand. NST also increases the release of water recycled in tailings ponds, speeding up tailings consolidation, and ultimately accelerate the reclamation process. However, the detailed chemistry of NST has not been well characterized and their potential phytotoxicity is not known. This is a crucial undertaking due to the large scale and risks associated with subsequent site revegetation.

Revegetation of oil sands reclamation sites is often impacted by high soil pH and elevated salinity (Renault et al., 1998, 2000; Howat, 2000; Calvo-Polanco et al., 2017). While the pH of undisturbed soils in the boreal forests is typically below 6.0, the soil pH in reclamation areas frequently exceeds 8.0 (Howat, 2000). High root zone pH can impair water and nutrient uptake (Zhang et al., 2013), causing reduced growth and leaf chlorosis (Zhang et al., 2015; Zhang and Zwiazek, 2016b). Fe, Mn, P, and Zn tissue deficiencies commonly occur in plants growing in high pH soils (George et al., 2012; Zhang et al., 2013). Soil electrical conductivity (EC) values in some reclamation areas can exceed 5 mS cm^{-1} (Lazorko and Van Rees, 2012). The "Guidelines for Reclamation of Forest Vegetation in the Athabasca Oil Sands Region" sets an EC threshold of reconstructed soil at 2 mS cm^{-1}, as higher values can severely affect the establishment and reduce the productivity of vegetation (Alberta Environment, 2010). In addition, high soil salinity can inhibit growth, decrease the water potential of plants, induce nutrient imbalance, and cause ion toxicity in plants (Munns and Tester, 2008; Duan and Chang, 2017; Vaziriyeganeh et al., 2018). It can also suppress the growth and activity of soil microorganisms (Elmajdoub et al., 2014).

Paper birch (*Betula papyrifera* Marsh.), white spruce [*Picea glauca* (Moench) Voss] and green alder [*Alnus viridis* (Chaix) DC.] are native boreal tree species in the AOSR and are commonly planted at oil sands reclamation sites. Previous hydroponic studies showed that white spruce is more tolerant to high root zone pH that commonly occur at oil sands reclamation sites compared with paper birch and green alder (Renault et al., 1998; Zhang et al., 2013; Zhang and Zwiazek, 2016b). Green alder can fix nitrogen from the atmosphere and is used for afforestation on infertile soils (Vogel and Gower, 1998). The present study was carried out under controlled environmental conditions to: (1) characterize the effects and potential toxicity of NST on paper birch, white spruce and green alder seedlings; (2) examine effects of addition of top soil and peat mineral mix in NST on seeding growth; (3) help develop guidelines for reclamation of NST-affected areas. We hypothesized that as NST contain lower levels of salt than traditional oil sands tailings, amending NST with reclamation soil (either peat mineral mix, PMM, or top soil, TS) would largely alleviate its deleterious effects on reclamation plants. By monitoring the growth and physiological parameters, we also assessed the suitability of paper birch, white spruce and green alder for the reclamation of sites affected by NST.

2. Materials and methods

2.1. Plant material and growth conditions

One-year-old, container-grown (415D styroblock, Beaver Plastics, Acheson, Canada) paper birch, white spruce, and green alder dormant seedlings were obtained from Smoky Lake Forest Nursery in Alberta, Canada. These seedlings were grown from seeds collected in undisturbed boreal forest near the CNRL oil sands mines. The forest LFH topsoil (TS), 0–50 cm below the forest floor layer, was collected from a boreal forest site (57°34′N and 111°75′W) near Fort McMurray, Alberta. Peat-mineral soil mix (PMM) and non-segregated tailings (NST) were obtained from CNRL oil sands mining areas. The study was conducted in a controlled-environment growth chamber. Environmental conditions in the growth chamber were: 22/18 °C (day/night) temperature, 65 ± 10% relative humidity, and 16-h photoperiod. The photosynthetic photon flux density (PPFD), provided by full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada), was 300–350 μmol m^{-2} s^{-1} measured at the top of the plants.

Roots were gently washed and the plants placed in individual 1000 ml pots. The plants were grown in five types of media: topsoil (TS), peat-mineral soil mix (PMM), non-segregated tailings sediment (NST), a mixture of TS and NST (1:2, v/v), and a mixture of PMM and NST (1:2, v/v). Each pot was filled with 800 ml of medium. Eight seedlings per species were grown in each type of growth medium ($n = 8$) for eight weeks. The seedlings were watered every other day and fertilized weekly with 50% modified Hoagland's solution (Epstein, 1972).

2.2. Growth medium analysis

One hundred grams of growth medium was collected from six replicates ($n = 6$) and ground into fine powder with a mortar and pestle. Thirty grams of powder was mixed with 60 ml of distilled water and shaken for 1 h. A solution was then obtained by vacuum filtration of this slurry. The electrical conductivity (EC) and pH of the solutions were measured with an Orion STAR A111 pH meter (Thermo Fisher Scientific Inc., Waltham, MA) and a Traceable 15-077-977 EC m (Thermo Fisher Scientific Inc., Waltham, MA), respectively. Total C and N concentrations of growth media were determined with a Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific Inc., Waltham, MA). The concentrations of other elements in the growth media solutions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) at the Canadian Center for Isotopic Microanalysis (CCIM) facility at the University of Alberta. The sodium adsorption ratio (SAR) was calculated using the following equation:

$$\mathrm{SAR} = \frac{Na^+}{\sqrt{\frac{1}{2}\left(Ca^{2+} + Mg^{2+}\right)}}$$

2.3. Mortality rate, height growth, and plant dry weights

The mortality rate was calculated by dividing the number of dead plants at the end of the experiment in each treatment by eight. Seedling shoot heights were measured from the root collar to the shoot tip both at the beginning and end of the experiment. The relative shoot height growth (RSHG) was calculated by dividing the difference of initial and final heights by the initial height. As green alder has many branches and it was difficult to identify a main stem, the shoot height of seedlings was not measured. Roots and stems were dried in an oven at 70 °C for 72 h. To avoid chlorophyll degradation at high temperature, separated leaves were freeze-dried for 72 h. Weights of all leaves and stems from each plant were combined to calculate shoot dry weights.

2.4. *Net photosynthesis and transpiration rates*

After eight weeks of growth in the different media, six seedlings were randomly taken from each treatment to measure net photosynthesis (A) and transpiration (E) rates. The measurements were carried out between 9:00–12:00 h using a LI-6400XT Portable Photosynthesis System (Li-Cor, Lincoln, Nebraska, USA). The reference CO_2 concentration was set to 400 μmol mol^{-1}; the flow rate was 200 μmol s^{-1}. The leaf chamber temperature was maintained at 20 °C, and PPFD was 400 μmol m^{-2} s^{-1}. For white spruce, a 3-cm distal section of needles was inserted into the leaf chamber. The needles inserted in the leaf chamber were severed with scissors, removed and scanned. Needle areas were then calculated using Sigmascan Pro 5.0 software (Systat Sofware).

2.5. *Foliar chlorophyll and elemental concentrations*

Leaf chlorophyll-a and chlorophyll-b concentrations were determined in six randomly selected seedlings per treatment ($n = 6$). The leaves were lyophilized and ground in a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). Chlorophyll was extracted from the leaf samples (10 mg dry weight) using 8 mL dimethyl sulfoxide (DMSO) at 65 °C for 22 h and the extracts filtered. Chlorophyll concentrations were measured with a spectrophotometer (Genesys 10 S-UV-VIS, Thomas Scientific, NJ, USA) at 648 and 665 nm. Total chlorophyll concentration (chlorophyll *a* and *b*) was calculated using Arnon's equation (Sěsták et al., 1971). Since the DMSO extracts of green alder leaves turned black, which interfered with chlorophyll analysis, chlorophyll was extracted from green alder leaves with 8 mL methanol at 55 °C for 24 h and measured at 652 and 665 nm for chlorophyll *a* and *b*, using the spectrophotometer as mentioned above (Wellburn, 1994).

For the elemental analysis of Na, Mg, P, K, Ca, Fe, Mn, Cu, Zn, pulverized leaf samples (0.2 g dry weight) were microwave-digested with 10 mL 70% HNO_3 for 10 min at 185 °C (Mars 5 Microwave Accelerated Reaction System, CEM), and diluted to 40 mL with Milli-Q water. After filtering the extracts were analyzed by ICP-MS at the Canadian Center for Isotopic Microanalysis (CCIM) at the University of Alberta (Zarcinas et al., 1987).

2.6. *Statistical analysis*

To reveal statistically-significant differences between treatments ($p \leq 0.05$), all data were fitted to GLM models using SAS (version 9.3, SAS Institute Inc.). Data that did not meet the ANOVA assumptions for normality of distribution and homogeneity of variance were transformed with a log10 function. Comparisons between different treatment means were conducted using the Student-Newman-Keuls (SNK) test.

3. Results

3.1. *Chemical properties of growth media*

The pH of NST was about 9, while that of TS and PMM was about 6. The pH of mixtures of NST + TS and NST + PMM was approximately 7 (Fig. 1a). There were no significant differences in EC between different types of growth media; EC ranged from 0.8 to 1.7 mS cm^{-1} (Fig. 1b). However, The SAR of NST was about 40-fold higher than that of TS and PMM, and the SAR of NST + TS and

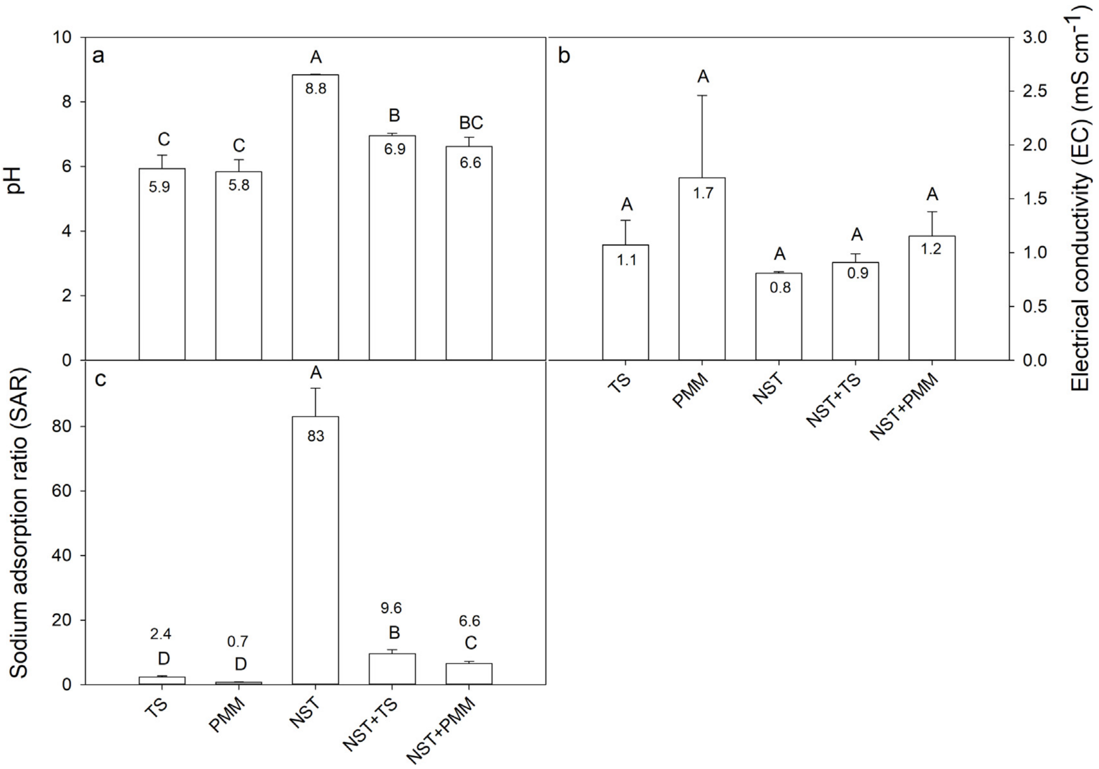

Fig. 1. Soil pH (a), electrical conductivity (EC) (b), and sodium adsorption ratio (SAR) (c) of plant growth media: top soil (TS), peat mineral mix (PMM), non-segregated tailings (NST), mixture of NST and TS (2:1, v/v), and mixture of NST and PMM (2:1, v/v). Means ($n = 6$) ± SE are shown. Different letters indicate significant difference at $p < 0.05$.

Table 1
Soil soluble elemental concentrations of Na, Mg, P, K, Ca, Fe, Mn, Cu, and Zn (Mean ± SE, n = 6). Different letters indicate significant differences in element concentration between different growth media at $p < 0.05$. TS, PMM, and NST stand for top soil, peat-mineral soil mix, and non-segregated tailings, respectively. NST + TS means mixture of NST and TS (2:1, v/v). NST + PMM means mixture of NST and PMM (2:1, v/v). TN, TC and DL stands for total nitrogen, total carbon and detection limit, respectively.

Media	TN	TC	Na	Mg	K	Ca	P	Fe	Mn	Zn
	%	%	ppm	ppm	ppm	ppm	ppb	ppb	ppb	ppb
TS	0.19 ± 0.02 c	4.33 ± 0.52 c	96.64 ± 15.93 b	40.83 ± 10.2 a	8.33 ± 0.82 a	82.49 ± 21.33 a	1211 ± 361 a	2245 ± 1162 a	3175 ± 612 ab	288 ± 45 a
PMM	1.07 ± 0.17 a	21.78 ± 3.98 a	47.21 ± 15.46 c	86.31 ± 42.08 a	8.17 ± 3.27 ab	248.86 ± 122.74 a	950 ± 384 a	1551 ± 618 a	3826 ± 2910 a	237 ± 81 a
NST	0.01 ± 0.00 d	0.82 ± 0.02 d	175.04 ± 2.91 a	0.18 ± 0.02 b	3.07 ± 0.06 b	0.12 ± 0.03 b	60 ± 17 b	<DL	3 ± 0.33 b	<DL
NST + TS	0.13 ± 0.02 c	3.17 ± 0.34 c	172.72 ± 9.52 a	7.6 ± 1.99 ab	5.61 ± 0.5 ab	19.97 ± 6.18 ab	293 ± 95 ab	550 ± 189 a	290 ± 38 ab	147 ± 31 a
NST + PMM	0.72 ± 0.11 b	14.86 ± 2.66 b	176.63 ± 16.27 a	19.03 ± 8.48 ab	8.18 ± 1.82 ab	55.05 ± 21.6 ab	149 ± 25 ab	354 ± 70 a	634 ± 413 ab	179 ± 14 a

NST + PMM was about 5- to 10-fold higher than TS and PMM (Fig. 1c).

The total nitrogen and carbon concentrations of NST were considerably lower than those of PMM and TS. However, NST + PMM and NST + TS mixtures had significantly higher total nitrogen and carbon concentrations than NST (Table 1). Overall, variations in TS and PMM elemental concentration were relatively high (Table 1). TS contained two-fold higher Na than PMM, while for other elements (Mg, K, Ca, P, Fe, Mn, Zn), there were no significant differences (Table 1). The Na concentrations of growth media containing NST (i.e. NST, NST + TS, and NST + PPM) were about two-fold higher compared with TS and three-fold higher than those of PMM (Table 1). The concentrations of all other elements (Mg, K, Ca, P, Fe, Mn, Zn) in TS and PMM were significantly higher than in NST, particularly those of Mg, Ca, Fe, Mn and Zn (Table 1). The mixtures of NST + TS and NST + PMM contained much higher levels of Fe and Zn compared with pure NST (Table 1).

3.2. Plant mortality and growth

For all three species, the highest mortality rate occurred in plants growing in NST with 37%, 75%, 17% mortality for paper birch,

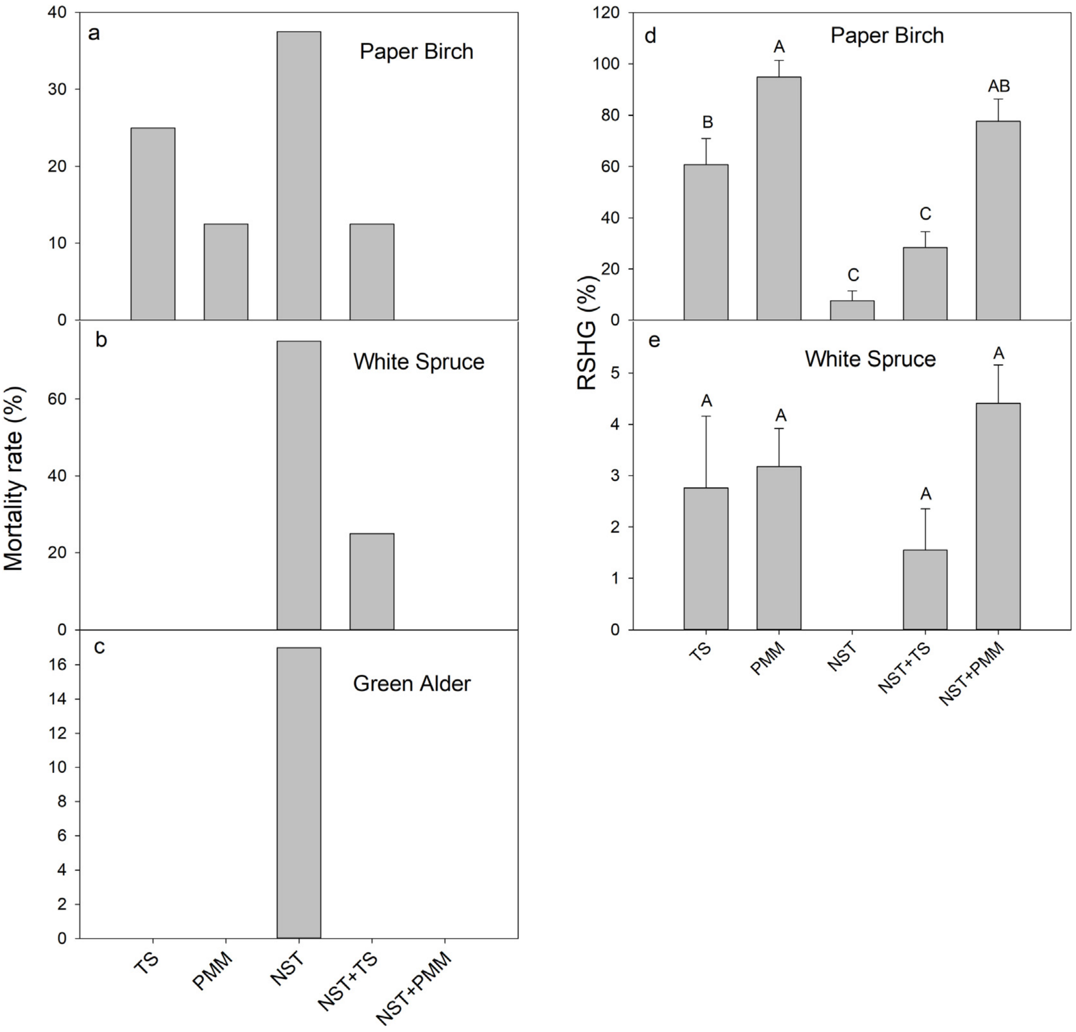

Fig. 2. Mortality rate (%) (n = 8) of paper birch (a), white spruce (b) and green alder (c) by the end of experiment; and relative shoot height growth (RSHG) (%) of paper birch (d) and white spruce (e). Means (n = 8) ± SE are shown. Different letters indicate significant difference at $p < 0.05$.

white spruce, and green alder, respectively (Fig. 2a, b, c). For paper birch growing in TS, PMM and NST + TS, between 10% and 25% seedlings died (Fig. 2a). In paper birch, the RSHG of plants growing in TS and PMM was significantly higher compared with plants growing in NST and NST + TS (Fig. 2d). White spruce grown in NST did not show a measurable increase in shoot heights, and the RSHG of white spruce in other growth media were similar (Fig. 2e).

For all three species, dry weights were highest in plants growing in PMM, and lowest in plants growing in NST (Fig. 3a, b, c). In addition, the dry weights of paper birch and white spruce growing in NST + PMM were significantly higher compared with plants in pure NST (Fig. 3a and b). Green alder dry weights were significantly higher for plants in PMM than in the other growth media (Fig. 3c). Paper birch shoot:root DW ratios did not differ between treatments (Fig. 3d). However, the shoot:root DW ratios of white spruce growing in NST were about two-fold higher than in the other media, and were significantly higher for green alder growing in NST compared with NST mixtures (Fig. 3e and f).

3.3. *Gas exchange and chlorophyll*

The net photosynthesis rates were significantly higher for paper birch growing in PMM compared with NST, although there were no differences in transpiration rates (Fig. 4a, d). White spruce growing in NST had significantly lower net photosynthesis and transpiration rates compared with plants growing in the other media (Fig. 4b, e). Green alder growing in TS and PMM had significantly higher net photosynthesis rates compared with NST, and the seedlings growing in NST mixtures had intermediate net photosynthesis rates (Fig. 4c). Transpiration rates were little affected by the type of growth medium. Only alder seedlings growing in PMM had significantly higher transpiration rate values compared with NST (Fig. 4f).

Leaf chlorophyll concentrations in paper birch varied little across the growth media (Fig. 5a). White spruce seedlings had significantly higher needle chlorophyll concentrations when growing in PMM compared with NST and NST + TS (Fig. 5b). In green alder, chlorophyll concentrations were the lowest for individuals growing in NST (Fig. 5c).

3.4. *Foliar elemental concentration*

Leaf Na concentrations in paper birch were about two-to three-fold higher for plants growing in NST and NST + TS compared with the other media. In addition, birch seedlings growing in NST had significantly lower leaf Ca concentrations than those growing in TS and PMM, whereas plants growing in NST + PMM had significantly higher K and Fe concentrations compared with plants growing in

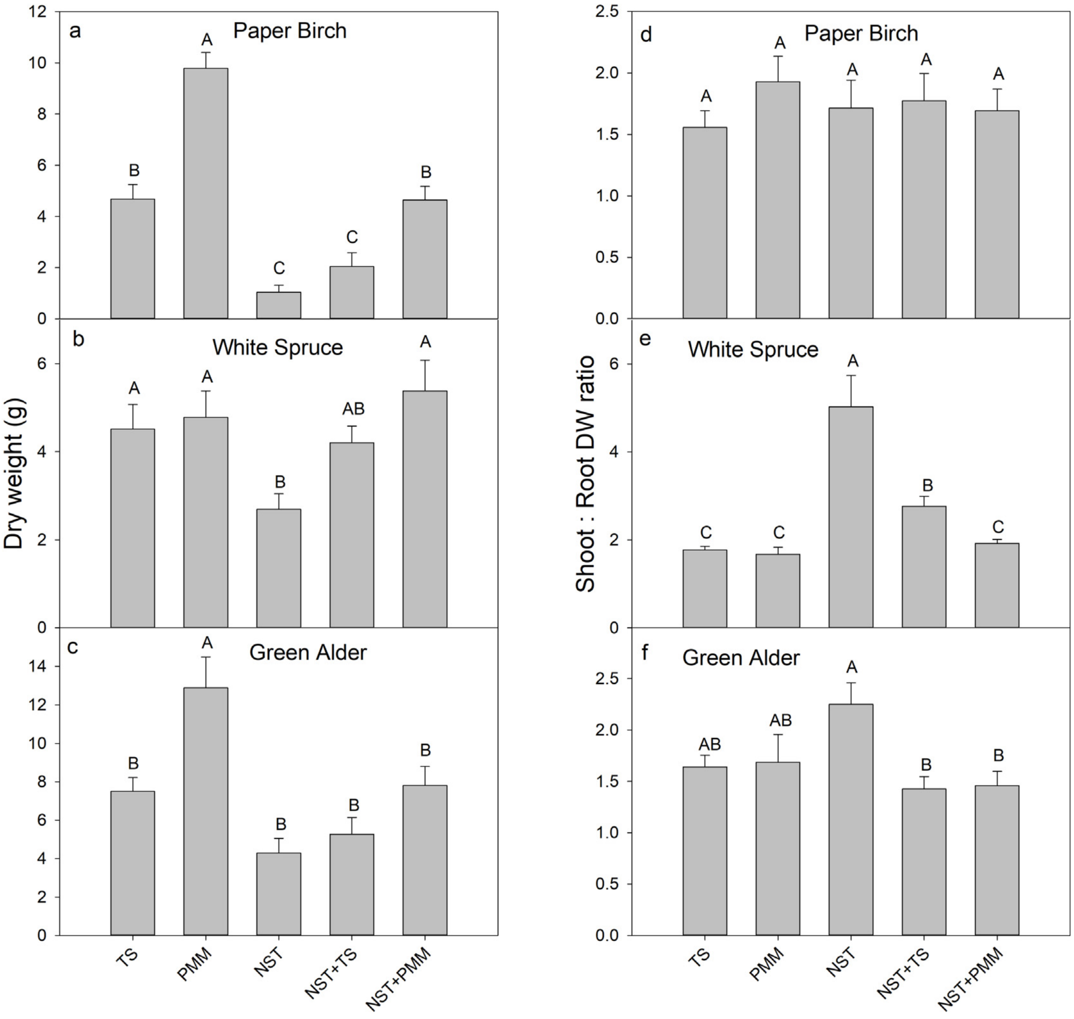

Fig. 3. Dry weight (DW) and shoot:root DW ratio of paper birch (a, d), white spruce (b, e) and green alder (c, f) seedlings at the end of experiment. Means ($n = 8$) ± SE are shown. Different letters indicate significant difference at $p < 0.05$.

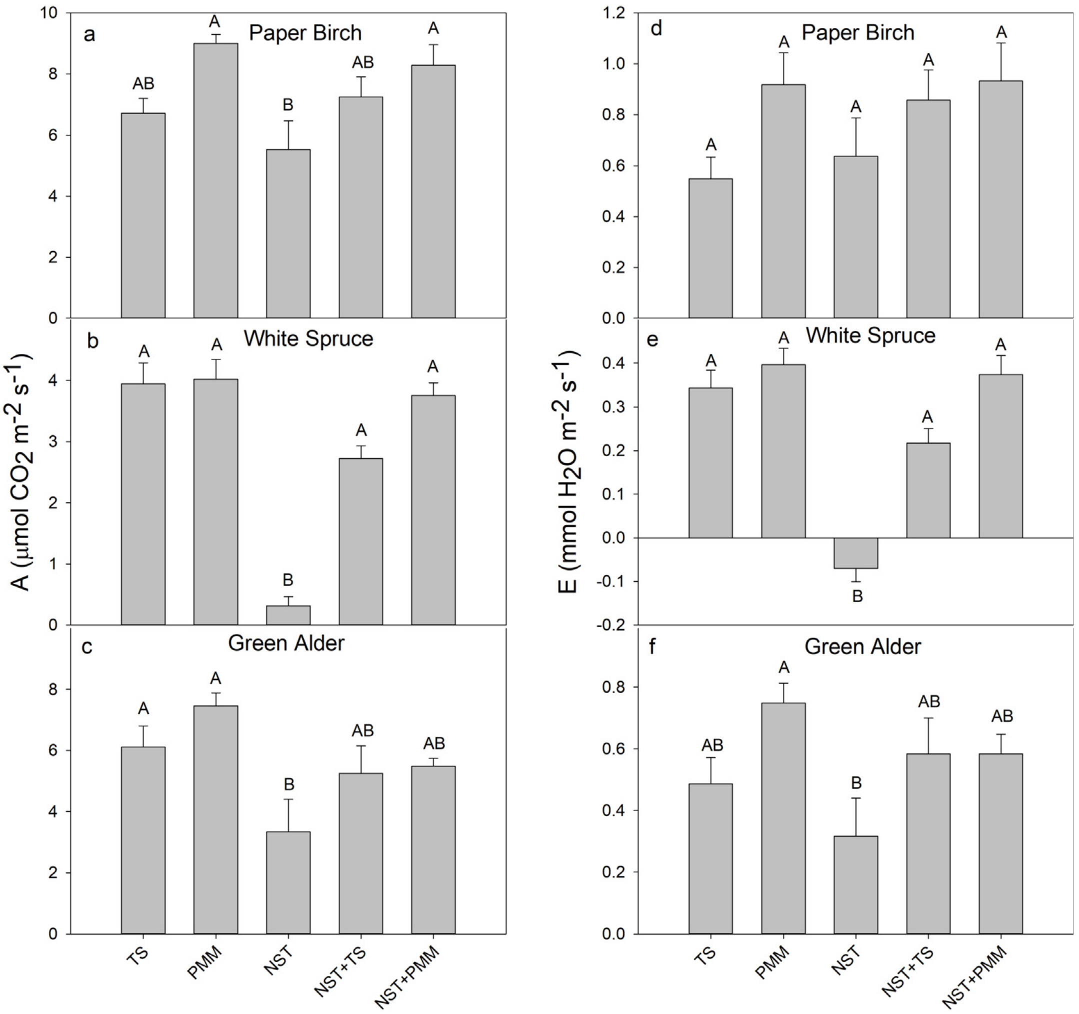

Fig. 4. Net photosynthesis (A) and transpiration (E) rate of paper birch (a, d), white spruce (b, e) and green alder (c, f) seedlings at the end of experiment. Means (n = 8) ± SE are shown. Different letters indicate significant difference at $p < 0.05$.

NST (Table 2).

White spruce seedlings growing in NST had significantly higher foliar concentrations of Na, P, K, Cu and Zn than those grown in the other media (Table 2). Needle concentrations of Na in white spruce seedlings growing in NST were over 100-fold higher compared with the seedlings growing in PMM and about five-to six-fold higher compared with the seedlings growing in the remaining growth media. Spruce seedlings also had significantly higher needle Ca and Mn concentrations when grown in PMM (Table 2).

Leaf Na concentrations in green alder were also highest in seedlings growing in NST compared with the other media. Seedlings growing in PMM had significantly higher leaf concentrations of Ca, Fe, Mn and Zn compared with the seedlings growing in NST (Table 2).

4. Discussion

The results clearly demonstrate that NST reduced growth and negatively affected the measured physiological processes in paper birch, white spruce and green alder seedlings. However, by mixing NST with PMM or TS, the growth and physiological responses of seedlings significantly improved. The harmful effects that NST imposed on plants can partly be attributed to high pH, elevated Na levels, and a resulting nutrient imbalance. However, it should also be noted that the reclamation top soil also contained naturally relatively high Na concentration. Therefore, we recommend that all reclamation soils are tested for their Na levels before using on the sites in which other sources of Na may be present.

However, unlike gypsum-amended composite tailings, the EC (0.8 mS cm^{-1}) of NST is within the acceptable range (0–2 mS cm^{-1}) for normal growth of reclamation plants (Alberta Environment, 2010). The Na concentration in the traditional alum- or gypsum-amended composite tailings can be higher than 1000 ppm (Renault et al., 2004) while in the NST, the Na concentration is lower than 200 ppm, that partially explains the relatively low EC of NST. Therefore, the low osmotic potential caused by high salinity that usually plagues traditional composite tailings reclamation sites (Duan et al., 2015), was not a major factor that impacted plant growth in this study. The low Na levels and EC of NST can offer advantages to revegetation at oil sands reclamation sites compared with traditional composite tailings. However, it is plausible that the more viscous texture of the NST medium may be a confounding factor and leading to poor root aeration and aggravating the effects of salt.

According to oil sands reclamation guidelines, a SAR of 12 and above is regarded as unsuitable for revegetation (Fung and Macyk, 2000). However, the SAR of the NST used in the study was greater than 80, which can be severely detrimental for the growth of

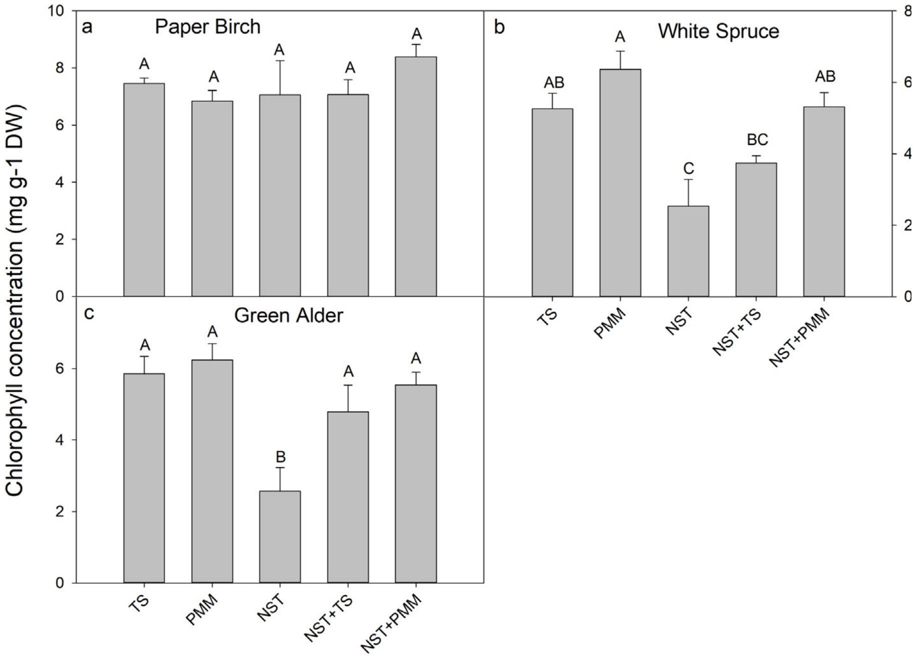

Fig. 5. Leaf chlorophyll concentration of paper birch (a), white spruce (b) and green alder (c) seedlings at the end of experiment. Means ($n = 8$) ± SE are shown. Different letters indicate significant difference at $p < 0.05$.

Table 2
Foliar element concentration of paper birch, white spruce and green alder grown in different media (Units for Mg, P, K and Ca is %; for other elements it is ppm. Mean ± SE, n = 6). Different letters indicate significant difference at $p < 0.05$. TS, PMM, and NST stand for top soil, peat-mineral soil mix, and non-segregated tailings respectively. NST + TS means mixture of NST and TS (2:1, v/v). NST + PMM means mixture of NST and PMM (2:1, v/v).

Media	Na	Mg	P	K	Ca	Fe	Mn	Cu	Zn
Paper Birch									
TS	211.4 ± 17.7 b	0.57 ± 0.04 a	0.1 ± 0 b	0.74 ± 0.06 b	0.73 ± 0.05 ab	103.8 ± 19.6 b	145 ± 30.7 b	2.7 ± 0.6 a	40.5 ± 3.9 b
PMM	154.9 ± 10.6 b	0.43 ± 0.02 b	0.18 ± 0.01 a	0.87 ± 0.03 ab	0.75 ± 0.04 a	93.5 ± 13.6 b	357.2 ± 95.7 a	2 ± 0.4 a	111.3 ± 11.4 a
NST	434.1 ± 95.1 a	0.42 ± 0.02 b	0.17 ± 0.03 a	0.77 ± 0.07 b	0.53 ± 0.03 c	94.4 ± 14.8 b	177.2 ± 58.1 ab	2.3 ± 0.2 a	28.5 ± 9 b
NST + TS	521.8 ± 121.8 a	0.46 ± 0.03 b	0.12 ± 0.01 b	0.91 ± 0.1 ab	0.59 ± 0.06 abc	90.3 ± 5.1 b	104.4 ± 18.2 b	2 ± 0.3 a	22.3 ± 8 b
NST + PMM	183.9 ± 23.6 b	0.41 ± 0.01 b	0.11 ± 0.01 b	1.09 ± 0.08 a	0.57 ± 0.02 bc	171.9 ± 26.7 a	285.4 ± 21.8 ab	1.2 ± 0.2 a	37 ± 4.6 b
White Spruce									
TS	1854.3 ± 314.9 b	0.21 ± 0.02 ab	0.14 ± 0.01 b	0.31 ± 0.02 c	0.55 ± 0.05 b	84.7 ± 3.3 ab	47.7 ± 5.7 b	2.6 ± 0.2 b	5.8 ± 0.5 b
PMM	125.5 ± 41.9 c	0.25 ± 0.02 a	0.16 ± 0.02 b	0.5 ± 0.04 b	0.71 ± 0.07 a	93.2 ± 6.9 a	78.8 ± 13.4 a	2.5 ± 0.3 b	7.4 ± 0.8 b
NST	13538.4 ± 2915.8 a	0.21 ± 0.01 ab	0.34 ± 0.03 a	1.2 ± 0.05 a	0.5 ± 0.03 b	85.9 ± 1.9 ab	46.9 ± 5.5 b	5.3 ± 0.3 a	16 ± 2.8 a
NST + TS	3007.4 ± 508.3 b	0.14 ± 0.02 b	0.12 ± 0.01 b	0.29 ± 0.04 c	0.31 ± 0.03 c	73.9 ± 2.6 b	28 ± 2.5 b	2.7 ± 0.5 b	7.4 ± 1.8 b
NST + PMM	2133.8 ± 324.6 b	0.19 ± 0.02 ab	0.14 ± 0 b	0.41 ± 0.03 bc	0.51 ± 0.03 b	87.3 ± 3.5 ab	40.2 ± 5.7 b	2.4 ± 0.1 b	6.4 ± 1 b
Green Alder									
TS	138.6 ± 27.4 b	0.35 ± 0.02 a	0.07 ± 0.01 b	0.78 ± 0.07 b	0.67 ± 0.05 b	82 ± 7.2 b	77.3 ± 19 ab	7.4 ± 1 a	13.6 ± 1.3 bc
PMM	103.3 ± 24.2 b	0.37 ± 0.02 a	0.13 ± 0.02 ab	0.93 ± 0.07 ab	0.99 ± 0.08 a	108.4 ± 9.3 a	103.6 ± 18 a	7.4 ± 0.8 a	32.4 ± 4 a
NST	772.9 ± 244.5 a	0.35 ± 0.03 a	0.2 ± 0.02 a	0.99 ± 0.14 ab	0.66 ± 0.06 b	68.6 ± 7.5 b	20.9 ± 6.9 c	7.3 ± 0.5 a	8.8 ± 0.8 c
NST + TS	330.9 ± 68.2 b	0.43 ± 0.04 a	0.16 ± 0.03 a	1.12 ± 0.07 a	0.83 ± 0.04 ab	73.7 ± 7 b	35.6 ± 7.9 bc	6.7 ± 0.3 a	9 ± 0.8 c
NST + PMM	167.9 ± 32.5 b	0.37 ± 0.04 a	0.14 ± 0.01 ab	1.15 ± 0.08 a	0.76 ± 0.07 b	132.9 ± 11.2 a	102.4 ± 19.7 a	7.1 ± 0.5 a	18.2 ± 2.7 b

reclamation plants. The foliar Na concentrations of paper birch, green alder, and white spruce seedlings in particular, were significantly higher when growing in NST than in TS and PMM. Surprisingly, the NST medium's very low soluble Mg and Ca concentrations were not reflected in their foliar concentrations of white spruce and green alder. It could be that since the growth of seedlings in NST was stunted, the nutrients stored in plant tissues prior to the start of the experiment were sufficient to support some limited growth.

As only one or two paper birch seedlings died in the first two weeks of the study, the mortality seen in TS and PMM might be the result of transplantation shock. However, birch seedling mortality in the NST substrate occurred after four weeks of growth. White spruce showed the highest mortality rate and lowest gas exchange values among all three species when grown in NST. Though not unexpected, as conifers are generally considered to be more susceptible to salt stress than deciduous trees (Renault et al., 1998), this could be related to an inability to prevent Na transport from roots to shoots (Redfield et al., 2004). The much higher Na concentration recorded in white spruce needles of seedlings growing in NST when compared to those grown in TS or PMM supports this assumption.

The shoot:root DW ratios were significantly higher in NST than in TS and PMM for white spruce seedlings only. However, when taking into account the lower dry weights of white spruce seedlings growing in NST, it can be inferred that the medium simply inhibited root growth to a larger extent than shoot growth. It appears NST inhibited paper birch shoot and root growth in a similar fashion. At the other end of the scale, paper birch and green alder dry weights were highest in seedlings grown in PMM, likely due to the low Na and high nitrogen and carbon content of PMM. Nitrogen availability is a major factor limiting growth of boreal trees due to its high demand (Duan et al., 2018). In addition, organic carbon found in PMM can improve nutrient cycling, water holding capacity, cation exchange capacity and microbial activities in reclaimed soil (Rowland et al., 2009). Furthermore, although all of the NST-containing media had similar soluble Na concentrations, only in the leaves of plants growing in NST + PMM was the Na concentration lower. Thus, it appears that the addition of PMM can also help plants growing in NST uptake mineral nutrients in a more balanced manner.

Although dry weights of paper birch were lowest when grown in NST, the A, E, and chlorophyll concentration of birch seedlings grown in NST were similar to those grown in TS. This suggests that paper birch grown in NST allocated more carbon resources to maintain physiological processes at the cost of growth. Deciduous trees and conifers use different strategies to utilize carbon resources both internally and externally (Dickson, 1989). Thus, unlike paper birch, white spruce roots might not be able to obtain enough photosynthates to cope with high pH and SAR stress of NST. This can explain why the shoot:root DW ratios were higher, and why injury was more pronounced compared to the two studied deciduous species.

The high pH (>9) of NST was another important stress factor affecting the growth and physiology of the studied seedlings. High soil pH is commonly associated with reduced availability of Fe, Mn, P, and Zn (George et al., 2012; Zhang et al., 2013). However, likely due to the relatively short timeframe of the study and slow growth of plants, we did not observe deficiencies of these elements in plant tissues. For the white spruce plants growing in NST, as the seedlings had the lowest dry weight among all treatments, the higher foliar concentration of P, K, Cu and Zn can be explained by the biomass dilution effect (Jarrell and Beverly, 1981). Green alder seedlings had the highest levels of Fe and Mn when grown in PMM-containing substrates. Typically, these are the most commonly deficient nutrients for plants grown in alkaline soils and can severely constrain chlorophyll synthesis and photosynthesis (George et al., 2012). High pH may also inhibit shoot and root growth (Tang et al., 1992; Zhang et al., 2013; Zhang et al., 2015) and result in reductions in stomatal conductance (Tang et al., 1993; Kamaluddin and Zwiazek, 2004) and shoot water potential (Tang et al., 1993; Zhang et al., 2013). Moreover, high pH can also reduce root water flux, likely by affecting the function of aquaporins (Kamaluddin and Zwiazek, 2004; Tournaire-Roux et al., 2003; Zhang and Zwiazek, 2016a). White spruce has been reported to be more resistant to high pH than trembling aspen (*Populus tremuloides*) and tamarack (*Larix laricina*) (Zhang et al., 2013). Therefore, it could be concluded that the injury and growth inhibition of white spruce grown in NST in this study was likely caused by the salt toxicity.

In conclusion, our results demonstrated that NST significantly inhibited growth of paper birch, white spruce and green alder and the effects were most prominent in white spruce. The stress that NST imposed on plants was likely caused by high levels of Na accumulation in tissues, which disrupted photosynthesis and water uptake processes. However, by mixing NST with TS or PMM, growth performance was significantly improved. PMM shows promise, as it enabled plants a more balanced uptake of mineral nutrients. Further studies should be conducted to monitor reclamation plant responses in NST-affected soils under field conditions. It appears that white spruce may be less suitable for planting at reclamation sites containing NST compared with the two studied deciduous tree species.

Author contribution statement

Wen-Qing Zhang: Investigation, Data curation, Formal analysis, Writing - original draft. Killian Fleurial: Writing - review & editing. Ira Sherr: Conceptualization, Resources, Writing - review & editing. Robert Vassov: Writing - review & editing. Janusz J. Zwiazek: Conceptualization, Resources, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Research funding for the study was provided to JJZ by Canadian Natural Resources Limited (CNRL), Canada's Oil Sands Innovation Alliance (COSIA), and the Natural Sciences and Engineering Research Council of Canada (NSERC), Collaborative Research and Development Grant.

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