Root hypoxia aggravates the effects of saline tailings water on the growth and physiology of woody plants

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Abstract

Aims

Oil sands mining in the boreal forest produces large volumes of liquid tailings. Research has generally focused on the thickness and composition of the soil layers to be placed on top of the tailings during reclamation. However, tailings release water, which may seep into the root zone and affect plants. Furthermore, the interactions and combined effects of root hypoxia and root substrate chemistry on plant responses are poorly understood.

Methods

The effects of the aqueous phase of novel tailings (Non-Segregating Tailings—NST) were studied under well-aerated and hypoxic conditions in three relatively hypoxia resistant tree species [tamarack (*Larix laricina*), black spruce (*Picea mariana*), and balsam poplar (*Populus balsamifera*)] and three relatively sensitive tree species [lodgepole pine (*Pinus contorta*), jack pine (*Pinus banksiana*), and aspen (*Populus tremuloides*)] by growing them in hydroponic solutions in a controlled environment.

Results

Root hypoxic conditions further reduced the survival, growth, and physiology of plants exposed to NST. Our results confirm that NST water produces the same deleterious effects in plants as previously reported in the amalgamated oil sands tailings. In trembling aspen, salt sequestration was inhibited, and in black spruce needles hypoxia may have prevented an osmoregulative mechanism.

Conclusions

Our results highlight the potential impact of water seepage from buried tailings on reclamation success. Furthermore, hypoxic conditions can aggravate these effects by inhibiting salt stress mechanisms. We suggest that the preparation of reclamation sites impacted by tailings water should involve efforts aimed at improvement of soil aeration to minimize the detrimental effects on plants.

Introduction

Oil sands mining produces large volumes of tailings, which may potentially affect the growth and survival of plants in reclamation areas if present in the root zone. Therefore, in sites where mining operations have ceased, oil sands companies must employ approaches to minimize the effects of these tailings on the vegetation used for mandated land reclamation and return these sites to their natural, pre-disturbance state (Government of Canada, 2013). The latest data available from Alberta Environment and Parks shows that, as of 2020, 159,961.9 ha of Athabasca oil sand land had been approved for mining use, 105,541.6 ha of which were actively disturbed by mining related operations. However, only 8,730.1 ha (15% of which are wetlands) are being reclaimed by mining operators and, of those, only 104.3 ha (> 1%) have been fully certified as having completed the process by government regulators and returned to the crown (Alberta Environment and Parks, 2020).

Previous studies have generally focused on the thickness, composition, and layering of the capping material placed on top of the tailings during reclamation (Macdonald et al., 2015; Pinno et al., 2012; Sorenson et al., 2011; Zhang et al., 2020). However, tailings release saline water, which over time may seep into the root zone and affect plants. This potentially constitutes an environmental liability, as the underlying consolidated tailings may continue to infiltrate the upper layers of reclamation substrates, including the root zone of plants used for reclamation. An initially vigorous reclamation project

could, therefore, find its species composition significantly altered in subsequent years if some of the species used are unable to cope with the compounds that are commonly found in oilsands tailings.

The elevated levels of Na, boron, and naphthenic acids present in tailings, are especially detrimental to the growth and establishment of reclamation plants (Kamaluddin and Zwiazek, 2002; Renault et al., 1999). However, the existing knowledge of how the chemical composition of oil sands tailings and tailings water affects the physiology of boreal plants is limited and more research is needed (Redfield et al., 2003; Zhang et al., 2020).

In addition to the potentially harmful chemical constituents of tailings water, soil saturation with water may lead to waterlogging, which reduces O_2 availability to plant roots. As hypoxic conditions set in, root respiration is inhibited, and cells undergo an anaerobic metabolic switch over to lactic and or alcoholic fermentation (Agarwal and Grover, 2006). Many plant-physiological processes are consequently affected, including photosynthesis, transpiration and stomatal conductance (Andersen et al., 1984; Pereira and Kozlowski, 1977), as well as carbohydrate metabolism (Huang and Johnson, 1995) and root water transport (Mohammed Kamaluddin and Zwiazek, 2002). To avoid or mitigate damage from a prolonged switch to fermentation pathways, plants must increase the influx of O_2 to the roots and reduce the accumulation of toxic fermentation products. In some hypoxia-tolerant species, morphological changes such as, the production of aerenchyma, hypertrophied lenticels, or adventitious roots are triggered (Calvo-Polanco et al., 2012; Islam and Macdonald, 2004; Wang et al., 2013). However, the potential interactions and combined effects of root hypoxia and root substrate chemistry on plant responses are poorly understood. This study aims to help fill this knowledge gap.

The present investigation focused on the effects of root hypoxia in combination with Non-Segregating Tailings (NST) developed by Canadian Natural to accelerate tailings consolidation and utilize less water during bitumen extraction. These tailings are created by removing water from the tailings stream, injecting carbon dioxide (CO_2), and adding thickeners to densify and ultimately reduce the final volume before their deposition in the tailings ponds (CNRL, 2020; COSIA, 2020). Once tailings solids are settled, the top liquid layer is removed and referred to as tailings water.

The study was carried out with six common boreal species which account for much of the dominant vegetation in the Canadian boreal forest where oilsands mines are located. The species included trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), lodgepole pine (*Pinus contorta*), and tamarack (*Larix laricina*). All six of these species are found in the northern boreal forest of North America growing alongside each other in various combinations of dominant, understory, codominant, or successional stages (Burns and Honkala, 1990). Due to their growth habits and the ecological niches that they occupy, tamarack, black spruce, and balsam poplar are considered to be relatively resistant tree species to flooding and root hypoxia, whereas lodgepole pine, jack pine, and trembling aspen are generally considered to be more sensitive (Johnston, 1990; Lotan and Critchfield, 1990; Perala, 1990; Rudolph and Laidly, 1990; Viereck and Johnston, 1990; Zasada and Phipps, 1990).

The main objectives of this study were to: 1) Assess how well different tree species used in the reclamation of consolidated oil sands tailings can tolerate the presence of oil sand tailings water in the presence and absence of root hypoxia 2) Identify potential chemical components in oil sands tailings water that may contribute to phytotoxicity and interact with hypoxia, and 3) Examine the underlying physiological mechanisms that may help plants tolerate these conditions. Here we evaluate the hypothesis that hypoxic conditions aggravate the effects of NST tailings water more severely in plant species that are less tolerant of root hypoxia and thus, the expectation that the effects of NST water under well-aerated and hypoxic conditions would be less detrimental to the flooding tolerant species (tamarack, black spruce, and balsam poplar) than the flooding sensitive species (lodgepole pine, jack pine, and trembling aspen).

Materials And Methods

Plant material and growth conditions

One-year-old, container-grown trembling aspen (*Populus tremuloides* Michx.) and balsam poplar (*Populus balsamifera* L.) dormant seedlings were obtained from the Smoky Lake Forest Nursery, Smoky Lake, AB, Canada. Tamarack [*Larix laricina* (Du Roi) K. Koch], jack pine (*Pinus banksiana* Lamb.), lodgepole pine (*Pinus contorta* Dougl.), and black spruce [*Picea mariana* (Mill.) B.S.P.] were obtained from the Bonnyville Forest Nursery, Bonnyville, AB, Canada. Fresh undiluted Non-Segregating Tailings (NST) were obtained directly from a pipe during discharge at the Canadian Natural oil sands mining area near Fort McMurray. After brief settling the liquid phase—NST water—was then collected for use. The study was conducted in a controlled-environment growth room. Environmental conditions in the growth room were maintained at 22/18°C (day/night), 70 ± 10% relative humidity, and 16-h photoperiod. The photosynthetic photon flux density (PPFD) was 300 µmol m^{-2} s^{-1} at the top of the seedlings provided by full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada).

The study was carried out in hydroponic media as earlier described (Redfield et al., 2004). Prior to the application of treatments, the seedlings' roots were gently washed free of soil and were then placed in well aerated 50% modified Hoagland's solution (Epstein, 1972) for two weeks to flush buds and acclimate to experimental conditions. Four treatments were then applied to the seedlings for two months: 1) Aerated control (50% Hoagland's solution, 7–8 mg O_2 l^{-1}), 2) Hypoxic control (50% Hoagland's solution, 2–3 mg O_2 l^{-1}), 3) Aerated NST (50% NST water in 50% Hoagland's solution at 7–8 mg O_2 l^{-1}), 4) Hypoxic NST (50% NST water in 50% Hoagland's solution at 2–3 mg O_2 l^{-1}). Each treatment was applied using two separate air pumps and tanks (100 l in volume) containing 50% NST water in 50% Hoagland's solution or 50% Hoagland's solution. Each tank was connected to three containers (30 l in volume) holding 18 randomly placed seedlings (3 plants per species) for a total of 24 containers and 332 plants (Figure S1).

Treatment solutions were aerated using air pumps connected to air stones placed in the tanks and strong solution circulation to maintain 7–8 mg O_2 l^{-1} solution whereas hypoxia (2–3 mg O_2 l^{-1}) was induced in the other treatments by limiting solution circulation and aeration. Solutions were fully replaced one month into the experiment. Dissolved oxygen levels were monitored daily using a Fisherbrand Traceable 06-662-66 portable dissolved oxygen meter (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) to ensure constant oxygen levels. The pH of the solutions was measured weekly using an Orion STAR A111 pH meter (Thermo Fisher Scientific Inc.) and adjusted when necessary to pH 5.5 in aerated and hypoxic control (no NST water) solutions using KOH.

Growth medium and NST water characterization

The electrical conductivity (EC) was measured with a Traceable 15-077-977 EC meter (Thermo Fisher Scientific Inc.).

Elemental analysis (Na, K, Mg, Ca, Cl, P, Fe, S, B) of NST water was conducted at the Natural Resources Analysis Laboratory (NRAL) at the University of Alberta. Elemental concentrations were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES) with a Thermo iCAP6300 Duo (Thermo Fisher Scientific Inc.) following US EPA methods 6010d and 200.7 (n = 6).

Fluoride concentrations were determined by ion chromatography using a Dionex DX-600 Ion Chromatography system (Dionex, Sunnyvale, USA) following the US EPA method 300.1 at the Biogeochemical Analytical Service Laboratory (BASL) facility at the University of Alberta (n = 6).

To determine naphthenic acid concentrations, NST water samples were first filtered (0.45 µm Teflon membrane), then extracted using dichloromethane and sodium sulphate (Scott et al., 2008). Concentrations were then obtained by Fourier-transform infrared spectroscopy (FTIR; n = 3).

The average pH, EC, elemental concentrations, and naphthenic acid concentrations of pure NST and of the treatment solutions used in the experiment are provided in Table 1.

Table 1
pH, electrical conductivity (EC, mS cm-1), elemental concentrations (mg L-1) and naphthenic acid (Naph, mg L-1) concentrations of 100% Non-Segregating Tailings (NST) water, 100% NST water in 50% Hoagland's solution (1:1, by volume) and 50% Hoagland's solution (n = 8 for pH and EC, n = 6 for 100% NST water elemental concentrations, and n = 3 for 100% NST water naphthenic acid concentrations, standard errors are shown).

	pH	EC	Na	K	Mg	Ca	Zn	F	Cl	P	Fe	S	B	Naph
100% NST water	9	5.5 ± 0.2	1095.7 ± 2.6	34.0 ± 0.7	7.3 ± 0.3	5.8 ± 0.4	0	2.6 ± 0.0	NA	0.1 ± 0.1	1.1 ± 0.4	34.9 ± 0.6	7.5 ± 0.1	133.0 ± 5.4
100% NST water in 50% Hoagland's	8.0–8.7	4.1 ± 0.1	547.9	75.8	9.7	42.9	0	1.3	0.4	15.6	0.8	25.5	3.8	66.5
50% Hoagland's	6–5.5	1.2 ± 0.0	0	117.5	12	80	0	0	0.9	31	0.6	16	0.1	0

Survival rate, root collar diameter growth, height growth, and plant fresh and dry weights

The survival rate was calculated by dividing the number of surviving plants at the end of the experiment in each treatment by the total number of plants for each species. The root collar diameter and heights of seedlings from the root collar to the shoot tip were measured at the beginning, after one month, and at the end of the experiment (two months of treatments). The relative root collar diameter growth and shoot height growth over the first month and two months were calculated by dividing the difference between the initial and final measurements by the initial measurements. After fresh weights were determined for 9 individuals per species, harvested roots and stems were dried in an oven at 70°C for 72 h, whereas leaves were separately freeze-dried for 72 h to avoid heat-induced breakdown of chlorophyll. To determine shoot dry weights, the dry weights of leaves and stems from each plant were added. Water content was determined by dividing the difference between the fresh weight and the dry weight of seedlings by their fresh weight and expressed as a percentage.

Foliar chlorophyll and elemental concentrations

Leaf chlorophyll-a and chlorophyll-b concentrations were determined in seven randomly selected seedlings per species per treatment (n = 7). Living fully expanded uppermost leaves were frozen, freeze-dried, and pulverized with a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA).

Chlorophyll was extracted from the pulverized leaf samples (10 mg dry weight) with 8 ml dimethyl sulfoxide (DMSO) at 65°C for 22 h. After filtering, chlorophyll concentrations were measured in DMSO extracts with a spectrophotometer (Genesys 10S-UV-VIS, Thomas Scientific, NJ, USA) at 648 and 665 nm. The total chlorophyll concentration was then calculated using Arnon's equation (Wellburn, 1994).

Elemental (Na, K, Mg, Ca, Zn, Fe, P, Mn, S, Cu, and B) analysis of pulverized leaf samples was conducted by inductively coupled plasma-optical emission spectroscopy (ICP-OES) using a Thermo iCAP6300 Duo (Thermo Fisher Scientific Inc.) following US EPA methods 6010d and 200.7 at the Natural Resources Analysis Laboratory (NRAL) at the University of Alberta.

Total foliar nitrogen of the pulverized leaf samples was determined by flash combustion using a Thermo FLASH 2000 Organic Elemental Analyzer, (Thermo Fisher Scientific Inc.) and following the methods outlined in (EPA, 2002; Thomas, 1996).

Relative electrolyte leakage rate

Leaf electrolyte leakage was measured as a rate using a modified version of Zwiazek and Blake (1991). In brief, two-cm diameter leaf disks were removed with a cork borer from 6 individuals per species per treatment and immediately immersed in collection tubes containing 10 ml milli-Q water. For black spruce, jack pine, lodgepole pine, and tamarack, 6 needle sections, each 1 cm long, were cut from each plant with scissors. An initial electroconductivity reading (EC_0) was immediately taken and samples were set aside to incubate at room temperature. Electrolyte leakage was subsequently measured every hour for the next 5 hours (EC_1 EC_5) and the collection tubes were then immersed in a boiling water bath for 20 min; a final EC_{total} measurement was taken when samples had returned to room temperature.

The relative electrolyte leakage rate was then calculated as follows:

$$EC_{Rate} = \frac{slope\left(\left\{EC_1, EC_2, EC_3, EC_4, EC_5\right\}, \left\{1,2,3,4,5\right\}\right)}{\left(EC_{total} - EC_0\right)} * 100$$

Net photosynthesis and transpiration rates

Net photosynthesis (A) and transpiration (E) rates were measured between 9:00 and 12:00 h immediately prior to the start (n = 6 per species), in the middle (after one month, n = 4 per species per treatment), and at the end of the experiment (after 2 months, n = 6 per species per treatment) with an infrared gas analyzer (LI-6400, LI-COR). The reference CO_2 concentration was set to 400 µmol mol^{-1} and the flow rate was 200 µmol s^{-1} in the leaf chamber. The leaf chamber temperature was kept at 20°C, and PPFD was set to 400 µmol m^{-2} s^{-1}. For black spruce, jack pine, lodgepole pine, and tamarack measurements, a 3-cm distal section of needles was inserted into the leaf chamber and then severed with scissors and scanned. Needle areas were then calculated using Sigmascan Pro 5.0 software (Systat Software, San Jose, USA).

Statistical analysis and graphs

To reveal statistically significant intraspecies differences between treatments ($p \leq 0.05$), data were fitted to linear mixed effect models and analyzed in R using the lme4, lmertest, and emmeans packages (Bates et al., 2019; Core Development Team, 2020; Kuznetsova et al., 2017; Lenth et al., 2019). As relative shoot height and diameter growths were proportions and presented many null values, data were fitted to a zero-inflated beta mixed model using the glmmTMB R package. (Magnusson et al., 2019). Water content were also fitted to a beta mixed model using the same package. Circulating pumps, each of which fed 3 containers holding seedlings, were treated as random effect factors and containers as a random nested factor within pump. Thus, mixed models were constructed as follows: Response variable ~ Aeration * Media + (1 |Pump/Container). When repeated measurements were present, a “Time” fixed effect factor was added. Foliar elements and N were analyzed separately per species per element. However, to investigate aeration type and media type interactions across all elements and N, an “element/N” fixed effect factor was added.

When models were ill fitting, pump was removed as a random effect and the new models were compared using the Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Tukey tests were used for post-hoc analysis of estimated means. When data did not meet the assumptions for normality of distribution and homogeneity of variance, they were log or box-cox transformed using the MASS package and assessed further graphically using Q-Q plots (Ripley et al., 2019). Type 3 analyses of variance tables using the Satterthwaite method were constructed for each species and analysis, relative growth and water content excepted. For these, analysis of deviance tables using type 2 Wald chisquare tests were used.

Estimated means (except for survival rates, where observed values were used) and standard errors are graphed using Sigmaplot 14.5 (Systat Software Inc., San Jose, USA).

Results

Survival rate

Seedling survival was only reduced for trembling aspen, balsam poplar, black spruce, and tamarack in treatments with NST water. When all species were grouped, survival rate was twofold lower in the hypoxic NST water treatment compared with the other treatments (Figure S2).

Relative root collar diameter growth (RRCDG)

The effect of medium type on RRCDG was significant for trembling aspen and balsam poplar (Tables S1 and S2). This was due to reduced RRCDG in NST water treatments for these species (Fig. 1A). Black spruce also showed differences in RRCDG between treatments. However, this effect was due to hypoxia (Fig. 1A, Table S3). There was a significant time effect on RRCDG for jack pine with a decrease observed in the second month (Fig. 1A, Table S4). There was a significant interaction effect between medium and time factors for trembling aspen, balsam poplar, and lodgepole pine (Table S1, S2, and S5) indicating that medium type influenced RRCDG changes over time in these species (Fig. 1A). In addition, the medium type to aeration type interaction effect was significant for black spruce seedlings (Table S3) and there was a significant three way medium-time-aeration effect for balsam poplar (Table S2). Trembling aspen and balsam poplar seedlings showed an increase in RRCDG in the second month of the experiment in the aerated control treatments and balsam polar seedlings showed significantly lower RRCDG over the second month when growing in an aerated NST solution (Fig. 1A).

Relative shoot height growth (RSHG)

There was a significant effect of the type of medium on RSHG of trembling aspen, balsam poplar, black spruce, lodgepole pine, and tamarack seedlings (Tables S7, S8, S9, S11, and S12) and seedlings from these species with their roots in NST solutions had reduced RSHG (Fig. 1B). There was a time effect on RSHG for black spruce and tamarack seedlings (Tables S9 and S12) which is reflected in the significantly smaller RSHG that occurred over the second month of the experiment in the aerated and hypoxic control treatments for black spruce and the hypoxic NST solution treatment for black spruce and tamarack (Fig. 1B). Lodgepole pine seedling RSHG had a significant medium-aeration type interaction effect (Tables S11) and there was a significant medium-time interaction effect for black spruce and tamarack (Tables S9 and S12) as RSHG was reduced over the second month in NST treatments (Fig. 1B). In parallel, the aeration-time interaction effect was significant for trembling aspen, black spruce, and tamarack (Tables S7, S9, and S12) as RSHG was also reduced over the second month in hypoxic treatments (Fig. 1B). Finally, RSHG in trembling aspen seedlings increased significantly over the second month of growth in the aerated control treatment (Fig. 1B).

Root to shoot ratio, dry weight, and water content

The effect of medium type on the root to shoot ratio was significant for trembling aspen and balsam poplar (Table S13 and S14). By the end of the experiment, root to shoot ratios in trembling aspen and balsam poplar were significantly higher in treatments where NST water was present in the hydroponic medium, remaining unchanged from the initial ratios (Fig. 2A). Black spruce, jack pine, lodgepole pine, and tamarack showed no significant differences or factors for their root to shoot ratios across all treatments (Fig. 2A, Tables S15–S18).

There was a significant effect of medium type on trembling aspen and balsam poplar dry weights (Tables S19–S20). Correspondingly, dry weights were significantly lower for trembling aspen and balsam poplar seedlings grown in 50% Hoagland's with NST water. (Fig. 2B). Jack pine dry weights showed a significant aeration effect and an interaction between aeration and medium type (Table S22) and had significantly higher dry weights when grown in the aerated control treatment (Fig. 2B). There was no treatment effect on dry weights in black spruce, lodgepole pine, and tamarack (Tables S21, S23, and S24; Fig. 2B).

Medium type had a significant effect on the water content of balsam poplar, black spruce, jack pine, and tamarack seedlings (Tables S26, S27, S28, and S30). In these four species, water content was significantly lower in treatments

containing NST water (Fig. 2C). In addition, trembling aspen, balsam poplar, and black spruce showed a significant effect of aeration (Tables S25, S26, and S27) and thus had lower water contents when exposed to hypoxic conditions (Fig. 2C). Aeration-medium interaction was significant for black spruce and jack pine (Tables S27 and S28) and seedlings of these species grown in hypoxic NST water did not show significantly lower water content than seedlings in the hypoxic control and aerated-NST media. (Fig. 2C).

Relative electrolyte leakage rate (RELR)

The type of hydroponic medium significantly affected the RELR of trembling aspen, balsam poplar, and tamarack seedlings (Tables S31, S32, and S36). Whereas for trembling aspen and balsam poplar seedlings the aerated NST treatment showed the highest rates, tamarack seedlings had significantly higher RELR in the aerated control treatment (Fig. 3). As the RELR of trembling aspen in aerated NST was significantly greater compared with the seedlings in hypoxic NST (Fig. 3), the effect of aeration was also significant (Table S31). Finally, there was a significant medium-aeration interaction effect on RELR in lodgepole pine seedlings (Table S35) with significantly higher rates in the hypoxic NST treatment compared with the hypoxic control treatment (Fig. 3).

Foliar chlorophyll

Although the effect of aeration on chlorophyll concentrations was not significant, there was a significant effect of the type of media on foliar chlorophyll concentrations in all species (Tables S37–S42). In effect, this translated to higher chlorophyll concentrations in the treatments without any NST water for all species except for lodgepole pine (Fig. 4). In addition, the aeration-medium interaction effect was significant for black spruce and jack pine (Tables S39 and S40).

Net photosynthesis (Pn) and transpiration (E)

In all species, Pn was significantly affected by the type of media (Tables S43–S48). Correspondingly, Pn was lower when seedlings were grown in hydroponic solutions with NST water (Fig. 5). In addition, the Pn of trembling aspen, balsam poplar, jack pine, and tamarack seedlings was significantly influenced by the level of aeration of the growth medium (Tables S43, S44, S46, and S48). Thus, in parallel when in the same growth solution, the Pn of seedlings of these 4 species was lower in the hypoxic treatments (Fig. 5A, B, D, and F). Time was a significant factor for Pn in trembling aspen, balsam poplar, black spruce, jack pine, and lodgepole pine (Tables S43–S47). There was a significant medium-aeration interaction effect on Pn rates in trembling aspen, balsam poplar, jack pine, and tamarack seedlings (Tables S43, S44, S46, S48). The medium-time interaction also had a significant effect on Pn in balsam poplar and black spruce seedlings (Tables S44 and S45). The three-way medium-aeration-time interaction only had a significant effect on trembling aspen Pn (Table S43)

Transpiration rates (E) in seedlings of all species were significantly affected by the type of media (Tables S49–S54). This was reflected in significantly lower E for seedlings grown in NST treatments (Fig. 6). In addition, E for trembling aspen, balsam poplar, jack pine, lodgepole pine, and tamarack seedlings was significantly affected by the level of aeration of the hydroponic media (Tables S49, S50, S52, S53, and S54). Thus, seedlings grown in hypoxic media showed lower rates of E than their counterparts grown in aerated solutions (Fig. 6). In addition, time had a significant effect on the E of trembling aspen and black spruce seedlings only (Tables S49 and S51). Furthermore, the medium-aeration interaction had a significant effect on trembling aspen, jack pine, and tamarack seedlings E (Tables S49, S52, and S54). Likewise, the interaction between hydroponic medium and time was significant for trembling aspen, and black spruce E (Tables S49 and S51). Finally, the aeration-time interaction and the three-way medium-aeration-time interaction had a significant effect on jack pine E (Table S52).

Foliar elements

The overall media-element interaction, indicative of the effect of medium type on individual elements or N, was highly significant for all species (Tables S55–60). Consequently, all species showed significant foliar accumulation differences

between seedlings grown in Hoagland's solution only and those grown with NST water added (Table 2). Broadly, the addition of NST water to growth solutions accounted for large increases in foliar Na and B concentrations in all studied species (Tables 2). In addition, NST water caused: i) an increase in N and reductions in Zn, Fe, P, S, and Cu concentrations in trembling aspen, ii) a reduction in Ca, Fe, P, Mn, and S in balsam poplar, iii) an increase in N and a reduction in all other elements in black spruce, iv) a decrease in all other elements for jack pine, v) a decrease in K, Mg, Ca, Zn, Fe, Mn, S, and N for lodgepole pine, and vi) a decrease in all other measured elements in tamarack (Table 2).

Table 2: Foliar elemental concentrations (mg kg^{-1} and g 100 g^{-1} for N) after two months of treatments (n = 7 per species per treatment). Estimated menas are shown and different letters indicate significant posthoc differences between treatments (from largest to smallest , $P < 0.05$). Color indicates significant factors after a two-way ANOVA: Yellow for an effect of growth medium, blue for an aeration effect, and green when both medium and aeration effects occurred, with '*' indicating a significant medium-aeration interaction ($P < 0.05$).

		Na	K	Mg	Ca	Zn	Fe	P	Mn	S	Cu	B	N
Trembling Aspen	Aerated	35.8 bc*	17829 a	1850 ab	10061 a*	57.7 a	80.5 a	2934 a	48.7 a	2951 a	7.7 a	38.8 c*	2.3 a
	Hypoxic	9.3 c*	17810 a	1468 b	8498 a*	29.0 ab	72.6 a	2932 a	30.8 a	2407 a	6.0 ab	39.9 c*	2.4 a
	Aerated/NST	264.8 b*	21291 a	2288 a	10923 a*	21.6 b	40.9 b	1770 b	43.8 a	1552 b	3.6 b	292.5 a*	3.0 a
	Hypoxic/NST	3558 a*	15957 a	1313 b	5466 b*	25.9 ab	48.5 ab	1922 b	28.2 b	1734 b	4.8 ab	104.8 b*	2.8 a
Balsam Poplar	Aerated	4.4 b*	28062 a	1902 ab*	10249 a*	28.2 a*	85.2 a	5433 a	34.2 a*	3213 a	1.8 a	26.2 c*	3.2 a
	Hypoxic	47.7 b*	31580 a	2008 ab*	8712 a*	17.3 a*	78.1 a	4037 ab	20.7 b*	3202 a	4.3 a	27.0 c*	2.51 a
	Aerated/NST	19435 a*	27862 a	2528 a*	9263 a*	17.9 a*	29.0 b	2496 bc	15.7 b*	1342 b	1.4 a	314.1 a*	3.07 a
	Hypoxic/NST	7807 a*	24898 a	1595 b*	4580 b*	19.1 a*	34.9 b	2197 c	18.3 b*	1279 b	3.0 a	171.9 b*	3.35 a
Black Spruce	Aerated	38.7 b	9599 a	1094 a	3717 a	35.7 a*	52.9 a*	2194 a	105.3 a	1071 a*	4.4334 a	23.3 b	1.1 b*
	Hypoxic	40.2 b	9213 a	884 a	3210 a	26.5 b*	33.7 a*	1708 b	82.3 a	772 b*	3.1757 a	20.0 b	1.3 b*
	Aerated/NST	2785 a	4966 b	476 b	938 b	12.3 c*	13.5 b*	1089 c	25.3 b	421 c*	0.00 b	57.6 a	2.3 a*
	Hypoxic/NST	2825 a	4336 b	462 b	871 b	14.8 c*	18.3 b*	986 c	21.6 b	470 c*	0.7577 b	56.9 a	1.4 b*
Jack Pine	Aerated	28.9 b	8230 b	1182 a	2362 a	58.0 a	55.2 a	1989 a	124.2 a	1453 a	6.4 a	29.0 b	1.3 a
	Hypoxic	78.8 b	12138 a	1093 a	2404 a	48.6 a	36.1 ab	2271 a	135.5 a	1748 a	8.0 a	25.4 b	1.5 a
	Aerated/NST	5933 a	4408 c	689 b	859 b	30.6 b	18.7 c	1345 b	52.5 b	701 b	4.0 b	49.6 a	1.8 a
	Hypoxic/NST	7977 a	6601 bc	668 b	920 b	26.7 b	20.1 bc	1519 b	44.3 b	900 b	5.7 ab	55.8 a	1.6 a
Lodgepole Pine	Aerated	59.9 b	10838 a	738 a	1800 a	36.7 ab	27.7 a	1749 a	66.9 ab	1098 a	4.6 ab	16.2 b	1.5 a*
	Hypoxic	78.7 b	10145 a	750 a	1926 a	43.8 a	25.3 ab	1665 a	79.3 a	1176 a	5.7 ab	16.0 b	1.4 a*
	Aerated/NST	4993 a	6603 b	551 a	732 b	27.9 b	12.7 b	1410 a	47.7 b	663 b	3.8 b	46.4 a	1.0 b*
	Hypoxic/NST	4612 a	8690 ab	628 a	845 b	27.7 b	17.0 ab	1709 a	56.0 ab	907 ab	6.2 a	41.6 a	1.2 ab*
Tamarack	Aerated	13.0 b	9229 ab	1118 a	2606 a	26.9 a	31.6 a	1996 a	126.6 a	1130 a	3.9 a*	26.4 b	1.7 a
	Hypoxic	26.3 b	10890 a	1054 a	2409 a	20.7 ab	32.4 a	2043 a	100.4 a	840 a	3.9 a*	22.5 b	1.0 b
	Aerated/NST	4227 a	8103 ab	766 ab	1073 b	13.0 b	13.9 b	1538 a	43.8 b	528 b	< 1.5 b*	91.3 a	1.6 a
	Hypoxic/NST	5488 a	5570 b	463 b	691 b	13.7 b	18.6 b	1484 a	37.3 b	506 b	1.7 ab*	74.4 a	1.1 b

The effect of the aeration-elements interaction, i.e. the overall effect of hypoxia on individual elements or N, was significant in balsam poplar, jack pine, and tamarack but not for trembling aspen, black spruce and lodgepole pine (Tables S55–S60). On an element per element basis, hypoxic solutions led to a i) reduction in foliar Mg, Ca, Mn, B, and N in trembling aspen seedlings (Table 2), ii) a reduction in Na, Mg, Ca, and B, and an increase in Cu in balsam poplar (Table 3), iii) a decrease in Mg, P, and S in black spruce (Table 4), iv) an increase in K, S, Cu, in both media and N in the control medium only for jack pine (Table 5), v) an increase in Cu for lodgepole pine (Table 6), and vi) a decrease in S and N for tamarack (Table 7).

The overall aeration-media-element interaction was significant for trembling aspen, balsam poplar, black spruce, and tamarack (Tables S55, S56, S57, and S60). Specifically, NST water influenced the effects of hypoxia, or vice-versa, on i) foliar concentrations of Na, Ca, and B in trembling aspen seedlings (Table 2), ii) Na, Mg, Ca, Mn, and B in balsam poplar (Table 3), iii) Zn, Fe, S, and N in black spruce (Table 4), iv) N only in lodgepole pine (Table 6), and v) Cu only in tamarack (Table 7).

Discussion

Except for electrolyte leakage, the measured stress responses including plant survival, growth, and physiology, were most negatively affected by NST water when root hypoxic conditions were present. Of the two studied factors, growth medium (50% Hoagland's or NST water amended with 50% Hoagland's) and level of aeration (aerated or hypoxic), the type of growth medium had the greatest impact on the parameters measured in the seedlings. The presence of NST water in the growth medium had an overwhelming effect on the proportions and types of mineral elements accumulated in the foliage and, thus, had a significant effect on the chlorophyll concentrations, net photosynthesis, and transpiration of seedlings of all species, which unsurprisingly led to significantly lower root collar diameter, shoot height growth, and dry weights. These results confirm that NST water produces similar deleterious effects in plants as have been previously reported for consolidated NST (Zhang et al., 2020), and underlines the potential risks of water seepage from buried NST layers into the root area to reclamation success. The elevated pH, EC, Na, B, and naphthenic acids, that were measured in NST water, have been all previously identified as responsible for reductions in growth and increased mortality in seedlings used for reclamation in prior studies involving oil sands tailings (Croser et al., 2001; Kamaluddin and Zwiazek, 2002; Redfield et al., 2003; Renault et al., 1999; Zhang et al., 2020). These factors were also likely detrimental to plants in the present study. In contrast, the overall effects of hypoxia, though measurable and significant, had an order of magnitude less of an impact on the measured parameters. Nevertheless, hypoxia interacted strongly with the NST water leading to an aggravation of its deleterious effects on plants, notably in aspen and black spruce.

For this study, balsam poplar, black spruce, and tamarack were selected due to their geographical range and their prevalence in many boreal land reclamation projects, and because they are considered to be relatively tolerant to soil waterlogging (Johnston, 1990; Viereck and Johnston, 1990; Zasada and Phipps, 1990). Thus here, as expected, under hypoxic conditions, both balsam poplar and black spruce seedlings were able to recover and maintain elevated levels of photosynthesis and transpiration by the end of the experiment. However, unexpectedly, tamarack seedlings were unable to recover, whereas trembling aspen, a relatively hypoxia sensitive species, was able to do so. It has been reported that tamarack relies on stem hypertrophy and adventitious root production to facilitate root aeration and maintain respiration under flooding conditions (Calvo-Polanco et al., 2012; Islam et al., 2003). In our hydroponic setup, seedlings were supported in hydroponic solutions at the root collar by foam inserts in Styrofoam insulation trays, and the experiment only lasted two months; this may have inhibited or provided insufficient time for these morphological responses to successfully develop. Furthermore, hypoxia disrupts root water uptake by inhibiting the function of aquaporins and triggering stomatal closure (Tan et al., 2018). Reactive oxygen species and ethylene are produced in response to oxygen deficiency in the roots and have been linked to flood adaptive mechanisms across a wide range of plants and climates (Voesenek and Bailey-Serres, 2015). Still, ethylene has been recorded to trigger a range of different responses in species subjected to hypoxia. In trembling aspen, under hypoxic conditions ethylene enhances aquaporin expression in the roots, stomatal conductance, and water transport (Kamaluddin and Zwiazek, 2002; Tan et al., 2021). In tamarack, ethylene leads to reductions in net assimilation and stomatal conductance (Islam et al., 2003). Ethylene production may thus explain the responses observed in trembling aspen and tamarack and are likely reflective of different physiological strategies to flooding. Indeed, in our experiment, water use strategies in balsam poplar, tamarack, trembling aspen, and black spruce varied greatly, resulting in different tradeoffs. To maintain water content and thus turgor under hypoxic conditions, balsam poplar and tamarack reduced their transpiration rates, but trembling aspen and black spruce exhibited a more anisohydric behavior leading to a lower water content.

The addition of NST to the media increased foliar Na concentrations in all studied species. However, although no differences due to aeration or factor interactions were observed in any of the conifer species, hypoxic conditions severely aggravated Na uptake in trembling aspen, leading to foliar concentrations more than 10-fold greater than in seedlings exposed to NST under aerated conditions. This suggests effective oxygen-dependent root Na sequestration or exclusion in trembling aspen, similar to what has previously been reported for other poplar species (Chen and Polle, 2009). Indeed, the ability of roots to store salt is a strong indicator in woody perennials of salt resistance (Redfield et al., 2003). However, excluding ions from the xylem sap is an energy dependent process which is inhibited under hypoxic conditions (Buwalda et al., 1988). In parallel, increases in foliar K have been associated with an Na tolerance mechanism in trembling aspen (Yi et al., 2008), as they compete for similar uptake sites and K is crucial for cells to maintain homeostasis in growing tissues (Chen et al., 2014). Our results may corroborate this as foliar concentrations of K were indeed 25% higher in the aerated compared with hypoxic NST water treatments (Table 2). Furthermore, aerated trembling aspen seedlings in NST water were also able to maintain elevated levels of Ca which is essential to maintain selective membrane permeability and thus K/Na selectivity in the roots (Hanson, 1984; Tozlu et al., 2000). In our study, at the energy expense of a higher root-to-shoot ratio, elevated electrolyte leakage and reduced growth, this mechanism likely allowed trembling aspen seedlings growing in the aerated NST water to maintain similar levels of photosynthesis, transpiration and water content as the seedlings grown in the control treatments. However, the combination of hypoxic conditions and NST water appear to have prevented the root growth essential for this tradeoff to occur. Nevertheless, this exclusion mechanism was not effective for boron since its foliar concentrations were all significantly greater under aerated conditions, supporting previous findings that show there is generally little control over boron uptake (Apostol et al., 2002). Similarly, in balsam poplar, where no salt exclusion mechanism was evident, greater transpiration driven water uptake led to greater levels of Na and boron in the leaves of seedlings growing in aerated NST water media. Still, it is worth noting that, even when aggravated by hypoxic conditions, the trembling aspen foliar Na levels remained lower than in balsam poplar, suggesting that although trembling aspen may be more sensitive to root hypoxia, it is still more resistant to the salinity in tailings than balsam poplar. This supports earlier reports showing that trembling aspen can be a viable species that meets commercial forestry requirements, even when growing in moderately saline sites (Lilles et al., 2012).

Although the studied conifer seedlings growing in the NST treatments had much greater foliar concentrations of Na and boron than those growing in the control solutions, contrary to the two broadleaf species, they were less prone to media-aeration interaction effects. Only relatively minor differences were apparent in foliar elemental concentrations between conifer seedlings grown in the aerated or hypoxic NST water amended treatments. This could partly be due to their slower growth rates and the overwhelming effects of NST media on all the measured parameters, but also be caused by an inability to prevent the uptake of salts by the roots (Zhang et al., 2020). Nevertheless, black spruce seedlings growing in aerated NST had twofold greater needle N concentrations than those subjected to the other treatments. In tamarack seedlings growing in aerated media. N concentration was 50% greater compared with the hypoxic media, regardless of the presence of NST water. This was in contrast to lodgepole pine needles, which had lower N concentrations when growing in the NST treatments. Flooded tamarack and black spruce have been reported to have less foliar nitrogen than those accessing water from lower in the water table (Lieffers and Macdonald, 1990), which may explain the observed results in tamarack, but not in black spruce. The increased N in black spruce needles could instead be a mechanism associated with increased foliar accumulation of proline and stress protective proteins (El Moukhtari et al., 2020). If this was indeed the case, then hypoxic conditions inhibited the production of these compounds aggravating the hypoxic effects. It would then also seem probable that foliar N differences between the media types did not occur in the broadleaf species and tamarack even if they were salt tolerant as their deciduous nature could allow them to lose their foliage and produce new salt-free leaves making such a mechanism unnecessary (Renault et al., 1999). More research is needed to investigate the potential mechanisms that allow black spruce to commonly grow in nutrient poor environments.

Conclusion

Prior to the start of this study, we hypothesized that root hypoxic conditions would aggravate the effects of NST tailings less severely in plant species that are more tolerant to root hypoxia. However, we observed that root hypoxia (< 3 mg O_2 l^{-1}) aggravated the effects of the saline NST water more severely in species that are reported to be more tolerant to salt stress. Our analysis of the liquid phase of NST also revealed elevated levels of naphthenic acids along with levels of B and Na, similar to those found in solid NST tailings and which are known to be detrimental to the growth and establishment of plants. Thus, we suggest that the development of hypoxic conditions in root zones where tailings water or soils of similar chemical composition are present, should be avoided or mitigated as hypoxia will interfere with root and leaf ionic toxicity tolerance mechanisms. Although the transpiration driven uptake of phytotoxic ions occurs more greatly under aerobic conditions in species bereft of these tolerance or ionic sequestering mechanisms, the additional deleterious effects caused by hypoxia led to greater mortality, and reductions in photosynthesis, growth, and dry weight. Further research should investigate the precise nature of the increased nitrogen accumulated in the needles of black spruce growing in NST water amended media and the salt sequestration mechanism in trembling aspen.

Declarations

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Author Contribution Statement

Killian Fleurial: Conceptualization, Methodology, Investigation, Writing—original draft, Wen-Qing Zhang: Methodology, Writing—review & editing, Robert Vassov: Resources, Writing—review & editing, Janusz J. Zwiazek: Conceptualization, Supervision, Writing—review & editing, Funding Acquisition.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests

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Figures

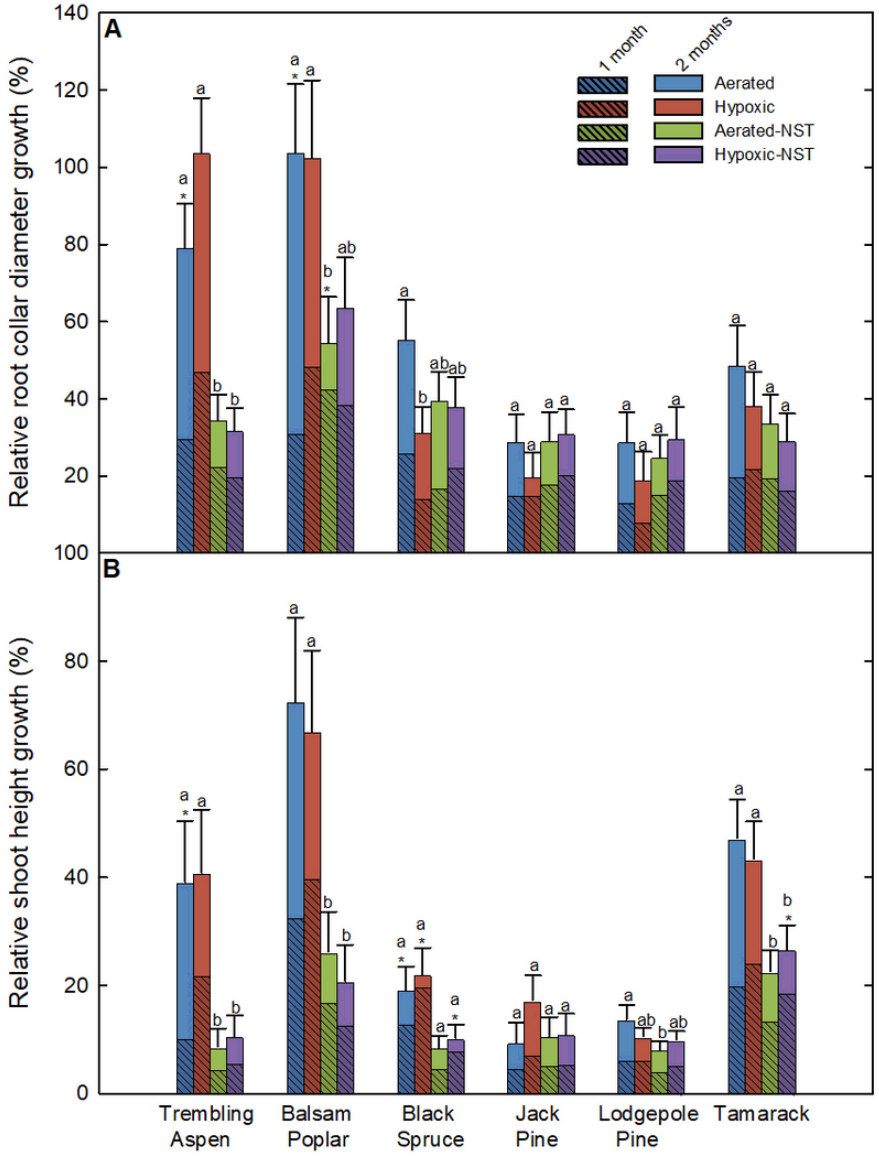

Figure 1

Relative root collar diameter growth (A), and relative shoot height growth (B) after the first and second month of the different treatments for plants of the studied species (n = 18). Values were obtained by dividing the difference between the initial and final measurements by the initial measurements. Estimated means and standard errors are shown. Letters indicate significant differences within species between treatments ($P < 0.05$), and "*" indicates a significant difference between the first and second month of growth ($P < 0.05$)

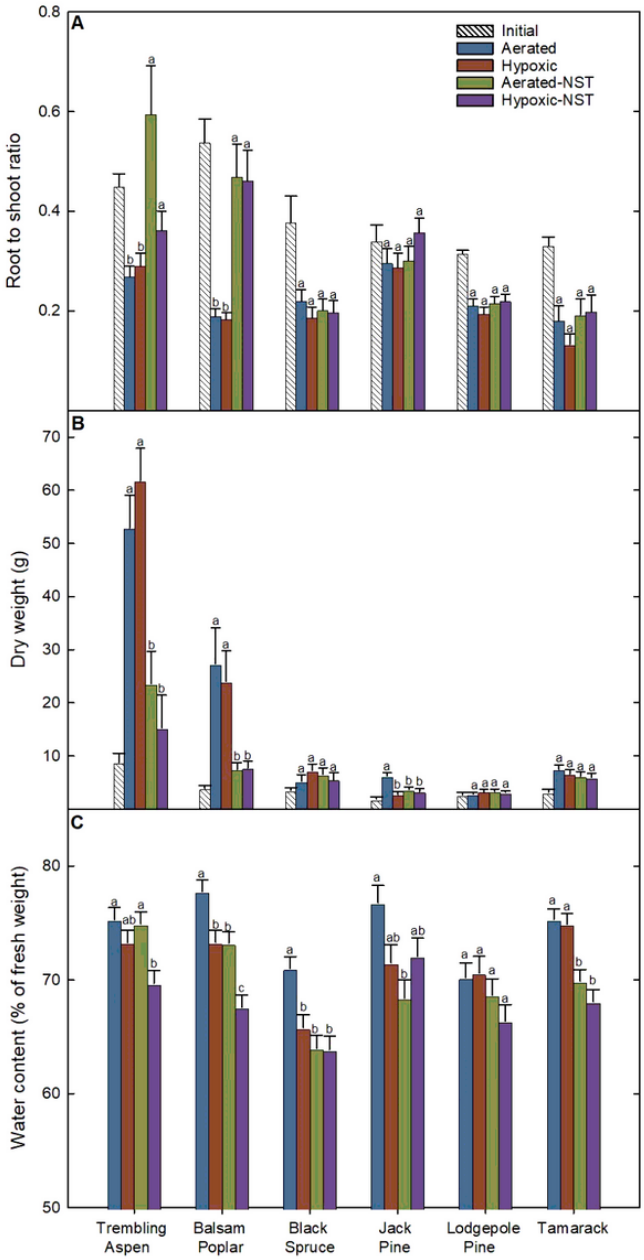

Figure 2

Root to shoot ratio (A), Dry weight (B), and Water content (C) after two months of different treatments for the plants of the studied species (n = 9). A and B include initial (prior to application of treatments, n = 4) root to shoot ratios and dry weights for reference. Estimated means and standard error bars are shown; different letters indicate significant differences between treatments of the same species ($P < 0.05$).

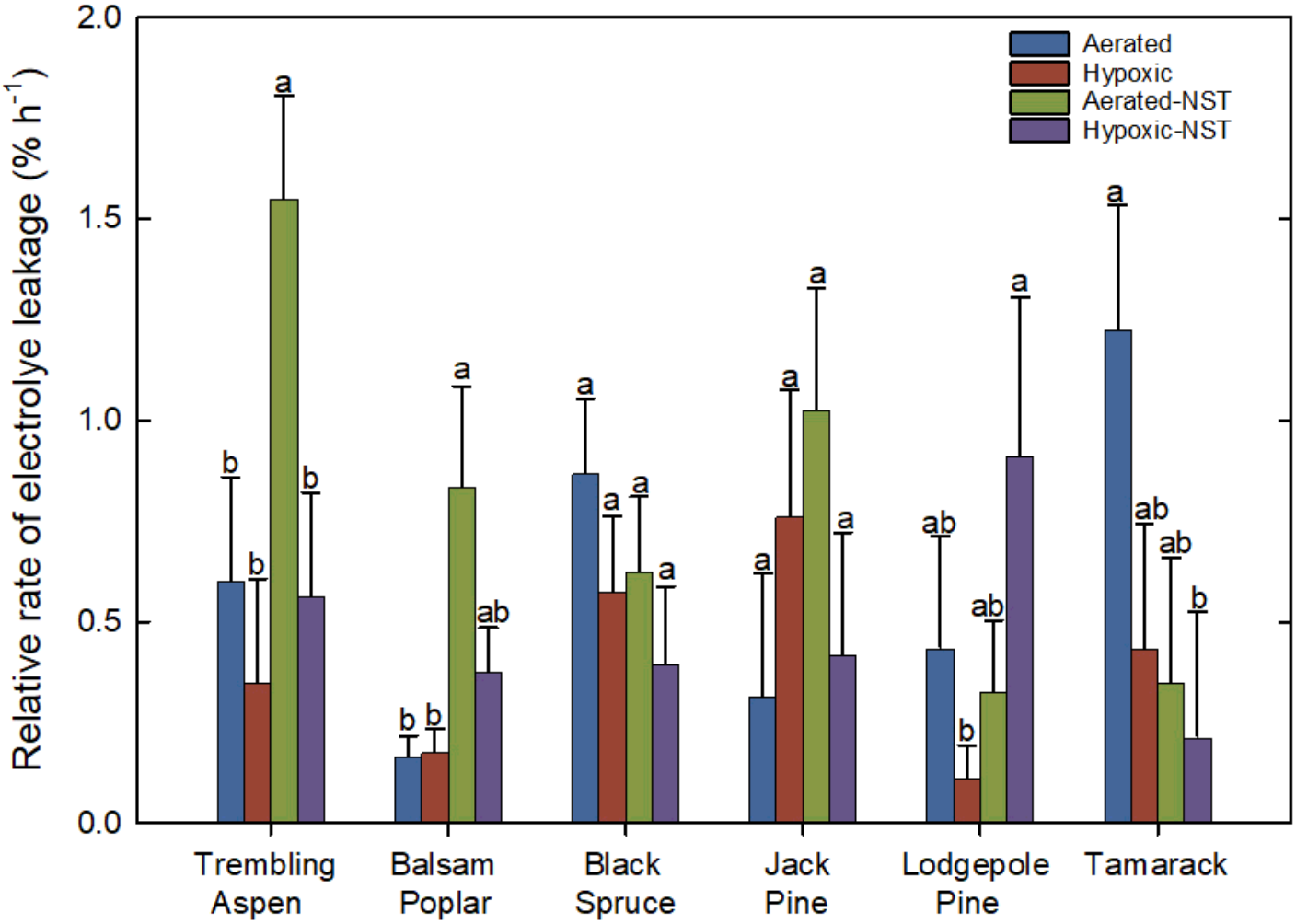

Figure 3

Relative rate of electrolyte leakage after two months of different treatments for the plants of the studied species (n = 6). Estimated means and standard error bars are shown; different letters indicate significant differences between treatments of the same species ($P < 0.05$).

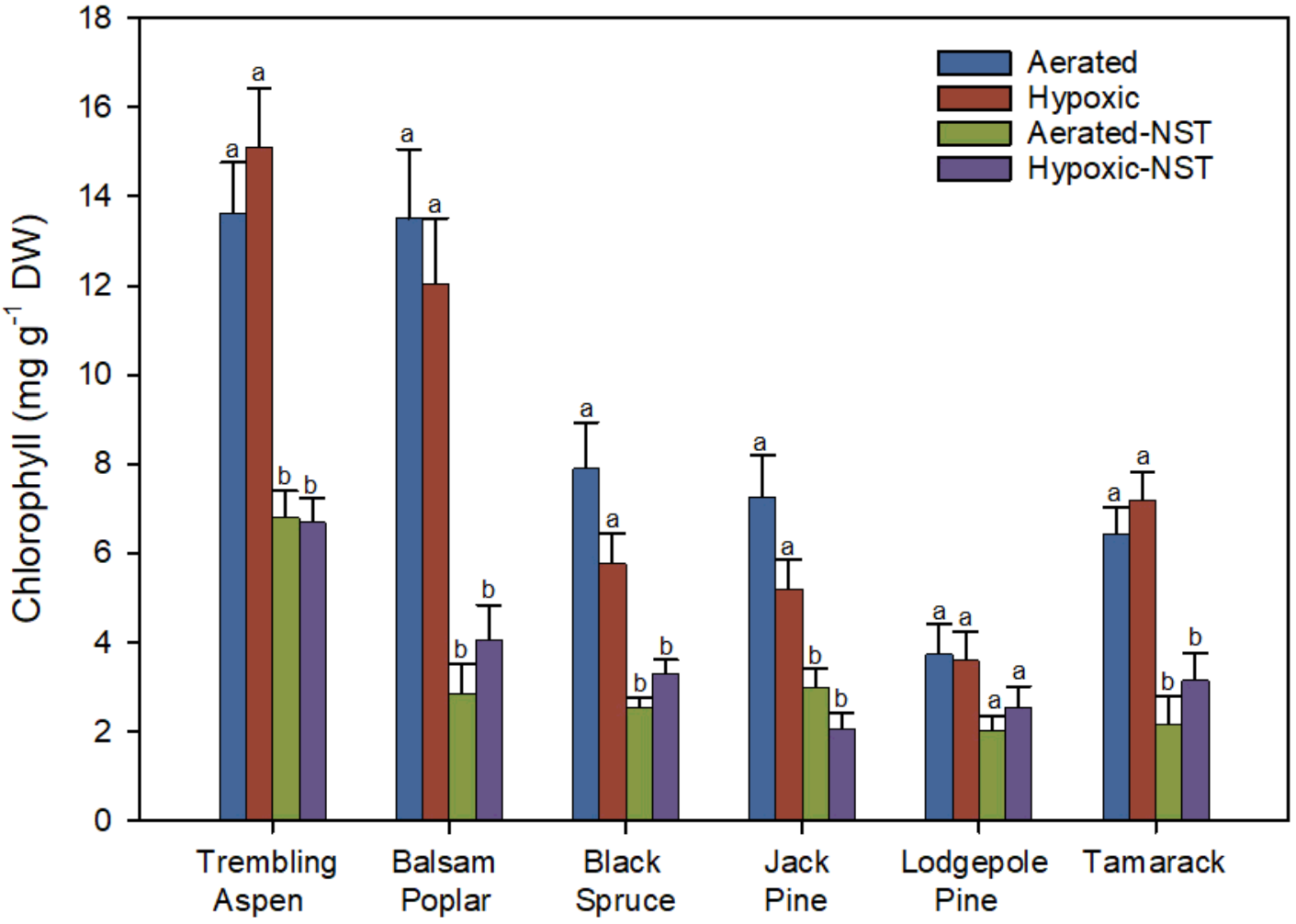

Figure 4

Foliar chlorophyll concentration (chlorophyll a + b) after two months of different treatments for the plants of the studied species (n = 7) Esimated means and standard error bars are shown; different letters indicate significant differences between treatments of the same species ($P < 0.05$).

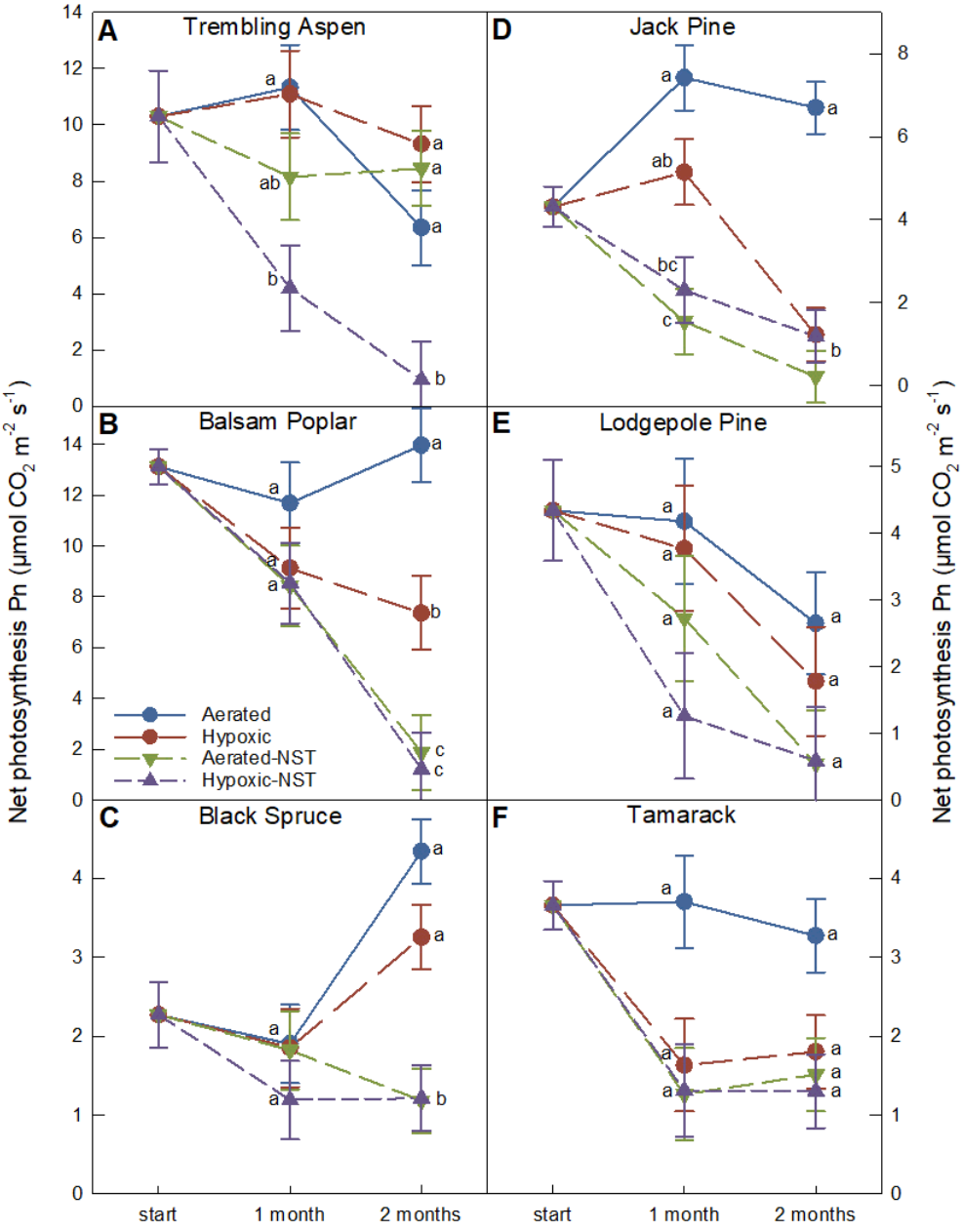

Figure 5

Net photosynthesis rate of trembling aspen (A), balsam poplar (B), black spruce (C), jack pine (D), lodgepole pine (E), and tamarack (F) at the start, after 1 month (n = 4), and after 2 months (n = 6) of the various treatments. Estimated means and standard error bars are shown; different letters indicate significant differences between treatments at the same time period ($P < 0.05$).

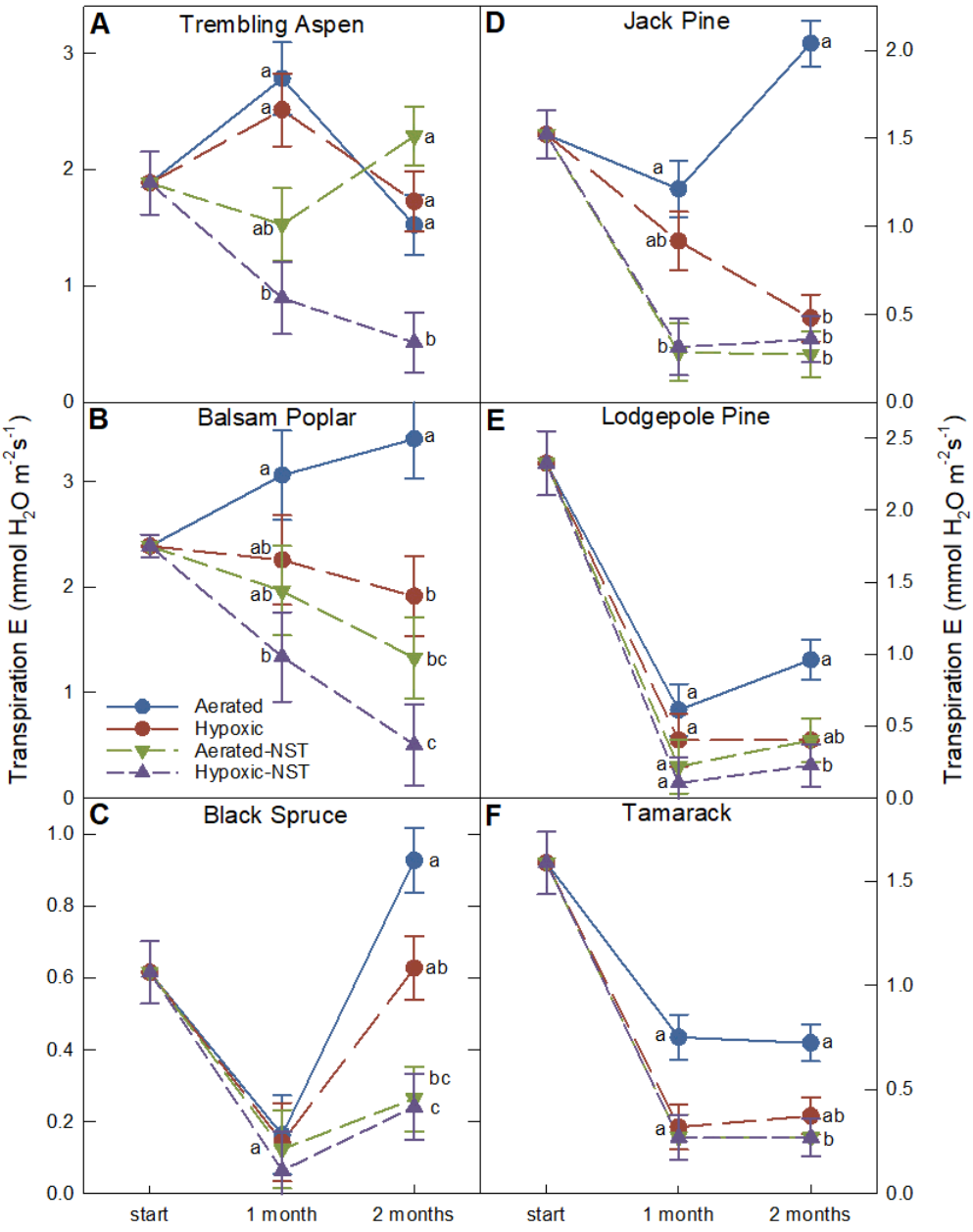

Figure 6

Transpiration rate of trembling aspen (A), balsam poplar (B), black spruce (C), jack pine (D), lodgepole pine (E), and tamarack (F) at the start, after 1 month (n = 4), and after 2 months (n = 6) of the various treatments. Estimated means and standard error bars are shown; different letters indicate significant differences between treatments at the same time period ($P < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HypoxiaNSTAppendix.docx