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Recovery of Trembling Aspen, Tamarack, And White Spruce Seedlings From NaCl Stress Following Winter Dormancy: Implications for Increased Foliar Potassium, Necrosis, And Sodium Management as Stress Resistance Mechanisms

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ORIGINAL ARTICLE

Recovery of trembling aspen, tamarack, and white spruce seedlings from NaCl stress following winter dormancy: implications for increased foliar potassium, necrosis, and sodium management as stress resistance mechanisms

Nathan Lauer[1]

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Abstract

Key message **Different tree species exposed to NaCl stress exhibited similar responses including elevated foliar K, increased foliar necrosis, as well as the exclusion or accumulation of foliar Na.**

Abstract Revegetation of boreal forest lands disturbed by surface mining for bitumen can be challenging due to fluctuating levels of soil NaCl and harsh winter temperatures. These stressors may hinder the growth and survival of planted tree seedlings. Two experiments were carried out to examine the processes of recovery from NaCl stress and overwintering in trembling aspen, tamarack, and white spruce seedlings. In the recovery experiment, seedlings were treated with 0, 50, or 100 mM NaCl for 60 days and then allowed to recover for 60 days. Most of the examined physiological variables (total dry weight, chlorophyll concentration, photosynthesis, and transpiration) in all examined species returned to control levels after 30 days of recovery from the NaCl treatment. In the overwintering experiment, seedlings were subjected to 0 or 50 mM NaCl treatment throughout the first growing season, overwintered, and treated with 0, 50, or 100 mM NaCl for 8 weeks during the second growing season. All tested species exhibited foliar chlorosis and necrosis from NaCl treatment in the first year. Several similarities were observed between species in both experiments, including increased foliar K and necrosis in trembling aspen and tamarack. Trembling aspen exhibited remarkably low foliar Na, whereas tamarack and white spruce had high concentrations of foliar Na despite the recovery of physiological variables to control levels. Elevated foliar K, necrosis, and Na management may constitute important salt resistance mechanisms for the tree species tested.

Keywords NaCl stress · Recovery · Overwintering · Resistance mechanisms

Introduction

Bitumen deposits located in northern Alberta that are extracted by surface mining involve the removal of all vegetation and soils from the site prior to mining (Berkowitz and Speignh 1975; Giese et al. 2010). Operators of bitumen surface mines are required by law to reclaim boreal forest lands to equivalent land capability once mining is complete (Government of Alberta 2010). Revegetation is challenging due in part to fluctuating levels of soil NaCl. Saline-sodic overburden is often used as backfill for open pits and can leach NaCl into reclamation soils. The levels of NaCl in the soil can be heterogeneous and transient because of upward water flux, variations in evapotranspiration, precipitation, and water table depth (Kessler et al. 2010; Carrera-Hernandéz et al. 2012). Since soil NaCl levels can be transient at reclamation sites, the ability of planted tree seedlings to recover following exposure to NaCl may be essential to their survival. Additionally, reclamation sites in northern Alberta experience harsh winters with temperatures reaching − 40 °C for prolonged periods of time. Although the effects of NaCl stress have been extensively studied in plants, very little research has been carried out to examine the recovery of plants from NaCl stress. Furthermore, studies on the

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effects of NaCl stress and overwintering on perennial plants are absent in the literature.

Salt stress involves a combination of osmotic and ionic factors, which elicit numerous physiological responses in plants. These responses include an almost immediate decline in root hydraulic conductivity that is linked to decreased water transport through aquaporins and results in rapid decreases in transpiration and photosynthetic rates. This is commonly followed by altered nutrient uptake, reduced growth, development of leaf chlorosis and necrosis and eventually plant mortality (Kozlowski 2000; Boursiac et al. 2005; Munns and Tester 2008; Lee et al. 2010; Shabala and Munns 2012; Arif et al. 2020). Trembling aspen (*Populus tremuloides* Michx), tamarack (*Larix laricina* (Du Roi) K. Koch), and white spruce (*Picea glauca* (Moench) Voss (Pinaceae)) are commonly used for oil sands reclamation and are known to have resilience to moderate levels of soil NaCl. Seedlings of these tree species have been reported to withstand at least 4 weeks of 60 mM NaCl stress (Renault et al. 1999; Renault 2005).

Many adaptive mechanisms are used by glycophytic plants to withstand elevated soil NaCl. One mechanism is maintaining a low Na:K ratio in foliar tissue by a combination of Na exclusion and K accumulation, which is essential for withstanding periods of NaCl stress. Increasing foliar cytoplasmic K is a mechanism of NaCl tolerance in glycophytic plants because elevated foliar Na negatively affects cellular metabolism by interfering with the subcellular role of K as an enzymatic cofactor necessary for nearly all growth processes in plants (Munns and Tester 2008). Elevated soil NaCl is known to trigger the release of K from the root cortex cells, which is then translocated to foliar tissue. This process facilitates osmotic adjustment, decreases oxidative damage from reactive oxygen species, and reduces the triggering of programmed cell death (Cakmak 2005; Chen et al. 2005; Escalante-Pérez et al. 2009; Wang et al. 2013; Wu et al. 2018). Accelerated foliar senescence is another commonly observed response of glycophytic plants to NaCl stress. Long-term Na toxicity in foliar tissue is manifested by yellowing (chlorosis) at the margins followed by necrosis and premature senescence. Although the loss of photosynthetic area decreases the photosynthetic capacity of plants, leaf necrosis and shedding of leaves may help eliminate some of the accumulated salt and remobilize nutrients to younger tissues (Munns and Tester 2008). On the cellular level, chlorosis is characterized by the degradation of chlorophyll and proteins followed by programmed cell death (Munné-Bosch and Alegre 2004; Wang and Blumwald 2014), suggesting that a plant's response to NaCl stress may be linked to complex cell signaling events.

Reports on the recovery of glycophytic plants from NaCl stress are underrepresented in the literature. In one example, spinach subjected to 100 mM NaCl for 3 weeks followed by a 4 week recovery exhibited initial decreases followed by the recovery of physiological variables such as RuBisCO activity, photosynthesis, stomatal conductance, Fv/Fm, and chlorophyll concentration (Delfine et al. 1999). In another example, ornamental brush cherry was exposed to NaCl stress up to 132 mM for 30 days followed by a 16 day recovery period. During the recovery period, Na and Cl continued to accumulate in the roots, stems, and leaves. Higher levels of NaCl treatment led to reduced foliar area during the recovery period (Acosta-Motos et al. 2015). In contrast to the limited number of recovery studies from NaCl stress, many reports exist for the recovery of plants from drought stress. Recovery from drought is characterized by a return of physiological function but almost never to full capacity (reviewed by Chaves et al. 2009). Drought and NaCl stress share some similarities due their osmotic effects on plants; however, direct ion toxicity in NaCl-affected plants may be expected to make recovery from NaCl stress more challenging compared to drought. Clearly, more studies are required to examine the processes of recovery from salt stress, especially in economically important plants.

In the current study, two separate experiments were conducted to explore the effects of long-term NaCl stress and recovery as well as the effects of NaCl stress and overwintering on trembling aspen, tamarack, and white spruce. The NaCl values of 50 and 100 mM used in this study represent values similar to saline conditions found in situ. The 50 mM NaCl treatment is slightly higher than the 4 dS m^{-2} soil surface EC threshold for boreal forest vegetation (Purdy et al. 2005; Lilles et al. 2010). The objectives of the two experiments were to examine the recovery of growth and physiological processes, including photosynthesis, transpiration, and chlorophyll, following exposure to NaCl in a deciduous angiosperm tree (trembling aspen), deciduous conifer (tamarack), and evergreen conifer (white spruce). Mechanisms of resistance to NaCl stress such as elevated foliar K, accelerated foliar necrosis, and Na exclusion or accumulation, were investigated in each species. It was anticipated that physiological variables would decrease because of NaCl stress but would increase during the recovery period for all species; however, since little is known about the stress recovery processes in trees, the degree and timing of recovery was a key question in this study. In a second experiment, nonlethal levels of NaCl were applied to trembling aspen, tamarack, and white spruce seedlings in one growing season. Seedlings were overwintered and again subjected to NaCl stress in the following growing season. The primary objective of the overwintering experiment was to investigate whether seedlings treated with sublethal levels of NaCl during the first year of growth would exhibit acclimation or cumulative NaCl injury when exposed to overwintering followed by NaCl treatment in the second year. It was hypothesized

that NaCl stress and overwintering in year 1 would hinder the ability of seedlings to recover from NaCl stress in year 2. The importance of foliar necrosis as a response to NaCl stress was studied in more detail for trembling aspen leaves. The results generated from this study are intended to generate fundamental knowledge on the ability of woody plants to recover from NaCl stress as well as to benefit reclamation efforts in northern Alberta.

Materials and methods

Plant material, growth conditions, and NaCl treatment

Recovery experiment

1 year-old dormant trembling aspen, tamarack, and white spruce seedlings were obtained from Smoky Lake Forest Nursery (Smoky Lake, AB, Canada). Seedlings were grown from seeds collected from open-pollinated wild tree stands in various locations within Alberta seed zone CM 2.2 by Tree Time Services Inc. (Edmonton, AB, Canada). Seedlings were planted in 4-L pots with a mixture of peat moss and sand (1:1, by weight) and grown for 6 weeks prior to treatments. The seedlings were watered every other day and fertilized with 250 mL of 3 g/L 20:20:20 (N:P:K) commercial fertilizer every 2 weeks prior to experimental treatments and once every month after the treatments commenced. The experiment was carried out in a controlled-environment growth room maintained at 22/18 °C (day/night) temperature, 65 ± 5% relative humidity, and 16 h photoperiod with 300 µmol m^{-2} s^{-1} PPFD using full spectrum fluorescent lights (Philips high output, F96T8/TL835/HO, Markham, ON, Canada). Immediately prior to NaCl treatments, seedlings from each species ($n = 3$) were measured for height and root collar diameter. No significant difference existed between treatments in all species (Tables S1 and S2). After 6 weeks of growth, seedlings were exposed to treatments with 0, 50, and 100 mM NaCl for 60 days by applying 250 mL of the respective NaCl solution once a week. After NaCl treatments, seedlings were thoroughly watered to flush out any remaining NaCl and allowed to recover by watering with 0 mM NaCl for the remainder of the experiment. Selected seedlings ($n = 6$) were taken for measurements immediately after 60 days of NaCl treatments as well as 30 and 60 days after the termination of NaCl treatments. Some trembling aspen seedlings treated with 100 mM NaCl completely defoliated during the NaCl treatment and re-flushed during the recovery period. These seedlings were sampled after 60 days of recovery.

Overwintering experiment

One-year-old dormant trembling aspen, tamarack, and white spruce seedlings were obtained from Smoky Lake Forest Nursery (Smoky Lake, AB, Canada). Seedlings were grown from seeds collected from open-pollinated wild tree stands in various locations within Alberta seed zone CM 2.2 by Tree Time Services Inc. (Edmonton, AB, Canada). Seedlings were planted in 4-L pots with a mixture of peat moss and sand (1:1 by weight) and grown outside for 1 year. During the first growing season, all seedlings were watered daily, and half of the seedlings for each species were treated with 250 mL of 50 mM NaCl every 2 weeks throughout the growing season. Seedlings were fertilized with 250 mL of 3 g/L 20:20:20 (N:P:K) fertilizer every 2 weeks from May to July and every 30 days from August to October. Before the onset of winter, seedlings were thoroughly watered to flush out remaining NaCl from the soil and left outside to overwinter. The pots were covered with soil and hay to prevent root freezing. After overwintering, seedlings were transported into a controlled-environment growth room several weeks before bud break in early May of the second growing season. The controlled-environment growth room was maintained at 22/18 °C (day/night) temperature, 65 ± 5% relative humidity, and 16-h photoperiod with 300 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) using full spectrum fluorescent lights (Philips high output, F96T8/TL835/HO, Markham, ON, Canada). Seedlings were watered every other day and fertilized with 250 mL of 3 g/L 20:20:20 (N:P:K) commercial fertilizer every 2 weeks. Seedlings were allowed to grow for 6 weeks before the NaCl treatments. Immediately prior to NaCl treatments in year 2, seedlings from each species treated with 0 mM NaCl or 50 mM NaCl from year 1 ($n = 3$) were measured for height and root collar diameter, and no significant differences existed between treatments in all species (Tables S3 and S4). Control and NaCl-treated seedlings were then subjected to weekly treatments with 0, 50, and 100 mM NaCl for 6 weeks. Each NaCl treatment was administered by applying 250 mL of the respective NaCl concentration to the soil. Selected seedlings ($n = 6$) were taken for measurements.

Measurements

Gas exchange

At the completion of the recovery and overwintering experiments, gas exchange measurements were carried out on living seedlings ($n = 6$). Net photosynthesis (A) and transpiration (E) rates were measured using an infrared gas analyzer equipped with a standard 6 cm^2 leaf chamber (Li-Cor 6400XT, Li-Cor Inc., Lincoln, NE, USA). Water use efficiency (WUE) was calculated by dividing A by E.

Measurements were conducted in the experimental growth room. Samples were allowed to equilibrate to a steady state for approximately 2 min prior to measurement. The light intensity for all measurements was 300 µmol m^{-2} s^{-1} PPFD provided by a red–blue light source (6400-02, Li-Cor Inc., Lincoln, NE, USA). The [CO_2] was maintained at 400 µmol/mol for all measurements. Light intensity and [CO_2] values were chosen to be the same as the plant growth conditions in the experimental growth room. Considering the relatively low light intensity, comparisons of gas exchange values were limited to within species. For white spruce and tamarack, the needle area was calculated using Sigmascan Pro 5.0 computer software (Systat Software, San Jose, CA, USA).

Foliar chlorophyll concentration and dry weight

After gas exchange measurements, a small number of leaves or needles were collected from mature foliar tissue on seedlings, lyophilized, and ground to a powder for chlorophyll extraction. Chlorophyll was extracted from tissue (10 mg DW) with 8 mL DMSO at 65 °C for 24 h. Chlorophyll concentration was measured with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Sweden) at 648 nm for chlorophyll-*a* and 665 nm for chlorophyll-*b*. Total chlorophyll was calculated using Arnon's equation (Sestak et al. 1971) and normalized by the dry weight of individual extracts.

For dry weight determination, seedlings were separated into foliar tissue, stems, and roots and oven-dried at 70 °C for 72 h before weighing. Lyophilized foliar tissue used for chlorophyll analysis was also weighed and added to the total dry weight measurement. For trembling aspen and tamarack in the recovery experiment and trembling aspen in the overwintering experiment, necrotic foliar tissue was first separated from living tissue prior to oven drying and weighed separately. Living and necrotic foliar tissue was ground to a fine powder using a Wiley mill (screen no. 40) and used for elemental analysis.

Foliar elemental analysis

For the determination of foliar concentrations of K, P, and Na, foliar tissue from all species as well as necrotic foliar tissue from trembling aspen in the overwintering experiment (200 mg) was digested with 10 mL 70% HNO_3 and diluted with deionized water up to 50 mL. Samples were analyzed by ICP–MS in the Radiogenic Isotope facility at the University of Alberta (Zarcinas et al. 1987). For the determination of foliar N concentration for all species as well as necrotic trembling aspen leaf tissue from the overwintering experiment, approximately 2 mg of dried ground samples were analyzed using a CE 440 CHN Elemental Analyzer (Exeter Analytical, MA, USA).

Measurements in green, chlorotic, and necrotic foliar tissue in trembling aspen

The process of accelerated foliar chlorosis was studied in further detail in trembling aspen from the overwintering experiment by analyzing both green and chlorotic foliar tissue for the presence of reactive oxygen species (ROS), relative chlorophyll:carotenoid ratio, and nighttime respiration. The presence of ROS in green and yellow foliar tissue was tested using the ROS-specific fluorescent probe 2,7-dichlorofluorescein-diacetate (DCFH-DA; Invitrogen, Carlsbad, CA, USA). Excised leaf segments were incubated in 5 µM DCFH-DA for 15 min and then washed three times with deionized water to remove unbound probe. Bright-field and fluorescent imaging (ex 488 nm/em 525 nm) was conducted on a Leica DMRXA compound light microscope with a QI click camera (Leica Microsystems, Buffalo Grove, IL). Hyperspectral imaging of green and yellow foliar tissue was taken using a Colorflow XR1 camera (Stream Technologies Inc., Edmonton, AB, Canada). The relative chlorophyll:carotenoid ratio was calculated using an equation simplified from Sims and Gamon (2002), where the relative chlorophyll:carotenoid ratio = 645 nm/531 nm. Respiration rates of green and yellow foliar tissue were measured at night using a Li-Cor 6400XT as described above but without light.

Statistical analysis

All data were analyzed using R (https://www.R-project.org). A P value of $P \leq 0.05$ was chosen for all analyses. For the recovery experiment, all dependent variables were analyzed using a type III two-way ANOVA linear fixed-effects model with NaCl treatment and day of recovery as fixed independent variables. The model equation used was $Y_{ijk} = \mu + S_i + T_j + (S * T)_{ij} + \mathcal{E}_{ijk,}$ where Y_{ijk} is the $_k$th observation of the $_i$th and $_j$th treatments, µ is the sample mean, S_i is the $_i$th NaCl treatment and T_j is the $_j$th day of recovery. The variable in parenthesis is the interaction between NaCl and the day of recovery. Tukey's HSD tests were used when significant differences were detected. To reduce the occurrences of type II statistical errors, only one-way post hoc analyses were conducted. Differences at a specific time point are represented by letters whereas differences from day 0 of recovery within the same treatment are represented by an asterisk (*). To compare the percent of necrotic foliar tissue to living tissue in trembling aspen and tamarack, data were pooled between sampling periods based on the NaCl treatment. The percent of necrotic foliar tissue for trembling aspen and tamarack was analyzed using a one-way ANOVA linear fixed-effects model with NaCl as a fixed independent variable. The model equation is $Y_{ij} = \mu + S_i + \mathcal{E}_{ij,}$ where S_i is the $_i$th NaCl treatment. Tukey's HSD post hoc test was used

when significant differences were detected. Significant differences are represented by letters. Data that did not meet the ANOVA assumptions of normality of distribution and homogeneity of variance were log10 transformed before analysis. Multivariate analysis was performed using principal component analysis (PCA) with physiological variables as vectors. Data from each treatment were averaged to create a single response point for each variable per treatment.

For the overwintering experiment, all dependent variables were analyzed using a type III two-way ANOVA linear fixed-effects model with NaCl treatment in year 1 and NaCl treatment in year 2 as fixed independent variables. The model equation is $Y_{ijk} = \mu + O_i + T_j + (O * T)_{ij} + \mathcal{E}_{ijk,}$ where Y_{ijk} is the $_k$th observation of the $_i$th and $_j$th treatments, μ is the sample mean, O_i is the $_i$th NaCl treatment in year 1 and T_j is the $_j$th NaCl treatment in year 2. The variable in parenthesis is the interaction between NaCl treatment in year 1 and NaCl treatment in year 2. To reduce complexity and the occurrence of type II statistical errors, Fisher's LSD post hoc test was used when significant differences were detected. Asterisks (*) represent differences between the control and NaCl stress treatments from year 2 for seedlings with the same watering regime from year 1. Carets (^) represent differences between NaCl treatments in year 1 within the respective NaCl treatments from year 2. To compare the elemental concentrations of green and necrotic foliar tissue in trembling aspen, data from NaCl treatments from year 1 was pooled based on the NaCl treatment in year 2. The data were then analyzed using a one-way ANOVA linear fixed-effects model with NaCl as a fixed independent variable. The model equation is $Y_{ij} = \mu + S_i + \mathcal{E}_{ij,}$ where Y_{ij} is the $_j$th observation of the $_i$th treatment, μ is the sample mean, and S_i is the $_i$th NaCl treatment from year 2. Significant differences between treatments were analyzed using Tukey's HSD test and are represented by letters. Data collected prior to the experiment, the relative chlorophyll:carotenoid ratio, and nighttime respiration were analyzed using Student's *t* test. Data that did not meet the assumptions of normality of distribution and homogeneity of variance were log10 transformed before statistical analysis. Multivariate analysis was performed using principal component analysis (PCA) with physiological variables as vectors. Data from each treatment were averaged to create a single response point for each variable per treatment.

Results

Recovery experiment

With the exception of dry weight in trembling aspen, all physiological variables in the examined species returned to control levels after 60 days of recovery from the 50 mM NaCl treatment. Recovery for seedlings exposed to 100 mM NaCl differed by species. Trembling aspen exhibited an ability to recover primarily through full defoliation during the stress period followed by re-flushing of new foliar tissue during the recovery period. New leaves had higher levels of the measured physiological variables. Tamarack exposed to 100 mM NaCl showed lowered physiological function after 60 days of recovery compared to 0 and 50 mM treatments. In trembling aspen and tamarack, seedlings treated with 50 mM NaCl exhibited physiological similarities to seedlings treated with 100 mM NaCl (brown ovals). However, after 30 days of recovery, seedlings treated with 50 mM NaCl exhibited physiological similarities to seedlings treated with 0 mM NaCl (blue and green ovals) (Fig. 1a and b). White spruce seedlings treated with 50 mM NaCl exhibited physiological similarities to seedlings treated with 0 mM NaCl after treatment and during the recovery period (green oval), whereas seedlings treated with 100 mM NaCl had lower physiological variables during the recovery period compared to seedlings treated with 0 mM NaCl (brown oval) (Fig. 1c).

Trembling aspen

In trembling aspen, 50 and 100 mM NaCl treatments reduced total dry weights compared to control treatments, and these reductions did not recover over time. The total chlorophyll concentration significantly decreased after treatments with 50 and 100 mM NaCl but recovered to control levels after 30 days of recovery. In most cases, A showed no significant differences between NaCl treatments at 0 and 30 days after recovery. Treatments with 50 and 100 mM NaCl caused E to decrease, but these values returned to control levels after 30 days of recovery. Interestingly, seedlings treated with 100 mM NaCl that re-flushed new leaves after 60 days of recovery had the highest chlorophyll concentration, A, and WUE values compared to other NaCl treatments (Table 1). Foliar Na levels were below 0.05% DW with no significant differences between treatments (Fig. 2a). Seedlings treated with 50 and 100 mM NaCl exhibited significant increases in foliar K. These values returned to control levels after 30 days of recovery in seedlings treated with 50 mM NaCl, whereas seedlings treated with 100 mM NaCl had significantly higher levels of foliar K during the recovery period (Fig. 3a).

Tamarack

In tamarack, both 50 and 100 mM NaCl treatments caused no significant changes in total dry weight and chlorophyll concentration at day 0 of recovery. All seedlings continued to increase in total dry weight during the recovery period; however, seedlings treated with 100 mM NaCl showed significantly lower total dry weight and chlorophyll concentration during the recovery period. The rate of A decreased in

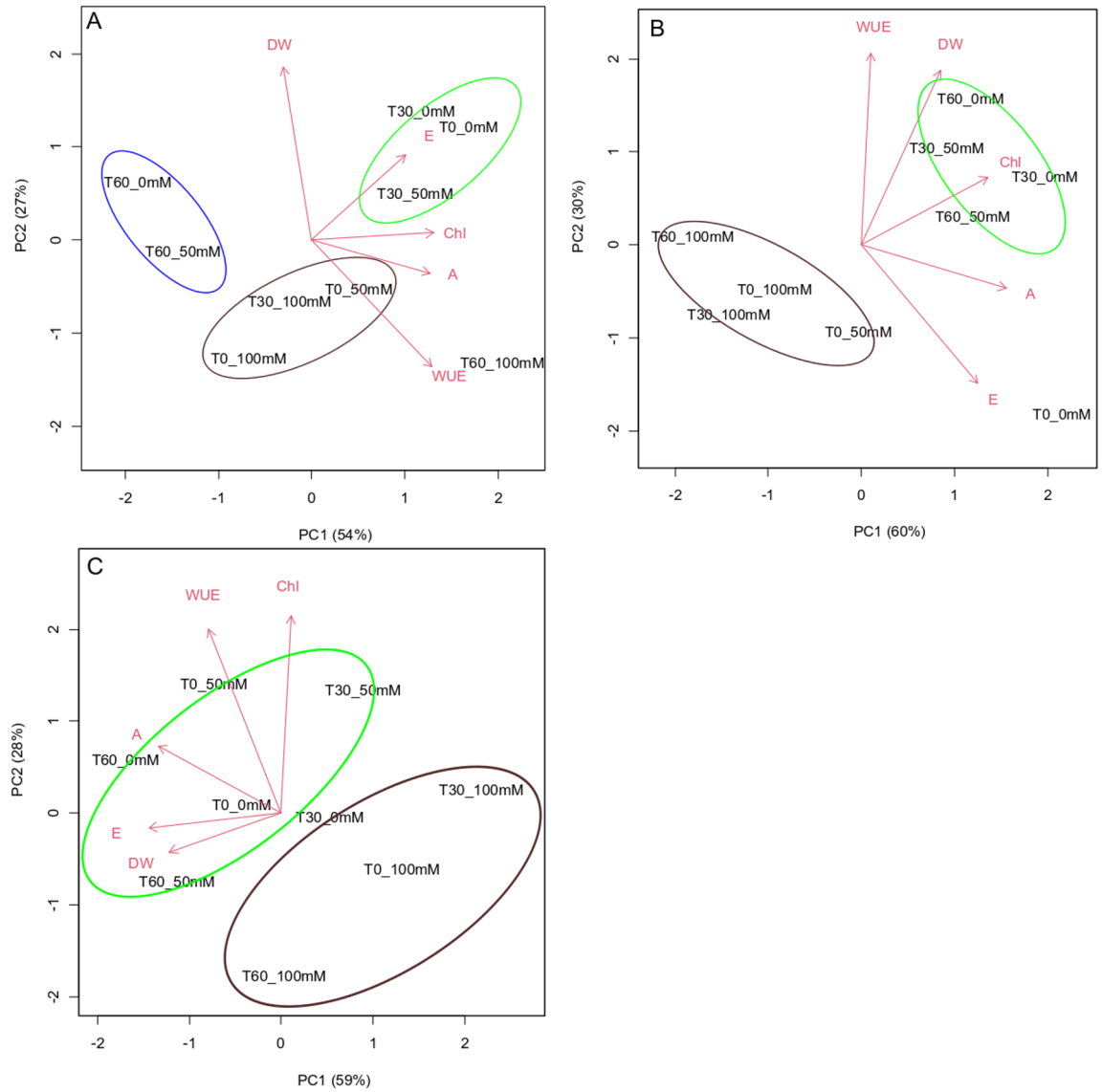

Fig. 1 Biplot of principal components 1 and 2 of physiological variables for trembling aspen (**A**), tamarack (**B**), and white spruce (**C**) for the recovery experiment. Ovals represent treatments with similar values for physiological variables

seedlings treated with 50 and 100 mM NaCl and remained lower than control values after 30 days of recovery. After 60 days of recovery, A returned to control levels in seedlings treated with 50 mM NaCl but not seedlings treated with 100 mM NaCl. Treatments with 50 and 100 mM NaCl caused significant decreases in E at day 0 of recovery; however, no differences in E were found after 30 days of recovery. The WUE increased in all treatments during the recovery period, but WUE was lower in seedlings treated with NaCl (Table 1). Foliar Na increased significantly and proportionally from NaCl treatments (Fig. 2b). Foliar K was elevated in seedlings treated with 50 and 100 mM NaCl compared to control seedlings. These values returned to control levels after 30 days of recovery (Fig. 3b).

White spruce

In white spruce, 50 and 100 mM NaCl treatments caused no significant changes in total dry weight and chlorophyll concentration. The rates of A were similar in the 0 and 50 mM NaCl treatments at all timepoints. Seedlings treated with 100 mM NaCl first showed a significant decrease in A but showed a full recovery after 60 days. Rates of E showed no differences between NaCl treatments for all timepoints. The WUE of seedlings treated with 100 mM NaCl was lower at all timepoints (Table 1). The foliar Na concentration increased in seedlings treated with 100 mM NaCl and remained high after 60 days of

Table 1 Effects of a 60 day NaCl treatment followed by a 60 day recovery period on total dry weight, chlorophyll concentration, A, E, and WUE in trembling aspen, tamarack, and white spruce seedlings

Species	Trembling aspen			Tamarack			White spruce		
Recovery (days)	0	30	60	0	30	60	0	30	60
Total DW (g)									
0 mM NaCl	144.9 ± 6.8[a]	41.8 ± 11.5[a]	47.9 ± 13[a]	16.9 ± 1.4	28.8 ± 2.6[a]*	31.6 ± 4.6[a]*	15.7 ± 0.4	12.5 ± 1.1	21.5 ± 2.5*
50 mM	28.8 ± 6.1[b]	29.1 ± 2.4[ab]	25.3 ± 3.8[b]	15.0 ± 2.5	24.9 ± 1.6[a]*	26.3 ± 3.8[a]*	12.3 ± 1.7	11.3 ± 1.1	19.9 ± 2.3*
100 mM	21.5 ± 3.3[b]	22.8 ± 8.5[b]	13.6 ± 5.0c	11.1 ± 1.7	14.2 ± 2.6[b]	18.3 ± 2.5[b]	13.3 ± 1.7	11.3 ± 1.1	15.8 ± 2.0*
Total Chlorophyll (mg/g DW)									
0 mM	13.4 ± 1.2[a]	12.1 ± 1.2	5.6 ± 0.8[a]	7.5 ± 0.5	8.5 ± 0.7[a]	7.4 ± 0.9[a]	6.1 ± 0.6	5.3 ± 0.5	7.0 ± 0.5
50 mM	10.1 ± 0.8[b]	10.7 ± 1.7	6.1 ± 0.6[b]*	6.2 ± 0.5	8.0 ± 0.9[a]	6.5 ± 0.7[a]	7.4 ± 0.4	7.6 ± 0.4	5.9 ± 0.4
100 mM	10.6 ± 0.4[b]	8.2 ± 0.9	13.1 ± 0.6[b]*	6.5 ± 0.6	3.8 ± 0.4[b]*	3.6 ± 0.3[b]*	5.7 ± 0.5	6.9 ± 0.6	4.1 ± 0.7
A (μmol m^{-2} s^{-1})									
0 mM	7.5 ± 0.6	6.1 ± 0.9	4.7 ± 0.9[a]*	5.8 ± 0.3[a]	4.4 ± 0.4[a]	4.0 ± 0.5[a]	4.2 ± 0.4[a]	3.1 ± 0.7	4.5 ± 0.6
50 mM	7.1 ± 0.8	5.6 ± 0.5	4.7 ± 0.8[b]*	3.4 ± 0.5[b]	2.8 ± 0.2[b]	4.5 ± 0.4[a]	4.8 ± 0.8[a]	3.4 ± 0.6	3.9 ± 0.3
100 mM	4.9 ± 1.4	5.6 ± 0.5	9.0 ± 0.4[b]*	2.5 ± 0.5[b]	2.6 ± 0.4[b]	2.2 ± 0.3[b]	2.7 ± 0.5[b]	1.6 ± 0.8	3.3 ± 0.6
E (mmol m^{-2} s^{-1})									
0 mM	2.8 ± 0.1[a]	3.1 ± 0.3	1.9 ± 0.3*	2.7 ± 0.1[a]	1.9 ± 0.2*	1.3 ± 0.2*	1.1 ± 0.1	0.8 ± 0.2*	1.3 ± 0.2
50 mM	2.2 ± 0.2[ab]	3.1 ± 0.1*	2.4 ± 0.2	1.9 ± 0.2[b]	1.4 ± 0.1	1.8 ± 0.2	1.3 ± 0.3	0.8 ± 0.1*	1.3 ± 0.2
100 mM	1.6 ± 0.4[b]	2.4 ± 0.1	2.2 ± 0.1	1.4 ± 0.2[b]	1.5 ± 0.2	1.2 ± 0.2	0.9 ± 0.1	0.6 ± 0.1*	1.3 ± 0.2
WUE (A/E)									
0 mM	2.7 ± 0.2	2.0 ± 0.2	2.5 ± 0.3	2.1 ± 0.1	2.4 ± 0.1	3.2 ± 0.3[a]*	3.8 ± 0.2	3.6 ± 0.2[a]	3.6 ± 0.2
50 mM	3.4 ± 0.3	1.9 ± 0.3*	2.0 ± 0.3*	1.7 ± 0.2	2.0 ± 0.2	2.5 ± 0.2[b]*	3.8 ± 0.3	4.2 ± 0.2[a]	3.2 ± 0.2
100 mM	3.0 ± 0.6	2.3 ± 0.2	3.3 ± 0.1	1.8 ± 0.1	1.8 ± 0.2	2.0 ± 0.3c	3.8 ± 0.2	3.6 ± 0.2[b]	3.6 ± 0.2

Values represent the mean ± SEM ($n = 6$). Letters represent a significant difference between NaCl treatments at one timepoint whereas asterisks represent a significant difference from day 0 of recovery within the same treatment at $P < 0.05$ using Tukey's HSD test

recovery (Fig. 2c). Treatment with 100 mM NaCl caused a decrease in foliar K that recovered after 30 days (Fig. 3c).

Foliar chlorosis/necrosis

Trembling aspen and tamarack exhibited chlorosis followed by necrosis staring at the foliar tips as a result of NaCl stress (Fig. 4). In both species, the percentage of necrotic foliar tissue increased with increasing NaCl treatment concentration. The increase in necrotic tissue was more pronounced in trembling aspen than in tamarack (Table 2).

Overwintering experiment

In most cases, treatment with NaCl in year 2 decreased the physiological variables in trembling aspen and tamarack seedlings. Multiple years of NaCl stress did not alter the total dry weight or any measured physiological variables in white spruce. Evidence of acclimation or cumulative salt injury was evident in all species exposed to 2 years of NaCl stress and overwintering. In trembling aspen, seedlings treated with 50 mM NaCl in year 1 or year 2 had lower physiological values than seedlings treated with 0 mM NaCl in both years (gray oval). The physiological variables of seedlings treated with higher levels of NaCl were even lower (red oval) (Fig. 5a). In tamarack, seedlings treated with 50 mM NaCl in year 1 showed a negative impact on physiological variables compared to those treated with 0 mM in year 1 (brown oval) (Fig. 5b). In white spruce, no clear trends were found from principal component analysis, perhaps due to mostly nonsignificant changes in physiological variables as a result of NaCl treatment (Fig. 5c).

Trembling aspen

Trembling aspen exhibited lower total dry weight after 50 and 100 mM NaCl treatment in year 2 regardless of NaCl treatment in year 1. Seedlings treated with 100 mM NaCl in year 2 had lower chlorophyll concentrations and A regardless of NaCl treatment in year 1. Seedlings treated with 0 mM NaCl in year 1 exhibited lower E when treated with NaCl in year 2; however, seedlings treated with 50 mM NaCl in year 1 had no changes in E when treated with NaCl in year 2. No changes to WUE were found (Table 3). Similar to the recovery experiment, foliar Na was remarkably low for all NaCl treatments (Fig. 2d). Seedlings exhibited a significant increase in foliar K concentration in 50 and 100 mM NaCl

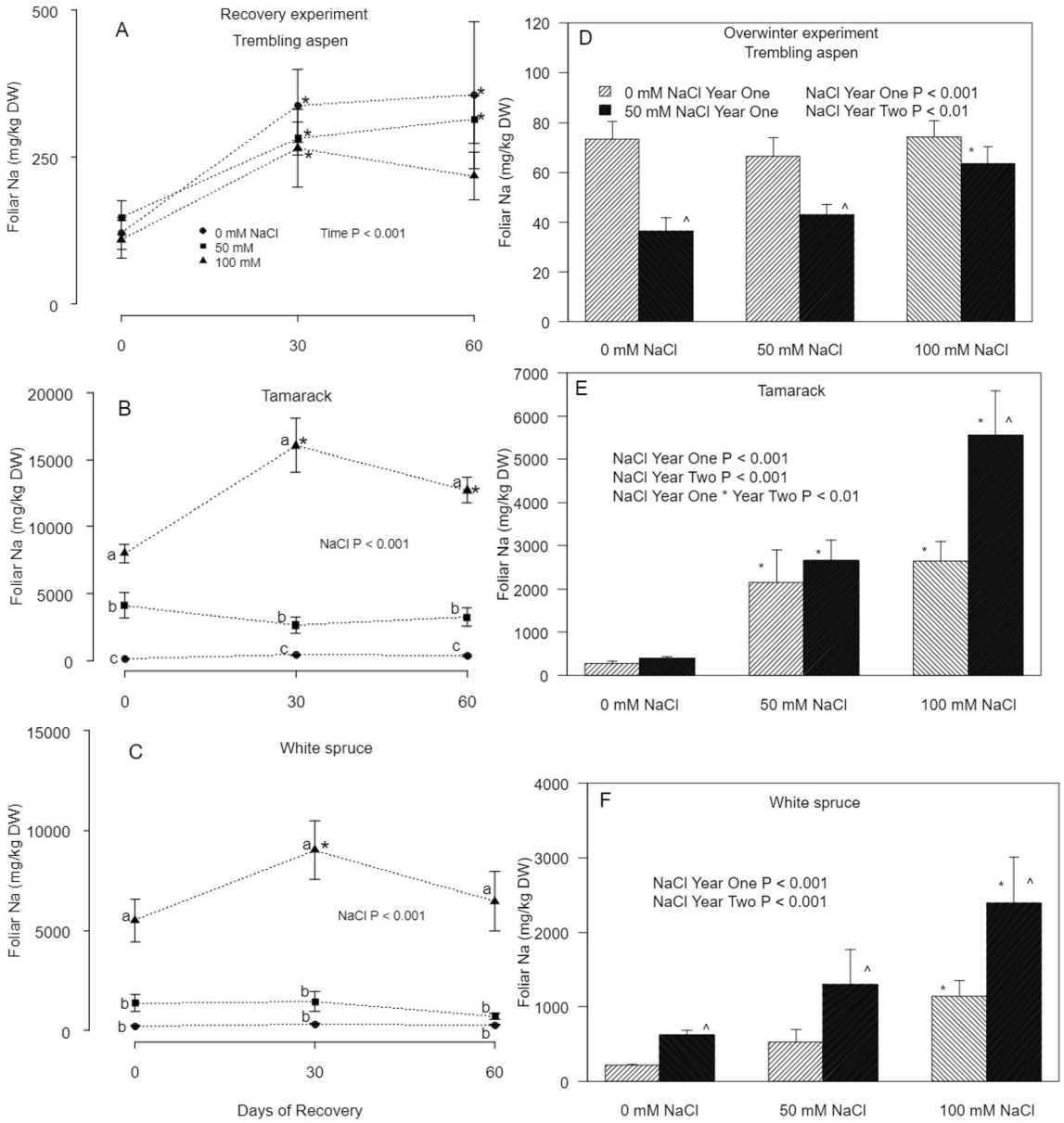

Fig. 2 Foliar Na for trembling aspen (**A** and **D**), tamarack (**B** and **E**), and white spruce (**C** and **F**) for the recovery and overwintering experiments. Values represent the mean ± SEM ($n = 6$)

treatments in year 2 treatments regardless of NaCl treatment in year 1 (Fig. 3d).

Tamarack

Tamarack treated with NaCl in year 2 exhibited decreases in total dry weight. This trend was enhanced in seedlings treated with 50 mM NaCl in year 1. No significant changes in foliar chlorophyll concentrations were observed between the treatments. Treatment with NaCl in year 2 caused decreases in A. Seedlings treated with 50 mM NaCl in year 1 were higher than those for seedlings treated with 0 mM NaCl when treated with NaCl in year 2. Seedlings treated with 0 mM NaCl in year 1 showed a decline in E when subjected to 100 mM NaCl treatment in year 2, whereas seedlings treated with 50 mM NaCl in year 1 showed no changes in 50 and 100 mM NaCl treatments in year 2. Treatment with NaCl in year 2 resulted in lowered WUE. Seedlings treated with 50 mM NaCl in year 1 exhibited higher WUE values compared to seedlings treated with NaCl only in year 2 (Table 3). Seedlings treated with NaCl in year 1 or year 2 exhibited higher concentrations of foliar Na (Fig. 2e). Foliar K increased as a result of NaCl treatment in year 2, regardless of NaCl treatment in year 1 (Fig. 3e).

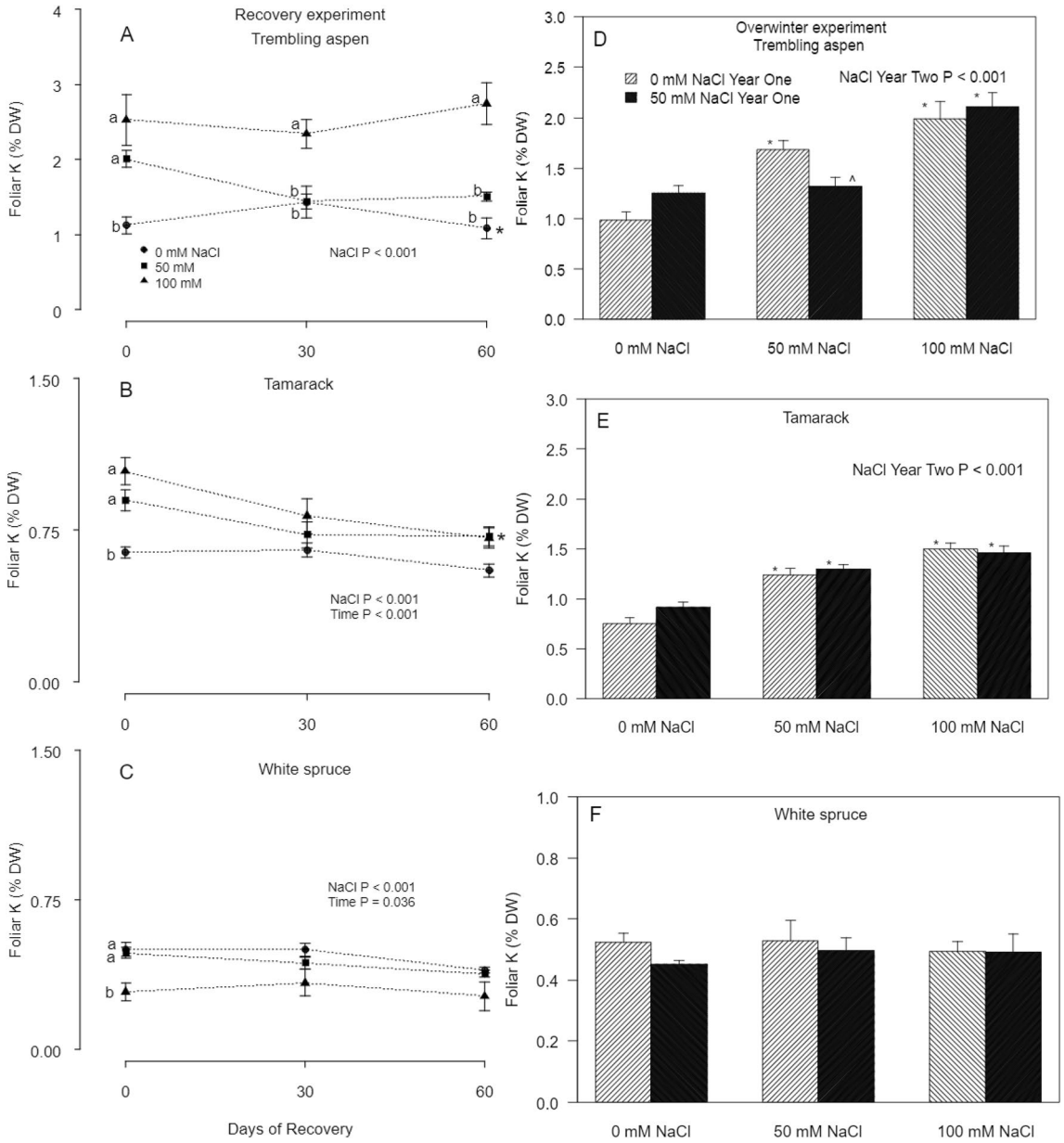

Fig. 3 Foliar K for trembling aspen (**A** and **D**), tamarack (**B** and **E**), and white spruce (**C** and **F**) for the recovery and overwintering experiments. Values represent the mean ± SEM ($n = 6$)

White spruce

NaCl treatment had relatively little effect on total dry weight, foliar chlorophyll concentration, A, and E in white spruce. The only exception being seedling dry weight which was lower when treated with 50 mM NaCl in year 1 and 100 mM NaCl in year 2. No changes to WUE were found (Table 3). Seedlings treated with NaCl showed increases in foliar Na concentration with increasing NaCl treatment levels (Fig. 2f). No changes in foliar K were observed (Fig. 3f).

Visible injury

All seedlings treated with NaCl had a similar visible injury pattern, which included yellowing at the foliar margins or tips and eventual death of older leaves or needles. Trembling aspen grew new leaves near the top of the seedling that appeared healthy. Tamarack showed a legacy of NaCl treatment from year 1, as some seedlings exhibited mortality of the terminal buds. White spruce showed significant needle chlorosis and death in the previous year's growth. The current year's needles in white spruce showed slight

Fig. 4 NaCl-induced foliar chlorosis and necrosis in trembling aspen (**A**) and tamarack (**B**) for the recovery experiment

chlorosis at the needle apex, and newly formed buds appeared healthy (Fig. 6).

Measurements in green, chlorotic, and necrotic foliar tissue in trembling aspen

In trembling aspen, the percentage of necrotic leaf tissue increased with increasing NaCl treatment in year 2. Necrotic tissue had higher concentrations of N, P and Na than green foliar tissue. The foliar K concentration increased in green foliar tissue following NaCl treatments but was lower in the necrotic tissue (Table 4). Compared to green tissue, chlorotic tissue showed an accumulation of reactive oxygen species, possibly near the chloroplasts (Fig. 7a). Chlorotic foliar tissue also had a lower relative chlorophyll:carotenoid ratio but a higher night-time respiration rate than green foliar tissue (Fig. 7b and c).

Table 2 Percent of necrotic foliar tissue for trembling aspen and tamarack from the recovery experiment

Recovery (days)	0	30	60
Trembling aspen			
0 mM NaCl	0^{a}	0^{a}	0^{a}
50 mM	33.6 ± 7.6^{b}	16.2 ± 4.2^{b}	25.3 ± 5.2^{b}
100 mM	43.0 ± 5.8^{b}	50.4 ± 9.2^{c}	0^{a}
Tamarack			
0 mM	0^{a}	0^{a}	0^{a}
50 mM	0^{a}	9.1 ± 2.3^{b}	2.7 ± 1.0^{b}
100 mM	15.3 ± 0.5^{b}	32.3 ± 6.8^{c}	20.4 ± 3.8^{c}

Values represent the mean ± SEM ($n = 6$), and letters represent a significant difference at $P < 0.05$ using Tukey's HSD test

Discussion

The purpose of this study was to generate fundamental knowledge that is either limited or absent in the literature. Investigations on the recovery of glycophytic plants are very limited, whereas studies on the effects of NaCl stress after overwintering are absent. It was anticipated that exposure of trembling aspen, tamarack, and white spruce seedlings to NaCl stress would elicit decreases in growth, chlorophyll concentration, A, and E as well as accelerated foliar senescence and that these variables would recover during the recovery period. The degree and timing of the recovery was a key question to be determined from the recovery experiment. The overwintering experiment was an investigation into whether seedlings treated with sublethal levels of NaCl followed by overwintering would exhibit an acclimation or cumulative injury when treated with NaCl in the second year. It was hypothesized that NaCl stress and overwintering in year 1 would hinder the ability of seedlings to recover from NaCl stress in year 2. Therefore, visible signs of injury were combined with physiological and elemental data to provide insight into potential mechanisms of acclimation and cumulative salt injury.

General observations

It is encouraging that the examined species in this study showed the ability to recover physiological variables after long-term exposure to 50 mM NaCl because this treatment level is slightly higher than the 4 dS m^{-2} soil surface EC threshold for boreal forest vegetation in natural settings (Purdy et al. 2005; Lilles et al. 2010). The findings for trembling aspen and white spruce from both studies are comparable to studies of naturally saline sites in boreal forests

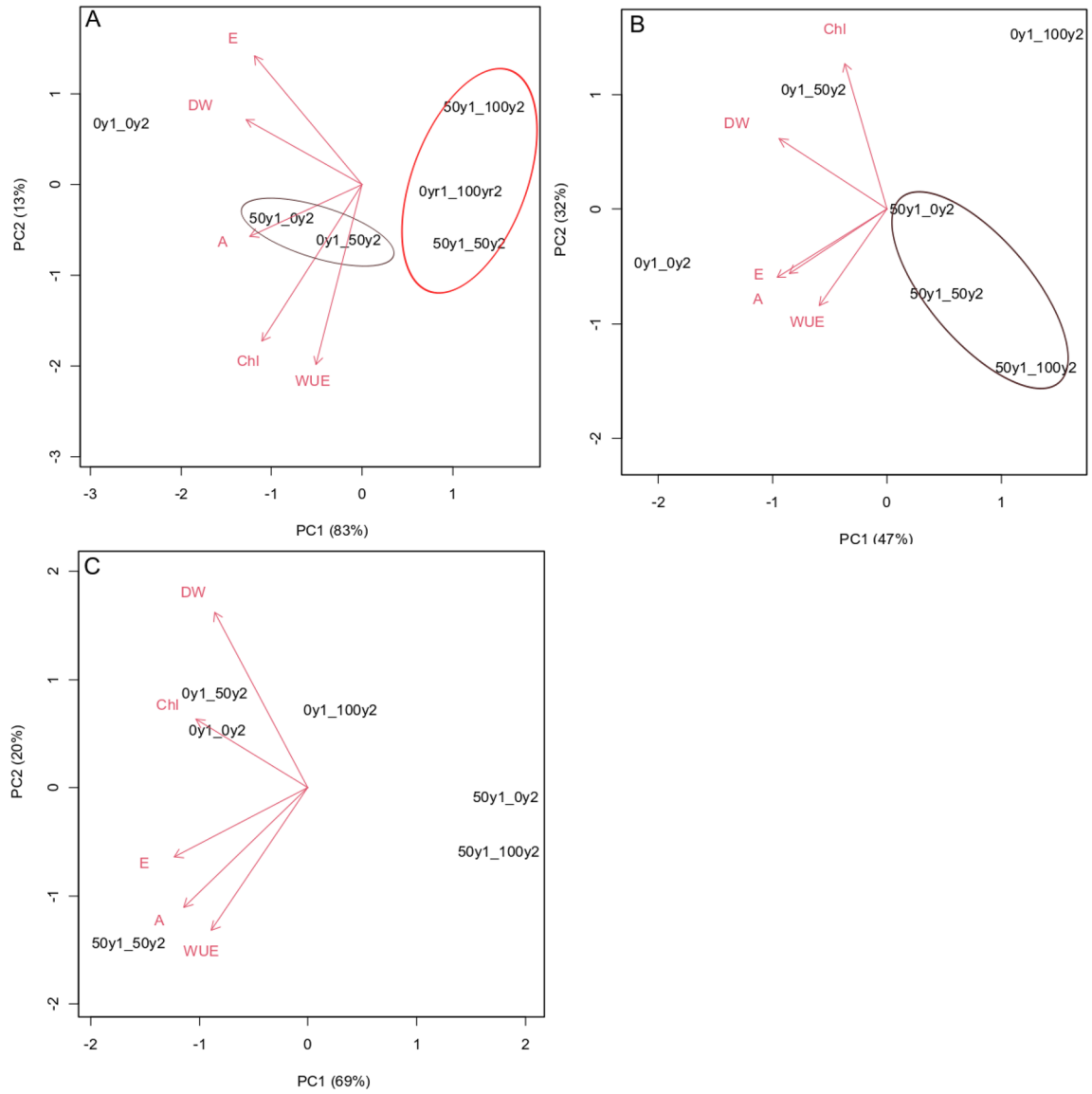

Fig. 5 Biplot of principal components 1 and 2 of physiological variables for trembling aspen (**A**), tamarack (**B**), and white spruce (**C**) for the overwintering experiment. Ovals represent treatments with similar values for physiological variables

where these species have shown productive growth on sites with soil EC as high as 7.8 dS m^{-2} (~ 80 mM NaCl). Trembling aspen grew on all sites but exhibited decreased growth as soil EC increased, whereas white spruce appeared to be unaffected (Lilles et al. 2012). Similar to a previous report, tamarack exhibited moderate tolerance to NaCl in this study (Renault 2005). All species examined in this study are feasible for use in reclamation. The effects of NaCl stress on tamarack are limited (Renault 2005); thus, observations from this study are highlighted below.

In both experiments tamarack seedlings exhibited significant decreases in A compared to E leading to lowered WUE from NaCl stress. This suggests the decreases in WUE were attributed to inhibition at the chloroplasts. Potential mechanisms for this trend may be increased photoinhibition and subsequent ROS production (Masojídek et al. 1991; Waraich et al. 2011). A notable trend was found in the overwintering experiment where seedlings exposed to 2 years of NaCl stress had elevated A and WUE compared to seedlings exposed to NaCl in year 2 alone. The partial recovery of WUE in tamarack seedlings exposed to NaCl in 2 consecutive years may be attributed to enhanced control over photoinhibition or ROS accumulation (Waraich et al. 2011). The partial recovery of A and WUE from multiple years of NaCl stress is encouraging and may represent an undescribed adaptive mechanism to cope with elevated soil NaCl.

Table 3 Effects of a 60 day NaCl treatment in year 2 on trembling aspen, tamarack, and white spruce seedlings treated with 0 or 50 mM NaCl in year one on total dry weight, chlorophyll concentration, A, E, and WUE

Species	Trembling aspen		Tamarack		White spruce	
Year 1 NaCl Treatment	0 mM	50 mM	0 mM	50 mM	0 mM	50 mM
Total DW (g)						
0 mM NaCl	109.7 ± 2.8	77.7 ± 6.4^	95.6 ± 5.7	70.7 ± 4.1^	42.9 ± 4.6	34.7 ± 3.2
50 mM	64.7 ± 6.6*	46.9 ± 3.9*^	76.6 ± 5.6*	66.3 ± 5.1	42.9 ± 7.7	38.4 ± 4.3
100 mM	44.7 ± 5.7*	48.4 ± 3.4*	78.4 ± 4.7*	55.3 ± 3.1*^	45.5 ± 3.8	32.0 ± 3.9^
Total Chlorophyll (mg/g DW)						
0 mM	9.2 ± 0.6	9.9 ± 1.0	7.7 ± 0.8	8.2 ± 0.8	6.6 ± 0.5	5.4 ± 0.6
50 mM	8.6 ± 0.8	7.8 ± 0.9	9.4 ± 0.9	7.5 ± 0.8	6.8 ± 0.8	6.2 ± 0.6
100 mM	6.6 ± 0.6*	5.1 ± 0.5*	8.5 ± 0.8*	6.6 ± 0.6	5.4 ± 0.5	5.3 ± 0.5
A (μmol m-2 s^{-1})						
0 mM	5.1 ± 0.7	3.5 ± 0.2^	5.1 ± 0.5	4.0 ± 0.6	1.1 ± 0.5	0.5 ± 0.3
50 mM	4.2 ± 0.6	3.4 ± 0.4	1.9 ± 0.5*	3.3 ± 1.0	1.0 ± 0.6	2.1 ± 0.9
100 mM	2.3 ± 0.4*	2.1 ± 0.3*	0.0 ± 0.6*	1.1 ± 0.6*^	0.9 ± 0.5	0.4 ± 0.2
E (mmol m-2 s-1)						
0 mM	2.0 ± 0.2	1.6 ± 0.1	2.8 ± 0.4	1.9 ± 0.1^	1.5 ± 0.4	0.9 ± 0.2
50 mM	1.4 ± 0.1*	1.3 ± 0.2	2.9 ± 0.3	2.2 ± 0.3	1.4 ± 0.4	1.9 ± 0.5
100 mM	1.2 ± 0.1*	1.4 ± 0.1	1.3 ± 0.2*	2.3 ± 0.2^	1.4 ± 0.2	1.0 ± 0.1
WUE (A/E)						
0 mM	2.7 ± 0.4	2.3 ± 0.3	2.0 ± 0.4	2.0 ± 0.3	0.9 ± 0.3	0.8 ± 0.4
50 mM	2.9 ± 0.2	2.8 ± 0.3	0.6 ± 0.2*	1.3 ± 0.4^	0.7 ± 0.5	1.4 ± 0.4
100 mM	1.9 ± 0.2	1.5 ± 0.2	− 0.2 ± 0.5^	0.6 ± 0.3^	0.6 ± 0.3	0.4 ± 0.2

Values represent the mean ± SEM ($n = 6$). Asterisks represent differences between the control and NaCl stress treatments from year 2 for seedlings with the same watering regime from year 1 and carets represent differences between NaCl treatments in year 1 within the respective NaCl treatments from year 2 at $P < 0.05$ using Tukey's HSD test

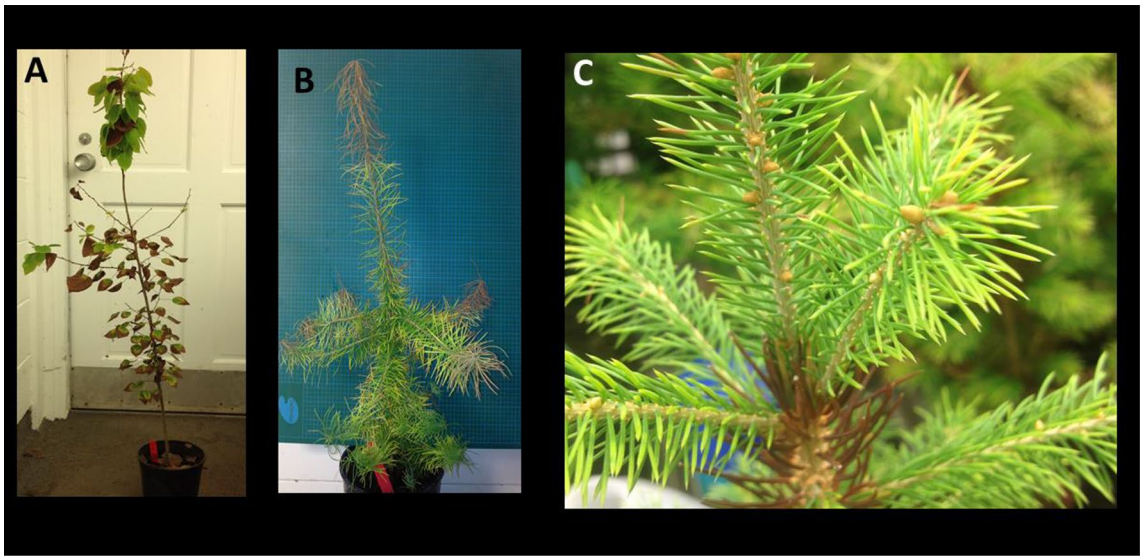

Fig. 6 NaCl-induced visible injury in trembling aspen (**A**), tamarack (**B**), and white spruce (**C**) for the overwintering experiment

Many tamarack seedlings exposed to NaCl in year one exhibited terminal bud dieback in year 2. Considering that tamarack exhibits an excurrent growth pattern with one dominant central stem, this defect caused by NaCl stress and overwintering could make a mature tree more susceptible to wind damage. It may be advantageous to correct this defect by pruning to create one dominant leading branch while the tree is young. Considering that the phenomenon of terminal bud dieback only occurred in seedlings exposed to NaCl stress in year 1, it was attributed to the combined stresses of NaCl and overwintering which may have interfered with cellular supercooling

Table 4 Percentage of necrotic tissue and foliar elemental concentrations of green and necrotic tissue in trembling aspen from the overwintering experiment

	0 mM NaCl	50 mM	100 mM	50 mM Necrotic	100 mM Necrotic
% Necrotic	N.A	N.A	N.A	34.9 ± 6.3^{b}	53.2 ± 7.3^{a}
N (% DW)	1.8 ± 0.1^{b}	2.1 ± 0.1^{b}	2.0 ± 0.1^{b}	2.2 ± 0.1^{a}	2.1 ± 0.1^{a}
P (% DW)	0.2 ± 0.01^{b}	0.2 ± 0.02^{a}	0.3 ± 0.01^{a}	0.3 ± 0.02^{a}	0.2 ± 0.01^{a}
K (% DW)	1.1 ± 0.1^{e}	1.5 ± 0.1^{c}	2.1 ± 0.1^{a}	1.2 ± 0.1^{d}	1.8 ± 0.1^{b}
Na (ppm DW)	54.9 ± 7.0^{c}	54.7 ± 5.4^{c}	68.9 ± 4.7^{b}	101.5 ± 12.9^{a}	97.7 ± 10.1^{a}

Values represent the mean ± SEM ($n = 12$), and letters represent a significant difference at $P < 0.05$ using Tukey's HSD test

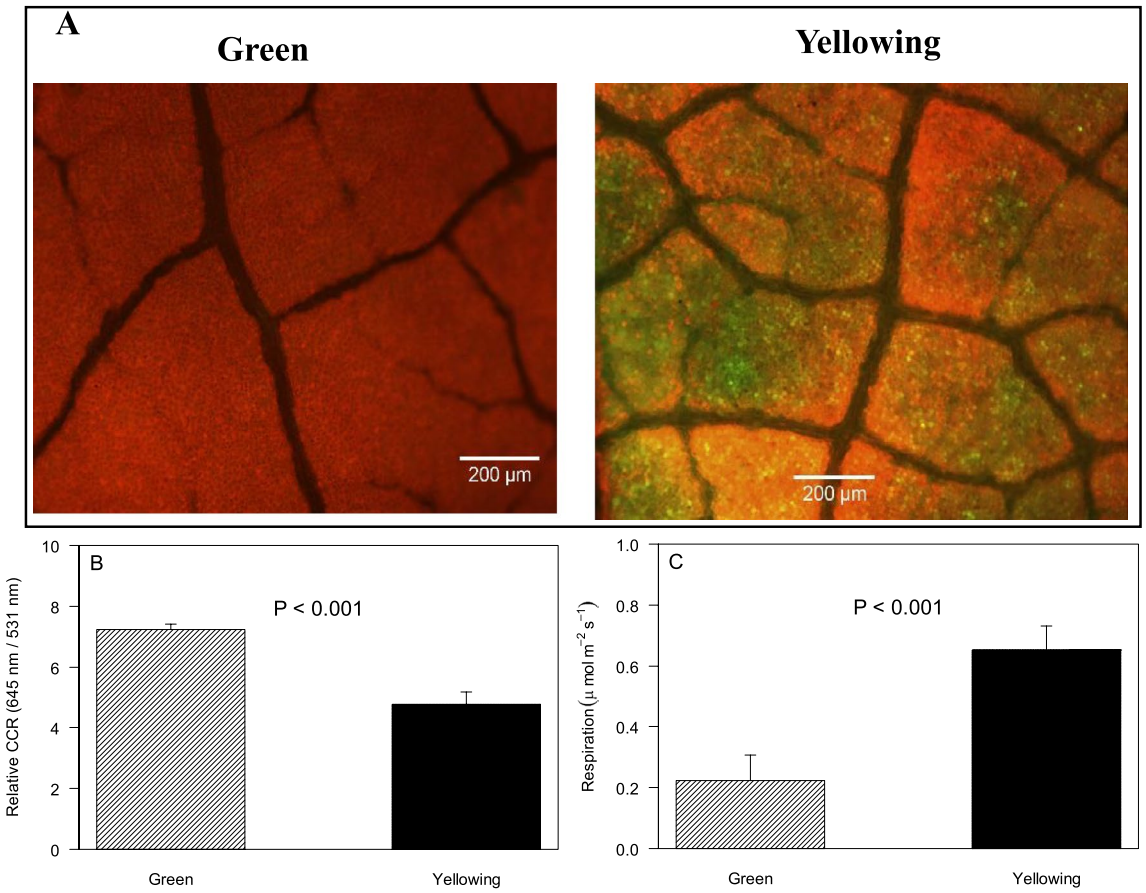

Fig. 7 Comparison of green and NaCl-induced foliar yellowing in trembling aspen from the overwintering experiment for the presence of ROS (**A**), relative chlorophyll:carotenoid ratio (**B**), and nighttime respiration (**C**). Tissue was stained for the presence of ROS using the fluorescent probe DCFH-DA. The red color represents the autofluorescence of chlorophyll, whereas the green color represents the presence of ROS. Values in bar graphs represent the mean ± SEM ($n = 6$)

mechanisms, resulting in terminal bud dieback. The predominant cause of frost injury in perennial plants is cellular desiccation caused by the formation of extracellular ice. Cellular supercooling is a response observed in woody plants to prevent ice nucleation within the cell. The process involves changes in cell membrane lipid content as well as increases in cytosolic sugars and hydrophilic polypeptides (Bertrand and Castonguay 2003). This process has been observed in many tissue types, including buds (George and Burkey 1984). The addition of Na within the tissue likely had negative effects on cellular function, potentially including cellular supercooling.

Mechanisms of acclimation to NaCl stress

Long-term exposure of plants to elevated soil Na leads to ion toxicity in plants and causes reduced physiological function (Munns and Tester 2008; Liang et al. 2018; Isayenkov and Maathuis 2019). Thus, the management of Na within plant tissue may facilitate the recovery

of physiological processes. Several similarities were observed between species in both experiments because of NaCl stress. First, trembling aspen and tamarack exhibited elevated foliar K in response to NaCl treatment. Second, all species exhibited accelerated foliar chlorosis and necrosis. Finally, trembling aspen exhibited remarkably low foliar Na, whereas tamarack and white spruce exhibited high levels of foliar Na, even as physiological variables recovered from the initial NaCl stress. The benefits and potential mechanisms of these observations are discussed below.

Increased foliar K

Trembling aspen and tamarack exhibited increases in foliar K from NaCl treatments in both experiments. In trembling aspen, the increase in foliar K and low Na concentration resulted in a lowered Na:K ratio in both experiments in response to NaCl stress. Elevated foliar K may have aided the recovery of A and E from NaCl treatment by out competing Na as a cofactor for enzymatic reactions. Foliar increases in cytoplasmic K from NaCl stress are proposed to be a fundamental mechanism of NaCl tolerance in glycophytic plants and maintaining a low foliar Na:K ratio can reduce physiological signs of NaCl stress (Munns and Tester 2008; Wang et al. 2013; Wu et al. 2018). The reason for this strategy has yet to be fully elucidated, but several lines of evidence suggest that elevated foliar K is beneficial for plants exposed to elevated soil NaCl. First, elevated levels of shoot K are positively correlated with higher salinity thresholds in a variety of plant species. Second, K acts as a cofactor for enzymes essential for nearly all growth processes in plants. Third, plants grown in soils with low K availability are more sensitive to both NaCl and drought stress. Finally, the exogenous application of KCl to the foliar tissue of plants can alleviate symptoms of poor health in soils with low potassium availability (Cakmak 2005; Chen et al. 2005; Escalante-Pérez et al. 2009; Wang et al. 2013; Zörb et al. 2014; Wu et al. 2018). Thus, it is advisable that reclamation sites have adequate levels of available soil K to help seedlings tolerate periods of elevated soil NaCl or other environmental stresses.

An interesting observation was made when green and necrotic foliar tissue was separated in trembling aspen from the overwintering experiment. The elemental concentrations in both green and necrotic foliar tissue were then measured. Compared to green tissue, the necrotic tissue had higher levels of most measured elements except for K. This trend may be an adaptation to retain foliar K in living tissue. Elevated respiration in the yellowing region between green and necrotic tissue may indicate the active transport of K to green tissue.

Foliar chlorosis/necrosis

All species exhibited chlorosis of older foliar tissue followed by necrosis staring at the foliar tips as a result of NaCl stress in both experiments. Accelerated foliar necrosis is a common response of glycophytic plants to Na toxicity and occurs after prolonged NaCl stress. This process aids the NaCl tolerance of plants by reducing the amount of toxic Na entering growing tissues (Munns and Tester 2008). The foliar yellowing process was studied in more detail in trembling aspen leaves from the overwintering experiment. It was found that yellowing foliar tissue exhibited common signs of leaf senescence, including elevated respiration but decreased chlorophyll:carotenoid ratios, compared to green tissue. Yellowing tissue also showed an accumulation of ROS in the cells, possibly surrounding the chloroplasts. This is intriguing because foliar yellowing can be induced by NaCl, leading to a cell signaling event to trigger the vesiculation and proteolysis of chloroplasts (Wang and Blumwald 2014). A thorough review of foliar yellowing from NaCl stress has yet to be conducted; however, similarities can be drawn to drought-induced foliar yellowing. Munné-Bosch and Alegre (2004) stated that foliar yellowing is characterized by elevated levels of ABA and ROS as well as an upregulation of senescence-associated genes and an organ-wide triggering of programmed cell death. The yellowing process is initiated by a cell signaling cascade that leads to decreased photosynthesis, chlorophyll degradation, and a loss of cellular integrity linked to nutrient remobilization from yellowing tissue to younger tissue. Interestingly, chloroplasts, which contain most proteins and lipids within the cell, are targeted for degradation. The current study did not directly show that programmed cell death was occurring in the chlorotic region of trembling aspen leaves, but the evidence suggests that it is likely the case.

Foliar Na management

In both experiments, foliar Na concentrations in trembling aspen were significantly lower than those in tamarack and white spruce. The low levels of foliar Na in trembling aspen may in part explain why A was mostly unaffected by NaCl treatment in both experiments. Accelerated foliar senescence may be one mechanism to maintain relatively low foliar Na levels. Another possibility for low foliar Na concentrations is ion exclusion via suberin deposition in the roots (Franke and Schreiber 2007; Munns and Tester 2008; Wu 2018). The mechanism of maintaining low levels of foliar Na in trembling aspen appears to be a physiological adaptation to NaCl stress.

Contrary to the low levels of foliar Na found in trembling aspen, both tamarack and white spruce appeared to accumulate Na in foliar tissue. In the recovery experiment,

foliar Na remained at the same level or increased during the recovery period for tamarack and white spruce seedlings. In most cases, growth, chlorophyll concentration, A, and E were similar to or returned to control values after 60 days of recovery. In the overwintering experiment, tamarack and white spruce seedlings exhibited significant increases in foliar Na concentration when treated with NaCl in year 2; however, physiological variables were not correlated with foliar Na concentration. In the case of tamarack, photosynthesis was enhanced when treated with NaCl for 2 years compared to seedlings treated with NaCl only in the second year. Considering that cytosolic Na is linked to decreases in growth, chlorophyll concentration, A, and E for many plant species, it is possible that mechanisms exist to reduce cytosolic Na while still accumulating in foliar tissue. Potential mechanisms of Na storage include vacuole sequestration or storage within cell walls (Munns and Tester 2008; Parihar et al. 2015; Solis et al. 2021). The mechanisms of foliar accumulation of Na in tamarack and white spruce represent a tolerance strategy that differs from trembling aspen.

Conclusion

From the recovery experiment, trembling aspen, tamarack, and white spruce subjected to a 60 day exposure to 50 mM NaCl exhibited a full recovery of growth, chlorophyll concentration, A, and E after a 60 day recovery. This result is encouraging because the 50 mM NaCl treatment is slightly higher than the 4 dS m^{-2} soil EC threshold for boreal forest vegetation. From the overwintering experiment, all species tested exhibited some injury in the form of foliar yellowing and necrosis. However, few deleterious effects on measured physiological variables were found for tree seedlings treated with NaCl in year 1 followed by overwintering. In both studies, trembling aspen and tamarack exhibited elevated foliar K and accelerated foliar senescence from NaCl stress. Both responses appear to be tolerance mechanisms designed to reduce the toxic effects of foliar Na. Foliar Na in trembling aspen was remarkably low and was attributed to accelerated foliar senescence and potentially ion exclusion by the roots. Both tamarack and white spruce had increased levels of foliar Na despite physiological variables returning to control levels during the recovery period. This was attributed to the potential storage of Na in the cell wall or vacuole. Taken together, all species tested can recover from moderate levels of soil NaCl if planted at reclamation sites with soil EC values at or below 4 dS m^{-2}. Furthermore, it is advised that reclamation sites have adequate soil K to facilitate the recovery of the tested species from NaCl stress.

Author contribution statement NL designed the study, conducted the experiments, analyzed the data, and wrote the manuscript.

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Declarations

Conflict of interest The author declares no competing interests.

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