Field Testing of Selected Salt-Tolerant Screened Balsam Poplar (*Populus balsamifera* L.) Clones for Use in Reclamation around End-Pit Lakes Associated with Bitumen Extraction in Northern Alberta

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Abstract: For the oil sands mine sites in northern Alberta, the presence of salty process affected water, a byproduct of the hot-water bitumen extraction process, is anticipated to pose a challenge on some reconstructed landforms. The fundamental challenge when re-vegetating these sites is to ensure not only survival, but vigorous growth where plants are subjected to conditions of high electrical conductivity owing to salts in process affected water that may be contained in the substrate. Finding plants suitable for high salt conditions has offered the opportunity for Alberta-Pacific Forest Industries Inc. (Al-Pac) to investigate the potential role of using native balsam poplar (*Populus balsamifera* L.) as a key reclamation species for the oil sands region. Two years of greenhouse screening (2012 and 2013) of 222 balsam poplar clones from Al-Pac's balsam poplar tree improvement program, using process affected discharge water from an oil sands processing facility in Ft. McMurray, has suggested an opportunity to select genetically suitable native clones of balsam poplar for use in reclamation of challenging sites affected by process water. In consideration of the results from both greenhouse and field testing, there is an opportunity to select genetically suitable native clones of balsam poplar that are tolerant to challenging growing conditions, making them more suitable for planting on saline sites.

Keywords: oil sands reclamation; end-pit lake; balsam poplar; salt tolerance

1. Introduction

In Canada, the Alberta oil sands region is located in the Cold Lake, Peace River, and Athabasca regions in the North America Boreal Plain and covers approximately 142,200 km^2 [1]. Currently, approximately 856,000 barrels of bitumen per day (bbl day^{-1}) are produced in the mineable portion of the Athabasca region [1]. Surface mining for oil sands production in the Athabasca Oil Sands Region (AOSR) of Alberta has resulted in a cumulative disturbance footprint of 895 km^2 (until 2013), with only 0.2% of the total land base disturbed by mining being certified as reclaimed by the Government of Alberta [2]. In addition, open-pit mining leaves a reconstructed landscape of overburden dumps and tailings deposits that require reclamation, targeting self-sustaining and locally common ecosystems [3]. The process of bitumen extraction requires vast amounts of water [4] and the resultant oil sands process water (OSPW) must be contained and not returned to the region's river system owing to a current zero-discharge policy by the Alberta Environmental Protection and Enhancement Act [5]. This requirement has resulted in over a billion cubic meters of tailings water being held through various types of containment systems [6], one of which is often referred to as an 'end-pit' lake. As development expands, large areas of

disturbed land in the oil sands region will require reclamation with suitable, well-chosen, native plant material. Reclamation, in the AOSR, is defined as the process to return the disturbed ecosystems to an "equivalent land capacity" as the pre-disturbance ecosystem [7]. This may involve practices such as recontouring the ground, replacing the subsoil and topsoil, revegetation, and monitoring the environmental conditions [7].

Soil salinity and sodicity in oil sands reclamation areas have been listed among the most challenging revegetation concerns [8–10]. Salt stress leads to reductions in growth, productivity, and survival in numerous plant species [11]. The stress induced by salinity on plants is the result of three mechanisms: osmotic stress due to a more negative soil water potential, accumulation of toxic ions, and disturbances in nutrient balance [11]. These effects, in turn, lead to reductions in growth, productivity, and survival in numerous plant species [11]. In the AOSR in Alberta, salinity problems associated with OSPW and exposed marine shale overburden are two major potential challenges when reclaiming upland land-scapes [9]. Salt-stress is particularly detrimental for boreal woody species as most exhibit relatively low tolerance to salinity [9]. The fundamental challenge when revegetating these sites is not only to ensure survival, but to achieve growth rates appropriate to the ecosystem class even where plants are subjected to conditions of high electrical conductivity (EC) owing to salts in process affected water that is contained in the substrate.

The challenge of finding plants suitable for high salt conditions has offered the opportunity for Alberta-Pacific Forest Industries Inc. (Al-Pac) to investigate the potential role of using native balsam poplar (*Populus balsamifera* L.) as a key reclamation species for the oil sands region. Poplars are used throughout North America to reclaim sites containing heavy metals, salts, pesticides, solvents, explosives, radionuclide hydrocarbons, and landfill leachates [12–14]. Many studies have suggested that *Populus* species are tolerant to salinity and can even lower soil salinity [15–18]. For example, Euphrates poplar (*Populus euphratica* Oliv.) can grow well in soils with up to 8000 mg L^{-1} salinity [15]. Moreover, Liu et al. (2001) [19] reported that white poplar (*Populus alba* L.) tolerated 2000 mg L^{-1} salinity irrigation in a sandy soil in a greenhouse for two years. Poplars are also well suited for phytoremediation thanks to their ability to uptake high levels of nutrients and mineral salts, accumulate above and below ground carbon, improve soil structure and function, and reduce erosion [12,16]. Balsam poplar is a desirable species for boreal forest reclamation thanks to its fast growth and ease of vegetative propagation [20], combined with its natural role as a pioneer species. In addition, the EC tolerance range for balsam poplar is very high, ranging from 14.58 to 31.38 mS cm^{-1}, whereas the tolerance of white spruce (*Picea glauca* (Moench) Voss) is 8.75 to 14.92 mS cm^{-1} and that of jack pine (*Pinus banksiana* Lamb.) is even lower at 1.02–6.33 mS cm^{-1} [9].

Al-Pac is a pulp company that manages a 6.37 million ha forest management agreement (FMA) area in northeastern Alberta with an overlapping tenure with the oil sands region of Alberta, Canada. Balsam poplar is native to the region and has been the focus of Al-Pac's controlled parentage (tree improvement) program (CPP) (PB1-Alberta-Pacific Controlled Parentage Program plan for balsam poplar (2011)). The CPP consists of clones selected from within the FMA area (also the CPP deployment region) and outside the FMA area, with a minimum of 10 clones per provenance and 52 provenances. Approximately 520 clones were selected and have been planted on six test sites throughout the FMA area, including extreme (i.e., dry) locations, to investigate both local adaptability and potential regional adaptability under climate change. While Al-Pac is testing these trees for their reforestation potential, they are also of significant interest for their oil sands reclamation potential. After two years of greenhouse screening (2012 and 2013) of 222 balsam poplar clones from Al-Pac's program and based on their responses to varying levels of exposure to OSPW, clones were grouped into three categories (see Section 2.2.1 for details) for further field testing.

Our objective for the greenhouse study was to identify clones through screening and select genotypes that would be expected to survive and grow when used for reclamation on sites affected by OSPW. For the field trial, our objective was to test and identify balsam

poplar clones, selected for salt tolerance from the greenhouse trials, exhibiting higher survival and increased growth (e.g., height and diameter) on reclamation sites compared with the following: (i) clones that did not exhibit tolerance to elevated salt levels in the greenhouse trials and (ii) a local seed zone Stream 1 wild balsam poplar cutting collection (local control).

2. Materials and Methods

2.1. Greenhouse Set-Up (2012&2013)

2.1.1. Plant Material and Growing Conditions

Two aeroponic greenhouse experiments were conducted in 2012 and 2013 using balsam poplar clones selected from Al-Pac's balsam poplar CPP. We selected 148 and then another 86 clones for screening in 2012 and 2013, respectively. In addition, 12 of the top clones from the 2012 experiment were retested in 2013.

All trees were propagated from 10 cm long dormant hardwood cuttings, from 1-year-old tissue, collected during the winter, prior to each experiment, from stooling beds grown at the Al-Pac mill site (54° 53′ N, 112° 51′ W, 575 m). Cuttings were stored in a chest freezer prior to commencement of the experiment. Cuttings were grown aeroponically in plastic containers filled with one of three treatment solutions and each aerated using a tubing system connected to a dedicated air compressor. The experiment was a completely randomized design with three treatments: (1) 100% reverse osmosis (RO) water (city water run through a reverse osmosis system); (2) 25% OSPW combined with 75% RO water; and (3) 50% OSPW with 50% RO water, with three replicates for each clone and water treatment combination (three containers per treatment) for a total of nine containers. Each treatment container held 80 L of solution.

The OSPW was collected directly from an outflow spout at a mine facility in Ft. McMurray, AB, into plastic jugs and transported to the Northern Forestry Centre (Natural Resources Canada, Canadian Forest Service) in Edmonton, AB, where the experiments were conducted each year. Prior to initiating the experiment, 170 mL of Hoagland's solution [21] was added to each treatment container. A near neutral pH was maintained 15 days prior to the start of the experiment and then monitored during the experiment for all water treatments by adding either phosphoric acid (H_3PO_4) (if higher than 7.5) or potassium hydroxide (KOH) (if lower than 6.5). Additional RO water was added to the containers at week four and week six to maintain adequate levels of liquid, compensating for water used by the plants and through evaporation. Additional OSPW was not added.

In 2012 and 2013, once pH was stabilized and after the experiment began (day 0), pH was maintained between 6.55 and 7.26 for all three water treatments for the duration of each experiment. The mean pH values for control (100% RO), 25% process water, and 50% process water were 6.91, 6.96, and 6.96, respectively, in 2012. In 2013, the mean pH values for control, 25% process water, and 50% process water were 6.96, 6.91, and 6.90, respectively. The mean electrical conductivity (EC) levels for control (100% RO), 25% process water, and 50% process water were 1.16 mS cm^{-1}, 2.14 mS cm^{-1}, and 3.28 mS cm^{-1}, respectively. In 2013, the mean EC levels for control, 25% process water, and 50% process water were 1.08 mS cm^{-1}, 2.25 mS cm^{-1}, and 3.31 mS cm^{-1}, respectively.

Containers (97 cm × 77 cm × 44 cm) used for this experiment had a cell arrangement of 11 cells long by 15 cells wide with a cell opening of 4 cm diameter into which a rubber bung, 4 cm long, was placed. Cuttings were placed into a hole in the middle of the rubber bung that fit into the cells in the lid of the container, suspending the cutting above the water. Cuttings were completely randomized for the location in each container. The experimental greenhouse had a day time temperature of approximately 24 °C and a mean night time temperature of 18 °C. Humidity was maintained at 65–85% with an 18 h photoperiod. In 2012, the cuttings were planted on 4 July (day 0) and grown until 17 August (day 44). In 2013, the cuttings were planted on 14 August (day 0) and grown until 18 October (day 65). An extended photoperiod was maintained with natural light supplemented with sodium vapor lamps at a light intensity of 250 µmol m^{-2} s^{-1}. Each container had its own water

pump that sprayed the solution into the air space where the cuttings were suspended, and the roots grew, every 30 min. In 2012, two water pumps failed (one for treatment 2 and one for treatment 3) just prior to harvest, resulting in only two replicates for each treatment at harvest, and in 2013, one water pump failed for treatment 2 at day 30. All data were collected up until the point of pump failure, which was day 22 in the 2012 experiment; therefore, only the final harvest and gas exchange data were affected.

2.1.2. Data Collection

In 2012, water samples were collected from the containers and analyzed three times during each experiment. Treatment water was sampled prior to planting the cuttings (day 15), near the middle of the growth period (~day 35), and at the end of the experiment (day 44, 2012 and day 65, 2013) and analyzed for basic nutrients, pH, and EC by the Biogeochemical Analytical Service Laboratory at the University of Alberta. Initial cutting diameters (mm) were measured for all trees at planting. Mortality was assessed prior to harvesting. To be considered DEAD, the cuttings were required to have had leaves emerge and then die; otherwise, a cutting that never flushed was considered a missing value. At the end of the experiment, prior to measurements and destructive sampling, a qualitative visual assessment of tree health was completed using the following scale: (1) dead tree; (2) tree was dying, leaves or stem were wilting and turning black; (3) tree appeared stressed, significant yellowing, or dropping of leaves; (4) tree showed signs of chlorosis, but otherwise looked healthy; (5) leaves were green and tree looked healthy; and (6) leaves were dark green and tree was thriving. Final height (cm) was measured from the base of the new growth (attachment of stem to cutting) or the rubber bung surface, whichever was higher, to the base of the terminal bud. Final basal stem diameter (mm) was measured at the base of the new growth (attachment of stem to cutting), or the rubber bung surface, whichever was higher. In 2012, photosynthesis rate (A) was measured using an infrared gas analyzer (LI-6400; Li-Cor, Lincoln, NE, USA) prior to the final harvest. Measurements were made on one fully expanded mature leaf per cutting between 08:00 and 13:30 with a supplied (saturating) light level of 1200 PAR. Following growth measurements, plants were destructively harvested and separated into leaf, stem, root, and original cutting components; these were oven-dried in paper bags for 72 h at 65 °C and then weighed and used to calculate biomass and root/shoot ratio.

2.2. Field Testing (2014–2019)

2.2.1. Treatment Groups

Thirty-five balsam poplar clones from the PB1-CPP were screened and assessed for salt tolerance according to the greenhouse study [22]. Twenty-five of the selected clones were the top performing clones in the 50% process affected water treatment (treatment 1) and were selected as the 'high salt tolerant' treatment group and 10 clones that were poor performing clones in the 50% process affected water treatment were selected as a 'low salt tolerant' control group (treatment 2). There was an additional Stream 1 vegetative control lot collected from the local seed zone (CM2.2) with a minimum of 75 genotypes [23], which was not screened previously in the greenhouse (treatment 3).

2.2.2. Plant Material

One-year-old whips were collected from the Al-Pac mill site in February 2014, processed into 10 cm long cuttings, and placed in freezer storage at −3 °C. Cuttings were removed from freezer storage and soaked for two days in cool, fresh water prior to striking into Beaver Plastics® 512A styroblock (Beaver Plastics Ltd., Acheson, AB, Canada) containers on 4 June 2014 at Bonnyville Forest Nursery. Stream 1 control cuttings were collected in the winter of 2013/14 and kept in freezer storage until being soaked for one day and struck into 512A styroblock containers on 13 June at Bonnyville Forest Nursery.

All cuttings were grown under commercial nursery growing conditions from June to September 2014. Once they were hardened off and set bud, rooted cuttings were sorted and labeled and transported to the mine site. Planting was completed by 15 October 2014.

2.2.3. Testing Environment

The end-pit lakeshore used for this study is located north of Ft. McMurray (57°0′30″ N, 111°37′18″ W, 290 m a.s.l.) (Figure A2). The climate is considered a "warm-summer humid continental climate (Dfb)" according to Köppen climate classification [24]. The 20-year average (1999–2019) mean annual precipitation is 474 mm and the average temperature is 1.8 °C [24]. The site has a gentle slope running parallel to the water's edge with good nutrient condition. The former 50 to 60 m deep mine pit was largely filled with fluid fine tails (FFTs), and then capped with 4 m of process water and later 2 m of fresh water [25,26]. Given that the pore water of the FFT and the process water on top are brackish, vegetation on the shore of the lake was expected to be subjected to salty water (roughly 10% of the salinity of seawater) for the foreseeable future.

2.2.4. Experiment Design

The trial was a randomized block design and was planted on 15 October 2014. There were four ramets of each of 35 Al-Pac clones (25 treatment 1; 10 treatment 2) and 60 Stream I control trees (treatment 3) planted in each of three blocks on the south shore of the end-pit lake (Figure A3). Each block contained a total of 200 trees. In order to reduce within block variability, blocks were laid out with five trees running perpendicular from the lakeshore by 40 trees parallel to the lakeshore. Trees were planted 1 m apart in rows moving away from the lakeshore, with block 1 as close to the edge of the water as possible to maximize exposure to potentially saline lake water and ground water discharge from the adjacent hillside. All blocks followed the curving edge of the lake to keep them at as consistent an elevation and soil moisture condition within each block as possible. Rows up from the lake were tilled and covered with plastic mulch prior to planting. These rows were spaced approximately 3 m apart. Additional plastic mulch was placed manually between the rows to cover the entire trial area to minimize weed competition. Tree locations and identities were individually marked and mapped (Figure A3).

2.2.5. Growth and Survival Data Collection

Tree height (Ht) and basal root collar diameter (RCD) were measured according to protocols described in the trial measurement manual [27]. All trees were measured after installation for Ht, RCD in year 1, and diameter at breast height (DBH) was measured starting in year 2, and they were remeasured each fall in years 1, 2, 3, 4, and 5 (fall 2019). Survival was evaluated based on a visual assessment of the above ground stem from 2015 to 2019.

2.2.6. Tissue Nutrient Analysis

In summer 2016, two sets of leaf tissue samples were collected. Two leaves were collected from each live tree of the 35 clones and grouped into composite samples by clone and block. The Stream 1 control treatment trees had two leaves per tree collected from six randomly selected trees from each block and grouped to provide a single composite sample. A total of 108 samples were collected for the primary sample analysis. The Stream 1 control trees were chosen for the heavy metal analysis owing to the composition of this lot (i.e., 60 trees/block, minimum of 75 clones collected in the lot) representing a random, composite sample of multiple clones and collected from trees not used for the primary tissue analysis.

All 108 tissue samples were analyzed at Exova Laboratories, Surrey, British Columbia, to determine the uptake of nutrients and other compounds from the site (including boron, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorous, potassium, sodium, sulfur, zinc, and nitrogen). The six Stream 1 control tree samples were also

analyzed for 33 heavy metals (including aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, mercury, molybdenum, nickel, phosphorus, potassium, selenium, silicon, silver, sodium, strontium, sulfur, thallium, tin, titanium, vanadium, zinc, and zirconium). The heavy metal analysis was completed as an indicator only of potential heavy metal accumulation.

2.3. Data Analysis

For both greenhouse and field studies, all growth and nutrient data were analyzed by two-way mixed model analysis of variance (ANOVA) using SAS 9.4 [28].

For the greenhouse study, treatment (0, 25%, or 50% OSPW) was considered a fixed effect and clone and container were considered random effects. The treatment × clone interaction was also included in the model and initial diameter of the cutting was used as a covariate. Following significant main effects analysis, multiple comparisons among means were completed using the Student–Newman–Keuls (SNK) test. We used $p \leq 0.05$ to determine significance.

For the field testing, treatments were considered a fixed effect and block considered as a random effect. Multiple comparisons among means were completed using the Student–Newman–Keuls test with $p \leq 0.05$ used to determine significance.

3. Results

3.1. Greenhouse Study

3.1.1. Survival and Growth

In both 2012 and 2013, there were significant effects of treatment ($p < 0.001$, 2012; $p < 0.001$, 2013) and clone ($p < 0.001$, 2012; $p = 0.036$, 2013) on visual health assessment. The average ratings of the visual health assessment for the 2012 experiment, done at the end of the experiment, were 3.84, 3.39, and 2.81 for the control, 25%, and 50% treatments, respectively, out of a maximum score of 6. The control was significantly healthier ($p < 0.05$) than those in either the 25% or 50% treatments, which also differed from one another ($p < 0.05$). Most of the trees in the 25% and 50% treatments showed signs of chlorosis in both the younger and older leaves, and between the leaf veins. Some trees that ranked as a 3 or lower had necrotic leaf spots, were losing leaves, or in the most severe cases were dead.

In the 2013 experiment, the average visual health assessment ratings were 4.48, 3.95, and 4.38 for the control, 25%, and 50% treatments, respectively. The control and 50% treatments were significantly healthier ($p < 0.05$) than the 25% treatment; however, there was no significant difference between control and 50%. Observation of mortality showed that overall survival for the control treatment was 77.0%, the 25% solution was 80.4%, and the 50% solution was 64.9%. Survival ranged from 0 to 100%.

In both 2012 and 2013, there were significant effects of treatment and clone for final stem height, stem basal diameter and stem, and root and leaf biomass, with no clone by treatment interaction effect (Table 1). In 2012, the overall mean stem height for all clones did not differ between the control and 25% OSPW treatment, measuring on average 26 cm. Mean stem height for the 50% treatment was 30% significantly lower than both the control and 25% treatment. In 2013, the overall mean stem height for all clones in the control treatment was significantly greater than those in either the 25% or 50% treatments, which also differed from one another.

For stem basal diameter in the 2012 experiment, there was a decreasing trend from the control to the 25% to 50% treatments, with means averaging about 3.40 mm (Table 1). The trees in the 50% treatment were significantly smaller than in the control and 25% treatments. In the 2013 experiment, mean stem basal diameter did not differ between the control and 25% treatment; however, stem basal diameter in the 50% treatment was significantly lower ($p < 0.001$) than either the control or 25% treatment. Initial cutting diameter was used as a covariate for both the final stem height and final basal diameter analyses; it was not

significant for the final stem height and final basal diameter in 2012 ($p = 0.06$; final height, $p = 0.052$; basal diameter), but it was significant in 2013 ($p = 0.034$; final height, $p = 0.003$; basal diameter).

Table 1. Mean values (±SE) for growth and biomass measurements of balsam poplar in 2012 (top) and 2013 (bottom) for all three treatments. Significant differences between treatment means within each row are indicated by different letters based on results of analysis of variance followed by post-hoc Student–Newman–Keuls (SNK) tests.

2012	Treatment		
	Control	**25% process water**	**50% process water**
Final stem height (cm)	26.49 ± 0.73a	26.74 ± 0.80a	18.08 ± 0.62b
Stem basal diameter (mm)	3.46 ± 0.06a	3.40 ± 0.07a	3.20 ± 0.06b
Stem biomass (g)	0.37 ± 0.02a	0.31 ± 0.02b	0.16 ± 0.01c
Root biomass (g)	0.19 ± 0.01a	0.13 ± 0.01b	0.09 ± 0.01c
Leaf biomass (g)	0.86 ± 0.04a	0.73 ± 0.04b	0.52 ± 0.03c
Total biomass (g)	1.42 ± 0.08a	1.17 ± 0.06b	0.77 ± 0.04c
2013	**Treatment**		
	Control	**25% process water**	**50% process water**
Final stem height (cm)	39.79 ± 1.28a	33.70 ± 1.43b	31.15 ± 0.98c
Stem basal diameter (mm)	4.69 ± 0.09a	4.62 ± 0.11a	4.06 ± 0.07b
Stem biomass (g)	1.06 ± 0.07a	0.86 ± 0.07b	0.54 ± 0.03c
Root biomass (g)	0.60 ± 0.04a	0.47 ± 0.04b	0.30 ± 0.02c
Leaf biomass (g)	1.63 ± 0.08a	1.30 ± 0.09b	1.04 ± 0.05c
Total biomass (g)	3.32 ± 0.19a	2.88 ± 0.21b	1.90 ± 0.10c

In the 2012 experiment, leaf, stem, root, and total biomass decreased significantly from the control to the 25% and 50% treatments (Table 1). In the 2012 experiment, the control treatment had the highest root/shoot ratio (0.15 ± 0.005) followed by the 50% treatment (0.14 ± 0.005), while the 25% treatment had the lowest root/shoot ratio (0.12 ± 0.006). There was no significant difference between the control and 50% treatment, although both differed from the 25% treatment. In the 2013 experiment, the control treatment had the highest root/shoot ratio (0.19 ± 0.008) followed by the 25% treatment (0.17 ± 0.008) and then the 50% treatment (0.15 ± 0.005).

Photosynthesis rates (A) were significantly influenced by both clone and treatment ($p < 0.001$) in 2012. Post-hoc comparisons ($p < 0.05$) showed that the control treatment had a significantly higher A than either the 25% or 50% process water treatments, which did not differ from one another. Pearson's correlation coefficient (r) between A and overall biomass was 0.435 for control, 0.434 for the 25% process water, and 0.435 for the 50% process water treatments. Significantly positive correlations ($p < 0.001$) were detected between all the treatments' total biomass and A. Despite some clones performing better under the OSPW treatments, the control water treatment plants had the highest rates of photosynthesis as well as the highest visual score for plant health at 3.84 in 2012 (vs. 3.39 in 25% and 2.81 in 50%).

Nitrogen and magnesium levels appeared to be adequate as compared with the control for all treatments (Table 2); however, there were noticeable decreasing trends with control > 25% > 50% process-affected water for iron, indicating that an iron deficiency may have been present. It was observed that the iron levels in the 25% and 50% process water samples were very low by the end of the experiment (Table 2). It is likely that the elevated phosphate levels, which were due to the addition of H_3PO_4 to reduce or maintain a stable pH, caused the iron to precipitate out of solution, making it unavailable to the plants. 'Rust' observed on the bottoms of both the 25% and 50% process water treatment containers supports this hypothesis.

Table 2. Results of analysis of the water solution (means) for the control (reverse osmosis: RO), 25% process water treatment (process H_2O 25%), and 50% process water (process H_2O 50%) treatments before and after the addition of Hoagland's solution (day 15), day 35, and day 44 in 2012. Values for undiluted process water (process H_2O 100%) are shown for comparison. Note: TDN = total dissolved nitrogen; TDP = total dissolved phosphorus.

	NH_4^+ (Nµg/L)	NO_2^+ NO_3 (Nµg/L)	TDN (Nµg/L)	TDP (Pµg/L)	Cl (mg/L)	SO_4 (mg/L)	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Al (mg/L)
Minimum level of detection	2	1	10	3	0.03	0.04	0.016	0.009	0.005	0.01	0.016	0.004
RO pre H * Day 15	27	289	<LOD ***	<LOD	5.51	31.03	13.05	0.90	31.75	7.61	<LOD	<LOD
RO post H ** Day 15	428	3800	<LOD	<LOD	5.72	34.28	13.19	7.47	32.03	8.61	0.06	<LOD
RO Day 35	17	42,867	<LOD	45,181	9.92	179.60	21.85	183.99	63.32	43.33	0.43	<LOD
RO Day 44	43	37,933	<LOD	52,183	8.38	135.47	20.04	122.40	41.98	31.54	0.21	<LOD
Process H_2O 100%	5787	16	<LOD	<LOD	717	397	1195	15	21	11	<LOD	<LOD
Process H_2O 25% pre H Day 15	2140	701	<LOD	<LOD	195.16	131.12	269.37	5.37	27.58	8.21	<LOD	<LOD
Process H_2O 25% post H Day 15	2390	2910	<LOD	<LOD	194.52	132.66	270.05	10.02	29.45	9.53	0.04	<LOD
Process H_2O 25% Day 35	10	50,567	<LOD	102,986	206	303	306	180	42	43	0.10	10
Process H_2O 25% Day 44	33	38,933	<LOD	109,417	198	257	275	120	30	32	0.06	<LOD
Process H_2O 50% pre H Day 15	4020	870	<LOD	<LOD	367.83	231.82	540.19	10.09	25.80	9.68	<LOD	<LOD
Process H_2O 25% post H Day 15	4060	2880	<LOD	<LOD	365.58	233.04	529.52	14.53	26.55	10.53	0.04	<LOD
Process H_2O 50% Day 35	26	33,933	<LOD	151,163	365.17	380.95	524.48	187.20	22.96	39.34	0.02	<LOD
Process H_2O 50% Day 44	27	53,467	<LOD	174,730	397.45	388.63	575.50	160.50	19.87	36.45	0.02	<LOD

* pre H = before addition of Hoagland's solution. ** post H = after addition of Hoagland's solution. *** LOD = limit of detection.

3.1.2. Clonal Variation

Fourteen clones consistently ranked in the top 30 (30 was selected as a target number of clones to ensure genetic diversity standards would be met [23]; Ne = 18 for operational deployment of native species from a CPP program onto public lands in Alberta) for stem height growth across all three treatments (Figure 1a). For stem basal diameter growth, 12 clones ranked consistently in the top 30 in all three treatments (Figure A1a). The same trends were observed for the top 12 clones in the 2013 experiment (Figure 1b; Figure A1b).
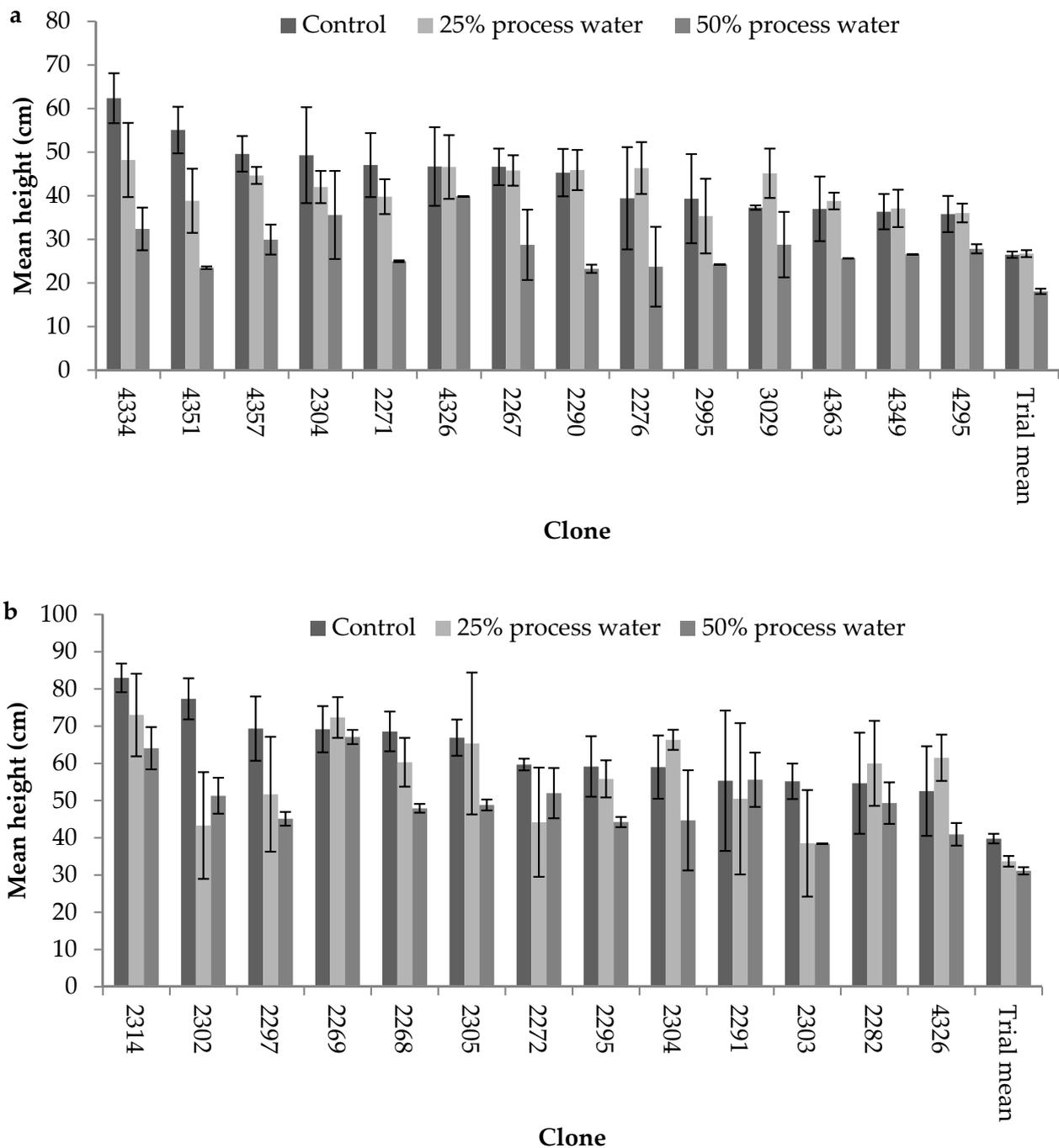
Figure 1. (**a**) Mean height (±SE) of 14 balsam poplar clones that ranked in the top 30 for all three water treatments as compared with the overall trial mean for 148 clones after 44 days of growth in the 2012 experiment. (**b**) Mean height (±SE) of 13 balsam poplar clones that ranked in the top 30 for all three water treatments as compared with the overall trial mean for 86 clones after 65 days of growth in the 2013 experiment.

Within the 148 clones tested in 2012, there were 38 clones that had higher total biomass in the 25% process water treatment than in the control treatment, while 24 clones had a higher mean total biomass in the 50% process water treatment than the control, and five clones ('AP2309', 'AP2453', 'AP3033', 'AP3127', and 'AP4356') showed the opposite trend with the 50% process-affected water treatment > 25% process-affected water treatment > control. Clones 'AP2453' and 'AP4356', which exhibited this reverse trend, also ranked within the top 30 clones for total biomass in all three treatments.

In the 2013 experiment, there was, again, a significant decrease in leaf, stem, root, and total biomass from the control to the 25%, and 50% treatment (Figure 2). Within the 86 clones that were tested in 2013, there were 27 clones that had higher total biomass for the 25% process water treatment than control (3.32 g $\pm$ 0.19), and 10 clones had higher total biomass in the 50% process water treatment than the control. See Appendix A Tables A1 and A2 for summary total biomass data for the 30 top performing clones in 2012 and 2013. Overall performance showed similar trends across both years for the top 10 clones (Figure 3). However, owing to the longer growth period in 2013, the biomass totals for 2013 were higher overall than those in 2012. There were, however, exceptions to this trend on an individual clone basis. In addition, some of the highest root/shoot ratios were observed in clones that had below average total biomass growth.

3.2. Field Testing

3.2.1. Survival and Growth

Overall, all trees grew well at the edge of the end-pit lake. There was no significant difference between treatments for survival in 2019, which overall remained very high at 82%, 84%, and 85% for treatments 1, 2, and 3, respectively (Table 3). However, mortality rates increased from 11% (2015) to 17% (2019), which was likely due to higher mortality in the first row of trees adjacent to the lakeshore, with some having almost eroded into the lake.

Table 3. Per cent (%) survival rate ($\pm$SE) of balsam poplar among treatments (1 = 25 selected tolerant clones, 2 = 10 selected control clones, 3 = Stream 1 vegetative lot clones) in the fall of each year.

Treatment	Year (Age)				
	2015 (Age 1)	2016 (Age 2)	2017 (Age 3)	2018 (Age 4)	2019 (Age 5)
1	87% $\pm$ 2%	87% $\pm$ 2%	86% $\pm$ 2%	83% $\pm$ 3%	82% $\pm$ 3%
2	92% $\pm$ 2%	90% $\pm$ 3%	90% $\pm$ 3%	87% $\pm$ 4%	84% $\pm$ 4%
3	89% $\pm$ 4%	89% $\pm$ 4%	89% $\pm$ 4%	87% $\pm$ 5%	85% $\pm$ 5%

The average height and DBH at year five for treatments 1, 2, and 3 were 3.75, 3.58, and 3.61 m for height (Figure 4) and 27.88, 27.02, and 26.38 mm for DBH, respectively (Figure 5). However, there were no significant differences in height and DBH among treatments. Mean growth increments for both height (Figure 4) and basal RCD or DBH (Figure 5) showed similar growth trends across all three treatments from 2015 to 2019. The largest annual height increment was in 2016, which averaged approximately 1 m for all three treatments (Figure 4).
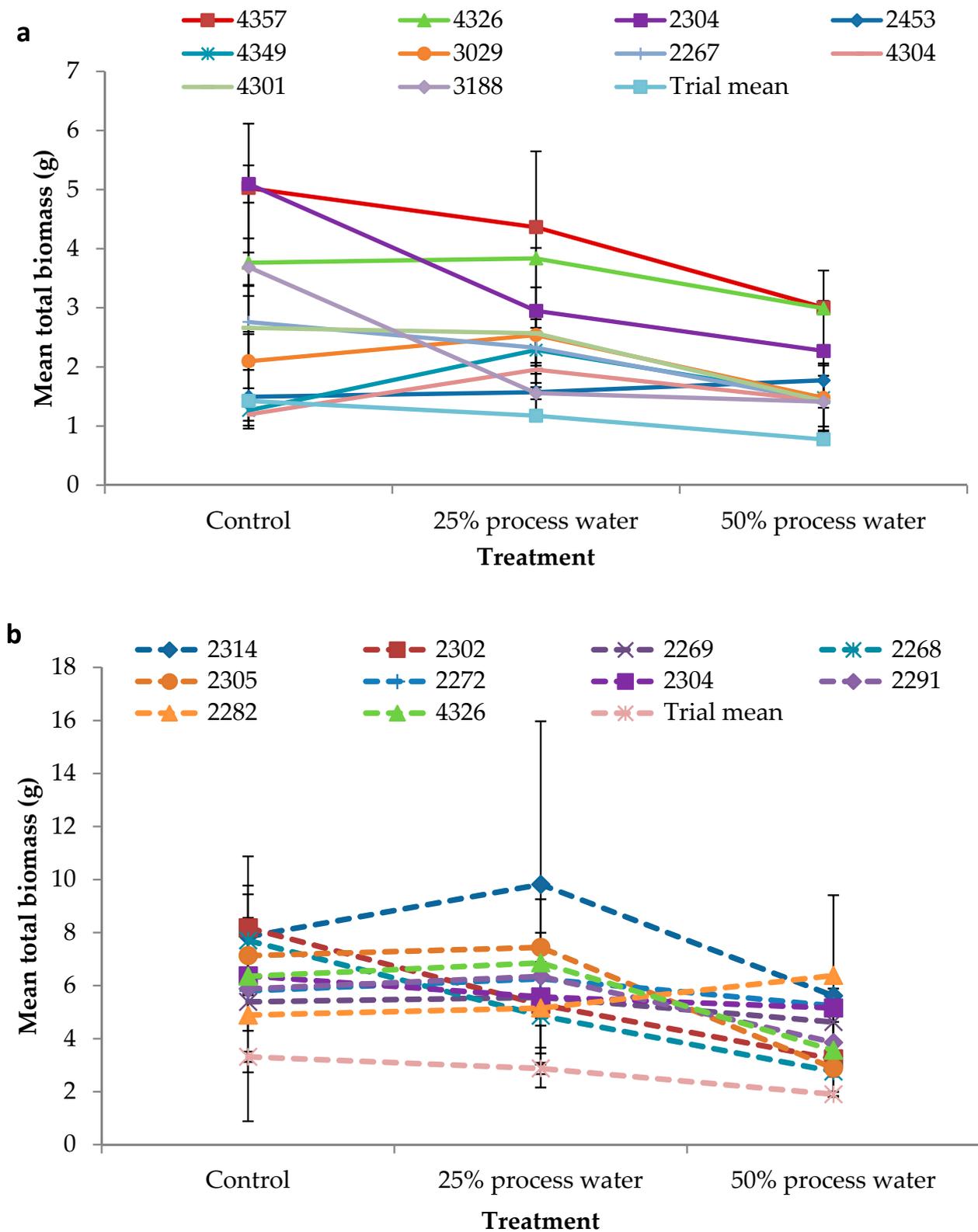
Figure 2. (**a**) Total biomass (mean dry weight ± SE) in each water treatment for the top 10 balsam poplar clones in the three water treatments and the overall means for all trees after 44 days of growth in the 2012 experiment (solid line). (**b**) Total biomass (mean dry weight ± SE) vs. treatment for the top 10 balsam poplar clones in the control, 25%, and 50% process water treatment solutions and the overall treatment mean after 65 days of growth in the 2013 experiment (dash line). Clones tested in both years (4326 and 2304) are in the same colour.
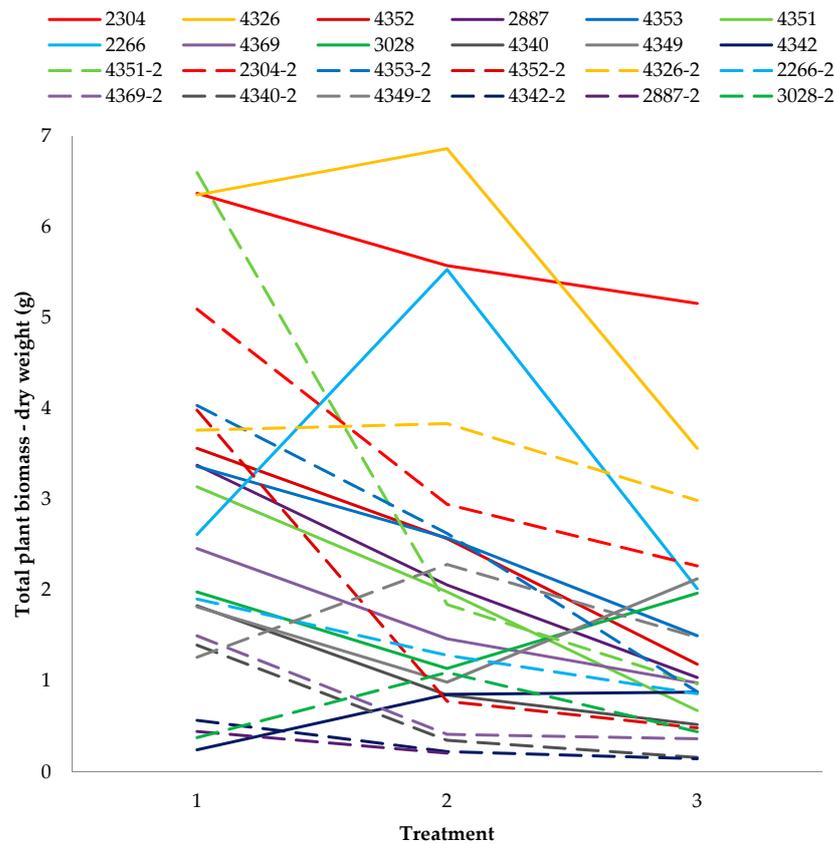
Figure 3. Performance (total plant biomass (g)) of the 12 balsam poplar clones grown in both 2012 and 2013 trials (2013 = solid line, 2012 = dashed line) under three treatments (1 = control; 2 = 25% process water; 3 = 50% process water).
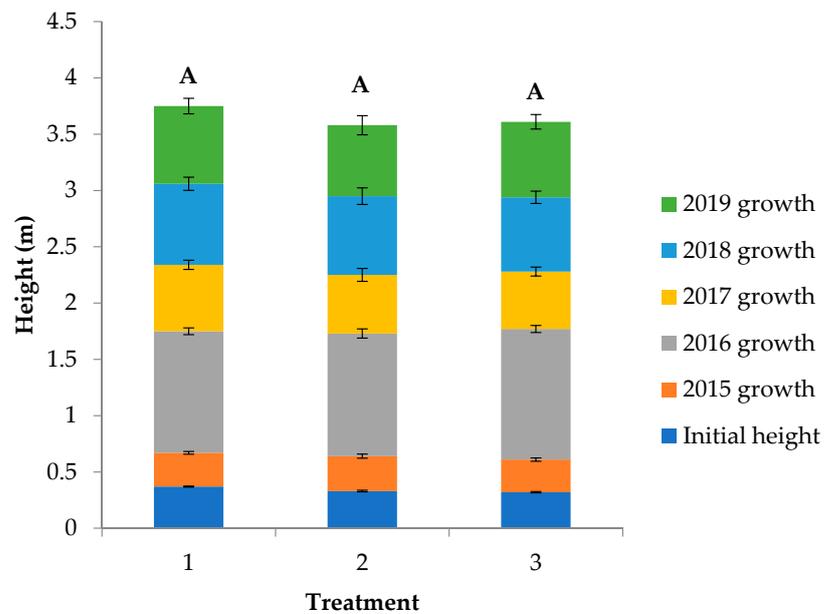
Figure 4. Mean initial height and mean annual height increment (±SE) (year 1 = 2015 growth; year 2 = 2016 growth; year 3 = 2017 growth; year 4 = 2018 growth; year 5 = 2019 growth) (m) (±SE) for balsam poplar trees planted in three treatments (1 = 25 selected tolerant clones, 2 = 10 selected control clones, 3 = Stream 1 veg. lot clones). Significant differences between treatment means for height are indicated by different letters at $p \leq 0.05$.
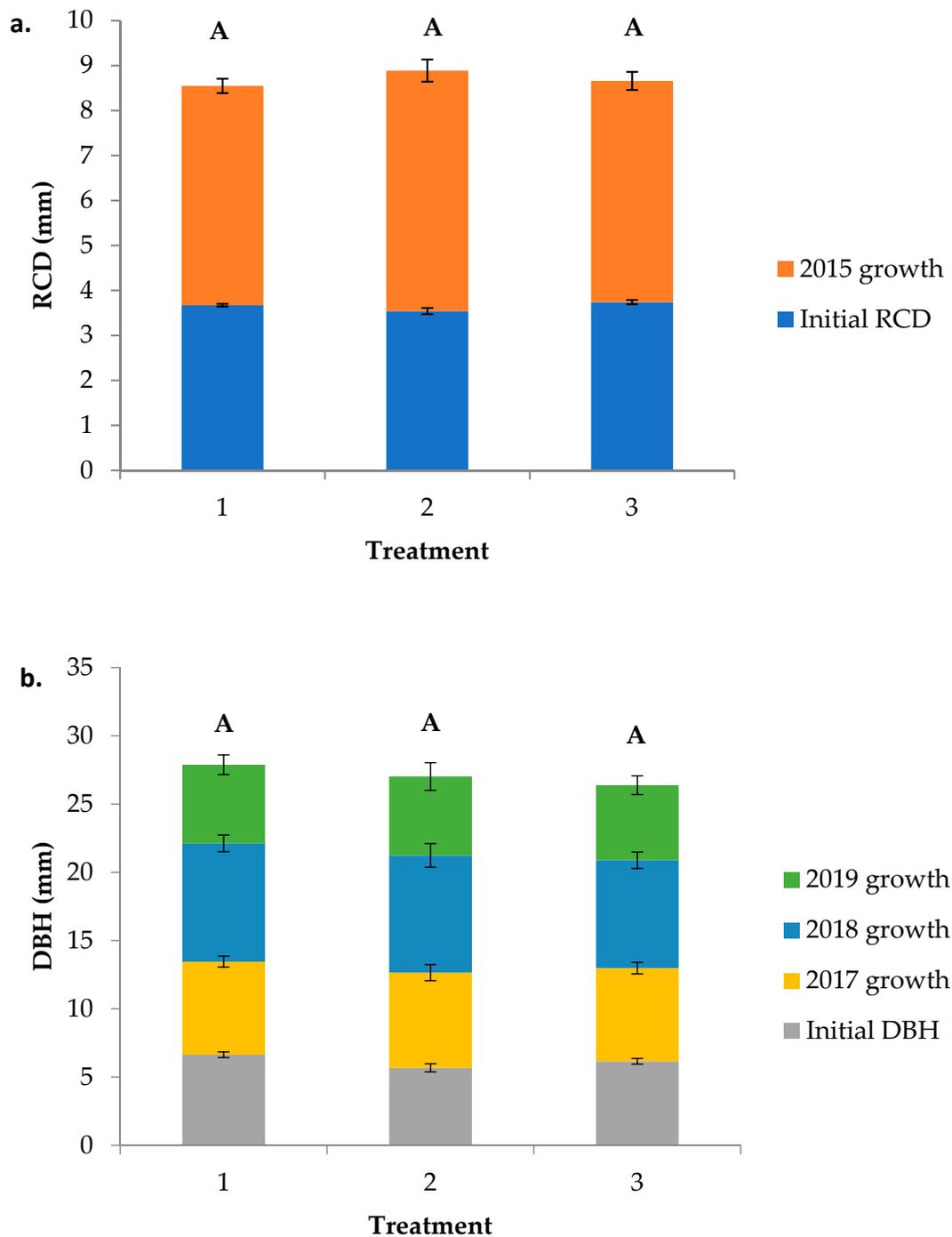
Figure 5. Mean initial diameter and mean annual growth increment (±SE) (year 1 = 2015 growth; year 2 = 2016 growth; year 3 = 2017 growth; year 4 = 2018 growth; year 5 = 2019 growth) (cm) (±SE) for balsam poplar trees planted in three treatments (1 = 25 selected tolerant clones, 2 = 10 selected control clones, 3 = Stream 1 veg. lot clones). (**a**) Basal root collar diameter (RCD mm) (2014–2015); (**b**) diameter at breast height (DBH mm) (2016–2019). Significant differences between treatment means for DBH are indicated by different letters at $p \leq 0.05$.

Significant differences were found among different blocks for both height and DBH growth parameters (Figure 6). The distance to the shoreline was used to determine the block design running parallel to the shore. Trees in Block 2 (10 m away to the lake edge) showed the best tree performance and this block represented the middle distance from the shoreline (between Block 1, closest to the water's edge, and Block 3, furthest up the slope from the water's edge).
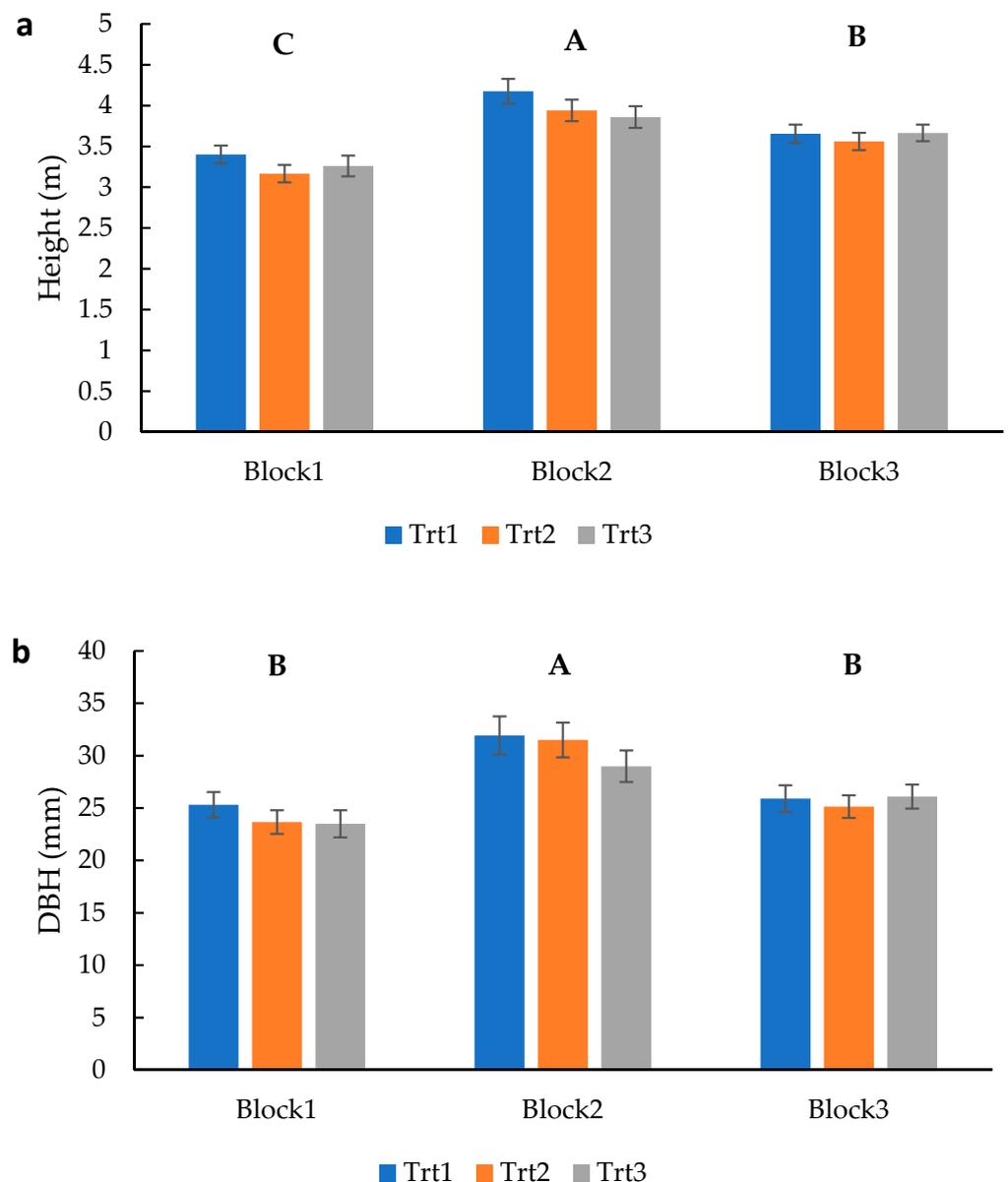
Figure 6. Treatment means (1 = 25 selected tolerant clones, 2 = 10 selected control clones, 3 = Stream 1 vegetative lot clones) of growth parameters for balsam poplar trees planted in three blocks (Block 1 = 5 m from the lake edge; Block 2 = 10 m from the lake edge; Block 3 = 15 m from the lake edge) in 2019. (**a**) Height (m); (**b**) DBH (mm). Significant differences between block means are indicated by different letters. Significant differences between treatment means for height or DBH in each block are indicated by different letters at $p \leq 0.05$.

Growth in height ranged from 2.5 m to more than 5 m across all clones (Figure 7a), while DBH ranged from 17 mm to more than 36 mm (Figure 7b). The Stream 1, treatment 3 clones showed average growth when compared with the 35 Stream 2 selected clones (treatment 1 + treatment 2) for both height and DBH (Figure 7a,b). Not surprisingly, there was a strong correlation (r = 0.925) between height and DBH by the fall of 2019, indicating that the taller trees also had, in general, great DBH.
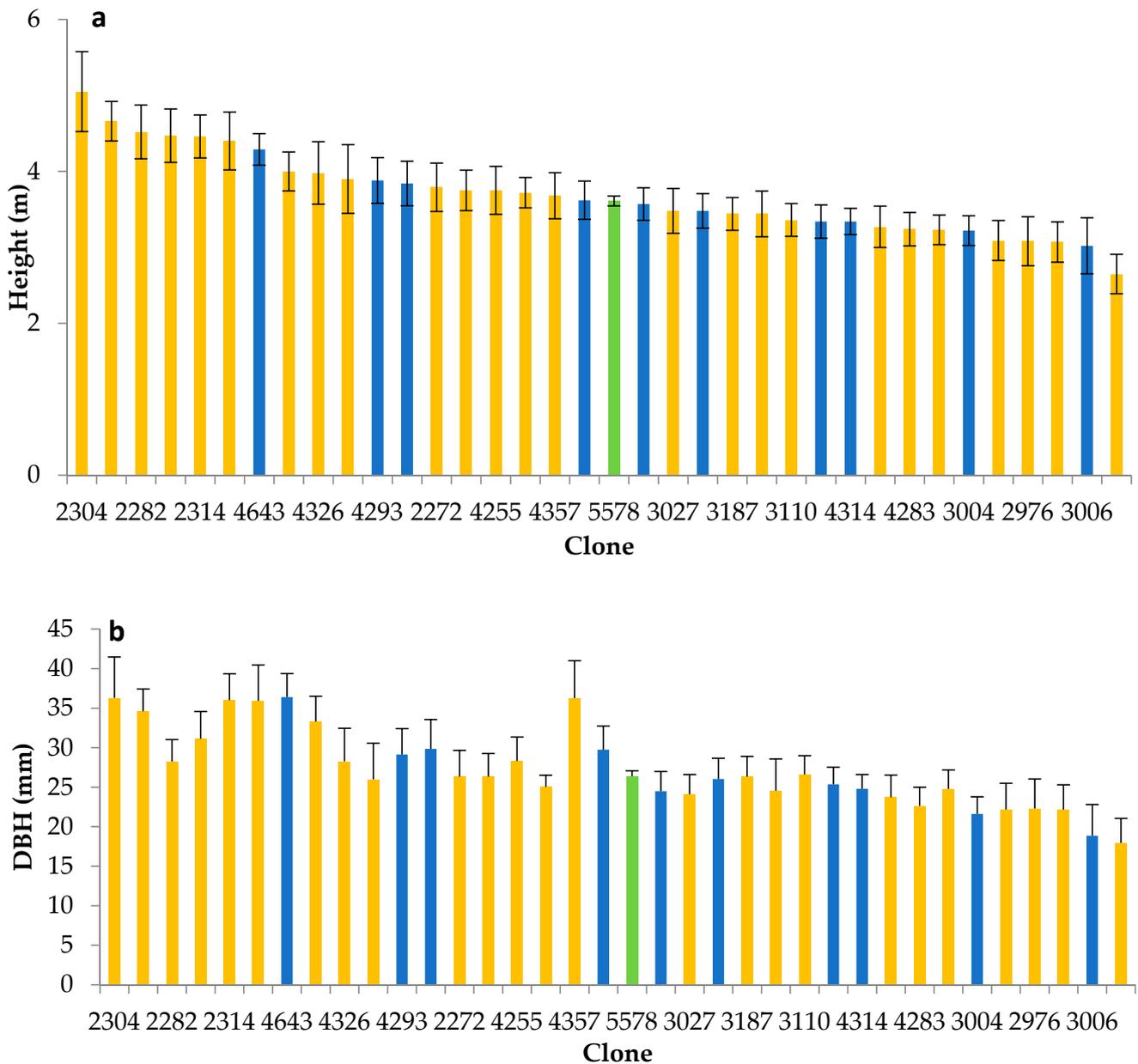
Figure 7. Mean growth (±SE) by balsam poplar clone (fall 2019). The yellow bars are treatment 1 clones, the dark blue bars are treatment 2 clones, and the green bar is treatment 3 (treatment 1 = 25 selected tolerant clones, treatment 2 = 10 selected control clones, treatment 3 = Stream 1 vegetative lot clones; # 5578 was the lot number for all Stream 1 clones). (**a**) Height (m); (**b**) DBH (mm).

When considering the tallest 18 clones from treatment 1 (where 25 clones were tested), which is the minimum number of clones required for unrestricted registration of a Stream 2 'lot' to be deployed operationally (i.e., Ne = 18) as determined by the government standards [23], the results showed a significant difference in height, but not DBH, when compared with treatment 2 (10 'low salt tolerant clones' from 50% process water testing) and, more importantly, the Stream 1 local 'wild' collection (treatment 3), for both height and DBH (Table 4).

Table 4. Growth data (height and diameter at breast height (DBH)) ($\pm$SE) of balsam poplar in 2019 for all treatments (top 18 clones from treatment 1 and all clones from treatment 2 and treatment 3). Significant differences between treatment means are indicated by different letters.

Treatment/Lot Type	Height (m)	DBH (mm)
1 (top 18 clones)/Stream 2	4.01 ± 0.08 [a]	29.99 ± 0.86 [a]
2 (10 control clones)/Stream 2	3.58 ± 0.09 [b]	27.02 ± 1.00 [b]
3 Local control/Stream 1	3.61 ± 0.06 [b]	26.38 ± 0.69 [b]

Stem volume was calculated for each tree using fall 2019 data based on the following equation: $V = A_b \times H/3$ (where V: stem volume (cm^3), A_b: basal area $= \pi \times DBH^2$ (diameter at breast height)/4 (cm^2), and H: height (cm)) [29] (Figure 8). Although no significant differences were found in either height or DBH, when stem volume was calculated, trees in treatment 1 (including all 25 clones) (1060.47 ± 68.24 mm^3) had a larger stem volume than trees in treatment 3 (814.87 ± 54.25 mm^3) (Figure 8). However, when considering the tallest 18 clones from treatment 1 (where 25 clones were tested), the stem volume of the top 18 clones was 1254.55 ± 86.71 mm^3, which is significantly greater than ($p < 0.05$) treatment 2 (893.39 ± 80.67 mm^3) and treatment 3.
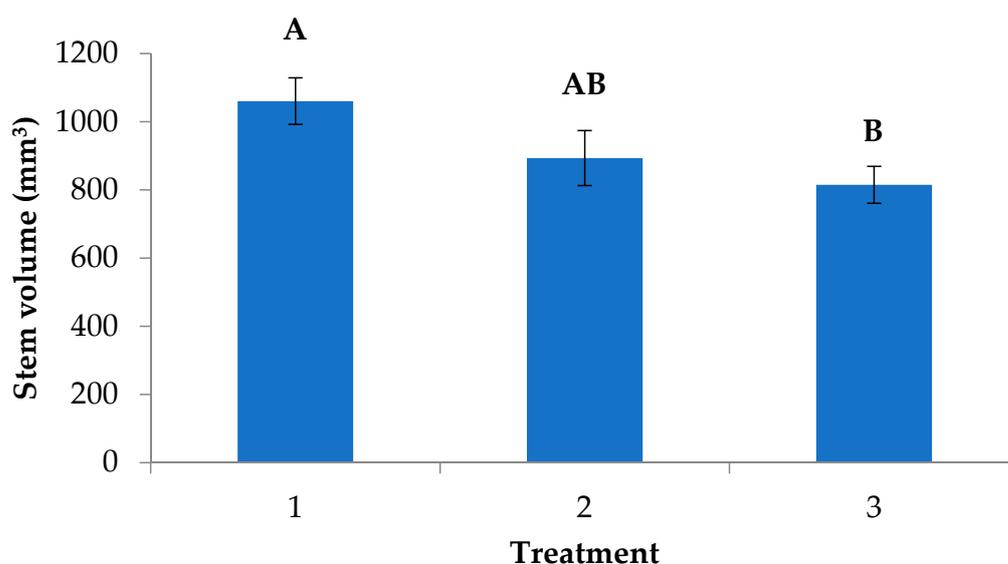
Figure 8. Mean stem volume (mm^3) ($\pm$SE) for five-year-old balsam poplar trees planted in 2019 (1 = 25 selected tolerant clones, 2 = 10 selected control clones, 3 = Stream 1 vegetative lot clones). Significant differences between treatment means for stem volume are indicated by different letters at $p \leq 0.05$.

3.2.2. Tissue Nutrient Analysis

Overall, nutrient analysis from bulk leaf tissue samples showed a low sodium level and a high calcium level (Table 5). There were no significant differences found for any of the nutrients by treatment except for magnesium, where treatment 3 had significantly higher levels compared with treatments 1 or 2 (Table 6). Significant differences were found among different blocks for some tissue nutrients (i.e., Cu, Fe, Mn, P, Zn, and N) for both treatments (Table 7).

Table 5. Mean tissue nutrient (a) and heavy metal (b) analysis (±SE) based on leaf tissue samples in two-year-old balsam poplar trees.

a. Tissue nutrient						
Site	**B(ug/g)**	**Ca (%)**	**Cu (ug/g)**	**Fe (ug/g)**	**Mg (%)**	**Mn (ug/g)**	**Mo (ug/g)**
End-pit lake	33.44 ± 0.82	1.05 ± 0.02	4.69 ± 0.12	136.06 ± 4.84	0.282 ± 0.004	9.65 ± 0.26	0.51 ± 0.01
	P (%)	**K (%)**	**S (%)**	**Zn (ug/g)**	**N (%)**	**Na (%) ***	
	0.164 ± 0.003	1.45 ± 0.03	0.36 ± 0.01	127.48 ± 5.51	1.38 ± 0.06	-	
b. Heavy metal **						
Site *	**Al (ug/g)**	**Ba (ug/g)**	**Cd (ug/g)**	**Cr (ug/g)**	**Co (ug/g)**	**Li (ug/g)**	**Ni (ug/g)**
End-pit lake	55.90 ± 14.70	1.87 ± 0.27	0.20 ± 0.00	0.79 ± 0.26	0.38 ± 0.13	0.45 ± 0.05	0.74 ± 0.11
	Si (ug/g)	**Sr (ug/g)**	**Sn (ug/g)**	**Ti (ug/g)**	**V (ug/g)**		
	270.67 ± 47.10	11.73 ± 1.53	1.40 ± 0.06	2.30 ± 0.30	3.83 ± 0.12		

* Na levels were all <0.01, so no statistical analysis could be completed. ** Heavy metal analysis was only conducted on Stream 1 control trees. *** Metal levels <0.5 (ug/g) are not shown.

Table 6. Mean leaf tissue nutrient analysis (±SE) results by treatment in two-year-old balsam poplar trees. Significant differences between treatment means at $p \leq 0.05$ are indicated by different letters. Treatment 1 = 25 selected tolerant clones, treatment 2 = 10 selected control clones, treatment 3 = Stream 1 vegetative lot clones.

Treatment	B(ug/g)	Ca (%)	Cu (ug/g)	Fe (ug/g)	Mg (%)	Mn (ug/g)	Mo (ug/g)
1	33.62 ± 1.02	1.04 ± 0.02	4.42 ± 0.13	139.83 ± 6.47	0.283 ± 0.004 [ab]	9.56 ± 0.33	0.48 ± 0.04
2	32.88 ± 1.52	1.07 ± 0.04	5.39 ± 0.21	127.00 ± 6.16	0.275 ± 0.007 [b]	9.67 ± 0.44	0.55 ± 0.04
3	33.70 ± 0.55	0.97 ± 0.05	4.61 ± 0.57	132.33 ± 14.44	0.32 ± 0.026 [a]	8.70 ± 1.79	0.47 ± 0.03

Treatment	P (%)	K (%)	S (%)	Zn (ug/g)	N (%)	Na (%)
1	0.161 ± 0.003	1.46 ± 0.03	0.35 ± 0.01	119.06 ± 6.61	1.35 ± 0.07	-
2	0.172 ± 0.006	1.44 ± 0.04	0.37 ± 0.01	149.42 ± 9.48	1.43 ± 0.13	-
3	0.160 ± 0.011	1.46 ± 0.12	0.33 ± 0.04	118.47 ± 10.90	1.38 ± 0.31	-

* Na levels were all <0.01, so no statistical analysis could be completed.

Table 7. Block means of tissue nutrient analysis for each treatment in two-year-old balsam poplar trees. Significant differences between block means at $p \leq 0.05$ are indicated by different letters. Block 1 = 5 m from the lake edge; Block 2 = 10 m from the lake edge; Block 3 = 15 m from the lake edge.

a. Treatment 1 (25 selected tolerant clones)						
Block	**B (ug/g)**	**Ca (%)**	**Cu (ug/g)**	**Fe (ug/g)**	**Mg (%)**	**Mn (ug/g)**	**Mo (ug/g)**
1	34.14 ± 1.74	1.06 ± 0.04	5.19 ± 0.20 [a]	178.44 ± 6.29 [a]	0.270 ± 0.008	10.96 ± 0.69 [a]	0.58 ± 0.03
2	33.65 ± 1.50	1.03 ± 0.05	4.46 ± 0.19 [b]	123.68 ± 6.58 [b]	0.280 ± 0.008	8.94 ± 0.43 [b]	0.53 ± 0.04
3	33.16 ± 2.07	1.02 ± 0.04	3.65 ± 0.18 [c]	115.36 ± 7.47 [c]	0.300 ± 0.008	8.77 ± 0.47 [b]	0.42 ± 0.04
Block	**P (%)**	**K (%)**	**S (%)**	**Zn (ug/g)**	**N (%)**	**Na (%)**	
1	0.170 ± 0.007 [a]	1.55 ± 0.04	0.36 ± 0.01	137.54 ± 9.69 [a]	1.81 ± 0.07 [a]	-	
2	0.170 ± 0.007 [a]	1.44 ± 0.07	0.36 ± 0.02	115.71 ± 8.26 [b]	1.34 ± 0.09 [b]	-	
3	0.140 ± 0.005 [b]	1.41 ± 0.07	0.33 ± 0.02	100.94 ± 7.71 [c]	0.89 ± 0.14 [c]	-	
b. Treatment 2 (10 selected control clones)						
Block	**B (ug/g)**	**Ca (%)**	**Cu (ug/g)**	**Fe (ug/g)**	**Mg (%)**	**Mn (ug/g)**	**Mo (ug/g)**
1	35.65 ± 2.70 [a]	1.09 ± 0.06	6.22 ± 0.38 [a]	166.80 ± 4.50 [a]	0.290 ± 0.010	10.98 ± 0.92 [a]	0.62 ± 0.02
2	32.39 ± 2.29 [b]	1.08 ± 0.09	5.10 ± 0.28 [b]	112.40 ± 5.62 [b]	0.270 ± 0.020	9.36 ± 0.74 [a]	0.53 ± 0.03
3	30.60 ± 2.91 [b]	1.03 ± 0.05	4.84 ± 0.33 [b]	101.80 ± 6.70 [b]	0.270 ± 0.009	8.67 ± 0.44 [b]	0.57 ± 0.03
Block	**P (%)**	**K (%)**	**S (%)**	**Zn (ug/g)**	**N (%)**	**Na (%)**	
1	0.180 ± 0.012 [a]	1.48 ± 0.07	0.39 ± 0.02	183.00 ± 8.17 [a]	1.77 ± 0.25 [a]	-	
2	0.172 ± 0.012 [a]	1.47 ± 0.06	0.39 ± 0.02	152.00 ± 9.23 [b]	1.56 ± 0.15 [b]	-	
3	0.160 ± 0.007 [b]	1.36 ± 0.09	0.34 ± 0.03	113.30 ± 11.01 [c]	0.95 ± 0.17 [c]	-	

4. Discussion

4.1. Greenhouse Study

Interest in and acceptance of poplar and willow for use in reclamation and phytoremediation have been increasing in the last 15 years [30,31]. Salinity is known to reduce water absorption and cause water stress [32]. Salts are taken up by plants and the increasing tissue ion concentration contributes to a decrease in water potentials. In addition, some plant species accumulate solutes under stress to maintain a positive water balance [33]. This osmotic adjustment allows plants to maintain turgor in saline environments. Approximately 1% of all plant species are halophytes and can complete their life cycle in relatively high saline environments, such as 200 mM NaCl or more [34]. The ability of poplars to grow on harsh sites, along with being relatively easy to propagate, through the use of cuttings, makes them an ideal candidate species for use in reclamation, more specifically with respect to this study, on reclaimed oil sands mine sites in northeastern Alberta.

In both years of the greenhouse studies, clear differences in the phenotypic growth response of the tested balsam poplar clones were observed, indicating tolerance for OSPW by native balsam poplars and clonal variability in that tolerance. These findings support the assertion that the opportunity exists to select and propagate an easily propagated native species for use in reclaiming these challenging sites. More specifically, there was a high degree of genetic (clonal) variability in survival, height, diameter, and biomass growth in response to the control, 25%, and 50% process water treatments. Most clones performed more poorly in the 25% and 50% process water solutions as compared with the control. There were several clones, however, that performed consistently better than the average for all of the traits measured across all three treatments, exhibiting desirable traits for selection from the population of clones tested. These results suggest that genetic differences in clones should be considered in the selection of genetic materials for use on reclamation sites impacted by high salt-containing tailings generated from oil sands operations.

Clone 'AP4357', tested in both years of the study, is an example of an OSPW-tolerant clone that consistently performed at or near the top for all traits measured, and in all three water treatments across both years. There were also a number of clones that performed better in the 25% process water treatment, 50% process water treatment, or both as compared with their control treatment performance. These tolerant clones appeared to actually prefer the saline conditions, which indicates that there are balsam poplar clones that are salt loving or 'halophiles'.

Significant positive correlations between height growth and rooting traits such as root length and root dry weight of poplar have been reported [35]. Thus, we believe height growth can be used as a surrogate measure of root development, which may have increased associated microbial activity in the rhizosphere. Therefore, our better-rooting clones (i.e., clones 'AP4326' and 'AP2304') may exhibit greater remedial potential. In addition, root/shoot ratios are often very useful in determining if plants have healthy root systems relative to above ground biomass [36]. Because salty soils often limit root penetration and inhibit root growth [37], clones that have higher root growth are likely going to have increased performance in saline environments. Therefore, higher root/shoot ratios would be desirable. However, one must be careful not to look at root/shoot ratios as a single trait for selection as it gives no indication of the actual growth performance of the plant. Ideally, in the selection of suitable clones from this experiment, clones that have high total above ground biomass with an above average root/shoot ratio would be considered desirable.

4.2. Field Testing

4.2.1. Survival and Growth

The initial high survival rate of all treatments at the end-pit lake (Table 3) indicated these trees were well adapted to the reclamation mine site. Additionally, survival through the first two years was high, indicating that early survival is an important indicator of later survival and growth. Owing to good water availability and sufficient nutrients throughout

each growing season, trees grew very well (Figures 4 and 5). The trees also experienced little to no competition owing to the installed black plastic, which also likely made the soil warmer, although this was not measured. In this trial, distance to the shoreline had a significant impact on the trees' height and DBH (Figure 6). For trees planted adjacent to the shoreline, the trees had ready access to the water table while also being more vulnerable to shoreline erosion.

As no significant differences were found between treatment 3 (Stream 1 vegetative lot clones) and the other two plant treatment groups for height and DBH, these results suggested the growth rates of the Stream 1 vegetative lot clones might be considered acceptable when compared with the Al-Pac selected clones (salt-tolerant and controls from Al-Pac's program) on a site with 'ideal' growing conditions. However, mean stem volume showed a significant difference between treatments (treatment 1 $\geq$ treatment 2 $\geq$ treatment 3), indicating the selected Stream 2 clones from Al-Pac's CPP program, overall, grew better than the Stream 1 vegetative lot clones. In addition, analysis of the top 18 clones from the Stream 2 lot selected from the treatment 1 group showed significant differences for both height and DBH when compared with treatments 2 and 3 (Table 4). This suggests that there is potential to plant groups of selected clones that would outperform wild clonal collections of Stream 1 native balsam, with the added advantage of not being restricted to deployment only within their local seed zone, but taking advantage of the entire region associated with the balsam poplar controlled parentage program.

Interestingly, despite clones selected from treatment 2 being chosen from the group of clones that did not exhibit superior salt tolerance (i.e., greatest growth) in the initial greenhouse screening trials (2012 and 2013), they still showed a level of tolerance to salinity testing [22]. It is worth noting that the selected high tolerance clones (treatment 1) were not necessarily the tallest trees overall in the initial screening experiment; some of the clones performed well in all three treatments, while others were selected because they performed better in the 50% process water treatment than in the 25% and control treatments. Moreover, the EC level of the 50% process water in the greenhouse was between 3 and 3.6 mS cm^{-1} [22]; however, the EC level of surface water from the end-pit lake was only 2.7–3.0 mS cm^{-1} (salty water) [37], which suggests that the Al-Pac selected clones did not experience the same level of stress in the field as their previous screening showed they had the capability to sustain. Therefore, if the end-pit lake site was not, in fact, heavily impacted by salts, the selected high salt tolerant treatment clones that did the best in the greenhouse trial might not have had the opportunity to exhibit their superior 'salt tolerance', and thus to this point in time, looked similar to the clones represented in treatment 2 and in the Stream 1 vegetative lot.

From the current data, Stream 2 clones, selected for salt tolerance, had greater volume when compared with the Stream 1 wild lot, even though for height and DBH alone, clones from treatment 1 and treatment 2 showed growth increments that were interspersed with each other, and treatment 3 was close to the median in performance (Figure 7). As treatment 1 (Stream 2) clones showed salt tolerance in the greenhouse study and performed well in the field study, selecting and planting these trees may prove beneficial in the future if reclamation sites become more challenging. In addition, access to the Stream 2 clones from stoolbeds and/or existing trees could simplify collections while also ensuring the material can be planted over a much wider area (i.e., no seed zone restrictions) associated with the CPP.

4.2.2. Nutrient Analysis

Sodium and calcium were of particular interest in this experiment as they are the main drivers of potential 'salinity' conditions on reclamation sites [38]. White and Liber (2018) [37] characterized the chemical constituents in surface water from this end-pit lake, and found sodium was one of the main ions that contributed to salinity. However, foliar sodium levels in our current study were below any accurately detectable level and, as such, were reported as being <0.01% (Table 5a). Foliar calcium levels, which were in the

normal value range (from 0.1% to 5%) [39], however, were higher than sodium levels in the samples (Table 5a). The low sodium levels measured may suggest that sodicity is currently not a concern, or that the poplars did not accumulate it in their leaves. The heavy metal accumulation patterns were similar to the published results under field conditions [40,41] (Table 5b), where Zn and Cd were accumulated in the leaves, indicating the phytoextraction ability of balsam poplar. The significant differences found in magnesium (Mg) levels among the three treatments indicate that the Stream 1 vegetative lot clones could be potentially used in a high Mg contaminated field. Overall, the trend from tissue nutrient analysis using block means (Table 7) showed the nutrient levels were higher in the block closest to the water. This finding indicated that a high-water table might also offer the opportunity for salt accumulation in both the immediately adjacent surrounding soil and eventually in the plant tissues that could affect tree growth. In addition, the data from the heavy metal analysis could be used as supplementary data that might be useful in the future as a source for comparison. However, there is very limited published literature that outlines the range of acceptable nutrient concentrations in balsam poplar leaves.

5. Conclusions

In consideration of the results obtained from both the greenhouse and field studies, there is an opportunity to select genetically suitable native clones of balsam poplar that are tolerant to challenging growing conditions, making them more suitable for planting in reclamation efforts on potentially saline sites than unselected clones or populations. The field testing indicated the potential use of selected Stream 2 clones (selected high salt-tolerant clones) from Al-Pac's balsam poplar controlled parentage program for oil sands reclamation sites in northeastern Alberta. In addition, the Stream 1 wild lot showed comparable growth performance under "ideal" conditions. However, if reclamation were being conducted on challenging, salty sites, clones from the Al-Pac selections are recommended. Furthermore, the selected salt-tolerant clones showed greater stem volumes, which indicates that they are potentially the most flexible trees as they will likely do better under higher salt conditions, and they will have a greater volume even when conditions are favourable. Balsam poplar has shown considerable genetic diversity in growth performance in this study and such results are encouraging in light of an expanding industrial energy sector footprint. Moreover, poplars are well known for their ability to tolerate salinity [17,18,42] and, therefore, screening clones for salt tolerance, and maximizing the potential use of the tree improvement program trees available through Al-Pac, while meeting government regulations for genetic diversity, could provide a significant opportunity for reclamation in the oil sands region in Alberta. Reclamation challenges are in their infancy in Alberta and adjacent regions, and selected material from native species may provide greater benefit as a source of reclamation materials than untested material to help meet those challenges.

Appendix A

Table A1. Mean total biomass (±SE, grams) of the top 30 balsam poplar clones in the 50% process water treatment with corresponding means (±SE) for the control and 25% process water treatments in 2012. Clone numbers in bold identify clones that ranked in the top 30 for all three water treatments, while underlined clone numbers indicated consistent performance in both years' experiments. Periods indicate dead plants.

Clone	Control Mean (g)	±SE	25% Process Water Mean (g)	±SE	50% Process Water Mean (g)	±SE
4357	5.03	0.63	4.36	0.26	3.00	0.63
4326	3.76	1.65	3.84	0.25	2.99	0.85
2304	5.10	0.32	2.95	1.07	2.27	1.36
2453	1.49	0.05	1.57	1.11	1.77	0.45
4349	1.27	0.07	2.29		1.48	0.68
3029	2.10	0.54	2.54	0.36	1.47	
2267	2.76	0.45	2.32		1.43	
4304	1.20	1.96	1.96	0.28	1.43	
4301	2.66	0.46	2.57	0.81	1.42	0.55
3188	3.69	0.21	1.56	0.76	1.41	0.12
2995	3.26	0.47	2.60	0.40	1.39	
4363	2.47	0.15	2.02	0.11	1.38	
4255	1.92	0.49	1.02	0.10	1.36	0.10
4296	3.93	0.18	1.07	0.01	1.36	0.08
4334	5.62	0.15	2.58	0.47	1.35	0.07
4277	1.16	0.34	2.57	0.48	1.34	0.25
4295	1.63	0.29	1.26	0.73	1.32	0.61
2288	4.01	0.27	1.86	0.28	1.26	0.08
3110	1.70	1.24	0.99	0.09	1.22	0.08
4285	1.53	0.22	4.17		1.19	
4315	0.23	0.71	2.15	0.24	1.19	0.43
3187	0.70	0.19	0.62	0.71	1.14	.
2447	1.63	0.03	0.24	0.25	1.13	0.17
4249	0.91	0.21	1.61	0.32	1.11	0.30
2976	1.26	1.18	0.54	1.81	1.09	
2312	0.41	0.97	2.47	0.91	1.08	0.31
4317	1.56	0.17	1.23	0.70	1.06	
4297	0.61		0.36		1.02	0.06
4356	0.66	1.09	0.89	0.02	1.02	0.12
3106	1.02	0.91	2.30	0.74	1.01	0.13
Treatment mean	1.42	0.08	1.17	0.06	0.77	0.04

Table A2. Mean total biomass (±SE, grams) of the top 30 balsam poplar clones in the 50% process water treatment with corresponding means (±SE) for the control and 25% process water treatments in 2013. Clone numbers in bold identify clones that ranked in the top 30 for all three water treatments, while underlined clone numbers indicated consistent performance in both years' experiments. Periods indicate dead plants.

Clone	Control Mean (g)	±SE	25% Process Water Mean (g)	±SE	50% Process Water Mean (g)	±SE
2282	4.89	2.16	5.16	0.40	6.39	3.02
2314	7.85	0.56	9.82	6.16	5.62	0.27
2287	8.91	5.21	9.85	3.71	5.55	0.68
2272	5.80	0.39	6.26	0.98	5.24	0.87
<u>2304</u>	6.37	1.65	5.57	1.39	5.16	2.02
2313	3.92	1.21	4.81	2.03	5.01	0.24
2269	5.39	1.09	5.56	1.07	4.63	0.88
2291	5.88	4.99	6.35	2.91	3.86	0.98
<u>4326</u>	6.35	3.52	6.86	1.03	3.56	0.37
2301	3.40	1.95	2.26	1.63	3.43	0.72
2302	8.18	1.26	5.29	2.27	3.24	1.40
2289	3.67	1.89	2.97	1.13	3.14	1.34
2305	7.13	1.42	7.44	0.55	2.89	0.32
2300	6.42	1.91	5.61	1.34	2.88	0.47
2268	7.70	2.07	4.86	2.71	2.76	0.77
2278	4.85	1.46	2.81	1.15	2.63	0.77
3027	5.33	1.17	1.46	0.013	2.59	0.30
2307	4.71	2.63	3.86	1.47	2.58	0.64
2997	2.68	1.90	1.26	0.72	2.47	0.44
2284			1.985		2.3	
2297	8.50	1.93	8.71	0.64	2.22	0.14
2295	4.20	1.55	3.27	0.35	2.21	0.32
4274	1.05	0.43	1.55	0.12	2.16	
<u>4349</u>	1.82	1.23	0.99	0.64	2.13	0.28
4283	3.05	0.64	0.88	0.51	2.08	0.41
2266	2.62	0.27	5.53	2.93	2.02	0.68
2303	4.93	0.75	4.16	0.90	1.99	
2293	3.61	0.47	3.52	0.87	1.99	0.26
915	4.27	0.86	2.70	1.63	1.97	0.30
3028	1.98	0.24	1.14	0.048	1.97	0.68
Treatment mean	3.32	0.19	2.88	0.21	1.90	0.10

Table A3. Growth variables of balsam poplar tested by ANOVA indicating the source of variation, F-value, degrees of freedom (df), and *p*-value for the 2012 and 2013 greenhouse experiments for different clones, treatments, and interaction effects. Trt = treatment.

		Growth Season					
		2012			*2013*		
Growth variable	**Source of variation**	F	df	*p*-value	F	df	*p*-value
Stem height (cm)	Clone	5.26	144	<0.001	9.24	85	<0.001
	Trt	85.78	2	<0.001	31.64	2	<0.001
	Clone * Trt	1.17	237	0.091	0.83	169	0.929
Basal diameter (mm)	Clone	3.83	144	<0.001	6.24	85	<0.001
	Trt	7.86	2	0.004	23.28	2	<0.001
	Clone * Trt	1.01	237	0.471	0.92	169	0.730
Stem biomass (g)	Clone	4.65	144	<0.001	6.36	85	<0.001
	Trt	51.64	2	<0.001	35.82	2	<0.001
	Clone * Trt	1.45	237	0.007	0.84	169	0.897
Root biomass (g)	Clone	4.6	144	<0.001	4.29	85	<0.001
	Trt	39.32	2	<0.001	25.4	2	<0.001
	Clone * Trt	1.23	237	0.039	0.75	169	0.985
Leaf biomass (g)	Clone	4.51	144	<0.001	5.62	85	<0.001
	Trt	39.56	2	<0.001	30.31	2	<0.001
	Clone * Trt	1.17	237	0.082	0.99	169	0.538
Total biomass (g)	Clone	4.97	144	<0.001	5.77	85	<0.001
	Trt	47.06	2	<0.001	34.60	2	<0.001
	Clone * Trt	1.28	237	0.016	0.87	169	0.841

Table A4. ANOVA for mean stem volume (mm^3) of balsam poplar in 2019 at the end-pit lake (treatment 1 = 25 selected tolerant clones, treatment 2 = 10 selected control clones, treatment 3 = Stream 1 vegetative lot clones).

Source	**DF**	**F Value**	***p*-Value**
Block	2	19.95	<0.0001
Treatment	2	5.03	0.0076
Clone	33	4.04	<0.0001
Error	458		
Total	495		
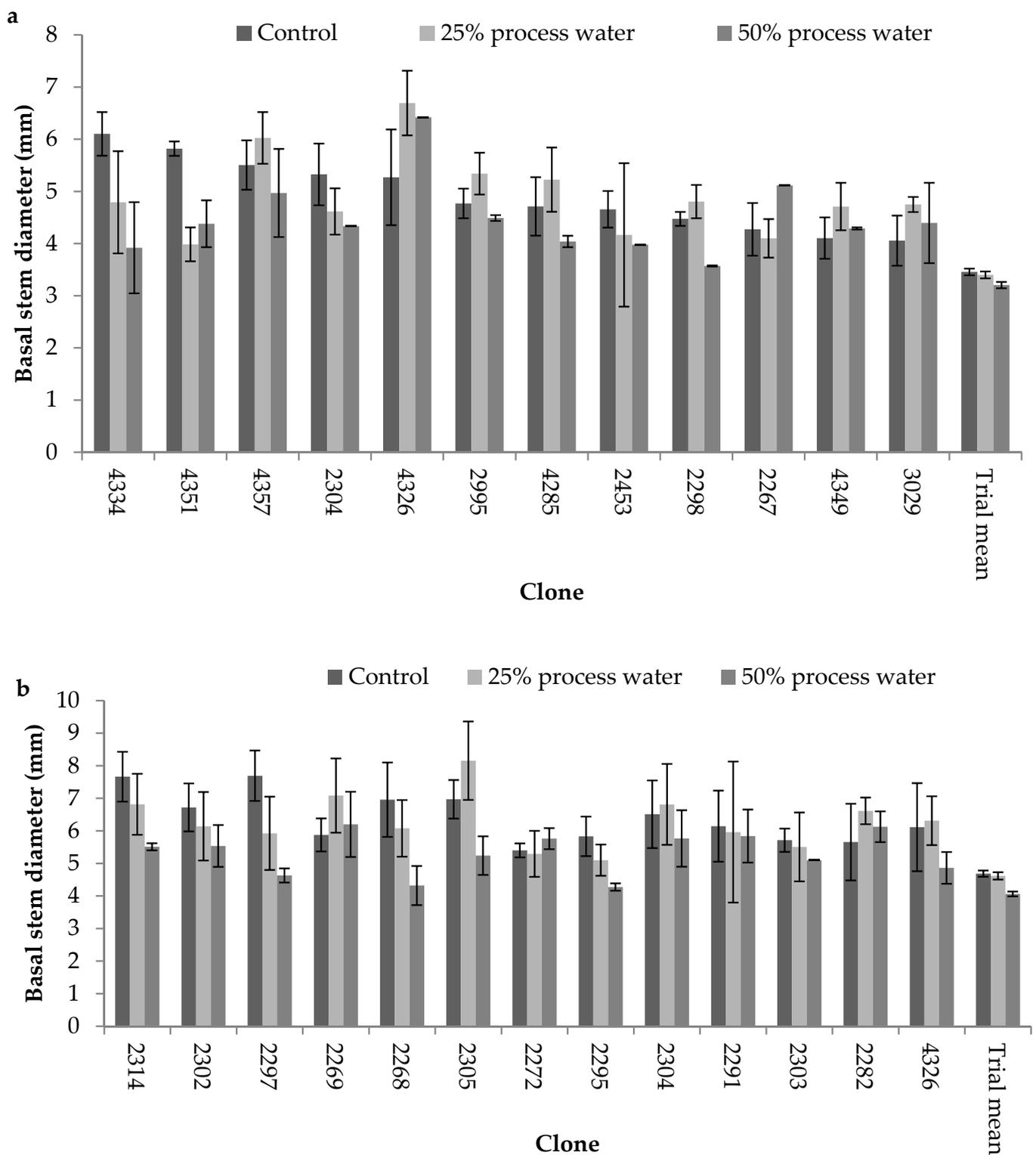
Figure A1. (**a**). Mean basal stem basal diameter (±SE, mm) of 12 balsam poplar clones that ranked in the top 30 for all three water treatments as compared with the overall trial mean for 148 clones after 44 days of growth in the 2012 experiment. (**b**). Mean basal stem basal diameter (±SE, mm) of 11 clones that ranked in the top 30 for all three water treatments as compared with the overall trial mean for 86 clones after 65 days of growth in the 2013 experiment.

Figure A2. Aerial view in winter of the end-pit lake showing the location (marked in red) of the trial adjacent to the lake edge.

End-pit Lake

North ↑

Rep 1

4283 361	2314 362	5578 363	5578 364	947 365	5578 366	3027 367	4643 368	3027 369	2304 370	5578 380	5578 379	4357 378	4304 377	5578 376	5578 375	4285 374	4274 373	2272 372	4296 371	2452 381	2887 382	5578 383	4643 384	4283 385	2453 386	4249 387	4255 388	4293 389	4314 390	3006 400	2997 399	4314 398	4277 397	5578 396	5578 395	3006 394	3006 393	4274 392	3110 391
3188 440	2314 439	4301 438	5578 437	4249 436	2452 435	4295 434	4295 433	947 432	5578 431	3027 430	4304 429	3002 428	4301 427	2997 426	5578 425	2282 424	5578 423	4277 422	3029 421	5578 411	2272 412	2304 413	4277 414	2976 415	5578 416	5578 417	2272 418	5578 419	5578 420	2976 410	4314 409	947 408	5578 407	5578 406	2314 405	5578 404	4357 403	5578 402	5578 401
4274 441	2887 442	4296 443	4301 444	3188 445	2995 446	4283 447	3004 448	4357 449	4301 450	3110 451	3002 452	4277 453	4285 454	2272 455	2887 456	4283 457	5578 458	5578 459	4352 460	2976 461	4296 462	2314 463	3110 464	5578 465	4295 466	5578 467	5578 468	5578 469	4326 470	5578 471	3004 472	2452 473	5578 474	4352 475	5578 476	3002 477	5578 478	2995 479	3187 480
5578 520	5578 519	2452 518	2453 517	3029 516	3188 515	4304 514	2453 513	3110 512	4285 511	5578 510	3187 509	3029 508	2304 507	5578 506	5578 505	5578 504	4255 503	3004 502	4255 501	2282 500	2997 499	5578 498	2304 497	5578 496	4295 495	2997 494	3188 493	5578 492	2282 491	5578 490	4249 489	2453 488	4293 487	4352 486	5578 485	5578 484	5578 483	5578 482	5578 481
5578 521	5578 522	3187 523	3027 524	5578 525	4293 526	4285 527	5578 528	4249 529	4326 530	4274 531	3029 532	4296 533	947 534	2995 535	4643 536	4293 537	5578 538	4352 539	2887 540	3187 541	5578 542	4255 543	5578 544	4326 545	5578 546	4326 547	3004 548	3006 549	4314 550	2995 551	4643 552	2976 553	4357 554	2282 555	5578 556	5578 557	5578 558	4304 559	5578 560

Rep 2

5578 561	2887 562	4301 563	4293 564	4249 565	5578 566	4326 567	3029 568	4296 569	2314 570	3187 571	5578 572	3188 573	4357 574	5578 575	5578 576	4283 577	2453 578	5578 579	2997 580	4301 581	4352 582	3006 583	3029 584	2282 585	5578 586	2452 587	3002 588	4326 589	2453 590	4357 591	2995 592	5578 593	4301 594	3110 595	5578 596	4296 597	2997 598	4274 599	5578 600
2995 640	5578 639	2997 638	3027 637	5578 636	5578 635	5578 634	5578 633	4314 632	5578 631	5578 630	2272 629	4277 628	2314 627	2452 626	2452 625	5578 624	5578 623	5578 622	2453 621	4643 620	3027 619	2304 618	5578 617	3188 616	3006 615	4255 614	5578 613	5578 612	5578 611	5578 610	4296 609	5578 608	4249 607	3004 606	2976 605	5578 604	4277 603	5578 602	5578 601
5578 641	4295 642	5578 643	4326 644	2272 645	4643 646	5578 647	4301 648	3004 649	4283 650	2997 651	4255 652	5578 653	2304 654	5578 655	3006 656	3002 657	4293 658	4274 659	5578 660	2995 661	2314 662	2453 663	4285 664	5578 665	2887 666	4255 667	4304 668	5578 669	4296 670	4277 671	4643 672	4314 673	2314 674	947 675	2976 676	4357 677	4314 678	5578 679	5578 680
4352 720	4643 719	4314 718	2304 717	4285 716	5578 715	3110 714	4274 713	4285 712	4249 711	3029 710	4283 709	5578 708	4295 707	4357 706	3027 705	4293 704	5578 703	4274 702	5578 701	4352 700	4304 699	4283 698	4326 697	5578 696	5578 695	2272 694	4304 693	2976 692	2976 691	5578 690	2272 689	2282 688	3187 687	947 686	5578 685	4295 684	3002 683	3110 682	5578 681
5578 721	4352 722	2282 723	4285 724	2304 725	5578 726	5578 727	5578 728	5578 729	2452 730	947 731	3002 732	3110 733	4304 734	4277 735	3004 736	2995 737	2887 738	2282 739	4295 740	4249 741	3188 742	3187 743	3029 744	4293 745	5578 746	5578 747	4255 748	3187 749	3006 750	3027 751	2887 752	5578 753	3188 754	5578 755	5578 756	5578 757	5578 758	947 759	3004 760

Rep 3

5578 761	4357 762	5578 763	4304 764	5578 765	4293 766	4285 767	4285 768	3188 769	2304 770	4357 771	5578 772	5578 773	4277 774	4301 775	3110 776	4301 777	4293 778	5578 779	2887 780	5578 781	2314 782	5578 783	4295 784	5578 785	5578 786	4296 787	2453 788	4295 789	5578 790	3002 791	5578 792	4314 793	3002 794	5578 795	3004 796	5578 797	2997 798	4643 799	3029 800
4277 840	4283 839	4285 838	3188 837	4249 836	4296 835	5578 834	4352 833	2995 832	4357 831	3002 830	4314 829	2314 828	2997 827	4304 826	2452 825	4352 824	4643 823	3029 822	5578 821	5578 820	4352 819	4274 818	3006 817	947 816	5578 815	4357 814	2453 813	3110 812	4296 811	5578 810	2452 809	2282 808	5578 807	5578 806	4285 805	5578 804	5578 803	4296 802	2272 801
4304 841	5578 842	5578 843	4283 844	5578 845	4295 846	3110 847	5578 848	3004 849	3006 850	2995 851	947 852	5578 853	4277 854	3027 855	2976 856	5578 857	5578 858	5578 859	2887 860	5578 861	947 862	2453 863	2452 864	2272 865	5578 866	4326 867	5578 868	4326 869	2282 870	5578 871	4255 872	5578 873	5578 874	4326 875	2314 876	3188 877	2887 878	5578 879	4314 880
5578 920	5578 919	2997 918	5578 917	5578 916	4283 915	5578 914	3187 913	5578 912	5578 911	2976 910	2997 909	3027 908	5578 907	2453 906	2304 905	2995 904	2282 903	2282 902	5578 901	4274 900	4249 899	4326 898	3029 897	2887 896	5578 895	3187 894	4643 893	5578 892	2272 891	4293 890	3006 889	3027 888	4643 887	5578 886	4314 885	5578 884	5578 883	5578 882	2272 881
4249 921	4301 922	2976 923	3187 924	3006 925	5578 926	2314 927	2976 928	4283 929	5578 930	5578 931	4352 932	2995 933	4295 934	3029 935	5578 936	3187 937	4301 938	4304 939	4274 940	3004 941	4274 942	947 943	4249 944	3004 945	3002 946	5578 947	5578 948	4277 949	2304 950	3110 951	5578 952	3188 953	5578 954	4293 955	2452 956	4255 957	5578 958	4255 959	2304 960

XXXX	← Clone Number
xxx	← Position Number

Treatment 1 (25 selected tolerant clones): 2272, 2282, 2304, 2314, 2453, 2976, 2995, 2997, 3027, 3029, 3110, 3187, 3188, 4249, 4255, 4274, 4277, 4283, 4285, 4295, 4296, 4301, 4304, 4326, 4357

Treatment 2 (10 selected control clones): 947, 2452, 2887, 3002, 3004, 3006, 4352, 4293, 4314, 4643

Treatment 3 (Stream 1 vegetative lot clones): 5578

Figure A3. Map of the field trial site showing individual tree locations within each block (rep).

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