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Tree–Substrate Water Relations and Root Development in Tree Plantations Used for Mine Tailings Reclamation

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Abstract

Tree water uptake relies on well-developed root systems. However, mine wastes can restrict root growth, in particular metalliferous mill tailings, which consist of the finely crushed ore that remains after valuable metals are removed. Thus, water stress could limit plantation success in reclaimed mine lands. This study evaluates the effect of substrates varying in quality (topsoil, overburden, compost and tailings mixture, and tailings alone) and quantity (50- or 20-cm-thick topsoil layer vs. 1-m^2 plantation holes) on root development and water stress exposure of trees planted in low-sulfide mine tailings under boreal conditions. A field experiment was conducted over 2 yr with two tree species: basket willow (*Salix viminalis* L.) and hybrid poplar (*Populus canadensis* Moench × *Populus maximowiczii* A. Henry). Trees developed roots in the tailings underlying the soil treatments despite tailings' low macroporosity. However, almost no root development occurred in tailings underlying a compost and tailings mixture. Because root development and associated water uptake was not limited to the soil, soil volume influenced neither short-term (water potential and instantaneous transpiration) nor long-term ($\delta^{13}C$) water stress exposure in trees. However, trees were larger and had greater total leaf area when grown in thicker topsoil. Despite a volumetric water content that always remained above permanent wilting point in the tailings colonized by tree roots, measured foliar water potentials at midday were lower than drought thresholds reported for both tested tree species.

Core Ideas

- Planted trees developed roots in the tailings underlying the soil treatments.
- Root development was limited in tailings underlying a compost and tailings mixture.
- Trees were larger and had greater total leaf area when grown in thicker topsoil.
- Trees showed low foliar water potential despite root access to tailings water reserve.
- The volume of the soil treatment did not affect tree foliar water potentials.

Water stress is often reported as a possible explanation for plantation failure in reclaimed mine lands (Moffat, 1995; Hutchings et al., 2001; Nicoll et al., 2006). Tree water uptake relies on well-developed root systems, but certain characteristics of mine substrates can prohibit root growth: no structure, no organic matter, extreme pH, elevated electrical conductivity, and trace metal concentrations (Tordoff et al., 2000; Burger and Zipper, 2002). However, studies dealing with plantations to reclaim mine wastes often evaluate revegetation success based on aboveground productivity (Drake, 1986; Kost et al., 1998; Angel et al., 2006; Emerson et al., 2009; Landhäusser et al., 2012; Sloan and Jacobs, 2013; Mosseler et al., 2014) and rarely account for tree root development. Moreover, only a few available studies examine tree planting in metalliferous mine tailings (Bjugstad, 1986; Renault et al., 2008; Boyter et al., 2009; Asensio et al., 2011; Larchevêque et al., 2013, 2014).

Among mine wastes, metalliferous mill tailings consist of the finely crushed ore (70–80% of particles between 2 and 80 μm) (Aubertin et al., 2002) that remains after valuable metals are removed. They are transported from the mine plant usually by pumping with water and deposited as slurry in tailings storage facilities. Among milling wastes, thickened tailings (Robinsky et al., 1991) have a solid content of 50 to 70% (on a mass basis) when deposited. Their basic properties are similar to those of conventionally deposited tailings (slurried) (Bussière, 2007), but they have a more uniform (homogeneous) grain size distribution in the tailings facility (Al and Blowes, 1999). In metalliferous mine tailings, root development seems restricted to cover soils (Borgegard and Rydin, 1989; Zhang et al., 2001; Larchevêque et al., 2013), probably because the underlying tailings have unsuitable properties for root growth (Meredith and Patrick, 1961; Evanylo et al., 2005; Michels et al., 2007), in particular low air-filled porosity (Larchevêque et al., 2013). Impeded root development may result in tree exposure to water stress despite tailings' richness in water due to low hydraulic conductivities ($k_{sat} = 10^{-6}$ to 10^{-8} m s^{-1}) (Barbour et al., 1993; Aubertin et al., 1996).

Efficient root systems optimize the contact surface with the substrate and the explored volume of substrate. Fine roots (i.e.,

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Abbreviations: MS20, treatment of 20 cm mineral soil mixed with compost; RLD, root length density; SRL, specific root length; SSA, specific surface area; T, tailings alone treatment; TC, compost and tailings mixture treatment; TS20, topsoil layer 20 cm treatment; TS50, topsoil layer 50 cm treatment; TSho, topsoil plantation holes treatment; VWC, volumetric water content.

diameter <1 mm) are the most active part of the root system in water uptake (Leuschner et al., 2004). When adapted plants are water stressed, they can increase the surface contact with the substrate with a relatively small decrease in fine root diameter and an increase in their specific root length (SRL) (i.e., root length per dry mass), specific surface area (SSA) (i.e., root surface area per dry mass), root length density (RLD) (i.e., root length by unit of soil volume), or proportion of fine to coarse roots, especially at depths in soil where water is available (Comas et al., 2013). Root mycorrhizae, frequently observed in boreal forests (Read et al., 2004), also greatly participate in water uptake by increasing the contact surface of the root system with the substrate (Garg and Chandel, 2010). Poplar (*Populus* sp.), a pioneer tree species of Canadian boreal region, may be an interesting indicator of water stress exposure on mine wastes because its growth is very sensitive to water availability (van den Driessche, 1999; Larchevêque et al., 2011).

In low-sulfide tailings, the toxicity of trace metals toward plant roots may be limited (Bagatto and Shorthouse, 1999; Trüby, 2003) because acidification is limited. Thus, adapted trees could be directly planted in low-sulfide tailings. The sulfide minerals of the tailings, like pyrite, react with water and oxygen when exposed to atmospheric conditions to produce sulfuric acid. Acidification of the tailings increases trace metal mobility toward the plant-available fraction. Willow (*Salix* sp.) is one of the first species to colonize mine wastes (Gibson, 1982) and seems well adapted to plantation on mine substrates (Mosseler et al., 2014; Mosseler and Major, 2014). In new mine projects, however, topsoil (i.e., superficial soil containing organic matter) and overburden (i.e., soil overlying bedrock) are usually saved for revegetation purposes (Cooke and Johnson, 2002). They are used above tailings to create an appropriate substrate for plant root development. Because the use of soil layers above tailings may reduce evaporation from the tailings' surface, water saturation of tailings under soil layers may be greater, and aeration lower, than that of tailings without soils. This may affect water availability and tree root growth in tailings under soils.

The available quantity of topsoil is often limited compared with the vast mine surfaces that require revegetation. Thus, the thickness of the layers of topsoil used and the area of the treated surfaces are likewise limited, and complementary solutions using overburden and amendments are necessary. Several studies showed that mine wastes used in combination with low-cost organic amendments, such as sewage sludge, domestic refuse, peat, or topsoil were suitable substrates for plant growth (Tordoff et al., 2000). Tailings mixtures with amendments have increased air-filled porosity and decreased density (Larchevêque et al., 2012) that may favor root development.

The main objective of this study was to evaluate the effect of substrates varying in quality (topsoil, overburden, compost and tailings mixture, and tailings alone) and quantity (50- or 20-cm-thick topsoil layer vs. 1-m^2 plantation holes) on root development and water stress exposure of trees planted in low-sulfide mine tailings under boreal conditions. It was previously demonstrated in a greenhouse experiment that the used tailings had no trace element toxic effects on basket willow and a DN×M poplar clone (Larchevêque et al., 2013). A 2-yr field experiment was conducted with these same two trees. All trees were fertilized with mineral N and P at planting to limit the effects of varying availability of nutrients among substrates on trees. Our working hypotheses were that (i) trees would not survive when directly planted in the tailings, even with mineral fertilization, due to low air-filled porosity of the tailings, which impedes root growth; (ii) the presence of soil layers above tailings would increase the tailings' volumetric water content (VWC) due to limited evaporation; (iii) trees would not develop any root in the tailings under the soil or amendment layers due to their low air-filled porosity; and (iv) the more limited the available volume of soil for tree root growth (i.e., from plantation holes to thin and thick soil layers), the more trees would be exposed to water stress.

Materials and Methods

Site Description

The Canadian Malartic gold mine in Malartic, Quebec, Canada (48°13′ N, 78°12′ W; property of Canadian Malartic GP) is a large open-pit mine that began production in 2011. It is located in the Northern Clay Belt region of Quebec and Ontario. Typical forest vegetation surrounds the mine and includes jack pine (*Pinus banksiana* Lamb.), black spruce [*Picea mariana* (Mill.) Britton], trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marsh.), tamarack or eastern larch [*Larix laricina* (Du Roi) K. Koch], and balsam fir [*Abies balsamea* (L.) Mill.]. In this boreal region, the growing season typically begins in mid-May and ends in early October, with a mean temperature during the three warmest months (June, July, and August) of around 18 or 19°C. Average annual temperature is 1°C, and the average number of frost-free days is 80. Mean annual precipitation is approximately 900 mm (Government of Canada, 2004).

Tailings, Soils, and Compost

Canadian Malartic ore is a mineralized greywacke. The tailings are low in sulfide (around 1% S) and contain calcite, which can neutralize acidity. The tailings consist of finely milled wastes (86% particles <80 μm) from the gold extraction process (cyanide leaching). They were deposited in the facility as thickened tailings (around 60% of solids in mass) less than 6 mo before the experiment took place. The tailings had undergone a cyanide destruction process (SO_2/O_2 technology) that left free CN^- concentrations lower than 20 mg kg^{-1}. The tailings' chemical characteristics are reported in Table 1. They have trace metal concentrations below the Quebec regulation thresholds for residential lands, except for total Cd, which appears slightly above the threshold (5 mg kg^{-1}).

Tailings were transported by truck from the tailings facility to the experimental zone in April 2012 and deposited above older tailings. The experimental cell was 120 m × 50 m and was contained by waste rock walls (0.5–1.5 m high) covered with a geotextile liner, which allowed water drainage but retained tailings.

The overburden soil used to cover the mine wastes was a luvic gleysol (Agriculture and Agri-Food Canada, 2010). The overburden topsoil consisted of the uppermost 30 cm of dark (organic-rich) soil layers (O and A horizons) that had been set aside before excavation of the open pit. The overburden subsoil consisted of the remaining mineral sandy clay loam that was excavated down to bedrock after the overburden topsoil had been removed. Overburden topsoil and mineral soil were stocked for 24 to 36 mo in separated piles of 7 m high with a 2.5:1 slope. During storage, the piles were seeded with fast-growing herbaceous species to protect the soils from erosion. The greenwastes' compost used as an amendment to improve the mineral soil and the tailings' structure came from the St-Henri-de-Lévis facility in Quebec (Biogénie). It was principally made from leaves and lawn residues as well as a small proportion of small branches and tree bark. It was sieved to 1.25 cm before use. Soils and compost characteristics are presented in Table 1.

Plant Material and Growth Conditions

Trees were adapted to boreal conditions and locally produced by the Ministère de l'Énergie et des Ressources Naturelles du Québec. Hybrid poplar and willow stock consisted of clonally propagated 1-yr-old whips (1-m-long cuttings) from *Populus canadensis* Moench × *Populus maximowiczii* A. Henry (DN×M, clone number 916004) and *Salix viminalis* L. (basket willow).

The field experiment was set up on thickened tailings in mid-June 2012. Unrooted whips were planted to a depth of 30 cm in the soil or the tailings. All trees were fertilized at planting with 15 g ammonium nitrate (34.5–0-0) and 15 g triple superphosphate (0–45–0) by placed fertilization (van den Driessche, 1999), which involved inserting the fertilizer into a slit made with a spade near the base of each tree, 20 cm from the tree and 15 cm deep.

Experimental Design

A split-plot design was used: 18 experimental plots = 3 blocks (replicates) × 6 treatments (whole plot factor) × 2 tree species (subplot factor: DN×M poplar, basket willow) × 9 trees per factor combination (pseudo-replicates). The six substrate treatments are represented in Fig. 1 and are defined as follows: MS20, 20 cm overburden mineral soil layer and compost; T, direct planting in tailings; TC, tailings mixed with compost; TS20, 20 cm overburden topsoil layer; TS50, 50 cm overburden topsoil layer; and TSho, plantation holes filled with topsoil. Each plot covered 11 m × 11 m and was separated from the others by a 3-m-wide zone of bare tailings. Tree spacing was 1 m × 1 m, and a 3-m buffer zone was kept free of trees at the edge of the different treatments. However, for the MS20 and TSho treatments, trees were spaced at 2 m × 2 m to separate the 1-m^2 plantation holes, resulting in a 16 m × 16 m plot size. The soil layers (TS50, TS20, MS20) were applied on the tailings by a mechanical shovel in May 2012. The mineral soil (MS20) was then mixed with 0.1 m^3 of fresh greenwaste compost (corresponding to 65 kg and a proportion of 23% of compost in the mix on a dry mass basis) by a mechanical shovel in 1 m^2 × 20 cm deep plantation holes. For the TSho treatment, plantation holes (1 m^2 × 20 cm) were dug in tailings and filled with topsoil by a mechanical shovel. Finally, the TC treatment consisted of a 6-cm-thick layer of compost superficially deposited

Table 1. Initial soil, tailings, and compost characteristics.

Characteristics	Overburden topsoil	Overburden mineral soil	Compost	Tailings	Regulation thresholds† (residential lands)
pH	5.1 (0.1)‡	7.2 (0.1)	6.7 (0.01)	7.9 (0.1)	
Clay,§ %	42 (5)	33 (5)		12 (1)	
Silt,¶ %	27 (1)	15 (1)		50 (1)	
Organic matter, %	17 (3)	1.1 (3)	41 (1)	0.1 (2)	
C/N	22 (7)	17 (5)	20 (2)	4 (1.5)	
Electrical conductivity	7 (1)	10 (1)	21 (0.5)	10 (1)	
N, g kg^{-1}	4.3 (0.3)	0.4 (0.3)	12 (1)	0.1 (0.5)	
P g kg^{-1}	0.6 (0.04)	0.6 (0.04)	2.5 (0.2)	0.7 (0.01)	
Available P, mg kg^{-1}	3.6 (0.7)	5.1 (0.7)	252 (7)	8 (5)	
K, g kg^{-1}	3.6 (0.05)	2.7 (0.05)	6.5 (0.2)	9 (0.4)	
Ca, g kg^{-1}	11 (1)	9 (1)	31 (1.3)	17 (2)	
Mg, g kg^{-1}	11 (0.1)	11 (0.1)	4 (0.01)	14 (0.6)	
Na, g kg^{-1}	0.3 (0.02)	0.4 (0.02)	0.4 (0.01)	0.5 (0.05)	
Al, g kg^{-1}	17 (1)	13 (1)	3.5 (0.3)	14 (1)	
Fe, g kg^{-1}	27 (2)	24 (2)	9 (0.3)	34 (2)	
B, mg kg^{-1}	6.3 (0.3)	3.9 (0.3)	21 (0.6)	0.8 (0.5)	
As, mg kg^{-1}	7.7 (0.5)	5.7 (0.5)	5 (0.8)	5.4 (1.4)	30
Cd, mg kg^{-1}	0.6 (0.15)	0.4 (0.15)	2 (0.07)	6 (0.2)	5
Co, mg kg^{-1}	5.8 (0.4)	6.0 (0.4)	1.0 (0.15)	5.9 (1.5)	50
Cr, mg kg^{-1}	120 (14)	116 (14)	10 (0.1)	168 (10)	250
Cu, mg kg^{-1}	58 (12)	31 (12)	42 (4)	52 (2)	100
Mn, mg kg^{-1}	344 (26)	367 (26)	427 (11)	441 (16)	1000
Mo, mg kg^{-1}	2.3 (0.2)	1.6 (0.3)	1.1 (0.4)	8.6 (0.6)	10
Ni, mg kg^{-1}	64 (7)	51 (7)	11 (1)	69 (9)	100
Pb, mg kg^{-1}	22 (4)	13 (4)	24 (1)	18 (100)	500
Zn, mg kg^{-1}	83 (5)	62 (5)	151 (5)	73 (4)	500

† Source: Government of Quebec (2016).

‡ Values are mean (SE) (n = 3). All values are expressed on a dry matter basis.

§ Particles <2 μm.

¶ Particles <50 μm.

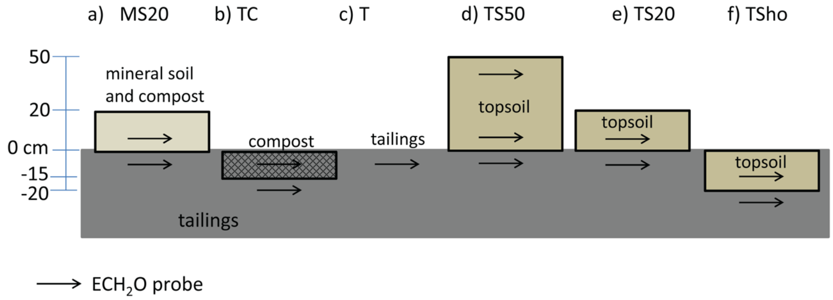

Fig. 1. Schematic representation of substrate treatments tested in the study with their respective ECH2O probes positioning. (a) Twenty centimeter mineral soil mixed with compost (MS20). (b) Compost and tailings mixture (TC). (c) Tailings alone (T). (d) Topsoil layer 50 cm (TS50). (e) Topsoil layer 20 cm (TS20). (f) Topsoil plantation holes (TSho).

above tailings and mixed at 15 cm depth by a compact loader (T110, Bobcat Co.) equipped with a rotovator. The compost amendment corresponded to 179 t ha^{-1} of dry compost and to a proportion of 13% of compost in the mix on a dry mass basis. Compost rates were calculated to provide around 4% of organic matter in the mixtures to improve the tailings and the overburden structure by providing a better infiltration rate and lower density (Peters, 1995; Marcus, 1997; Bendfeldt et al., 2001).

Measurements, Sampling, and Analysis

Substrate Measurements

Three random samples were taken at planting (June 2012) for soils, tailings, and compost characterization (Table 1) at 0 to 10 cm depth. Analyses were conducted on sieved (2 mm mesh), finely ground, oven-dried samples (50°C) (Lakehead University Centre for Analytical Services,). Total N was analyzed by the Dumas combustion method (CNS 2000, LECO) and organic carbon (C) by thermogravimetric method (LECO-TGA,). Organic matter concentrations were calculated as 1.72 × organic C. After HNO_3–HCl digestion, sample concentrations of total P, K, Ca, Mg, Na, Al, As, B, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn were determined by inductively coupled plasma–atomic emission spectrometry (Vista PRO, Varian Canada). Available P was determined in a sodium bicarbonate solution using spectrophotometry (Olsen et al., 1954). Finally, pH was determined in a saturated paste extract, and electrical conductivity was determined in 1:2 water solution. Soil texture was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962).

Undisturbed 100-cm^3 substrate samples were taken with a double cylinder soil sampler at 0 to 10 cm in each plot in July 2012 to characterize each treatment: one by tree species, taken at the foot of the fifth tree on each plot (one sample by tree species and treatment; n = 36). Additional samples were taken at the same location to measure permanent wilting point, electrical conductivity, and organic matter concentration. Water retention properties of the substrates were determined using procedures proposed by Klute (1986) and Cassel and Nielsen (1986). Undisturbed substrate samples (5 cm diameter, 100 cm^3) were brought to saturation in the lab under vacuum and weighed (W1). They were set on the porous surface of a sand box apparatus (Eijkelkamp Agrisearch Equipment) and brought to equilibrium with a tension value of −10 kPa (field capacity). They were weighed (W2) and oven dried (105°C, 48 h) before weighing one last time (W3). Total porosity (%), macroporosity or air-filled porosity (%), and bulk density (g cm^{-3}) were estimated with the following formulas:

$$\text{Total porosity} = (\text{W1} - \text{W3})/100\ \text{cm}^3 \times 100$$

$$\text{Macroporosity} = (\text{W1} - \text{W2})/100\ \text{cm}^3 \times 100$$

$$\text{Dry bulk density} = \text{W3}/100\ \text{cm}^3$$

For permanent wilting point measurements, bulk soil samples were sieved at 2 mm and immersed in water for 48 h. Then they were brought to equilibrium with a tension value of −1500 kPa using a pressure membrane apparatus (Soilmoisture Equipment). Samples were weighed (W4) and dried for 48 h at 105°C (W5). Permanent wilting point (PWP) on a percent volume basis was estimated with the following formula:

$$\text{PWP} = (\text{W4} - \text{W5})/\text{W5} \times \text{bulk density} \times 100$$

EC-5 probes (Decagon Devices) were placed in the substrates in the center of each experimental plot (n = 18) at several depths (Fig. 1) and connected to EM-50 dataloggers to measure the VWC evolution from August 2012 to September 2013. The first 18 probes were installed 5 cm deep in the tailings (5 cm from the surface in T treatment; 5 cm under the treatment layer for other treatments). Fifteen other probes were installed in the treatment layers, 5 cm above the underlying tailings. Finally, three more probes were installed at 10 cm from the topsoil layer surface in the TS50 treatment. Records were set at a 1-h frequency. The probes were calibrated for each substrate type (tailings and compost mix, topsoil, clay and compost mix, and tailings). Their accuracy was ±3%. Mean VWC values (mL mL^{-1}) were calculated for each week throughout the growing season (May–September) based on the hourly records. The substrate water saturation (%) was calculated as VWC/total porosity × 100.

Plant Measurements

Survival, stem height, and basal diameter were measured at planting and at the end of each growing season in 2012 and 2013 for each planted tree. On 23 Sept. 2013, the fifth tree of each treatment by species combination (n = 36) was harvested for aboveground biomass assessment. Trees were separated into stems and leaves. Total leaf areas of willow and poplar were measured with a LI-3100 C leaf area meter (LiCor) before drying. The plant parts were then oven dried at 50°C for 48 h and weighed. Specific leaf area (SLA; total leaf area/total leaf dry mass, cm^2 mg^{-1}) was calculated.

In July 2013, tree water relations (three replicates by species × treatment; n = 36) and gas exchange (poplar only, three replicates by treatment; n = 18) were assessed on the fifth tree of each treatment. Then the foliage was sampled for isotope ratio (13C/12C) analysis. Leaf water potential (MPa) was measured with a pressure chamber (Model 600, PMS Instrument Co.) on one fully mature leaf per tree in the middle of the stem between 10:00 and 13:00 h on sunny days. The measurements were done on 3

July and repeated on 5 July, without any rain between the two dates. On 5 July, instantaneous transpiration (*E*i, mmol m^{-2} s^{-1}) was measured on the poplars in the afternoon (13:00–15:00 h). Measurements were performed using a CIRAS-2 portable infrared gas analyzer equipped with a PLC6 broadleaf chamber illuminated by halogen lamps (photosynthetic photon flux density of 1400 μmol m^{-2} s^{-1} at leaf level) (PP Systems Inc.). Measurements were made on one leaf per tree at air CO_2 concentration of 360 μmol mol^{-1} and flow at 295 mL min^{-1}.

In July 2013, 10 to 20 fully matured leaves were sampled on the fifth tree of each treatment × species combination (n = 36). Sampled foliage was oven dried (50°C) and finely ground with a Brinkmann MM2 ball grinder (Brinkmann Instruments Ltd.). Stable isotope ratios (13C/12C) were determined using a Costech ECS 4010 Elemental combustion system (Costech Analytical Technologies Inc.) coupled to a continuous flow Finnigan Delta Plus Advantage IRMS (ThermoFinnigan). The relative abundance of 13C in leaves was expressed in terms of carbon isotope composition (δ13C) according to the following relationship: δ13C = [(Rl − Rs)/Rs] × 1000, where Rl and Rs refer to the 13C/12C ratio in the leaf sample and in the standards, respectively. BMO (grain), CS (corn stover), and NBS 1575 N (pine needle) (NIST, Standard Reference Materials) were used as calibrating standards and Red Clover as working standard, with C isotope compositions of −23.91, −12.5, −26.3, and −27.42‰ relative to Pee Dee Belemnite, respectively.

At the end of September 2013, tree roots were sampled with an 8-cm-diameter auger (core 750 cm^3) (Eijkelkamp Agrisearch Equipment) at two depths. The first core was taken at approximately 0 to 20 cm and corresponded to the total depth of the thin soil layers MS20, TS20, and TSho or to the tailings mixed with compost. The second core was sampled at 20 to 30 cm in the tailings underlying the thin treatments or in the thick topsoil layer (TS50). Soil cores were taken on the diagonals of the square formed by the four central planted trees of each plot, one tree per species, at 40 cm of the tree foot on the external side of the square. A total of 36 cores × 2 depths were sampled (three repetitions by tree species by treatment). Each core depth was carefully measured at sampling to calculate the core volume. In the laboratory, tree roots were washed from the soil above a fine metallic grid and separated manually from the weed roots. Scanned images of fresh roots were analyzed with Winrhizo software (regular version, Regent Instruments Inc.) for root length, surface, diameter, volume, and tip number. Then, RLD (cm cm^{-3} of substrate), root surface density (cm^2 cm^{-3} of substrate), root volume density (cm^3 cm^{-3} of substrate), and number of tips density (nb cm^{-3} of substrate) were calculated. Finally, the root samples were oven dried at 80°C, separated by fine (<1 mm diameter) and coarse (>1 mm diameter) roots, and weighed to measure root soil density (g cm^{-3} of substrate), SSA (m^2 kg^{-1}), and specific root length (SRL; m g^{-1}). Because few samples had roots with a diameter greater than 1 mm, the ratio of fine to coarse roots was not calculated.

Statistical Analyses

Survival data were compared using the χ^2 test. Because only one tree (one willow) survived the second year planted in tailings, the T treatment was removed from further statistical analysis regarding trees. For soil characteristics and poplar instantaneous transpiration, data were submitted to one-way ANOVA (treatment effect). For final height and diameter (height and diameter at planting as covariable), leaf water potentials, tree biomass, and δ13C, the data were submitted to two-way ANOVA (treatment × species). Two ANOVAs were performed on the root data: two-way (treatment × species) on the superficial samples (0–20 cm depth) and three-way (treatment × species × depth) on all the samples. No roots (one willow core with root among six sampled) were found in the tailings under the mixed tailings and compost. Thus, the TC treatment was removed from the analysis of the species by treatment by sampling depth effect. Substrate VWC and water saturation data of 2013 (during 22 wk) and 2012 (during 7 wk) were submitted to three-way ANOVA (depth × treatment × date). The seven common weeks of measurements for both years were submitted to four-way ANOVA (depth × treatment × date × year). Finally, the VWC and water saturation of the soil layers and mixed tailings and compost were submitted to two-way ANOVA (treatment × date). Two sets of data (VWC and water saturation) were considered separately for the 50-cm topsoil layer: at 10 cm from the surface and at 40 cm from the surface (5 cm above the underlying tailings).

All tested factors were fixed effects, and the block factor was considered a random effect. When effects were significant for a given variable, least-square means were estimated, and Tukey tests were conducted to separate the means. Overall significance for analyses was set to α = 0.05. All analyses were performed using SAS V.9.2 (SAS Institute Inc.), and repeated measures were used where appropriate.

Results

Tree Survival and Growth

After two growing seasons, all trees were dead when directly planted in the tailings (T treatment) (Fig. 2). For both species (poplar and willow), the survival rates were similar and greater than 90% in all soil treatments (TS20, TS50, MS20, TSho), whereas poplar survival declined in the mixed tailings and compost (TC) during the first year. Willow survival was maximal in all treatments except tailings for both years.

For both species, the thick layer treatment (TS50) always gave the highest final height, diameter (Fig. 3), and foliar biomass (Table 2), and the TC treatment gave the lowest performance compared with all the other treatments. The thin soil layer treatments (TS20, MS20) yielded intermediate results except for poplar diameter. In the short term (2 yr), no growth difference was found between trees planted in a 20-cm topsoil layer and topsoil plantation holes.

Tree Water Stress and Gas Exchanges

The water potential and gas exchange measurements were taken on 3 and 5 July 2013 after 2 wk with less than 10 mm precipitation. At both dates the volumetric water content of all the substrates (20–38%) was the lowest of all the growing season (from the beginning of June to the end of September) according to the EC-5 probes' continuous records (Fig. 4). The species or date did not influence the water potential of trees (i.e., there was no significant interaction between species, treatment, and date). Foliar water potential (both species) and instantaneous transpiration (poplar) were similar among treatments (Table 2). Poplar had stopped its photosynthesis at the time of measurements;

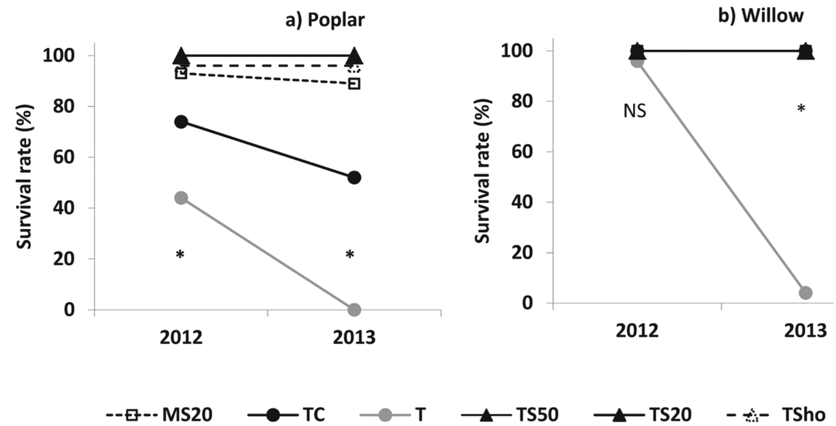

Fig. 2. Tree survival rate (%) among treatments (20 cm mineral soil mixed with compost [MS20], tailings alone [T], compost and tailings mixture [TC], topsoil layer 20 cm [TS20], topsoil layer 50 cm [TS50], topsoil plantation holes [TSho]) at each growing season (2012: first growing season; 2013: second growing season) for (a) DN×M poplar and (b) basket willow. Values are means (n = 27). Statistical comparisons were done among treatments at each date. *P < 0.05. NS, nonsignificant.

no net CO_2 assimilation occurred (results not shown). Both tree species showed no variation of $\delta^{13}C$ among treatments, but poplar had significantly greater total leaf area in TS50 (Table 2) compared with other treatments.

Root Development of Trees

For all thin soil layer treatments (TS20, TSho, MS20), tree roots were always found in cores taken in the underlying tailings, but this was not the case under the compost mixed with tailings (TC) treatment. Both species' responses to treatments were similar because there was no significant interaction between the treatment and species. We found no significant differences in root morphological parameters among the substrates above the tailings (soil layers or compost mixed with tailings) (Table 3). We also found no significant difference among root parameters when cores were taken deeper than 20 cm in the substrate profile compared with superficial (0–20 cm) cores, except for the root tip density, which was significantly lower in deeper cores (Table 3). However, regarding the cores at >20 cm depth, there was no difference between root tip density in the tailings under the soil layers and in the lowest part of the thick topsoil layer (TS50).

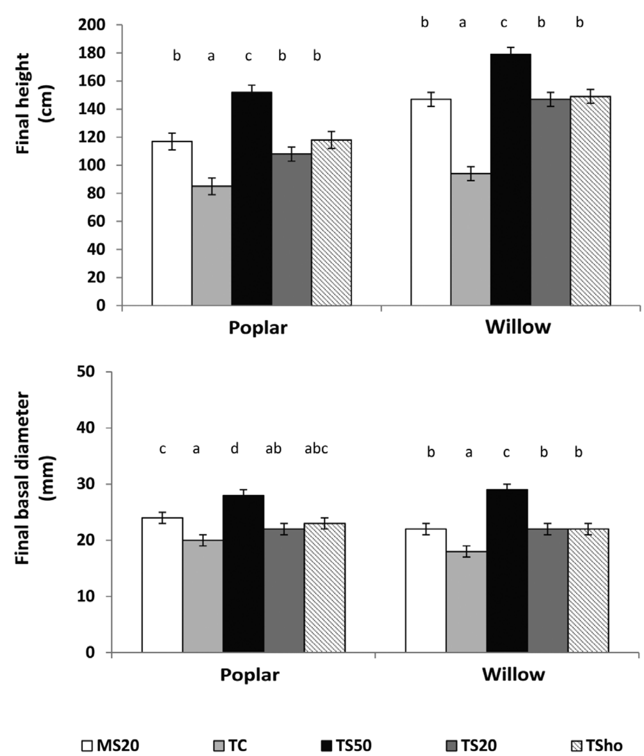

Fig. 3. Tree height (cm) and basal diameter (mm) among treatments (20 cm mineral soil mixed with compost [MS20], compost and tailings mixture [TC], topsoil layer 20 cm [TS20], topsoil layer 50 cm [TS50], topsoil plantation holes [TSho]) at the end of the second growing season (2013) for (a) DN×M poplar and (b) basket willow. Values are means (n = 27). Bars denote SE. Statistical comparisons were done among treatments at each date, and treatments denoted by the same letter do not significantly differ at P = 0.05.

Substrate Structure, Volumetric Water Content, and Saturation

Tailings (T) showed greater bulk density (1.33 g cm^{-3}) and lower total porosity (47%), macroporosity (6%), and permanent wilting point (2%) compared with the other treatments (Table 4). The substrates' electrical conductivity increased from topsoil to tailings or mineral soil mixed with compost (Table 4). The organic matter concentration of tailings or mineral soil mixed with compost did not differ significantly from those in tailings and remained lower than those found in the topsoils (Table 4).

All the probes installed in the tailings (tailings under a treatment layer or without any layer) gave similar VWC values (2013, 22 wk of measurement [Fig. 4]; 0.31–0.36 mL mL^{-1}). Regarding the treatment layers, the 10-cm-deep probes installed in the tailings mixed with compost (TC) and the thick topsoil layer (TS50) gave similar VWC and water saturation values in 2013 (Table 5), which were lower than values given by the probes installed at the same depth in the other treatment layers (MS20, TS20, TSho) or 40 cm deep in the thick topsoil layer (TS50). Volumetric water content and water saturation significantly decreased in the three topsoil layers (TS20, TS50, and TSho, −10% of water saturation) and their underlying tailings (−14 to −20% of water saturation) from 2012 to 2013, whereas they were similar for both years in the MS20, TC, and T treatments. During both years of the experiment and whatever the treatment, the water saturation of the tailings remained above 25% and near or above 20% in the soil layers and tailings mixed with compost (Fig. 4), which is above tailings, MS20, or TC permanent wilting point thresholds but closer to the one of topsoil (Table 4).

Discussion

Surprisingly, and contrary to our third hypothesis, both tree species developed roots in the tailings underlying the thin soil treatments. Moreover, the studied morphological characteristics of these roots were similar to those of roots sampled at the same depth in the thick soil layer treatment; however, large variability between replicates may have affected the treatment comparison. Yet, corresponding to our first hypothesis, tailings showed density close to the threshold impeding root growth (1.4 g cm^{-3}) (Archer and Smith, 1972), and air-filled porosity values were lower than the minimum required for root development (10% v/v) (Archer and Smith, 1972). However, tailings also showed a mean water saturation of 77% (Table 3), which left 23% of pores available for air circulation and which may have facilitated root

Table 2. Indicators of water stress exposure (measured in July 2013) and foliar characteristics (measured at the end of the second growing season, September 2013) for both planted tree species among treatments.

	Treatment†				
	MS20	TSho	TS50	TS20	TC
Indicators of water stress exposure					
Foliar water potential, MPa					
Poplar	−1.3 (0.1)a‡	−1.1 (0.1)a	−1.1 (0.1)a	−1.0 (0.1)a	−1.4 (0.1)a
Willow	−1.1 (0.1)a	−1.2 (0.1)a	−1.2 (0.1)a	−1.2 (0.1)a	−1.1 (0.1)a
Poplar instantaneous transpiration, mmol m^{-2} s^{-1}	3.3 (0.3)a	3.7 (0.3)a	4.0 (0.3)a	4.1 (0.3)a	3.8 (0.3)a
Carbon isotope composition					
Poplar	−27.4 (0.5)a	−27.6 (0.5)a	−27.8 (0.5)a	−26.5 (0.5)a	−25.6 (0.5)a
Willow	−29.1 (0.5)a	−29.7 (0.5)a	−29.3 (0.5)a	−29.8 (0.5)a	−29.8 (0.5)a
Foliar characteristics					
Total leaf area, cm^2					
Poplar	4034 (925)a	4356 (925)a	8992 (925)b	3929 (925)a	1319 (925)a
Willow	5115 (1433)a	4602 (1433)a	9322 (1433)b	3130 (1433)a	1238 (1433)a
Total leaf biomass, g					
Poplar	57 (9)b	46 (9)b	105 (9)c	32 (9)ab	16 (9)a
Willow	34 (9)b	44 (9)b	84 (9)c	45 (9)ab	11 (9)a
Specific leaf area, cm^2 mg^{-1}					
Poplar	0.09 (0.01)a	0.09 (0.01)a	0.08 (0.01)a	0.09 (0.01)a	0.09 (0.01)a
Willow	0.09 (0.01)a	0.10 (0.01)a	0.11 (0.01)a	0.10 (0.01)a	0.09 (0.01)a

† MS20, 20 cm mineral soil mixed with compost; TC, compost and tailings mixture; TS20, topsoil layer 20 cm; TS50, topsoil layer 50 cm; TSho, topsoil plantation holes.

‡ Values are mean (SE) (n = 3). Treatments followed by the same lowercase letter do not significantly differ at P = 0.05.

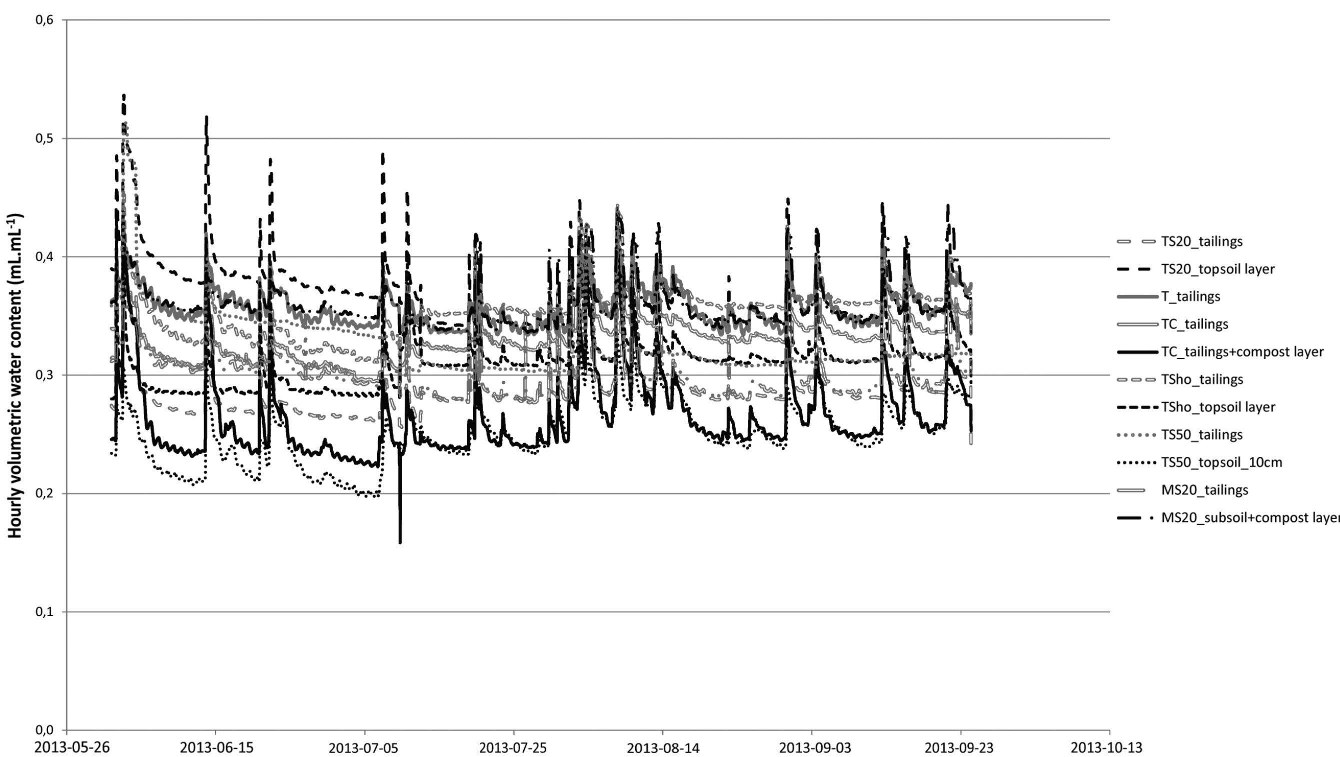

Fig. 4. Substrate volumetric water content (hourly measures) among treatments (20 cm mineral soil mixed with compost [MS20], tailings alone [T], compost and tailings mixture [TC], topsoil layer 20 cm [TS20], topsoil layer 50 cm [TS50], topsoil plantation holes [TSho]) during the second growing season (May–October 2013) measured by EC-5 probes placed in the treatment layer and in the underlying tailings (see Fig. 1 for details). Values are means (n = 3).

Table 3. Morphological parameters of roots measured at the end of the second growing season (September 2013) for bulk planted tree species among treatments (at two sampling depth (0–20 cm and >20 cm).

	Treatment†							
	TS20		TS50		TSho		MS20	
	Soil layer 0–20 cm	Tailings >20 cm	Soil layer 0–20 cm	Tailings >20 cm	Soil layer 0–20 cm	Tailings >20 cm	Soil layer 0–20 cm	Tailings >20 cm
Root mean diameter, mm	0.42 (0.04)a‡	0.57 (0.05)a	0.47 (0.04)a	0.42 (0.04)a	0.47 (0.04)a	0.40 (0.05)a	0.41 (0.05)a	0.42 (0.05)a
Root length density, cm cm^{-3}	0.55 (0.12)a	0.17 (0.14)a	0.49 (0.12)a	0.35 (0.12)a	0.49 (0.12)a	0.33 (0.14)a	0.36 (0.14)a	0.32 (0.14)a
Root soil density, g m^{-3}	196 (110)a	80 (136)a	455 (110)a	92 (110)a	370 (139)a	312 (136)a	151 (124)a	81 (124)a
Root tip density, nb cm^{-3}	3.1 (1.3)b	0.9 (1.5)a	4.4 (1.3)b	2.3 (1.3)a	2.6 (1.3)b	1.3 (1.5)a	4.2 (1.5)b	1.5 (1.5)a
Specific surface area (<1 mm), cm^2 g^{-1}	302 (145)a	285 (179)a	159 (145)a	606 (145)a	107 (183)a	420 (179)a	415 (164)a	426 (164)a
Specific root length (<1 mm), cm g^{-1}	3571 (1386)a	3002 (1709)a	2085 (1386)a	6785 (1386)a	1356 (1748)a	4775 (1707)a	4806 (1562)a	4540 (1562)a

† MS20, 20 cm mineral soil mixed with compost; TC, compost and tailings mixture; TS20, topsoil layer 20 cm; TS50, topsoil layer 50 cm; TSho, topsoil plantation holes.

‡ Values are mean (SE) (n = 6). Treatments followed by the same lowercase letter do not significantly differ at P = 0.05.

respiration. Based on these results, root development of trees is possible in tailings despite their poor structural properties.

However, this development may not be systematic because we observed very poor root development in tailings under the compost and tailings mixture. This difference in root development was not due to water saturation in tailings under the different surface treatments. Indeed, contrary to our second hypothesis, the presence of soil layers above tailings did not increase VWC by limiting water evaporation at the surface. Similarly, the compost and tailings mixture at the surface had no effect on the underlying tailings' VWC compared with tailings alone. The formation of a crust was observed at the surface of the tailings without treatment and could have limited water evaporation as well. Crust formation can occur at tailings surface due to salt accumulation in the first 10 to 20 cm of tailings and precipitation at the tailings surface after water evaporation (Newson and Fahey, 1997; Simms et al., 2007). This salt accumulation could have participated in tree death when planted in tailings by decreasing tailings' water potential. The lack of root development under compost and tailings mixture was not due to differing structure. Structural properties of the compost and tailings

Table 4. Substrate structural and water retention properties as well as electrical conductivity and organic matter concentration among treatments in July 2012.

Treatments†	Properties‡					
	Bulk density	Total porosity	Macroporosity	PWP	EC	OM
	g cm^{-3}	%			cS m^{-1}	%
T	1.3 (0.1)c§	47 (1)a	6 (1)a	2 (0.1)a	10 (1)bc	0.1 (2)a
TS50	0.9 (0.1)a	58 (2)c	15 (1)b	21 (5)b	7 (1)ab	14 (2)c
TS20	0.9 (0.1)ab	54 (2)bc	14 (1)b	18 (6)ab	5 (1)a	12 (2)c
TSho	1.0 (0.1)ab	55 (1)bc	13 (1)b	15 (1)ab	8 (1)abc	11 (2)bc
MS20	1.1 (0.1)bc	50 (3)ab	12 (1)b	7 (2)ab	10 (1)bc	5 (2)ab
TC	0.9 (0.1)a	57 (3)c	12 (1)b	8 (2)ab	13 (1)c	4 (2)a

† MS20T, 20 cm mineral soil mixed with compost; T, tailings alone; TC, compost and tailings mixture; TS20, topsoil layer 20 cm; TS50, topsoil layer 50 cm; TSho, topsoil plantation holes.

‡ EC, electrical conductivity; OM, organic matter; PWP, permanent wilting point.

§ Values are mean (SE) (n = 6). Treatments followed by the same lowercase letter do not significantly differ at P = 0.05.

Table 5. Substrate mean volumetric water content and water saturation among treatments (along the second growing season (May–Oct. 2013) measured by EC-5 probes at differing positions (above or under the tailings surface).

Treatment†	Substrate	Probe depth from the surface	Probe position relative to the underlying tailings	VWC‡	Water saturation
		cm		mL mL^{-1}	%
T	tailings	5	5 cm under the tailings surface	0.36 (0.02)c§	77 (3)d
TS20	topsoil	12	5 cm above the tailings surface	0.39 (0.02)d	67 (3)c
TS50	topsoil	40	5 cm above the tailings surface	0.38 (0.02)cd	66 (3)c
TS50	topsoil	10	35 cm above the tailings surface	0.27 (0.02)a	46 (3)a
TSho	topsoil	15	5 cm above the tailings surface	0.33 (0.02)b	57 (3)b
MS20	mineral soil + compost	16	5 cm above the tailings surface	0.37 (0.02)cd	74 (3)d
TC	tailings + compost	11	5 cm above the tailings surface	0.27 (0.02)a	48 (3)a

† MS20, 20 cm mineral soil mixed with compost; T, tailings alone; TC, compost and tailings mixture; TS20, topsoil layer 20 cm; TS50, topsoil layer 50 cm; TSho, topsoil plantation holes.

‡ Volumetric water content.

§ Values are mean (SE) (n = 3). Treatments followed by the same lowercase letter do not significantly differ at P = 0.05.

mixture were similar to those of topsoil or the mixture of mineral soil and compost (Table 4).

Contrary to our fourth hypothesis, neither short-term (water potential and instantaneous transpiration) nor long-term ($\delta^{13}C$) indicators of water stress exposure in trees were sensitive to the decrease of soil volume. Even at low foliar water potentials, poplars kept their stomata open (positive *E*i), showing an anisohydric behavior (Tardieu and Simonneau, 1998) that did not restrict CO_2 entry into leaves and therefore may have decreased the accuracy of $\delta^{13}C$ as a long-term indicator of water stress exposure (Farquhar et al., 1989) for this species. Indeed, $\delta^{13}C$ increases when stomata close due to water stress exposure because intercellular spaces beneath closed stomata become increasingly enriched in $^{13}CO_2$, which results in greater levels of its fixation (Farquhar et al., 1989). Concomitantly, no variation in tree root indicators of water stress exposure (e.g., SRL, SSA, and RLD) was evidenced among treatments in our study. Because trees were able to develop roots in tailings under the soils, water uptake was not limited to the soil, and its volume did not affect the measured indicators of tree water stress exposure. Thus, a thicker topsoil layer did not increase the available water reserve for trees, but trees had larger aboveground dimensions and had a greater total leaf area when grown in the thick topsoil compared with all other treatments. On compost and tailings mixtures, tree root development was restricted to the surface mixture, but this did not result in a significant decrease in foliar water potentials, probably due to decreased aboveground growth of the same trees. Indeed, under water stress, decreased aboveground growth and leaf area decreases the transpiring surface of woody species and allows water conservation (Pallardy, 2008).

Whatever the treatment, the values of foliar water potential measured in our experiment were lower than drought thresholds reported by several authors (−0.6 to 0.7 MPa for several poplars [Ridolfi and Dreyer, 1997; Larchevêque et al., 2011] and −1.0 MPa for willow [Ögren and Öquist, 1985]), especially for poplars. These results indicate that all planted trees showed symptoms of water stress when the measures were taken after 2 wk with low precipitation despite important water storage in tailings. The cause of low foliar water potentials of trees is unclear because water saturation of the tailings always remained far above the permanent wilting point with acceptable electrical conductivity levels (40 cS m^{-1} reported threshold for impeding root survival) (Epstein et al., 1976; Jordan et al., 2008). Thus, water was available from tailings for root uptake at all times during the experiment. Intense wind exposure on mine sites, vast surfaces without any sheltering vegetation, could have exposed trees to greater air temperature and vapor pressure deficit (Kapos, 1989), decreasing foliar water potential.

In compost mixtures with tailings or mineral soil, water saturation also remained clearly above the permanent wilting point, whereas it sometimes decreased at the permanent wilting point level in topsoil treatments. Trees growing in topsoils thus may have pumped more water from underlying tailings to compensate for lower available water in topsoil as well as greater leaf biomass (i.e., probable greater water loss; Table 2), which decreased water saturation of tailings. Thus, contrary to our second hypothesis, the presence of topsoil layers planted with broadleaf trees decreased water saturation of tailings after the first year of plantation.

Conclusion

Our main finding is that roots were able to colonize underlying mine tailings when trees were planted in soil layers. Moreover, the change of growing substrate for roots did not result in morphological changes of the roots. However, the nature of the substrate above tailings influenced root colonization of tailings because it was impeded when trees were planted in the compost and tailings mixture. In this mixture, trees, particularly poplar, were also smaller and had lower survival rates. Thus, the use of soil layers, even with limited volume (i.e., plantation holes or limited thickness), should be preferred to tailings improvement with organic amendments. To maximize tree aboveground growth, however, thick soil layers are recommended. Moreover, with soil layers (i.e., topsoil in our experiment) prone to occasional lack of water during the growing season, broadleaf trees could be used to decrease tailings water saturation by root pumping. Finally, trees showed symptoms of water stress despite available water in the tailings colonized by roots, and the causes of these symptoms should be further investigated.

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