The effects of agronomic herbaceous plants on the soil structure of gold mine tailings and the establishment of boreal forest tree seedlings.

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Abstract

In Canada, low-grade ore mines generate large amounts of mineral waste, such as mine tailings. To control erosion of the fine-grained tailings particles as quickly as possible, it is common practice for the mining industry to revegetate the mine tailings with agronomic herbaceous plants. However, it is unclear whether this practice is consequential to the natural establishment of boreal species. The first objective of this study was to evaluate which families of agronomic herbaceous plants (legumes or grasses) result in the most favorable physical and chemical soil properties for the establishment of boreal species. The second objective was to determine the effect of the agronomic herbaceous plants on the growth and foliar nutrient concentration on three indigenous boreal forest seedlings; jack pine (*Pinus banksiana* Lambert), tamarack (*Larix laricina* Du Roi), paper birch (*Betula papyrifera* Marshall) and a willow cultivar (*Salix miyabeana* Seemen).

In 2013, a one-hectare *in situ* experimental surface of mine tailings was set up on the gold mine site in Malartic, Abitibi-Témiscamingue, Quebec. The experimental site was subdivided into three blocks, each further divided in 5 plots. Each plot was randomly seeded as follows: 100 % grass, 100 % legumes, a mixture of both, topsoil, and a control (tailings only, no seeding). In the 2015 spring season, thirty seedlings of the three boreal tree species and cuttings of the willow cultivars were planted in each treatment plot. Seedling height and root biomass were measured at the end of the 2016 growing season.

Soil sample analyses indicated significant differences for bulk density, wilting point and organic matter content between the topsoil and the different agronomic herbaceous and control treatments, however no significant differences were found between the different herbaceous treatments and the control for soil pH, bulk density, wilting point, macroporosity and organic matter content. The mortality rate of jack pine, tamarack, and paper birch seedlings was higher in the control plots compared to all other treatments. Root biomass and height of the willow cultivar were significantly higher in the legumes compared with topsoil treatment. Among the four pioneer tree seedlings studied, this research indicates that the combination of the willow cultivar with the legumes treatment produces the best seedling growth and survival in the highly abiotic and stressful environments inherent to mine tailings.

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1 Introduction

The mining industry generates large amounts of mineral wastes during the extraction and treatment of commercial ore (Ripley et al., 1996; Bennett, 1995; Lottermoser, 2007; Dudka and Adriano, 1997). Among the different types of mine waste, mine tailings are of particular concern for their reclamation challenges. (Lottermoser, 2007).

Mine tailings are defined as fine crushed, milled, and grounded ore from which the valuable mineral has been chemically recovered. The mine tailings' mixture of sand grain to silt size particles is then pumped with water in a slurry paste with some chemical residues and deposited into a tailing storage facility (NRCan, 2017; Huang et al., 2012; Edraki et al., 2014). The physical and chemical soil properties (such as bulk density, macroporosity and pH) of the mine tailings depend on the processing technology, the parent ore, the size of particles resulting from the milling process, and the type of chemical used for the extraction of the valuable mineral (Lottermoser, 2007).

In 2016, 63 active metal ore mines were in operation in Canada, producing 122 million cubic meters of tailings ponds (NRCan, 2016; MAC 2017). Environmental impacts generated by the mine tailings include dust particle pollution, surface and groundwater contamination, acid leaching, wildlife habitat destruction (SMCA-1977; Donato et al., 2007) and human health impacts (Dudka and Adriano, 1997; Sanchez-López et al., 2015; Edraki et al., 2014). As such, mine tailings reclamation ("*Mine reclamation entails restoring these disturbed areas to a previous natural resource setting, such as forest or agricultural land uses, while minimizing environmental impacts". www.igws.indiana.edu/Reclamation, 2020)* is a crucial intervention to mitigate their environmental impacts, with revegetation being the most common and least expensive treatment option (Wang et al., 2017; Sheoran et al., 2010). Revegetation used for mine tailings reclamation offers many benefits, including the creation of a soil profile and structure, an increase in soil fertility through the plants' decomposition, and a reduction of airborne fine dust particles from wind erosion (Bradshaw, 1997; Bradshaw and Chadwick, 1980; Bradshaw et al., 1978).

After a mine's closure, or when the tailings facilities area is filled up, revegetation is commonly used to control erosion and to improve mine soil substrate. Since mine tailings are anthropogenic soils, they are devoid of most benefits and services inherent to natural soils such as soil carbon sequestration and nutrient supply (Bronick and Lal, 2005; Macdonald et al., 2012; Capra et al., 2015; Dazzi and Lo Pappa, 2015). Moreover, mine tailings offer poor soil structure, poor drainage and aeration, high salinity, and lack organic matter as well as some essential nutrients for plant growth (Wang et al., 2017; Bradshaw and Chadwick, 1980). With some exceptions, most vascular plants do not have the physiological traits to survive such soil conditions (Khasa et al., 2005; Khasa et al., 2002). Past research efforts thus focused on the addition of organic amendments to compensate for these deficiencies, with the objective of inducing some biotic soil functions and establishing the microbiome environment (Li and Fung, 1998). For examples, amendment with biosolids, composts, wood chips, municipal and pulp and paper sludge have all demonstrated some promising achievements in the pursuit of mine tailings reclamation (Young et al., 2015; Renault et al., 2007; Larchevêque et al., 2013; Guittonny-Larchevêque and Pednault, 2016; Asensio et al., 2013; Asensio et al., 2014; Sheoran et al., 2010).

Another technique used in mine tailings reclamation is the application of topsoil material (up to 50 cm depth). At the mine's location, topsoil amendments consist of the stripped, saved, and stockpiled upper layers of the soil (i.e., a mixture of wood debris, humus, organic soil, and some trace of mineral soil) once the initial vegetation is removed. There is an ongoing understanding from recent mining reclamation studies that topsoil salvage is a valuable amendment to increase the speed of the soil ecosystem recovery and function (Shrestha and Lal, 2011). Cooke and Johnson (2002) suggest that if the topsoil amendment is available, the post-mining reclamation can be achieved much faster with its use compared to alternatives. Similarly, Skousen et al. (2011) suggest that coal mine soil reconstruction in the Appalachian Forest ecosystem can also be achieved faster with topsoil amendments, including the addition of overburden weathered rocks to reclaim mine land. These authors explain that topsoil material, including logging residues, is a soil "living resource" inoculum that contains seed banks, organic matter, and microorganisms. Many studies have used topsoil as a reference (positive control) to weigh against other categories of amended mine tailings or to compare the vegetation response of the topsoil to other amended mine tailings (Bendfeldt et al., 2001; Shrestha et al., 2009; Boyter et al., 2009; Guittonny-Larchevêque and Pednault, 2016; Guittonny-Larchevêque et al., 2016). Importantly, while topsoil amendments show promising results for forest restoration, their availability can be limited, and challenges arise when large areas must be reclaimed. Moreover, its stockpile residence time and its manipulation with heavy equipment (soil compaction) can adversely affect its potential for ecological restoration. Indeed, the stockpile residence time may affect topsoil properties necessary for soil restoration, such as its biological activity (e.g., decreased mycorrhizal potential and earthworm population) (Abdul-Kareem and McRae, 1984; Sheoran et al., 2010; Shrestha and Lal, 2011).

In Canada, open-pit and underground mining operations are mostly conducted within the boreal shield ecozone (Government Canada, 2016; Brandt et al., 2013). In the province of Quebec, the mining industry is required to provide a reclamation plan and a financial guarantee to alleviate the environmental impacts of mining activities (Mining Act, chapter M-13.1). One of the reclamations objectives is to restore mining sites to a natural setting in harmony with the surrounding environment; in Canada, the surrounding environment is most often a boreal forest (MERN, 2017). Consequently, mining reclamation often aims to mitigate the environmental impacts of biodiversity loss, land fragmentation, and other ecosystems benefits associated with the boreal forest biome. As the first step of mine tailings reclamation, it is common practice for the mining industry to start the process by vegetating their mineral waste with agronomic herbaceous seeds (grasses and legumes), often referred to as "conventional seed mix" (Fields-Johnson et al., 2012; Sheoran et al., 2010). Furthermore, the cost, availability, physiological traits, and the ease of using agronomic herbaceous plants allow for rapid vegetation cover establishment and stabilization of the mineral surface.

Vegetation affects soil structure and chemistry through its root activities: root penetration capability, water extraction ability, soil aggregates, macroporosity formation, organic matter deposition (root turnover) and root exudates (Lafleur et al., 2013; Grevers and Jong, 1990; Ibrahim and Goh, 2005; Bardgett et al., 2014). In general, grasses (graminoids) are water demanding species producing large amounts of roots and fine roots (Robinson 1972). By growing within existing pores or through the soil matrix, roots exert compression and shear stress which in turn create macropores (Angers and Caron 1998). The stability of soil aggregates is largely dependent on organic materials (organic binding agents) such as polysaccharides, roots, fungal hyphae, and polymers (Tildall and Oades, 1982). Most grass species produce large above and belowground biomass that enrich tailings in organic matter (Cooke and Johnson, 2002). Grasses have the capacity of tailoring new shoots with their fibrous, adventitious roots (Freams, 1974). The fibrous roots activity of grasses changes soil properties by creating soil aggregates. Doormar and Foster (1991) observed that microaggregates (2-20 μm) were formed by perennial ryegrass (*Lolium perenne* L.) through roots associated to mineral particles, root gel, root fragments and microbial extra-cellular polysaccharides. In a controlled nursery condition, a study by Guittonny-Larchevêque et al. (2016) showed an increased benefit on mine tailing soil macroporosity related to perennial grass after two growing seasons. Despite the positive effects on soil structure, much of the literature has treated grasses as plant competition, limiting the woody species establishment (Sheoran et al., 2010; Carnevale and Montagnini, 2002).

The roots of agronomic herbaceous legumes can form symbiotic relationships with atmospheric nitrogen fixing *Rhizobium* bacteria that supply the plant with nitrogen (N). In return, legume decomposition enriches the soil with nitrogen (Ledgard and Steele, 1992). In general, most agronomic legume plants have tap roots for soil deep water intake (OMAFRA, 2017). Studies have shown that in a soil environment where there is a high amount of organic matter and nitrogen, the efficiencies of legumes as nitrogen suppliers can be inhibited. Conversely, in soils with low organic matter content and N stock, such as in mine tailing environments, legumes can be an effective agent for soil improvement and vegetative succession (Bradshaw 1997; Government of Saskatchewan, n.d.; Sheoran et al., 2010; Domingo and David, 2014; Maiti and Maiti, 2014).

However, in the context of mine tailing reclamation within the boreal forest biome, it is still unclear whether these agronomic herbaceous plants, grasses and/or legumes, are conducive or competitive towards the afforestation of shade intolerant boreal forest trees species.

This paper has two main objectives; first, we aimed to measure the effects of different agronomic herbaceous treatments (grass, legumes, or a mix of the two) and the topsoil treatment on selected physical and chemical soil properties, including macroporosity, bulk density, wilting point, organic matter, and pH. Second, we aimed to identify the effects of the different agronomic herbaceous treatments on the survival, height, root biomass, and foliar nutrition of three native planted boreal forest tree species and one willow cultivar. We used willow cuttings as one of the selected species because of their phytoremediation potential shown by previous studies (Mosseler and Major, 2017; Kuzovkina and Quigley, 2005; Boyter et al., 2009). We hypothesized that: (1) the grass treatment, with its greater roots volume and fibrous roots physiology potential, would have a greater effect on the mine tailing soil physico-chemical properties; (2) a higher nitrogen foliar concentration would be found in the legume treatments regardless of the tree seedling species and subsequently would translate in improved growth of the planted seedlings for the legume treatment.

2 Materials and Methods

2.1 Mine site

The *in situ* study site was located at an open pit gold mine site, south of Malartic, in the Abitibi-Témiscamingue region of northwestern Québec, Canada (48° 06'N, 78° 08'W). The study site is located within the balsam fir (*Abies balsamea* (L.) Miller) - paper birch (*Betula papyrifera* Marshall) bioclimatic domain (MFFP, 2003). The climate is characterized by long, cold winters, with short, moderately warm summers. For the 1981- 2010 period, mean annual rainfall is 985 mm with daily average temperature of -13.3, -17.2, -15.3 ^{0}C for December, January, and February and 14.4, 17.2, 15.8 ^{0}C for June, July, and August (Government of Canada, 2021). The area is included within the last glacial advance of Wisconsin glaciation region (Vincent and Hardy 1977) with a dominant grey luvisol soil type (Agriculture and Agri-Food Canada 2015).

The gold mine is still an active open-pit gold mine in 2021 with low grade ore and substantial deposition of mineral waste.

2.2 Experimental site description and design

The experimental site area (1 ha) is located on a flat surface of natural soil. Mine roads surround the north and west sides of the experimental site. Across the mine road at the west side (ca.75 meters from the experimental site) are patches of boreal forests consisting mainly of black spruce (*Picea mariana* (Miller) BSP (60-79 %)) and balsam fir (MFFP, 2021). Small slopes of waste rock berms surround the east side of the experimental site.

In May 2013, thickened tailings (50-70 % solids by mass at deposition, (Bussière, 2007)) were excavated from the gold mine tailings facility to fill the 1 ha area of the experimental site. Thickened tailings are a technology used by mining industries to decrease water consumption during transportation and deposition of the mine tailings (Robinsky et al., 1991). Thickened tailings are mainly composed of 86 % mineral particles (< 90 μm) and contain calcite and low sulfur content (~1 % S); the ore at the mine site consisted of a mineralized greywacke.

The experimental site was set up in a randomized complete block design, consisting of three blocks (Fig. 1A). Each block was further subdivided into five experimental plots (20 m × 15 m), each 5 meters apart. Each plot received one of the following treatments randomly applied within the block:

1) 100 % perennial Poaceae (hereinafter referred to as grasses)
2) 100 % perennial Fabaceae (hereinafter referred to as legumes)

3) An equal mix of grasses and legumes7

4) Topsoil amendment (details are provided in section 2.3.1)

Control (thickened tailings only)

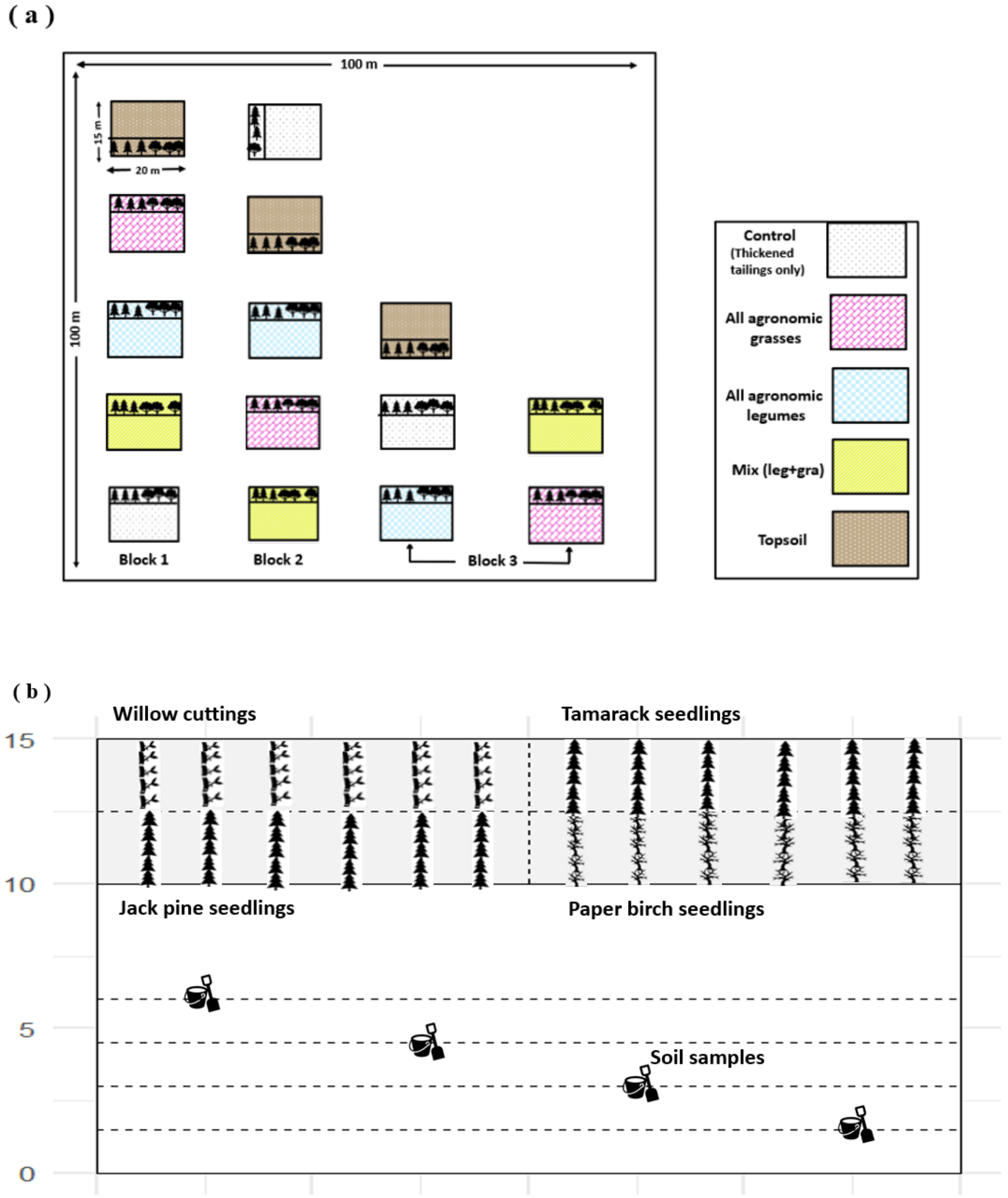

Fig. 1 (a) Experimental site layout of the treated plots in each block (set up in 2013, not drawn to scale). In 2015, one third of each treated plot were afforested with 30 seedlings of jack pine, tamarack, paper birch and willow (b) Treatment plot design (to scale) including 30 tree seedlings/species and soil samples' location.

2.3 Soil materials

2.3.1 Topsoil and thickened tailings (control treatment)

Prior to the gold mine exploitation, the existing forest stand was harvested, and the upper layers of organic soil (topsoil) and its underneath mineral soils were salvaged and stockpiled (for 30 to 36 months) in two separates 7-m-high piles (2.5H:1V slope) for future use. The topsoil stockpile consisted of the first 30-cm dark (20 % organic matter) soil layer (O- and A-horizons). The forest soil prior to mining was classified as a luvic gleysol (CSSC, 1998). In May 2013, the topsoil stockpile was excavated and spread over (2 cm thick layer) one plot (20 m × 15 m) of each block as showed in Fig. 1(A) and an example of one treatment plot design including the 30 tree seedlings/species and the location where the soil samples where pick up.

In June 2013, one soil sample (0-10 cm) of each topsoil treatment plot (1 plot per block, 3 blocks, n=3) and of each control treatment (thickened tailings only) (1 plot per block, 3 blocks, n=3) were collected for soil nutrient and metals analysis. Both topsoil and thickened tailings soil samples were oven-dried (at 50 ºC for 48 h), grounded and sieved (2 mm mesh) for total N (Dumas combustion method, CNS 2000; LECO) and organic C (thermogravimetric method, TGA; LECO) analyses. Organic matter concentration was computed as 1.72 × total C (Allison, 1965). Total cations and metals were extracted by HNO_3^- digestion and analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Total P was extracted using the Olsen method (Olsen et al. 1954) and analyzed by spectrophotometry. The initial topsoil and thickened tailings characteristics are summarized in Table 1.

Table 1

Characteristics of initial thickened tailings and topsoil

Parameters	Thickened tailings	Topsoil	Regulatory threshold [a] (Industrial lands)
pH	7.9 (0.4)	6.2 (0.2)	–
EC [b] (cS m^{-1})	8.3 (1.1)	6.6 (1.2)	_
OM [b] (%)	0.74 (0.67)	14.9 (1.5)	_
Total N (%) [c]	0.04 (0.01)	0.34 (0.02)	_
Total S (%) [c]	1.0 (0.1)	0.32 (0.05)	_
Total K (g kg^{-1}) [c]	7.6 (1.7)	3.5 (0.31)	_
Total Mg (g kg^{-1}) [c]	14.8 (0.3)	13.2 (1.4)	_
Total P (g kg^{-1}) [c]	0.68 (0.03)	0.65 (0.01)	_
Total Ca (g kg^{-1}) [c]	14.4 (2.6)	8.6 (0.20)	_
Total Al (g kg^{-1})	13.4 (0.4)	12.4 (0.62)	_
Total Fe (g kg^{-1})	32.0 (2.7)	28.6 (1.07)	_
Total Na (g kg^{-1})	0.54 (0.18)	0.30 (0.09)	_
Total B (mg kg^{-1})	2.4 (1.1)	3.2 (0.51)	_
Total As (mg kg^{-1})	5.1 (1.2)	6.8 (1.73)	50.0
Total Cd (mg kg^{-1})	0.11 (0.1)	0.21 (0.06)	20.0
Total Cr (mg kg^{-1})	194.0 (29.4)	197.4 (32.5)	800.0
Total Cu (mg kg^{-1})	53.4 (2.4)	45.1 (0.94)	500.0
Total Mn (mg kg^{-1})	442.1 (8.6)	459.6 (35.7)	2200.0
Total Ni (mg kg^{-1})	78.5 (12.5)	83.8 (11.0)	500.0
Total Pb (mg kg^{-1})	42.9 (22.7)	76.0 (15.8)	1000.0
Total Zn (mg kg^{-1})	83.9 (6.8)	105.0 (5.8)	1500.0

Note: Mean (SE); n=3. All values are expressed on a dry mass basis

[a] MELCC, 2021

[b] *EC:* Electrical Conductivity, *OM*: Organic Matter

[c] Plant macronutrients

Initial data from 2013

2.4 Plant materials

2.4.1 Agronomic herbaceous seeds

In June 2013, commercial agronomic herbaceous seeds (forage seeds) were obtained from Lanexco Inc. (Amos, QC). The company sowed the seeds using a manual sowing instrument according to the experimental design on the freshly hand-raked tailings. An annual nurse crop of barley seeds (*Hordeum vulgare*) was added to all seed mixes for soil stabilization, and to reduce weeds establishment (Espeland and Perkins, 2013). The legumes treatment plots included barley 10 % by mass, white clover (*Trifolium repens L.*) 45 % by mass, and bird foot trefoil (*Locus corniculatus*) 45 % by mass. Legume seeds used for the experimental site were off hand coated with soil bacterium *Rhizobium* inoculant to enhance biological nitrogen fixation. The grass treatment plots included barley 10 % by mass, ryegrass (*Lolium perenne*) 40 % by mass, redtop (*Agrostis gigantea*) 40 % by mass, reed canary grass (*Phalaris arundinacea* L.) 10 % by mass. The mix legumes/grasses treatment plots included barley 10 %, white clover 20 %, bird foot trefoil 20 %, reed canary grass 25 % and ryegrass 25 %. Seeding mixtures were applied at a rate of 100 kg ha^{-1}. Mineral fertilizer 8-32-16 (Nitrogen, Phosphorus, and Potassium) was applied once at 750 kg ha^{-1} rate to all treatment plots (except for the control and topsoil plots). Commercial MYKE® promycorrhizal inoculant (Premier Tech biotechnologies, Rivière-du-Loup) was also added to all treatment plots (except for the control and topsoil plots) in compliance with the manufacturer application chart.

2.4.2 Tree seedlings

In May 2015, two-year-old seedlings in containers (cells) of jack pine (*Pinus banksiana* Lambert) and tamarack (*Larix laricina* Du Roi) were obtained from the nearby Trécesson nursery of the Ministère des Forêts, de la Faune et des Parcs (MFFP). At the same time, two-years-old bareroot paper birch seedlings were obtained from the Berthier MFFP nursery. Willow cuttings (31 cm) were obtained from clones (*Salix miyabeana* Seemen, Sx64 clone) of a parent tree from a nearby nursery (La Morandière, QC, Canada). Tree seedlings were stored in a cold chamber until planting time. The initial average height (cm, measured above the soil) and the initial average diameter (mm, measured at 10 cm above soil) for the 450 seedlings/species are as followed: Paper birch (51 cm, 3.6 mm), Jack pine (34 cm, 4.6 mm), Tamarack (45 cm, 4.3 mm), willow's cutting (31 cm, 8.1 mm). In early June 2015, thirty seedlings (cuttings for Sx64) of each of the four tree species were planted by monospecific clusters (5 rows of 6 seedlings, planted 50 cm apart) at the end of each experimental plot (30 seedlings × 15 treatments × 4 species = 1800 seedlings) as per Fig. 1(b).

2.5 Soil measurements

2.5.1 Soil core sampling

In September 2016, after the removal of the top surface layer of grass or legumes material, using a double-cylinder soil sampler, two undisturbed soil cores (100 cm^3) of the 0-10 cm soil depth and two soil cores of the 10-20 cm soil depth were systematically collected on four parallel transects located at 1.5, 3.0, 4.5, and 6.0 meters from the bottom edge of the plot. Therefore, a total of 16 undisturbed soil cores (8 of 0-10 cm and 8 of 10-20 cm) per plot were collected for bulk density and macroporosity analyses. One bag of loose samples at the 0-10 cm and at the 10-20 cm depth soil layers were also collected for organic matter, permanent wilting point, and pH measurements. All samples were kept in a refrigerator (4 ^{0}C) until processed.

2.5.2 Soil physical and chemical properties

Soil bulk samples at the 0-10 cm and the 10-20 cm soil depth were randomly collected in each treatment plots. Samples were then air-dried and sieved (2 mm), and organic matter (OM) content was determined using the loss-on-ignition method (Ball 1964) for each soil depth sampled. Excess soil samples were used for pH determination (10 g soil: 5 ml demineralized water, 2w:1w) (Sobek et al., 1978). For permanent wilting point, extra samples were collected, dried and sieved (2 mm) before being saturated and brought to equilibrium with a pressure of -1500 kPa using a pressure membrane apparatus (Soil Moisture Equipment Corp., Santa Barbara, CA) and weighed (W_1). The samples were dried for 48 h at 105 ^{0}C and weighed again (W_2). The permanent wilting point (PWP) was estimated as:

$$PWP(\%\, w/w) = \frac{W_1 - W_2}{W_2} \times 100$$

For macroporosity and bulk density measurements, 16 undisturbed soil samples (5 cm diameter, 100 cm^3) collected in each of the fifteen plots were brought to saturation under vacuum and weighed (W_3, macroporosity). Samples were set on the porous surface of a sand-box apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), and brought to equilibrium at a tension of -10 kPa (field capacity). The samples were weighed again (W_4), then oven-dried (105 ^{0}C for 48 h) and weighed again for the last time (W_5). Macroporosity and bulk density (BD) were estimated as follows:

$$Macroporosity\ (\%) = \frac{W_3 - W_4}{100\ cm^3} \times 100$$

$$BD = \frac{W_5}{100\ cm^3}$$

2.6 Plant measurements

2.6.1 Above-ground measurements of tree seedlings

The 30 seedlings of each species planted in 2015 (Fig. 1) were measured in May 2016 and in October 2016 for height (cm), root collar diameter (mm) and seedling survival. The planted seedlings were marked as dead when they showed less than 5 % greenery (twigs and leaves included). The height difference (cm) was measured for all surviving seedlings from the base of the seedling to the top leader.

2.6.2 Root biomass of tree seedlings

In each plot, we unearthed seedlings of rows 2 to 4 of each species (three seedlings per species) chosen randomly on the row for root biomass measurement. Seedling roots were water washed to remove any extra dirt. Tangled roots from any other plants were removed. Seedling roots were cut from the main stem and placed in paper bags to dry for 48 hours at 60 ^{0}C and weighed for root biomass within the same day.

2.6.3 Foliar analysis

Foliar samples were collected on the experimental site at the end of the seedlings' growing season in 2016. Five to ten leaves were haphazardly sampled on each live stem of each plot and placed in brown paper bags for further foliar analysis. For the coniferous species, we took a random cluster of needles on different twigs and different twigs' height place on the seedling.

Leaves and needles from the same species and plot were combined, dried at 60 ^{0}C for 24 hours, then ground for analyses. Foliar samples were analyzed for total nitrogen and carbon (Carbon-Nitrogen-Sulphur (CNS) combustion by Elementar CHNS), total cations and metals (Nitric-Hydrochloric Acid Digestion and inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis method) and moisture content (% weight loss method). Foliar analyses and the initial soil analyses (before treatment) were conducted at the Forest Resources & Soil Testing laboratory (FoReST Laboratory) of Lakehead University (Thunder Bay, ON, Canada).

2.7 Statistical analyses

The additive effects of agronomic herbaceous treatments and soil depth (0-10 cm and 10-20 cm) on soil properties were analyzed using a two-way analysis of variance (ANOVA), followed by post-hoc comparisons with Tukey's range test.

The effects of herbaceous treatments on the root biomass, height and diameter increments of seedlings were estimated with linear mixed models, with plot as a random effect since the growing environment might be more similar between seedlings located in the same plot. Height increments were log(x+1) transformed to improve normality and homoscedasticity of the regression residuals. On the other hand, the effect of herbaceous treatments on seedling survival was estimated with a mixed effects logistic regression, with plot as a random effect. Separate models were fitted for each seedling species. All mixed effects models were fitted with the lme4 package (Bates et al., 2015) in R (R Core Team, 2019) version 3.6.2 and the emmeans package (Lenth, 2020) was used to perform post-hoc comparisons between treatments, with a Tukey adjustment applied for multiple comparisons. Foliar nutrient concentrations were compared across treatments with a one-way ANOVA, followed by post-hoc comparisons with Tukey's range test. We used a significance threshold of α = 0.05 for all analyses. In addition, principal component analyses using the FactoMineR package (Lê et al., 2008) were applied to the soil properties and foliar analysis results to visualize the multivariate structure of the data.

3 Results

3.1 Soil physico-chemical properties

The topsoil treatment showed a significantly higher content of organic matter, lower bulk density and significantly higher wilting point compared to all other treatments, whereas macroporosity did not differ among all treatments (Table 2). No significant differences in soil physico-chemical properties were found between the control and the grasses, legumes, and mix treatments. For the pH, the only significant difference occurred between the topsoil and control treatments (Table 2). All soil properties except macroporosity also show significant differences by soil depth (0-10 cm and 10-20 cm), however, among all the treatments, the topsoil treatment showed the highest difference between the two soil depths in regards of the physico-chemical soil properties (except for the macroporosity) (Fig. 2).

Table 2

Effects of the herbaceous treatments and depth on the mine tailing soil properties

	pH -	Organic Matter %	Bulk Density g/ cm^3	Macroporosity %	Wilting Point %
Treatment					
Control	7.37 (0.07) a	0.31 (0.12) b	1.36 (0.02) a	5.47 (0.60) a	0.65 (0.28) b
Grasses	7.36 (0.07) ab	0.60 (0.12) b	1.44 (0.02) a	4.63 (0.80) a	0.42 (0.05) b
Legumes	7.32 (0.05) ab	0.45 (0.07) b	1.36 (0.01) a	5.18 (0.58) a	0.43 (0.13) b
Mix	7.30 (0.08) ab	0.47 (0.11) b	1.34 (0.01) a	4.41 (0.32) a	0.59 (0.10) b
Topsoil	7.05 (0.10) b	9.14 (1.84) a	1.11 (0.07) b	6.48 (1.04) a	6.58 (1.50) a
Depth (cm)					
0-10	7.20 (0.05) B	2.92 (1.32) A	1.29 (0.02) B	5.82 (0.52) A	2.39 (0.99) A
10-20	7.35 (0.05) A	1.46 (0.63) B	1.35 (0.03) A	4.65 (0.35) A	1.09 (0.38) B

Mean (std. error) values shown for each property. Values followed by the same letter show no significant differences at $p < 0.05$ (two-way ANOVA followed by Tukey's range test). The organic matter content and wilting point were square root transformed for the ANOVA ($n = 5$ / treatment).

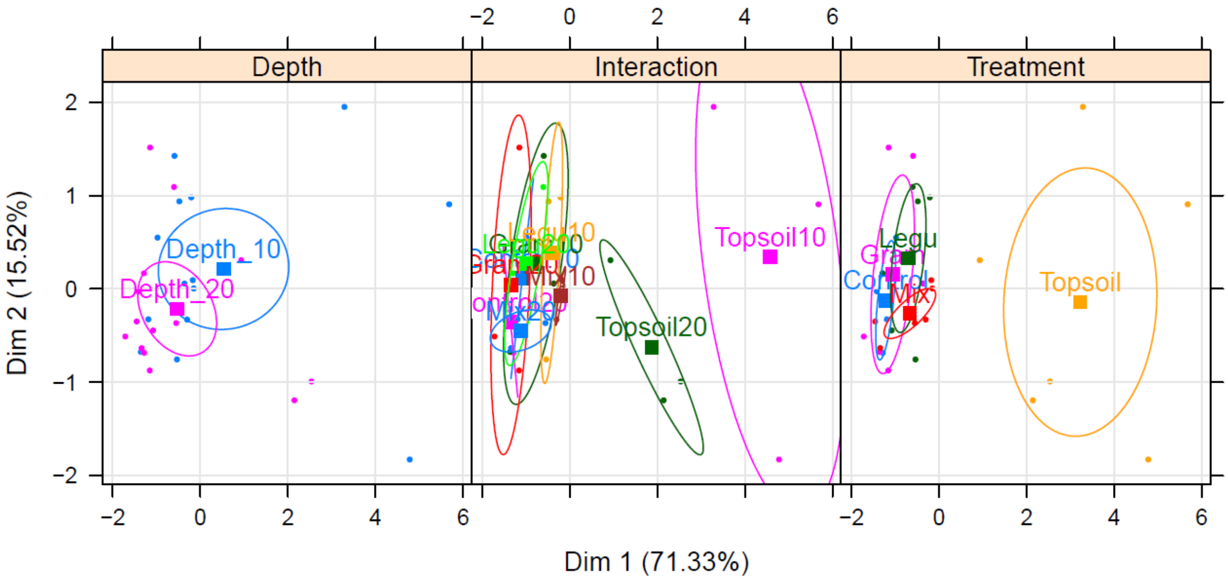

Figure 2. Principal component analysis of the physical and chemical soil properties measured for the Grasses (Gram), Legumes (Legu), Mix, Topsoil and Control treatments for each soil depth (0-10 cm and 10-20 cm). The Interaction panel shows the interaction of treatment and soil depth.

3.2 Seedling survival

Across all species, 626 of the 1800 planted seedlings were dead after two growing seasons in 2015-2016 (Table 3). The ranking of the treatments from the highest to lowest observed survival rate (irrespective of tree species) were as follows: topsoil > grasses > mixed > legumes > control (Table 3).

Figure 3 indicates the mean probability of survival estimated for tree seedlings within each treatment. For jack pine, a significant difference in the mean probability of survival was found between the grass, legumes, and control treatments (grasses > legumes > control). For tamarack seedlings, there was no significant difference among any of the treatments (except for the control treatment). For the willow and paper birch seedlings, results showed no significant difference in the survival probability between the control and legumes treatments. Furthermore, the probability of survival of the tree seedlings, for all species except jack pine, did not vary significantly between the topsoil treatment and the herbaceous (legumes, grasses, and mix) treatments.

Table 3

Number of dead seedlings (out of 90 seedlings per species and treatment combination

	Jack pine	Tamarack	Willow	Total

	Paper birch				
Treatments					
Topsoil	7	5	1	7	20
Grasses	12	25	16	18	71
Legumes	40	54	9	30	133
Mixed	22	29	12	37	100
Control	77	83	73	69	302
	158	196	111	161	626

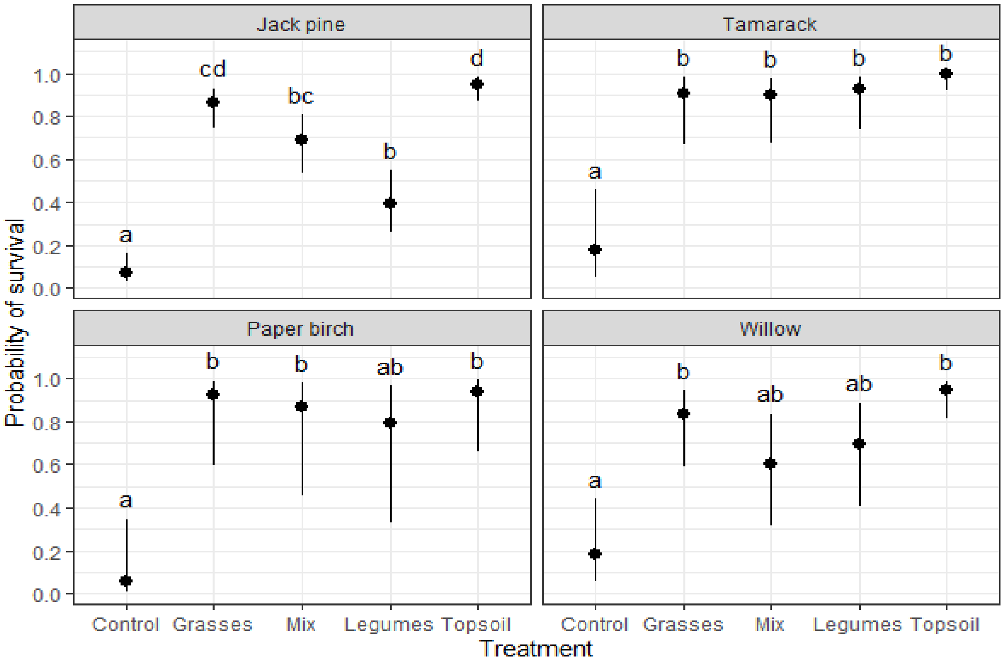

Figure 3. Mean probability of survival (with a 95 % confidence interval) of the tree seedlings of each species within each treatment after two growing seasons (n = 90 seedlings per treatment and species combination). Shared letters indicate no significant difference (α = 0.05).

3.3 Seedling growth

3.3.1 Seedling roots biomass

After two growing seasons, the dry root biomass of jack pine seedlings showed no significant difference among treatments (Fig. 4). For tamarack seedlings, only the topsoil treatment significantly differs from the control treatment, with a 89 % increase in root biomass. The willow seedlings (cuttings) had a significantly greater (over 100 % increase) root biomass in the legumes treatment compared with the topsoil treatment. However, the control treatment did not significantly differ from the legumes treatment. The roots of paper birch seedlings were very similar to the agronomic herbaceous plant roots and extremely tangled together. Therefore, were unable to accurately separate the paper birch roots from of the agronomic herbaceous roots and did not include paper birch in this analysis.

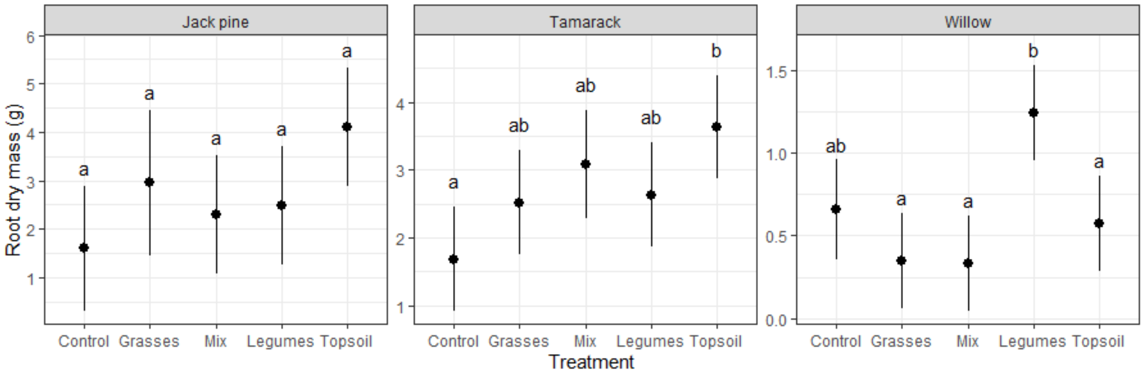

Figure 4. Mean root biomass (with a 95 % confidence interval) of the jack pine, tamarack and willow tree seedlings after two growing seasons within each treatment (n = 90 seedlings per treatment and species combination). Shared letters indicate no significant difference (α = 0.05).

3.3.2 Seedling height growth

After two growing seasons, the seedling diameter increment did not significantly differ among treatments for any species planted (results not shown). However, the aboveground height increment of the willow seedlings was significantly greater in the legumes treatment compared to the topsoil treatment (Fig. 5). No significant differences for the height increments were found in any other tree species between the different treatments.

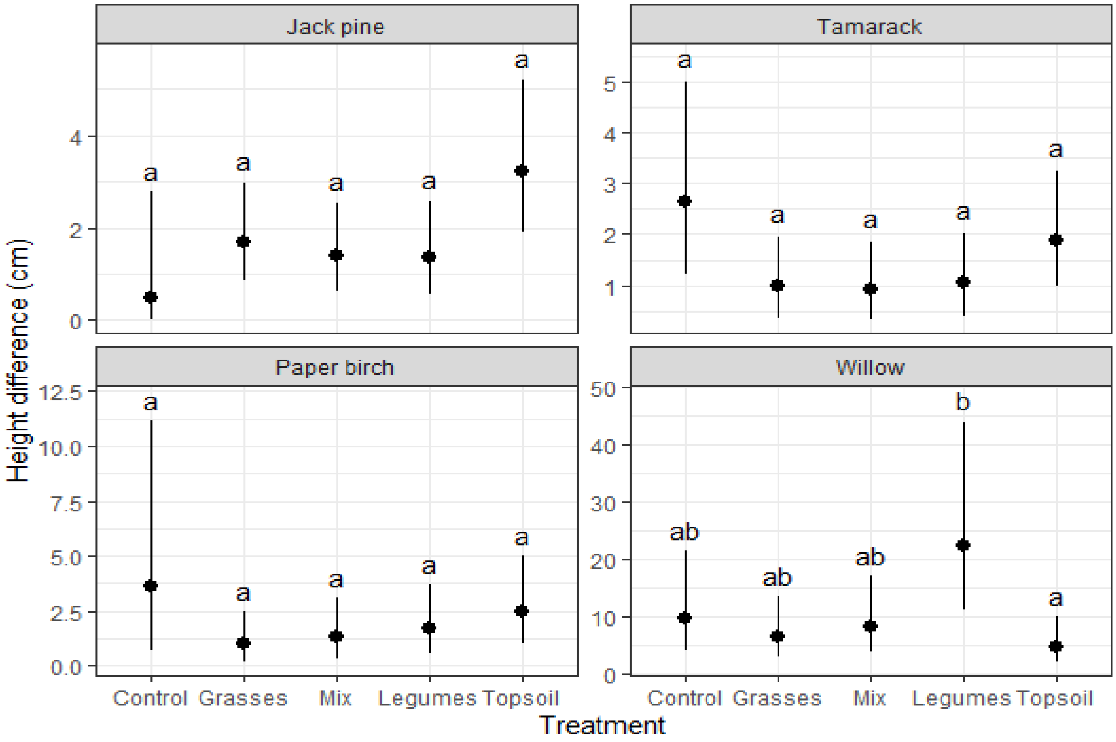

Figure 5. Mean height increment (with a 95 % confidence interval) of the tree seedlings of each species within each treatment after two growing seasons (n = 90 seedlings per treatment and species combination). Shared letters indicate no significant difference (α = 0.05).

3.4 Foliar analyses

3.4.1 Heavy metals

After four years of weathering on the experimental site, no heavy metal accumulation of arsenic (As), cadmium (Cd), cobalt (Co), lead (Pb), nickel (Ni), and thallium (Tl) were found in the leaf tissues based on foliar analysis, regardless of the tree seedlings species and the applied treatments (Appendix 1). Figure 6 indicates that the foliar concentration of calcium (Ca), copper (Cu), and zinc (Zn) showed a distinct pattern between the deciduous (willow and paper birch) compared to the coniferous needles (jack pine and tamarack). Furthermore, a higher portion of minerals are translocated to the leaves of the paper birch compared to the other species (Fig. 6).

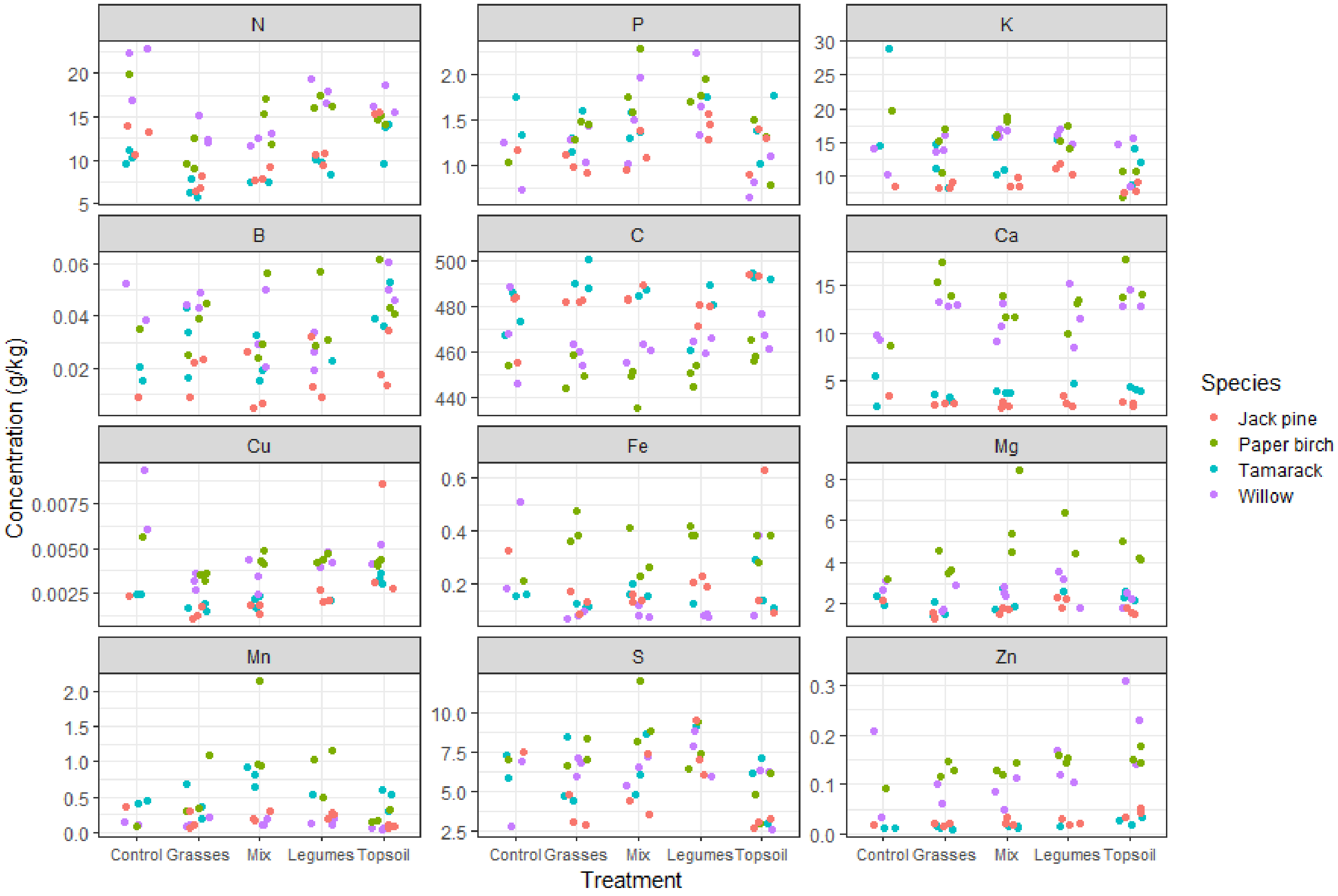

Figure 6. Foliar mineral concentration of the tree seedlings species. Elements N and C are in % and all other element in the figure are in g/kg. Foliar analysis of elements; Al, Cr, Mo, Na, Ni, Sr, are excluded because data were below the detection limit (bdl). No available data for chloride. Each point corresponds to the pooled leaves samples from one plot (see text for details).

3.4.2 Foliar nitrogen concentration

For the jack pine seedlings, foliar nitrogen concentration results indicate a significant difference between the legumes and the grasses treatment (over 50 %). However, the topsoil treatment had the most significant difference from the other treatments except with the control treatment; topsoil > legumes > mix > grasses (Fig. 7).

For the tamarack seedlings, foliar nitrogen concentration was not significantly different between the topsoil and the control, and there were no significant differences between the grass, legumes, and mix treatment (Fig. 7).

For both willow and paper birch, foliar nitrogen concentration results indicate no significant difference between the topsoil and the control. For the paper birch, but not the willow, there is a significant difference between the grasses and legumes treatment.

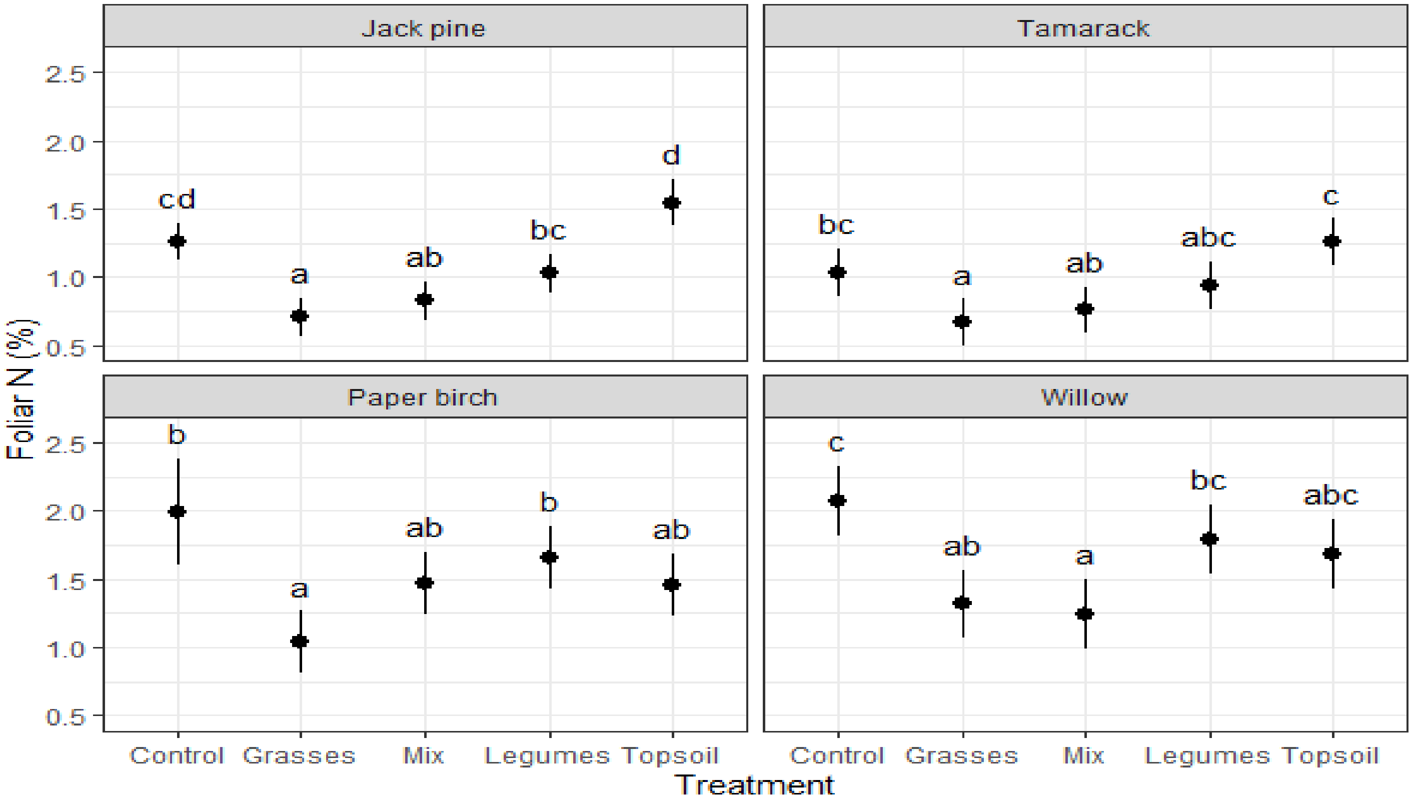

Figure 7. Mean foliar nitrogen concentration of the tree seedlings by species and treatment (with a 95 % confidence interval). Shared letters indicate no significant difference (α = 0.05).

4 Discussion

4.1 Soil physico-chemical properties

The results of this study confirm a significant difference for most of the mine soil physico-chemical properties (except the macroporosity) in the upper soil depth 0-10 cm compared to the 10-20 cm depth for all treatments considered. This concurs with similar studies (Filcheva et al., 2000; Shrestha and Lal, 2011; Shrestha and Lal, 2007, Liu et al., 2017).

The topsoil treatment which has a soil richer in organic matter and nutrients (Table 1) showed significant differences for most soil physico-chemical properties (except the macroporosity) compared to the grasses, legumes, mixed and control treatments (Table 2). Among the agronomic herbaceous plant treatments (grasses, legumes and mixed), we were unable to confirm our first hypothesis that the grass treatment with its greater roots volume and fibrous roots, would have a greater effect on the mine tailing soil physico-chemical properties. Guittonny-Larchevêque et al. (2016) conducted a study on the effect on soil macroporosity of metalliferous mine tailing using agronomic graminoids species in a controlled environment as well as *in situ*. The research has shown that within a controlled environment, the perennial grass has affected the mine tailing soil macroporosity with a significant difference even after two months growth. However, the study did not confirm any significant difference of the mine tailing soil macroporosity for the *in situ* experiment even after three growing seasons of the perennial grass.

Different factors may explain our results: there could have been unequal spreading and growth of the agronomic herbaceous grass, or the agronomic herbaceous grass species used for this research may not be optimal species for the mine tailings soil conditions. Further research is required to verify the efficiency of the agronomic herbaceous grass on the physico-chemical properties in an *in situ* mine tailing environment. For example, studies using indigenous grass species versus agronomic herbaceous grass, or different varieties of agronomic herbaceous grass with a longer pedogenic process time would be worth investigating.

4.2 Foliar nutrition

4.2.1 Foliar heavy metals

The pH of the tailings in our experimental site ranged from 6.6 to 7.7 for all included treatments. A low soil pH increases the solubility of most heavy metals, making metals available for plant uptake (Masindi and Muedi, 2018; Hodson, 2012). The mine tailings' neutral pH may have been a contributor in limiting heavy metal solubility and accumulation in plant tissue. We found no heavy metals or traces of heavy metals below the instrumentation detection limit in the foliage of any of the seedlings, regardless of applied treatments and despite two years of weathering (Appendix A). However, fast growing trees such as poplars, willows, and paper birch are known to have high rate of nutrient uptake (Lafleur et al., 2013) and the functional traits of paper birch may make them more sensitive to higher concentration of metal and metalloids as shown in Fig. 6.

4.2.2 Foliar nitrogen

Nitrogen is a macronutrient required in much higher quantities than micronutrient elements. Tree seedlings (except willow) that originated from nurseries may have been supplied with optimal nitrogen and soil conditions. Further, only the surviving seedlings were sampled, likely representing those strongest and best able to adapt in harsh environmental conditions. This may have created a bias in the results for the nitrogen foliar analyses, especially for the control treatment where mortality was high.

4.2.3 Foliar phosphorus

Phosphorus is an important nutrient for photosynthesis processes (Plaxton and Carswell, 2018). The phosphorus foliar analysis did not show any significant statistical difference for all treatments regardless of the seedling species. These results may be due to a sufficient concentration of phosphorus for plant growth even in the control treatment (mine tailings only) at the initiation of the experiment (Table 1). However, the mean phosphorus concentration is consistently slightly higher in the legumes treatment, suggesting a possible different association with plant roots and living organisms in this treatment.

4.2.4 Foliar potassium

The initial soil analysis (Table 1) indicates that the potassium was at a higher concentration in the control plot (7.6 g kg^{-1}) compared to the topsoil soil (3.5 g kg^{-1}). The potassium foliar analysis (Fig. 6) showed no significant difference in the leaf or needle content among the different treatments within the same seedlings' species, except for the paper birch and jack pine (Appendix A). However, it did not translate in height difference or root biomass increase across the treatment types suggesting that this nutrient was not one of the key element to account for the plant's growth (Fig. 5).

4.3 Seedling survival, seedlings root biomass and seedling height growth

Soil conditions of mine tailings are highly variable; depending on the season, they can have dry soil surfaces, or wet stagnant surfaces, or saline crusted surfaces. All four tree seedling species – jack pine, tamarack, paper birch and willow – were selected based on their ability to grow under such variable conditions. Their pioneer qualities, tolerance to full sunlight and some drought, and their capability to populate oligotrophic and hypoxic sites are key to their resiliency (USDA, n.d.; Kuzovkina and Quigley, 2005). Nevertheless, although these species (jack pine, tamarack, and paper birch) and the willow (Asian cultivar) may well be equipped with genotypes to sustain boreal forest climate and edaphic soil conditions, the study was able to identify which tree seedling species would be the most capable to survive and grow among agronomic herbaceous plants on a substrate such as mine tailing soil.

Jack pine

Among the four planted species, regardless of the applied treatment, results show that the jack pine seedlings had the lowest survival rate. The reasons for the poor jack pine survival on the mine soil could be related to the high mine tailing pH (ranging from 7.05 to 7.37) of this study. South (2016) conducted a review of studies on the optimum pH for growing pine seedlings. Based on the data, this author conclude that optimal pH would range from 4.5 to 5.0 for growing most common pine species (including jack pine) at sandy bareroot nurseries. Burger et al. (2007) conducted a study to evaluate seedlings responses of loblolly pine hybrid (*Pinus rigida* Mill. X *Pinus taeda* L.) on five different mine overburden mixes. Their results indicated that the loblolly pine hybrid grew much better on mine spoil that contained a high proportion of sandstone spoil rather than siltstone, and at a pH lower than 6.5. The authors indicated that pine growth decreased linearly as the pH increased within the 5.7 to 7.1 range.

Another reason that may also explain our results is that the mine tailing soil texture used for this study was not favorable to jack pine. Jack pines require a sandier soil with low organic matter (mineral soil) (South, 2016; USDA, n.d.). The combined effect of inadequate soil pH and soil texture may be to the reason for the jack pine's poor survival. The combined effect of inadequate pH and soil texture may have overshadowed any of the possible benefits of the applied treatments on the root biomass and seedling height (no significant difference within all applied treatment, Figs. 4 and 5).

Within the applied treatments, the probability of survival of jack pine showed a significant difference between the grasses and legumes treatments, but not between the grasses and the topsoil treatments. Since this was not translated by in any significant difference for the jack pine's growth or root biomass increases, it is difficult to explain these results without further research. Another avenue would be to study the interaction between grasses and jack pine seedlings at the rhizosphere level for possible mycorrhizal facilitation (Toju and Sato, 2018) between the two species.

Tamarack

Across all treatments, tamarack seedlings showed less mortality compared with paper birch, willow, and jack pine seedlings (Table 3). Tamarack seedlings showed a great resistance in surviving the mine tailing environment after two growing seasons. However, it was within the topsoil treatment that the tamarack seedlings performed best (only one of 90 initial tamarack seedlings was reported dead). The height increment and root dry mass of tamarack seedlings were unaffected by the different applied treatments (except for topsoil > control). Similarly, the probability of survival also seems unaffected by the treatment type (except that it was significantly lower in the control treatment than all other treatments). Garbarino and al. (2010) used the deglaciation of the Ventina glacier as a surrogate to study spatial and temporal chronosequence establishment of the larch (*Larix decidua* Mill). Results showed that the age of trees was highly influenced by the terrain age or time since deglaciation and not by plant cover. Since the tamarack seedlings were planted in new mineral soil i.e., a soil that is "young soil" in the context of a chronosequence, their survival and growth may also not be affected by the plant cover (grass, mix or legumes), consistent with the results found in Garbarino et al. (2010) (Fig. 3).

Paper birch

Paper birch seedlings on the experimental site did not perform well. It is a species sensitive to harsh environmental conditions and pollution (USDA, n.d.). The control treatment (thickened tailings only) in which we planted 90 paper birch seedlings showed the highest mortality and a significant difference in survival probability compared to the topsoil treatment (Table 3, Fig. 3). Those results may be attributed to a complexity of contributing factors such as low soil aeration and built-up salinity crust (field observations, results not shown), which may in return impede plant root development (Guittonny-Larchevêque et al., 2016). However, the main contributing factor that would explain our results is the low organic matter concentration found in the control treatment; 0.31 % compared to 9.14 % OM for the topsoil treatment (Table 2). Normal soil OM averages between 2 to 10 % OM (Bot and Benites, 2005); the control treatment had very little OM, therefore little humic substances as well. Humic substances play an important role in binding metal(loid)s (Gagnon et al., 2020), as well as improving saline soil properties (Ouni et al., 2014). The combined effect of high pH with low humic substances may explain our results. The study of Gagnon et al. (2020) also demonstrated higher nutrient concentrations in paper birch plant tissue growing on gold mine tailing versus natural boreal forest soil (like topsoil) and corroborates our results. Our experiment was based on a very short study period (only two growing seasons); however, we note that on the scale of many generations, trees of the *Betula* genus can develop genetic adaptations to grow in a metal-contaminated region (Kirkey et al., 2012).

Willow cultivar

For the willow cultivar, the highest mortality was observed in the control treatment and the lowest in the topsoil treatment (Table 3). However, the probability of survival (Fig. 3) showed no significant difference between the control, mix and legumes treatment, implying the ability of this species to survive within a soil that contains little organic matter, such as in the control and to a lower extent within the mix and legumes treatment (Table 2). Willows are renowned to survived in harsh conditions and have been a preferred species in many phytoremediation projects for their abilities to survive in heavy metal spoils, organic contaminants, and polluted wetland areas (Kuzovkina et al., 2004a; Kuzovkina et al., 2004b; Yergeau et al., 2014). A study from Doty et al. (2009) have isolated beneficial nitrogen-fixing (diazotrophic) endophytes bacteria in the stem of black cottonwood (*Populus trichocarpa*) and willow (*Salix sitchensis*) that may explained the ability of these tree species to survive in limited nitrogen medium. Our study showed the willow species were able to survive and grow in all treatments including the control treatment and these endophytes microorganisms may have play a role that would explain our results. However, the willow cultivar in this study showed a significant higher root biomass in the 100 % legumes treatment compared to all the other treatments suggesting two possibilities.1) The willow may have greatly benefited from the added nitrogen provided by the nitrification cycle processes of the legumes' decomposition. Jefferies *et al*. (1981) indicated that legumes are capable of nitrogen accumulation at a rate of approximately 100 kg ha^{-1} y^{-1} on derelict land, in a form which can be mineralized. The rhizospheres of the willow cultivars may be more capable to use this additional nitrogen whereas the other studied tree species grown in the legumes treatment may not have the same genetic background to efficiently use the added nitrogen; or other parameters, such as soil pH, may have impeded the uptake of the additional nitrogen from the legumes' nitrification processes.

2) Alternatively, the legumes' microorganism rhizosphere (including diazotrophic endophytes bacteria but not limited to *Rhizobium* sp.) provides a beneficial environment in which the willow unique genotype tree species is capable to harbor, exploit or interact to its full benefits (Doty et al., 2009; Mastretta et al., 2006; Afzal et al., 2019; Belimov et al., 2015; von Wuehlisch, 2015).The results of this study suggest this may be an important avenue for future research. Not all legume species act the same regarding nitrogen transfer, nor do all willow species have the same ability to interact efficiently with the legumes. However, within the realm of the study parameters, the willow cultivar and the legumes treatment showed the most promising results for improving the soil conditions and may facilitate an easier natural establishment of other plant species.

5 Conclusion

This study demonstrates a positive interaction between the legumes treatment and a willow cultivar despite only a few years of growth on anthropogenic gold mine tailings. Further investigation is required to determine if the increased willow biomass is associated with the nitrification benefits in the legumes treatment or the diazotrophic microorganisms enabled by the legumes' rhizosphere environment.

The study did not show soil contamination with heavy metals after three years of weathering conditions. However, monitoring and further analysis would be needed to verify if the concentration of toxic chemicals may accumulate in the plant tissue. This study did not show a clear distinction between the grasses, legumes, and mixed treatments in terms of the soil physico-chemical properties. For the seedling growth, the willow was the species that benefitted most from the legumes treatment. The foliar analysis shows that nitrogen may be appropriated by the agronomic grass, leaving little in the rhizosphere area for seedlings. Depending on the mine reclamation objective, the combination of the willow clones with the legumes treatment may be a good first step to facilitate the natural succession of pioneer tree establishment.

Further studies at the site will evaluate the effects of grass and legumes treatments on the mine tailings' plant biodiversity colonization on a mine tailing in situ conditions, as well as the microclimate and its potential for tree seed germination.

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The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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