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Organic Matter Additions for Improved Revegetation of Arsenic-Rich Waste Rock with
Planted Boreal Conifers: A Three-Year *In Situ* Monitoring Study

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Abstract

Mining waste creates challenging soil conditions that hinder tree establishment for boreal
forest restoration. This study investigated the effects of adding topsoil or ramial chipped
wood (RCW) on the physicochemical properties of waste rock and the growth and survival
of planted native tree species. An randomized-block setup with four treatments and

repetitions was established on a gold mine site in western Quebec, Canada in 2018, and planted with *Pinus banksiana* and *Abies balsamea*. Results demonstrated that topsoil addition significantly improved height and diameter growth, aerial and root biomasses, survival, and nutrient uptake (N, P, and S) in conifer seedlings, concomitantly to increased water content, decreased pH, and elevated nutrient concentrations in the substrate. However, multivariate analysis revealed that these improved soil conditions alone did not determine the survival and growth of conifer seedlings. In contrast, the application of RCW-based treatments had no discernible impact on the growth and survival of the planted trees. Additionally, topsoil addition effectively reduced the concentration of potentially phytotoxic elements in soil and needles, particularly arsenic. The total arsenic concentration in the mineral substrate (84.1 to 507 $\mu\text{g.g}^{-1}$) emerged as a growth-limiting factor for both conifer species. The total concentration of arsenic in the waste rock correlated positively with arsenic accumulation in the tree needles, indicating potential root uptake of this element. This study emphasizes the significance of addressing arsenic availability during reclamation efforts at mine sites. Nonetheless, further research is required to determine the phytotoxic thresholds of arsenic on conifers and its potential metabolic effects.

Keywords: aspen ramial chipped wood, topsoil, mine waste afforestation, *Pinus banksiana*, *Abies balsamea*, arsenic phytotoxicity.

1. Introduction

The mining industry generates a large quantity of mining waste, including waste rock, which refers to the non-ore or rocky mineral material that is removed during mining operations (Bian et al., 2012). The rehabilitation of mine sites is essential to allow the reestablishment of the forest ecosystem and the recovery of the original ecological services and biodiversity (Bussière and Guittonny, 2020; Macdonald et al., 2015; Tongway and Ludwig, 2011). However, there has been limited research specifically targeting the reforestation of waste rock in the boreal context (Babi et al., 2018; Bouchard et al., 2018; Larchevêque et al., 2015).

Efforts to rehabilitate waste rock can be limited by soil conditions that constrain the establishment of trees (Bouchard et al., 2018; Macdonald et al., 2015; Tongway and Ludwig, 2011). Among these conditions, the absence of organic matter often implies a low availability of micro and macro nutrients for plants, a low water retention capacity of the soil, and significant temperature variations during the period of plant growth (Bradshaw, 1992; Lal, 2001; Lal, 2020; Macdonald et al., 2015). Adding organic matter to waste rock can thus help afforestation.

In addition, the mobility of certain potentially phytotoxic elements contained in waste rock, such as arsenic, can reduce the chances of survival and growth of planted trees (Bradshaw, 1992; Macdonald et al., 2015), and can also result in implications for the food chain in the ecosystem (Peralta-Videa et al., 2009). Recent research has pointed towards the complex role that organic matter plays in the mobility of arsenic in mine wastes (Coudert et al., 2020; Wang and Mulligan, 2009). The presence of organic matter has been found to promote arsenic mobilization under certain conditions.

Conifer species in the boreal forest are not all equally sensitive to poor soils with low organic matter and fast drainage like waste rock. Some species, such as jack pine (*Pinus banksiana* Lamb.), are pioneer species that can survive and grow on mainly mineral substrates, despite low water retention conditions (Chrosciewicz, 1974, 1990; Weber et al., 1987). Other late-successional boreal conifers, such as balsam fir (*Abies balsamea* (L.) Mill.), are shade-tolerant and can survive and grow in forest conditions with high canopy cover (Asselin et al., 2001; Bergeron, 2000; Chen and Popadiouk, 2002). This canopy cover often implies a soil rich in organic matter and high water retention favorable to the development of these species (Gómez-Aparicio, 2009; Keenan and Kimmins, 1993; Wessman et al., 1988).

Several restoration approaches are possible on mine waste rock, including revegetation using an ameliorative approach (Bussière and Guittonny, 2020; Huebner et al., 2022; Prach et al., 2001; Prach and Hobbs, 2008; Singh et al., 2002). The ameliorative approach in a boreal forest context can consist of planting trees of native species combined with improving the substrate using organic matter, such as ramial chipped wood (RCW) or topsoil, as an amendment, mulch, or surface layer (Bussière and Guittonny, 2020; Gastauer et al., 2018; Larcheveque et al., 2013; Maiti et al., 2021; Taurines, 2019).

Topsoil is a material that is sometimes available from borrow pits near mine sites in the boreal region or conserved after on-site excavations associated with mining works. Topsoil is the fertile portion of the soil profile containing organic matter, which, when mixed with a mineral substrate, can create favorable conditions for pedogenesis and lead to the formation of a soil (Brevik and Lazari, 2014; Hugron et al., 2011). However, the cost of this material is often too high to restore all the waste rock accumulation areas at large-scale

mine sites. Moreover, this approach often involves borrowing soils from other ecosystems, which can lead to their disturbance.

Ramial chipped wood can also be used as part of mine restoration projects. It is a by-product of pruning cuts and is available at lower cost. RCW is a fresh or slightly decomposed organic material (Lemieux et al., 2000). It is sometimes laid out as mulch or mixed with mineral soil. RCW is used in agricultural and horticultural work, but very little mention has been found in the scientific literature for the revegetation of mine sites (Barthes et al., 2010; Germain and Eng, 2007; Lemieux et al., 2000; Taurines, 2019). Also, little research has focused on the most desirable way to use it as an organic matter input to promote soil formation (Fortin Faubert et al., 2021; Lemieux et al., 2000; Soumare et al., 2004).

The main objective of this four-year study was to investigate the effects of an organic matter supply in the form of topsoil or RCW (as mulch or directly mixed with waste rock) on *in situ* survival and growth (height, diameter, and biomass) of *A. balsamea* and *P. banksiana* individuals planted on waste rock, while determining the physicochemical conditions of the substrate that contributed to their growth and survival.

Three main hypotheses were formulated: (1) the supply of organic matter on waste rock increases the growth in diameter, height, and biomass, as well as the survival of seedlings of *A. balsamea* and *P. banksiana*, as compared to waste rock alone; (2) the supply of organic matter on waste rock improves the microclimatic conditions (volumetric water content and temperature) of the soil as compared to waste rock alone; and (3) the addition of organic matter to waste rock improves the chemical conditions (availability of nutrients, reduction of phytotoxicity risks) of the soil as compared to waste rock alone.

2. Materials and methods

2.1. Study area

The study was conducted on a mine site, located in Abitibi-Témiscamingue, Quebec, Canada (Fig. 1). The mine site was an underground gold mine that overlapped the Cadillac-Larder Lake fault zone. The mine site closed in 2018. The bioclimatic domain of the site is boreal mixedwood forest; more specifically, it is located in the balsam fir–white birch forest ecosystem (Québec, 2019). Climate records from 1981 to 2010 indicate an average annual temperature of 1°C, with average annual minimum and maximum values of −5°C and 7.1°C, respectively; this data was collected from the weather station closest to the site, at Mont-Brun, less than 40 km from the mine site. The mean monthly maximum and minimum values are −24.3°C in January and 23.2°C in July. The average annual precipitation is 707.7 mm of rain and 281.2 cm of snow (Québec, 2010).

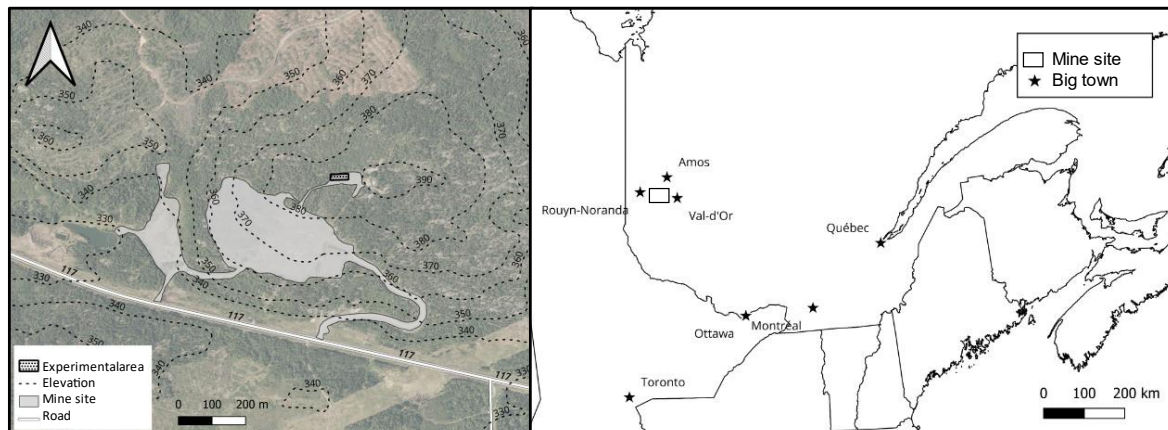


Fig. 1 On the left, the site map shows the position of the experimental setup, its proximity to the forest adjacent to the site, and the elevation. On the right, a rectangle indicates the approximate position of the mine site in the province of Quebec

2.2.Experimental setup

2.2.1. Setup construction

The experimental setup was installed on August 22nd, 2018, on a waste rock deposit at an elevation of 393 m. The setup used a fully randomized block design with four blocks (repetitions). Each block included four cells measuring 5 m by 5 m, separated by a 1 m interval. Each cell included one of the four treatment types. The position of the treatments in each block was determined randomly (Fig. 2).

The treatments were as follows:

- WR: waste rock from the mine site, spread without slope;
- RCW/WR: fresh ramial chipped wood deposited as mulch to a thickness of 2 cm above the waste rock;
- RCW×WR: fresh ramial chipped wood deposited as mulch to a thickness of 2 cm above the waste rock, then mixed with the waste rock using the crenellated edge of an excavator bucket manipulated by a mechanical shovel over 15 to 20 cm deep in the mineral material; and
- TS: topsoil from an excavation company close to the site, deposited on the surface of the waste rock to a thickness of 10 cm.

The RCW was composed of 100% freshly cut trembling aspen (*Populus tremuloides*) twigs (chips varying in length between 2 and 4 cm; supplied by Émondage Abitibi Inc. ©); it was stored for less than a week before use.

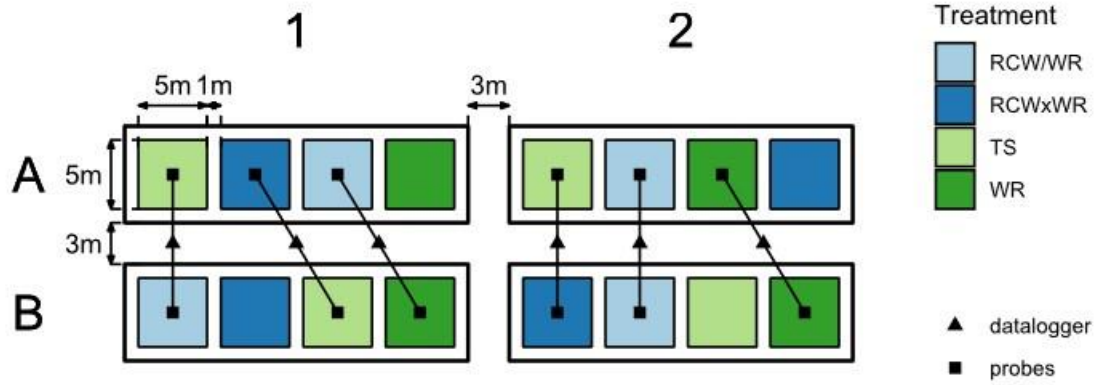


Fig. 2 Experimental setup with the four blocks (A1, A2, B1, and B2) containing the four treatments (RCW/WR, RCWxWR, TS, and WR). The position of the volumetric water content and temperature probes is represented by squares and the position of the dataloggers is represented by triangles. The spacing between the blocks was 3 m and the spacing of the cells within the blocks was 1 m. The cells measured 5 m square. WR: waste rock; RCW/WR: RCW over waste rock; RCWxWR: RCW mixed with waste rock; TS: topsoil

2.2.2. Material characterization

The materials were characterized by various methods. The particle size distribution of the waste rock was determined using a Malvern Mastersizer 3000 laser diffraction analyzer for the $< 80 \mu\text{m}$ fraction and a mechanical sieving device for the $\geq 80 \mu\text{m}$ fraction (ASTM, 2007, 2022).

Table 1: Granulometric distribution of the waste rock. D_x corresponds to the mesh size of the sieve at which $x\%$ of the particles pass. The uniformity and curvature coefficients are calculated as follows: $C_U = \frac{D_{60}}{D_{10}}$ and $C_C = \frac{D_{30}^2}{D_{10} \times D_{60}}$.

D_x	Particule size (mm)
D_{10}	0.315
D_{20}	2.5

D_{30}	5
D_{40}	10
D_{50}	14
D_{60}	20
D_{70}	28

Coefficients	-
Uniformity (C_U)	63.49
Curvature (C_C)	3.97

165

166 Based on the GSD (grain-sized distribution) parameters, the waste rock in the study area
 167 was considered “poorly graded gravel” (ASTM, 2020) (Table 1).

168 The *in situ* bulk density of the materials was determined for each cell using the following
 169 technique. A cylindrical soil sample with a diameter of 25 cm and a depth of 15 cm was
 170 extracted and the edges and bottom of the resulting hole were lined with a hydrophobic
 171 textile (Campbell, 1994). The volume of the soil sample was calculated by measuring the
 172 amount of water required to fill the hole. The soil sample was then oven-dried at 60°C for
 173 48 hours and its dry mass was recorded. The *in situ* bulk density was obtained by dividing
 174 the dry mass of the soil sample by its *in situ* volume (Table 2).

175 Table 2: Average bulk density of each treatment type (n = 4). The standard error (S.E.) is indicated in parentheses.

Treatment	Average bulk density (S.E.)
RCW/WR	1.81 (0.21)

RCW×WR	1.74 (0.08)
TS	0.61 (0.2)
WR	1.89 (0.12)

176

177 The soil water retention curve of the waste rock (alone or mixed with RCW) was obtained
 178 by fitting the Brooks and Corey equation to the measured data from a column experiment
 179 (Chapuis et al., 2007). A 70 cm-high column measuring 30 cm in diameter, containing
 180 waste rock placed at the average bulk density measured *in situ*, was used to determine the
 181 gravimetric water content (w) measurements following a water suction gradient (elevation)
 182 in the soil.

183 2.3.Conifer trees

184 2.3.1. Plantation of seedlings

185 The choice of planted conifer species was made according to the plant composition of the
 186 bioclimatic subdomain of the study area. Since the area is in the balsam fir–white birch
 187 forest, two species of conifers belonging to different stages of succession were planted: *P.*
 188 *banksiana* Lamb. And *A. balsamea* (L.) Miller.

189 Individuals were purchased in June 2018 and stored in an open warehouse until September
 190 5th, 2018, the date of the planting. According to the survival of individuals in the
 191 warehouse, the number of planted individuals of each species was 12 per cell for *A.*
 192 *balsamea* and 26 for *P. banksiana*. The seedlings came from the Ministère des Forêts, de
 193 la Faune et des Parcs of Quebec (MFFP) nurseries (from Trécesson, QC, Canada for *P.*
 194 *banksiana*; from the Abitibi-Témiscamingue Forestry Association for *A. balsamea*). In

each cell, the individuals were divided into four groups (two groups of each species) and were randomly arranged in four uniform squares with randomly selected positions. Each tree was planted with an inter-individual spacing of 1 m, ensuring appropriate space for growth and development.

2.3.2. Survival

After the first growing season (in 2019), the criteria for determining whether an individual was alive or dead in the study was based on the condition of the needles. If all an individual's needles were no longer green or they had no needles left, the individual was considered dead. However, if there were still green needles present, the individual was considered alive. The number of live individuals was counted at the end of each summer from 2018 to 2021 (mid-August, from plantation to after the third growing season).

The study measured survival in two different ways for all planted individuals. The first method (1) calculated survival difference ($\Delta_{Survival}$) by taking the difference between the number of individuals observed in the previous year (N_{Y-1}) and the number observed in the current year (N_Y).

$$\Delta_{Survival} = N_{Y-1} - N_Y \quad (1)$$

The second method (2) calculated survival rate ($R_{Survival}$) by dividing the number of individuals observed in the current year by the number observed in the previous year (N_{Y-1}).

$$R_{Survival} = \frac{N_Y}{N_{Y-1}} \quad (2)$$

2.3.3. Aerial growth

Aerial growth was measured annually for all planted seedlings. The measurements always took place at the end of the summer from 2018 to 2021 (mid-August, from plantation to after the third growing season). The initial measurements occurred in 2018, just after plantation. Annual aerial growth was determined with two measurements:

- Height from the base to the stem alive apex of the individual, folding all the branches towards the top of the individual to find its apical point (highest point of the individual). This measurement was collected using a flexible measuring tape.
- Diameter at the base of the soil. This measurement was collected using a caliper.

2.3.4. Biomass

The total, aerial, and root biomasses were measured after three growing seasons by excavating three randomly chosen individuals per cell in the last year of monitoring of the setup (2021). Only individuals of *P. banksiana* were excavated because the survival of *A. balsamea* did not provide an adequate representation of the species in each cell of the setup.

Individuals were first cut at the base of the stem and identified. Then, an excavation of the root system was carried out to recover all the roots, including the finest. The root samples were identified and associated with the corresponding aerial sample from each individual.

The plant material was brought back to the laboratory where it was washed with tap water, then placed in an oven for 64 hours to eliminate any residual water. A verification of the drying was carried out by ensuring that the mass no longer changed after 60 hours. The mass of the different samples (root and aerial) was measured with a Mettler PM4800 DeltaRange © scale and a total mass per individual was calculated.

2.3.5. Chemical composition of needles

In 2020, needles of the two species of conifers were collected selecting three living trees at random from each cell. Needles from the three trees in a single cell were then combined to form a composite sample. Since there were a total of 16 cells in the experimental area and two species, this resulted in a total of 32 composite needle samples. After being cleaned, the samples were placed in an oven to dry for 48 hours, until they no longer lost weight.

Samples were sent to the Lakehead University Forest Resources & Soils Testing Laboratory for analysis of total nitrogen (total N) and trace metal (Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Sb, Se, Si, Sn, Sr, Ti, Tl, V, and Zn) concentrations in the needles. The method of analysis for total nitrogen was combustion combined with a nitrogen analyzer. The analytical method for trace metals was microwave assisted acid digestion followed by metal determination using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (U.S.EPA, 2007).

2.4.Environmental variables

2.4.1. Volumetric water content and temperature

The volumetric water content and the temperature were measured hourly from 2019 to 2021 on 12 cells of the setup (three randomly selected cells for each treatment) (Fig. 2).

Two types of probes were used:

- 5TM (Meter Environment ©), which measured the volumetric water content by volume and the temperature at a depth of 10 cm below the surface of the waste

rock. The accuracy of these probes was $\pm 2\%$ after calibration and the temperature measurement range was -40 to $+60^{\circ}\text{C}$, with an accuracy of $\pm 1^{\circ}\text{C}$.

- EC-5 (Meter Environment ©), which measured only the volumetric water content by volume at a depth of 25 cm below the surface of waste rock. The precision of these probes was $\pm 2\%$ after calibration.

Since the probes were not buried in topsoil or RCW, we calibrated them for waste rock used in this study (waste rock alone and waste rock mixed with RCW), in the laboratory.

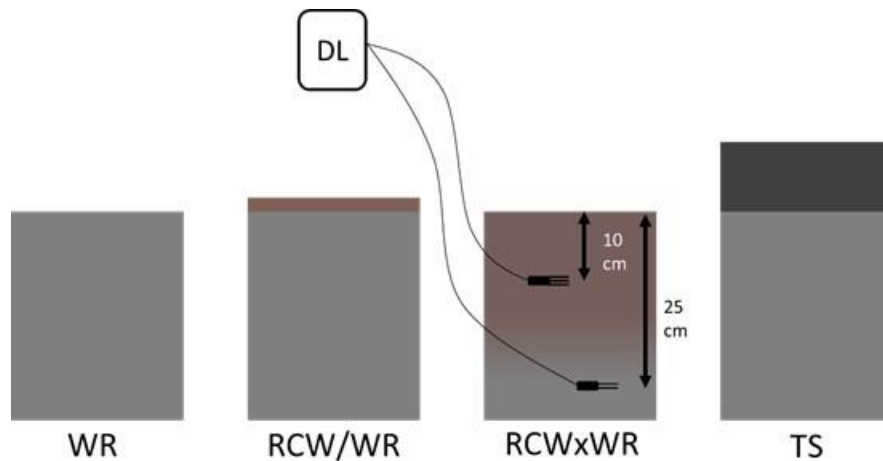


Fig. 3 Position of the probes in the soil. The 5TM (Meter Environment ©) probes, which measured volumetric water content and temperature, were located 10 cm below the surface of the mineral substrate, while the ECH₂O-EC5 (Meter Environment ©) probes, which measured the volumetric water content only, were located 25 cm below the surface. DL: data logger; WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil

2.4.2. Chemical analysis

Substrate samples were collected in September 2020 and sent to the Lakehead University laboratory for chemical analysis. The samples included bulk samples of topsoil and RCW mulch, and a sample of waste rock underlying each of the four treatments in each experimental cell (16 waste rock samples). The analyses carried out were as follows:

- 274 - electrical conductivity (1:1, H₂O:soil) with a conductivity meter;
- 275 - total nitrogen concentration by combustion with a nitrogen analyzer;
- 276 - total organic carbon concentration by combustion with a carbon analyzer;
- 277 - pH in saturated paste; and
- 278 - trace metals (Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P,
- 279 Pb, S, Se, Si, Sr, Ti, Tl, V, and Zn) with microwave assisted acid digestion followed
- 280 by metal determination using ICP-AES (U.S.EPA, 2007).

281 2.4.3. Colonizing plant cover

282 In 2021, during the dismantling of the experimental setup, the total and specific cover of
283 spontaneously colonizing plants were measured by the method of point interceptions along
284 a transect arranged diagonally on each cell. The measurement points were distributed every
285 5 cm along the transect. To facilitate the recognition of the plant species present, the
286 *Pl@ntNet*© application was sometimes used (Bonnet et al., 2015). Total plant cover (C_T)
287 corresponded to the quotient of the sum of the interception points (n_{T_s}) over the total
288 number of measurement points (n_T) on the transect (3). Specific cover (C_s) corresponded
289 to the quotient of the sum of interception points for a given species (n_s) over the total
290 number of measurement points (n_T) on the transect (4).

291

$$C_T = \frac{n_{T_s}}{n_T} \quad (3)$$

$$C_s = \frac{n_s}{n_T} \quad (4)$$

292

2.5. Statistical analysis

The statistical analyses were conducted with the R language in the RStudio development environment (R, 2023). The scripts used can be found in the Supplementary Material. The significance level for the analyses was $\alpha = 0.05$.

When the assumptions were met (normality of residuals and homoscedasticity), a repeated custom analysis of variance was performed on the conifer variables (response variables) and environmental variables (explanatory variables), followed by a post-hoc test with Tukey's correction (Rupert Jr, 2012) to test treatment effect. The treatment effect was tested for each species independently. When the assumptions were not met, a non-parametric Kruskal-Wallis test was performed (Hollander et al., 2013), followed by a non-parametric Dunn's multiple comparison test using Benjamini-Yekutieli adjustment (Benjamini and Yekutieli, 2001; Dunn, 1961, 1964). Tables summarizing the analysis results are available in the Supplementary Material.

Multivariable linear regressions were also modeled to determine by stepwise selection the model that best explained the response of the trees, based on the Akaike criterion (AICc) of the different models and by analyzing all the models, from the full model to the null model (Sakamoto et al., 1986). The null model was composed of all the variables identified in the scientific literature as being able to influence the response variable, while considering the collinearity of these explanatory variables. To determine the collinearity of the variables, the variance inflation factor (VIF) was calculated for the full model, in addition to the correlation coefficient between the explanatory variables (Salmerón et al., 2018). A VIF less than 2 was considered acceptable for the variables included in the full model. When a variable had a VIF greater than 2, variables with a correlation coefficient

greater than ± 0.5 were eliminated from the model (Fox and Monette, 1992). Finally, the collinearity was checked graphically by principal component analysis (PCA) and using scatterplot to ensure the linearity of the variables and that the correlation coefficient was not fortuitous (De Marco and Nobrega, 2018). The script for the analysis, as well as the data for the multivariate analysis, is provided in the Supplementary Material.

The *tidyverse* metapackage was used for the data cleaning, transformation, and visualization steps, as well as the functional programming steps (Wickham et al., 2019). The *multcomp*, *emmeans*, *lmerTest*, *lme4*, and *rstatix* packages were used for all statistical testing analysis steps (Bates et al., 2015; Hothorn et al., 2008; Kassambara, 2023; Kuznetsova et al., 2017; Lenth et al., 2023). The stepwise selection was carried out with the *step()* function of the *stats* package, with a bidirectional search (*direction = "both"*) (Venables and Ripley, 2002). The VIF was calculated using the *vif()* function of the *car* package (Fox and Weisberg, 2018). The PCA and the correlations were carried out respectively using the *prcomp()* function and the *cor()* function, both from the *stats* package (R, 2023).

3. Results

3.1. Tree responses

3.1.1. Tree survival

The number of dead individuals from year to year ($\Delta_{Survival}$) and the interannual survival rate ($R_{Survival}$) were significantly different between treatments only for *A. balsamea*. In 2021, the TS treatment showed a $\Delta_{Survival}$ significantly lower and a $R_{Survival}$ significantly higher for this species than the RCW×WR treatment (Table 3).

Table 3: Mean survival indices calculated in 2021 among treatments for *Abies balsamea*. $\Delta_{Survival}$: number of dead individuals from year to year; $R_{Survival}$: interannual survival rate; WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil. The letters represent the results of the post-hoc test performed. The standard error is indicated in parentheses.

	TS	RCW×WR	RCW/WR	WR
$\Delta_{Survival}$	1.5 (0.29) a	6.5 (1.66) b	4.75 (1.38) ab	2.5 (0.65) ab
$R_{Survival}$ (%)	83.6 (3.38) b	30.9 (14.93) a	44.9 (8.21) ab	70.5 (8.67) ab

3.1.2. Tree height and basal diameter

At planting in 2018, the average height of the *A. balsamea* individuals on the site was 21.5 cm (0.3) and their average diameter was 4.3 mm (0.01). The height and diameter of *A. balsamea* individuals began to significantly differ among treatments from 2020 onwards (Figure 4). In 2020 and 2021, the TS treatment exhibited *A. balsamea* individuals with a significantly larger diameter than the other three treatments. The TS and WR treatments had trees with a significantly greater height than the RCW×WR treatment in 2020. Then, in 2021, the TS treatment had a significantly greater height of *A. balsamea* individuals than

the other three treatments. The average diameter of *A. balsamea* individuals in 2021 was 10.6 mm (0.7) for the TS treatment, which was about twice that of the other treatments: 4.8 mm (0.4) for RCW×WR, 5.1 mm (0.4) for RCW/WR, and 5.4 mm (0.3) for WR. The average height of *A. balsamea* individuals in 2021 was also greater for the TS treatment, with 37.8 cm (1.9), as compared to 24.1 cm (1.6) for RCW×WR, 23.9 cm (1.4) for RCW/WR, and 27.5 cm (1.0) for WR.

The average height of the *P. banksiana* individuals at planting in 2018 was 23.5 cm (0.2), and their average diameter was 4.4 mm (0.04). *P. banksiana* individuals exhibited a significantly greater diameter on the TS treatment than on the other three treatments from 2019 until 2021. They had a significantly greater height on the TS treatment than on the RCW×WR and WR treatments in 2019; in 2020 and 2021, the height of *P. banksiana* on the TS treatment was greater than on all three other treatments. The average diameter of *P. banksiana* individuals in 2021 was 21.2 mm (0.3) for the TS treatment, three times greater than the average diameter of 7.1 mm (0.2) for RCW×WR, 6.6 mm (0.2) for RCW/WR, and 7.5 mm (0.2) for WR. The average height of *P. banksiana* individuals in 2021 was 77.1 cm (2.0) for the TS treatment, 37.7 cm (0.9) for the RCW×WR treatment, 38.4 cm (0.8) for the RCW/WR treatment, and 39.6 cm (0.8) for the WR treatment.

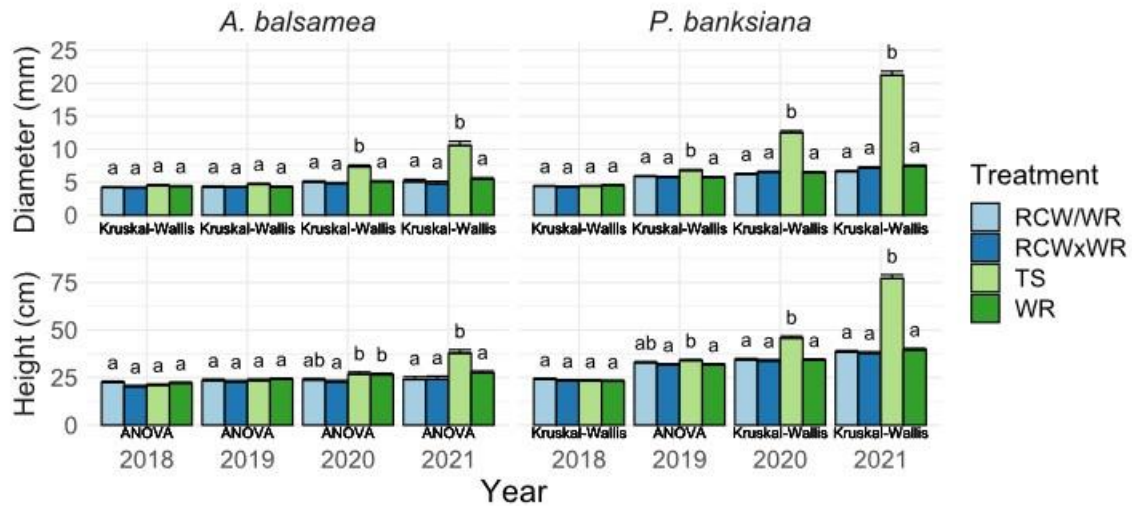


Fig. 4 Annual mean height (in cm) and basal diameter (in mm) of *A. balsamea* and *P. banksiana* planted individuals among treatments. WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil. The letters represent the results of the post-hoc test performed. The statistical comparison test is indicated under each barplot. Error bars represent standard error

3.1.3. *P. banksiana* biomass

After excavation of the *P. banksiana* individuals in 2021, at the conclusion of three growing seasons, the total, aerial, and root biomasses were found to all be significantly greater on the TS treatment than on the three other treatments. The average total biomass of excavated *P. banksiana* individuals was 300.82 g (43.8) on the TS treatment, more than 10 times greater than that on the other treatments: 20.53 g (2.59) on RCW×WR, 24.72 g (1.65) on RCW/WR, and 26.6 g (2.81) on WR.

3.1.4. Chemical composition of needles

For both planted species, the concentrations of total K, P, S, and N (essential elements that make up proteins) were significantly higher in the trees growing on the TS treatment than on the other treatments (Fig. 5.A).

For *P. banksiana*, the concentrations of potentially phytotoxic elements in the needles, such as Cr or As, were generally lower in the plants growing on the TS treatment than on the other treatments (not significant for As, partly significant for Cr) (Fig. 5.B). Nickel concentrations were not significantly different among the treatments. The variance of concentrations on each treatment was too large for the statistical comparison analysis to detect a possible significant difference among the treatments. Average As concentrations in the needles ranged from 2.25 to 6.43 $\mu\text{g/g}$ for *A. balsamea* and from 3.73 to 17.4 $\mu\text{g/g}$ for *P. banksiana*.

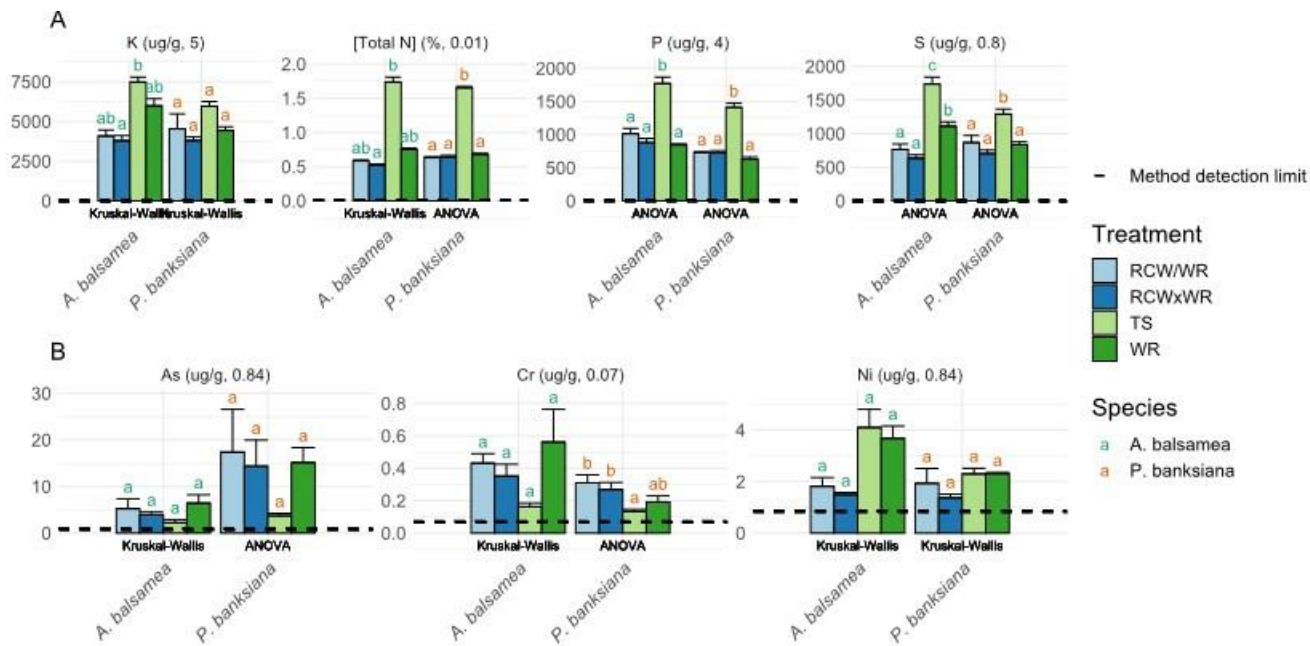


Fig. 5 Mean element concentrations in the needles of *A. balsamea* and *P. banksiana* individuals in 2020, compared among treatments. WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil. The barplots in A present the concentrations of essential elements (macronutrients), while the barplots in B present the concentrations of potentially phytotoxic elements. The name of the element is indicated above the barplot. The units are specified in parentheses, as well as the method detection limit (MDL). The MDL is also represented by a dashed line. The statistical comparison test is indicated under each barplot. The letters are the results of the post-hoc test, in blue for *A. balsamea* and in red for *P. banksiana*. Error bars represent standard error

3.2.Environmental variables

3.2.1. Microclimatic variables

3.2.1.1. Water content

The average volumetric water content measured at a depth of 10 cm in the waste rock was significantly different among treatments from 2019 to 2021 (Fig. 6). In 2019, the average volumetric water content was significantly higher in the TS treatment than in the RCW/WR treatment. In 2020, the TS treatment had a significantly higher average volumetric water content than the other three treatments. In 2021, the average volumetric water content was significantly higher in the TS treatment than in the RCW/WR and WR treatments.

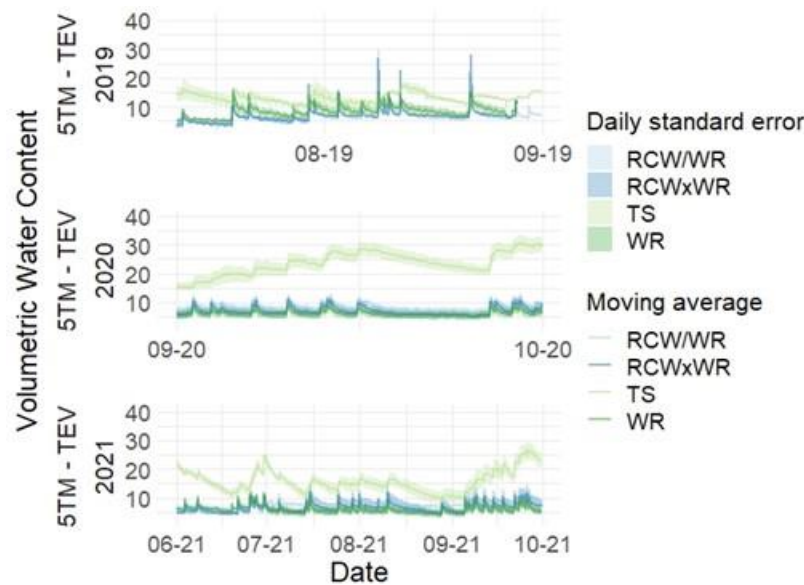


Fig. 6 Evolution of the volumetric water content at a depth of 10 cm in the waste rock substrate for each treatment, measured during the growing season in 2019, 2020, and 2021. The periods correspond to those where all the measurements were of sufficient quality. WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil

Water retention curve results are presented in the Supplementary Material. The number of days when the suction exceeded 100 kPa during the periods of volumetric water content measurement at a 10 cm depth were significantly different among the treatments in 2019 and 2021. In general, the TS treatment had the fewest number of days when the soil suction at a 10 cm depth was above 100 kPa.

The volumetric water content was not significantly different among the treatments at a depth of 25 cm. Average volumetric water contents at this depth in the waste rock during the measurement period (June to late September) were 12% (1.64) in 2019, 14.1% (2.37) in 2020, and 12.7% (2.83) in 2021.

3.2.1.2. Temperature

The mean temperature variation (difference between daily maximum and minimum temperature) at a 10 cm depth in the soil was significantly different among treatments in 2021. The difference between the daily maximum and minimum temperature was significantly lower on the TS treatment than on the other treatments in 2021 ($p < .05$). The average daily temperature differences were 7.73°C (± 0.08) on WR, 6.97°C (± 0.68) on RCW/WR, 7.02°C (± 0.04) on RCW $\times$ WR, and 4.38°C (± 0.89) on TS.

3.2.2. Chemical composition of mineral substrate

3.2.2.1. Nutrients

The total concentrations of nitrogen and phosphorus in the waste rock substrate were higher in the TS treatment than in the other treatments. The topsoil layer contained 1.2% total N on average. In contrast, the K concentration in the TS treatment was significantly lower as compared to the other treatments. The concentrations of potential micronutrients (Cu, Fe,

Mn, Mo, and Zn) were not necessarily different among the treatments, with the exception of Fe, which was lower in the TS treatment than in the other treatments. The average concentrations of all these elements are presented in table 3 in the Supplementary Material.

3.2.2.2. Potentially phytotoxic elements

The pH of the waste rock substrate under the TS treatment was significantly lower (slightly acidic) than the other three treatments (neutral) (Fig. 7), thanks to an acidic average pH of 4.5 in the topsoil layer.

The average total As concentrations in the waste rock substrates of all the treatments exceeded the regulatory thresholds (criterion C, for industrial land use) of Schedule 1 of the Land Protection and Rehabilitation Regulation (Quebec Environmental Quality Act Q-2, r.37).

The average total Cr concentrations exceeded the residential regulatory thresholds (criterion B) in the waste rock substrates of the RCW/WR, RCW×WR, and WR treatments.

The total Ni concentrations in the waste rock substrates of all the treatments also exceeded the residential regulatory thresholds (criterion B). Although not significant, the mean As, Cr, and Ni concentrations were lower in the TS treatment than the other three treatments.

The topsoil layer showed total As concentrations ($12 \mu\text{g.g}^{-1}$ on average) below the B criterion for residential land use, but the average total Hg concentration reached 90 ng.g^{-1} , which is above the C criterion for industrial land use.

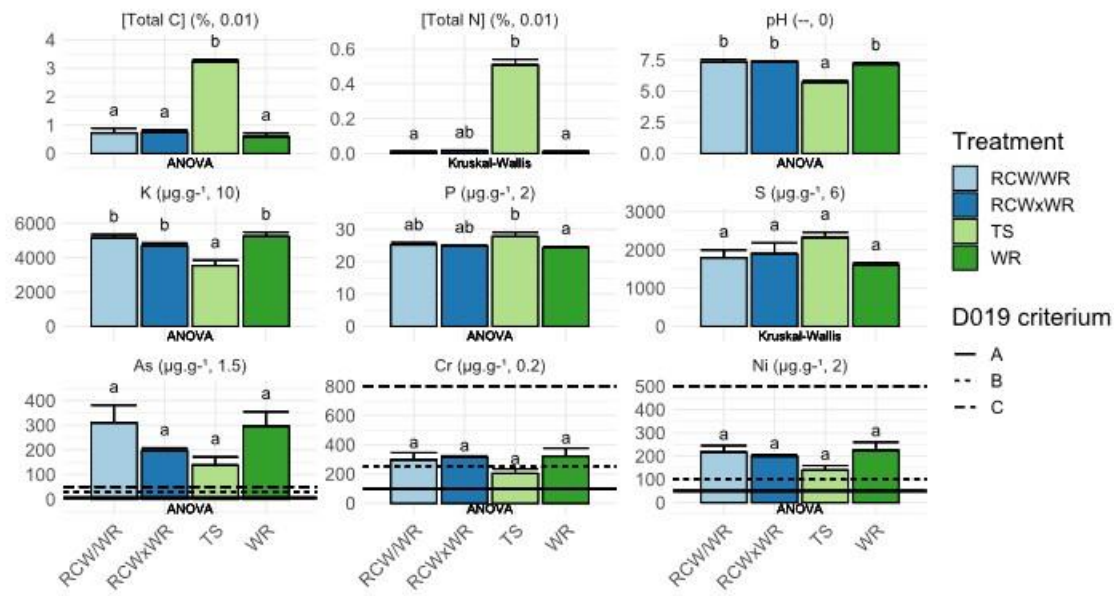


Fig. 7 Average total element concentrations in the waste rock substrate of all treatments in 2020. WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil. The units are specified in parentheses, as well as the method detection limit (MDL). The directive D019 criteria (A, B, and C) are represented by the different type of lines. The statistical comparison test is indicated under each barplot. The letters are the results of the post-hoc test. Error bars represent standard error

3.2.3. Colonizing plant cover

The total and specific cover of colonizing plants had no effect on tree response. Only the TS treatment had a total cover greater than 1%, with an average value of 70.5% (4.84). No alien species were observed on any of the cells in the experimental setup (Colautti and MacIsaac, 2004). The species present on the TS treatment were *Agrostis* spp., *Anaphalis* spp., *Artemisia* spp., *Cirsium* spp., *Eutamia graminifolia*, *Iris foetidissima*, *Leucanthemum vulgare*, *Linaria* spp., *Potentilla norvegica*, *Rumex* spp., *Solidago canadensis*, *Solidago* spp., *Taraxacum* spp., *Trifolium repens*, *Trifolium pratense*, and *Vicia sativa*. No woody

species were observed among the colonizing plants in 2021, three years after the construction of the experimental setup.

3.3. Multivariate relationships

The microclimatic variables (volumetric water content, temperature, and number of days when the suction exceeded 100 kPa) of the substrate did not explain the dynamics of the response variables. On the other hand, after having observed several correlations between response variables and physicochemical variables of the substrate, the analysis using a multivariate linear regression model and a stepwise selection based on the Akaike criterion made it possible to determine the model comprising the explanatory variables that best explained the response of the plants.

The model that best explained the tree responses (cumulative diameter of *A. balsamea* and average height of *P. banksiana*) contained only one explanatory variable: the total As concentration in the substrate. The bivariate linear relations of the modeled tree responses are represented graphically in the form of scatterplot in Fig. 8. The observed number of dead individuals for *P. banksiana* was notably lower than for *A. balsamea*. Consequently, the selected response variable for *P. banksiana* was the mean height of its individuals in 2020. For this species, height appeared to be more impacted by arsenic concentrations than diameter. For *A. balsamea*, the cumulative diameter—defined as the sum of diameters of living individuals on each experimental cell—was chosen as the response variable. This approach effectively highlights the combined influence of both mortality and diameter in relation to arsenic soil concentrations in 2020.

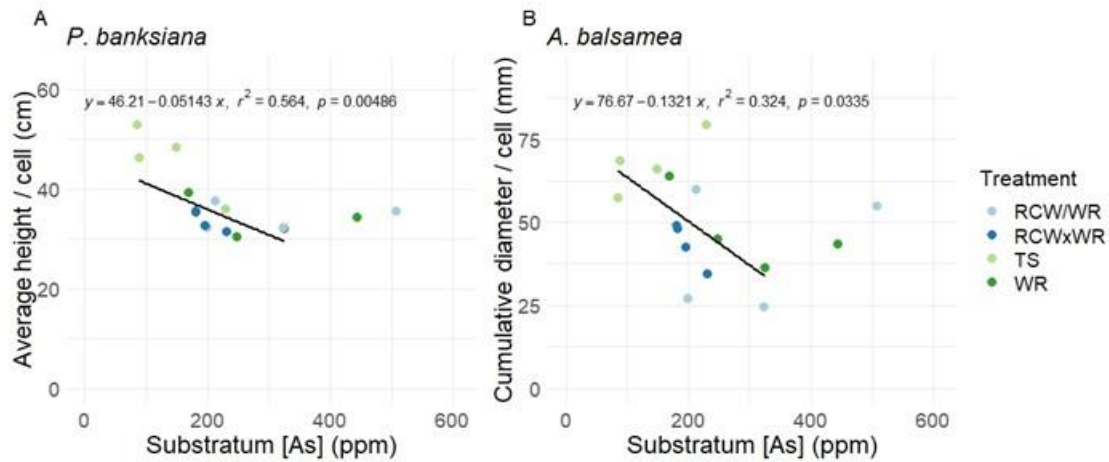


Fig. 8 Responses of the two tree species to the total arsenic concentrations of the waste rock substratum. A) Average height of *P. banksiana* individuals as a linear function of the arsenic concentrations of the mineral substrate. B) Cumulative diameter of *A. balsamea* individuals as a linear function of the arsenic concentrations of the mineral substrate. WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil

To illustrate that the total arsenic concentration in the substrate significantly influenced the morphological response of the trees, two scatterplots depict the relationship between the total arsenic concentration in the substrate and the arsenic concentration in the needles of coniferous trees (Fig. 9). Linear regressions with coefficients of determination over 0.5, combined with visible linear patterns among the data points and a gradation of arsenic concentrations, reveal clear linear relationships between these soil and plant variables.

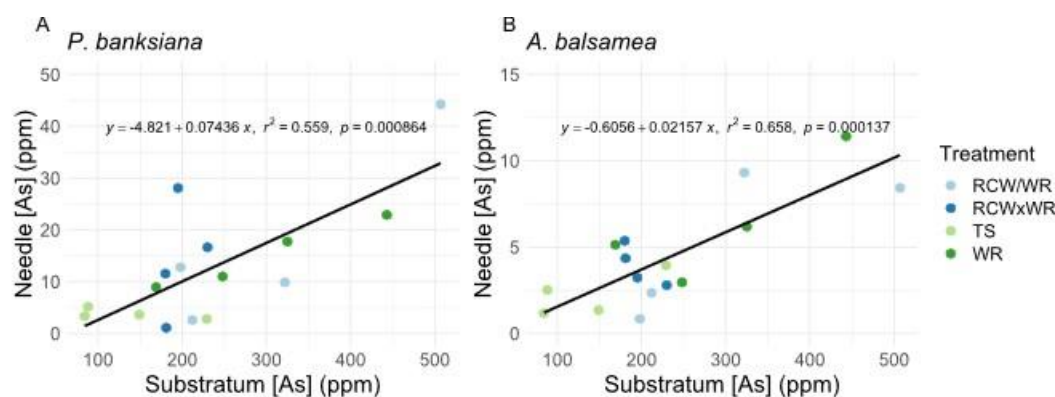


Fig. 9 Arsenic concentration in the needles of *P. banksiana* (A) and *A. balsamea* (B) individuals in 2020 as a linear function of the total arsenic concentration in the waste rock substrate. WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil

Finally, the total phosphorus and sulfur concentrations in the waste rock substrate showed negative correlations with the total arsenic concentration in the same substrate (Pearson correlations of -0.53 and -0.55 , with p-values of 0.04 and 0.03 , respectively). The concentrations of total P and S were also correlated with the arsenic concentrations in the needles of *A. balsamea* individuals (Pearson correlations of -0.56 and -0.64 , with p-values of 0.03 and 0.01 , respectively). Additionally, a mild negative correlation was observed between the total phosphorus concentrations in the waste rock substrate and the arsenic concentration in the needles of *P. banksiana* individuals (Pearson correlation of -0.5 and p-value of 0.048).

The biplot (Fig. 10), which illustrates the associations among the principal correlated variables, effectively projects a point cloud that is based on a reduction of the two primary components derived from the PCA. This visual representation delineates the relationships between the chemical composition of the substrate and that of the needles from the two studied species. Notably, it underscores the impact of the total As concentration in the waste

rock substrate on the corresponding As concentration in the plant needles. Arsenic concentrations appeared to be independent of treatment type, although the stepwise selected model previously showed that plants growing on the TS treatment seemed to accumulate less As than on the other treatments. The figure also shows that the TS treatment was characterized by greater accumulation of nutrients (N, P, K, S) in the needles of the seedlings.

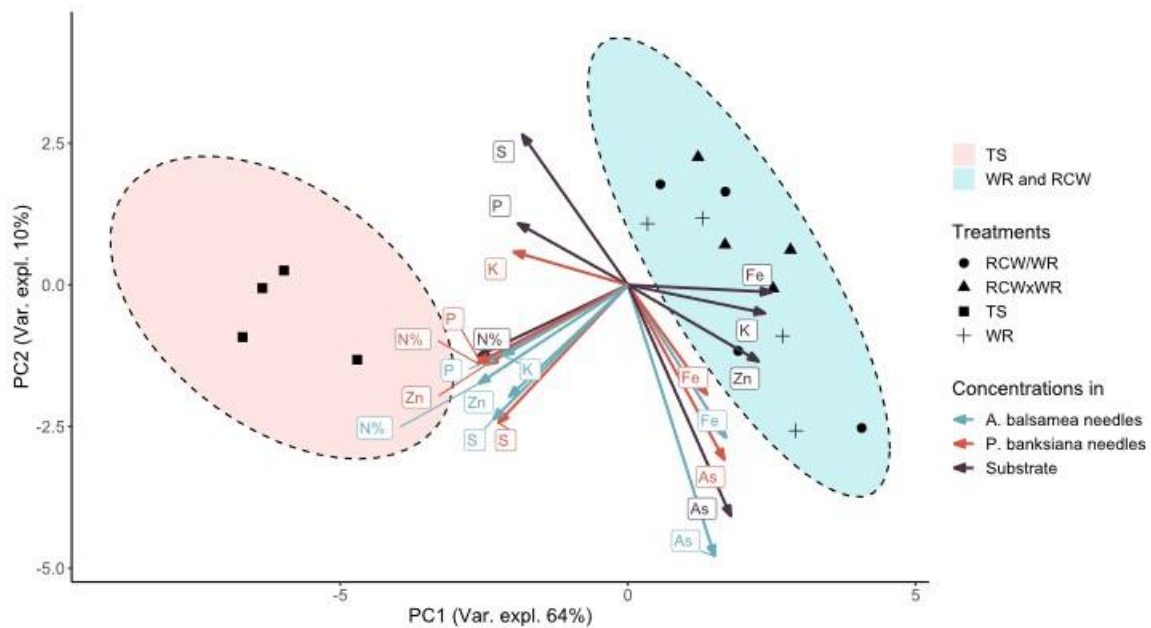


Fig. 10 PCA biplot showing the relationships between chemical variables with the strongest relationships to each other (Pearson correlation $|R| > .5$). Correlations between the element concentrations of the needles of the two species were not considered to avoid biasing the interpretation of PCA. The ellipses represent the multivariate t-distributions of two groups (TS cells and other treatment cells). The arrows represent the correlation between element concentrations in each group (*A. balsamea* needles, *P. banksiana* needles, and waste rock substrate). Finally, treatments are represented by different shapes: RCW/WR by circles, RCW×WR by triangles, TS by squares, and WR by crosses. WR: waste rock; RCW/WR: RCW over waste rock; RCW×WR: RCW mixed with waste rock; TS: topsoil. The cumulative proportion of variance explained by the graphical representation of the first two components is 74.6% (PC1: 64.4%, PC2: 10.2%)

4. Discussion

4.1. Positive effect of topsoil layer on waste rock substrate and development of planted trees

In agreement with our first hypothesis, a significant supply of organic matter (10 cm-thick layer) in the form of topsoil promoted growth in aerial and root biomasses, diameter, and height, and the survival of *A. balsamea* and *P. banksiana* individuals planted in waste rock. However, the RCW-based treatments (mixed or mulch) affected neither the growth of the planted trees nor their survival, while the topsoil treatment had a considerable effect on these variables. As expected, *P. banksiana* suffered less than *A. balsamea* from the early successional conditions present at the experimental site, in terms of survival and growth (Asselin et al., 2001; Bergeron, 2000).

At the soil level, the TS treatment had greater water retention capacity, lower pH and total concentrations of potentially phytotoxic elements, and greater soil essential nutrient concentrations (except potassium). Our hypothesis concerning the improvement of the microclimatic and physical conditions of the mineral substrate by organic matter addition is supported by the results of the volumetric water content, the number of days when the matrix pressure was higher than 100 kPa, and the soil temperature at a depth of 10 cm. These findings could be attributed to the ability of organic matter to improve soil structure, thus increasing water-holding capacity, which can support plant growth and improve overall soil health (Libohova et al., 2018; Naeth et al., 1991). However, based on the multivariate analysis, improving these conditions was not a determining factor in the survival or growth in biomass, height, or diameter of conifer seedlings.

As expected, the total concentrations of elements that can constitute macronutrients in plants were higher when a supply of already decomposed organic matter was present over the waste rock, such as topsoil. Although total element concentrations are not necessarily a good indicator of the availability of nutrients in the soil, it was noted that the total concentrations of these macronutrients were linearly related to the concentrations found in the needles of the trees, except for K. Accordingly, the concentrations of essential elements such as P, K, S, and N were significantly higher in the needles of the planted trees in the topsoil treatment. This suggests that, in this case, the total concentration of essential elements found in soil reflected the concentration bioavailable to plants. Interestingly, despite the lower concentrations of total K in the waste rock of the TS treatment, a higher concentration was observed in the seedling needles. This phenomenon could potentially be attributed to K not acting as a limiting factor within the context of this study (van der Ploeg et al., 1999). Furthermore, the concentration of K in the needle proteins may be influenced by the concentration of other constituent elements of these proteins, thus explaining the observed discrepancy.

Our results also revealed that the average pH in the TS treatment was around 5, compared to approximately 7.5 in the other treatment groups. The pH level in the TS treatment closely aligns with the optimum soil acidity range for the growth of both *P. banksiana* and *A. balsamea*, as documented in existing research. A review pointed out that *A. balsamea* grows in cooler, wet-mesic sites with acidity between 5.1 and 6.0 (Frank, 1990). *P. banksiana* is adaptable to different soil conditions, including slightly acidic environments (Rudolph and Laidly, 1990). Therefore, the lower pH substrate provided by the TS

treatment appeared more conducive for the growth of these species when compared to the other treatments with higher pH values.

4.2.Arsenic in waste rock and accumulation in trees

The concentrations of potentially phytotoxic elements such as chromium or arsenic were generally lower in the plants growing on the TS treatment (even if not statistically significant for As). However, for Cr, the needle concentrations were far below the 5 $\mu\text{g.g}^{-1}$ concentration reported to possibly induce phytotoxicity (Kabata-Pendias, 2010; Kabata-Pendias et al., 2017). The total arsenic concentrations were high in the waste rock, which constitutes the mineral growing substrate for the treatments. This is an important consideration as the mining industry increasingly exploits refractory ore deposits to extract gold, which often results in the release of a substantial proportion of arsenic (Coudert et al., 2020). Adding organic matter in the form of topsoil to the surface of the waste rock appeared to reduce the transfer of arsenic to planted tree needles. The root volume growing in the 10 cm-thick topsoil was indeed not in direct contact with the As-bearing waste rock. Moreover, total As concentrations were lower in the waste rock under the topsoil. Recent research has shown that adding organic cover material in the form of peat with an acidic pH can decrease the concentration of arsenic in desulfurized gold mine tailings with neutral-alkaline pH by making it mobile and more leachable, especially in the form of As(III) (Rakotonimaro et al., 2021).

Total arsenic concentrations are generally not a good indicator of the availability of this element for plant uptake since it may be non-leachable and retained in gangue. However,

the accumulation of As in the needles of the sampled trees that appeared proportional to the total As concentrations in the waste rock substrate suggests that, in this case, As was available for root uptake. In the supplementary material of a recent PhD thesis (Kalonji, 2020), the author reported averaged arsenic concentrations of 0.2 mg/L in leachates from a waste rock sample originating from the same mine site. These results were obtained through column leaching tests, which suggests that arsenic may be released into the water upon contact with the waste rock (Kalonji, 2020). This finding could potentially explain the uptake of arsenic by *P. banksiana* observed in our own investigation, as the arsenic may have been leached into the water and subsequently absorbed by the plants. However, it was noted that in another experimental setup on the same site, the waste rock did not contain any detectable total As (Taurines et al., 2023, unpublished results).

In the scientific literature, arsenic concentrations in the needles of conifers of the genus *Pinus* grown on disturbed sites never exceeded 8 ppm on average (Juranović Cindrić et al., 2018; Popovic et al., 2022; Shin et al., 2019; Wang et al., 2022; Yu et al., 2020). In natural forests (including forests adjacent to disturbed sites), the concentrations in *Pinus* spp. never exceeded 1 ppm (Juranović Cindrić et al., 2018; Shin et al., 2019). Data from this non-exhaustive literature review are presented in the Supplementary Material. In our study, the highest average arsenic concentration found in the needles was 17.4 ppm (± 9.21) for *P. banksiana*. Arsenic concentrations were therefore high in the needles of the trees in our experiment.

4.3. Arsenic phytotoxicity

The accumulation of arsenic in *A. balsamea* and *P. banksiana* needles negatively impacted their growth in diameter and height, respectively. Few research studies have focused on the

630 phytotoxicity thresholds of arsenic on conifers of the genus *Pinus* and it is difficult to
631 determine the effect of the concentrations attained here on the metabolism of the genus
632 (Juranović Cindrić et al., 2018). Moreover, very few studies present information on arsenic
633 concentrations in needles for the genus *Abies*. According to our analysis of the variables
634 that can explain the response of conifer seedlings (diameter of *A. balsamea* individuals and
635 height of *P. banksiana* individuals), the growth-limiting factor seemed to be the presence
636 of high arsenic concentrations in the waste rock.

637 In agreement with recent research on the bioavailability of arsenic in contaminated
638 agricultural soils in India (Singh and Srivastava, 2020), the higher total P and S
639 concentrations found in the waste rock of the TS treatment could be concomitant with a
640 decrease in the bioavailability of arsenic for the plants. The authors of that study identified
641 that an increase in the availability of phosphorus in the soil was correlated with a decrease
642 in the bioavailability of arsenic, because phosphorus and arsenic compete for sorption sites
643 in the soil (Singh and Srivastava, 2020). They also found that the arsenic concentrations in
644 the soil decreased with the sulfur concentrations. Sulfur and arsenic have similar and often
645 interconnected biogeochemical cycles (Singh and Srivastava, 2020).

646 When conducting revegetation work in waste rock including soil improvement techniques
647 with organic matter addition, it is recommended that the speciation of As and its available
648 concentration to plants be considered. Topsoil application, especially if it contains available
649 phosphorus, can effectively improve the physicochemical growing conditions for *P.*
650 *banksiana* and *A. balsamea* on waste rock, potentially reducing certain phytotoxicity risks
651 to some extent.

5. Conclusion

This study demonstrated that the addition of organic matter in the form of topsoil to coarse mineral substrates on mine sites can enhance the growth and survival of conifer seedlings such as *A. balsamea* and *P. banksiana*, resulting in improved access to water and nutrients from the substrate. However, mulched or mixed aspen RCW treatments had no effect on tree growth after three years of follow-up. RCW also did not have a major impact on improving the physicochemical and microclimatic conditions of the substrate favorable to the growth of the two species. Moreover, the results suggested that high concentrations of arsenic in gold mine waste rock may be an important factor limiting the growth of conifer seedlings. Further research should be conducted to explore the potential of organic matter-based treatments and phosphorus fertilization for improving the revegetation of arsenic-bearing gold mine waste rock. The development of substrate quality indicators reflecting As availability to planted trees should also be investigated to anticipate a possible decrease of afforestation success for mine sites in the longer term.

Declarations

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