Canadian Journal of Forest Research

Tree establishment on post-mining waste soils: species, density, and mixture effects

Journal:	<i>Canadian Journal of Forest Research</i>
Manuscript ID	cjfr-2021-0044.R1
Manuscript Type:	Article
Date Submitted by the Author:	08-Jun-2021
Complete List of Authors:	Asmara, Degi Harja; Laval University, Centre for Forest Research and Institute for Integrative and Systems Biology Allaire, Suzanne; GECA Environnement van Noordwijk, Meine; World Agroforestry Centre Southeast Asia Indonesia; Wageningen University & Research, Plant Production Systems P. Khasa, Damase; Laval University, Centre for Forest Research and Institute for Integrative and Systems Biology
Keyword:	overyielding, facilitation, Nelder plot, planting density, post-mining
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

SCHOLARONE™
Manuscripts

1 **Tree establishment on post-mining waste soils: species, density,**
2 **and mixture effects**

3 **Degi Harja Asmara¹, Suzanne Allaire², Meine van Noordwijk^{3,4}, Damase P. Khasa¹.**

4 ¹*Centre for Forest Research and Institute for Integrative and Systems Biology, Université*
5 *Laval, Québec, Canada G1V 0A6;*

6 ²*GECA Environnement, 4018 Ave des Pyrénées, Québec, QC, Canada G1P 3L9;*

7 ³*World Agroforestry (ICRAF), 16115, Bogor, Indonesia;*

8 ⁴*Plant Production Systems, Wageningen University, The Netherlands*

9 **Author for correspondence:**

10 Name: Degi Harja Asmara

11 Address: 18-2470 Ch. Quatre-Bourgeois, Ville de Québec, QC G1V1W7, Canada

12 E-mail: degiharja@gmail.com

Abstract

Tree establishment to restore degraded boreal post-mining lands is challenged by low soil productivity, a harsh microclimate, and potentially high contaminant levels. Use of mixed vegetation can facilitate microclimate but increase competition for soil resources. A statistical accounting of plant-plant interactions and adaptation to multi-species conditions is hard to achieve in field experiments; trials under controlled conditions can distinguish effects of planting density and species interactions in the early stages of plant establishment.

A greenhouse trial was established in containers ('mesocosms') with waste rock or fine tailings from gold mines. Pre-germinated (one-week-old) seedlings (*Alnus viridis* subsp. *crispa*, *Picea glauca*, *Populus tremuloides*, *Salix arbusculoides*) were planted using a Nelder density gradient design, modified for species combinations. A Relative Competition Effect (RCE) was estimated as a competitiveness index for each species combination, calculated as ratio of α coefficients in the Holliday growth equation. The Specific Leaf Area (SLA) was measured to indicate plant water stress adaptation.

All species grew better in monoculture on fine tailings, while only *P. tremuloides* grew better in all mixtures on waste rock. Although net positive effects of density on SLA increment during early growth suggested microclimate improvement on fine tailings, no mixture provided advantages for both species in paired combinations.

Keywords: overyielding, facilitation, Nelder plot, planting density, post-mining

32 **Introduction**

33 Challenges for the restoration of degraded post-mining lands include low soil
34 productivity and a high potential for metal toxicity (Markham et al. 2011). Low levels of
35 micro- and macronutrients and a lack of beneficial microbes in mining residues are a
36 challenge for revegetation (Bois et al. 2005). Fertilization can be costly and natural vegetation
37 can take decades to colonize and remediate post-mining sites in boreal ecosystems (Nadeau
38 et al. 2016). Cost-effective active restoration requires solving technical problems such as
39 species selection, plantation method, planting resources etc. (Bechara et al. 2016).

40 Post-mining sites can be very degraded, and conventional reforestation or afforestation
41 approaches may fail to successfully re-establish native vegetation. The physico-chemical and
42 microbiological characteristics of post-mining soil materials prevent desirable rates of plant
43 growth (Nadeau et al. 2018). For some tree species, mycorrhizal inoculation may improve
44 plant growth and health, together with plant survival, under these stressful conditions
45 (Nadeau et al. 2018). Another option could be to find an improved method for enhancing the
46 facilitative effect within the plants sharing the same space (Markham et al. 2011; Bertoncello
47 et al. 2016).

48 Facilitative effects play an important role in restoration of plant communities in severely
49 disturbed ecosystems with harsh microclimates (Brooker et al. 2008; Markham et al. 2011),
50 as found in boreal post-mining sites. The interactions between plant species can be very
51 complex however, requiring a deep understanding of the trade-offs between positive
52 facilitation and negative competition. These trade-offs need to be managed for successional
53 processes to proceed in severely disturbed ecosystems such as mining sites (Brooker et al.

2008; Markham et al. 2011). Mechanisms may vary, including classical nurse-plant effect interactions, soil nitrogen availability, common mycorrhizal networks, species-specific mutualism interactions, and several others (Holmgren et al. 1997; Brooker et al. 2008). The facilitative effect that emerges from cluster planting might be due, in part, to belowground mycorrhizal activities (Holmgren et al. 1997; Brooker et al. 2008; Markham et al. 2011; Bertoncello et al. 2016). However, the underlying mechanisms in restoration practices are site-specific and may depend upon species, planting configuration, intervention scenarios, and other factors. Thus, the facilitative effect that is observed in one situation may not be replicable elsewhere, given that it may be influenced by other site-specific factors.

Understanding the mechanisms on the interplay between facilitation and competition in plant communities is important (Holmgren et al. 1997). Different factors may influence species interactions at different densities. Monospecific density experiments are expected to show the “Allee” effect, a benefit of living in groups (Courchamp et al. 1999). The Allee effect is an ecological mechanism of facilitative behaviors in biology and environmental conditioning in plant ecology (Courchamp et al. 1999). The environmental conditioning can be affected by density and may be further enhanced by species mixtures.

Biodiversity is believed to have an important role in ecosystem productivity through complex interactions and facilitative mechanisms which are also known as complementarity and selection effects (Bechara et al. 2016). The complementary effect with niche partitioning in a mixture can lead to overyielding (Van de Peer et al. 2018). Knowledge of the facilitative mechanism of species mixtures is very important for designing an effective plantation method. The experimental separation of density effects and species combinations is required to understand trade-offs and competitive dominance.

Nelder wheel plot experimental designs may be used for efficiently testing the (aboveground) response of plants to planting density. Its purpose serves in comparing multiple tree spacings within a single plot (Nelder 1962). The plot is circular with the highest density in the center and outward radiating concentric rings, with spokes connecting the center with the furthest ring (Nelder 1962). The original method was developed for density effects in single species, but the design can also be applied to mixed-species plantings with some modifications (Wurtz 1995; Doran et al. 2001). Its application during initial planting stages of post-mining restoration is expected to indicate the best strategy for demonstrating net positive interactions both between and within species. A known challenge is that belowground zones of influence can be wider than those aboveground and plant growth responds to the gradient in density tested, rather than the local planting density (Pachas et al. 2018). In the current study, we evaluated combinations of four woody species that are native to boreal Canada: green alder (*Alnus viridis* (Chaix) DC. ssp. *crispa* (Aiton) Turrill); white spruce (*Picea glauca* (Moench) Voss); trembling aspen (*Populus tremuloides* Michaux); and littletree willow (*Salix arbusculoides* Andersson). These species have different growth forms and successional status. The objective was to determine the effects of mixing species along a density gradient on early growth after tree planting in post-mining waste tailings. We posed the following research questions: 1) Are there any advantages to mixing species over monocultural plantings? 2) Are the effects of mixing species on facilitation and competition consistent along density gradients?

Materials and methods

Tailings

Fine tailings and waste rock came from two gold mining sites, Sigma-Lamaque (now Integra Gold) and Metanor Resources (now BonTerra). These are located in the Abitibi-Témiscamingue administrative region of Quebec, Canada. Fine-tailing waste materials are generated during gold extraction, while waste rock spoil is unused rock from the mining processes. The fine-tailings are usually deposited in the form of a slurry into tailings ponds that are left to evaporate (Aubertin et al. 1996; Kossoff et al. 2014). These fine-tailing ponds and waste rock dumps are subject to remediation and reclamation upon mining closure. Tailings were composed primarily of biotite, an iron-rich mica mineral (Taner et al. 1986). Analyses of element concentrations that were performed by COREM Company-Group Roche Inc. (unpublished data, 2010) included S from 0.48 to 0.51%, Al from 5500 to 6100 mg kg⁻¹, Ca from 21000 to 23000 mg kg⁻¹, Fe from 14000 to 16000 mg kg⁻¹, Mg from 4000 to 4500 mg kg⁻¹, P from 0 to 560 mg kg⁻¹, and K from 86 to 100 mg kg⁻¹, together with other mineral elements that were important for plant growth in low concentrations, such as Zn, Mn, Cu, Mo, and Na. Macronutrients essential for plant growth, but absent from the waste rock and fine tailings included nitrogen (N) and phosphorus (P).

The pH of tailings was between 8.55 and 8.68. Arsenic (As) and cyanide concentrations were quite high at 8 to 9 mg kg⁻¹ and 3.7 to 6.3 mg kg⁻¹ (> standard of 2 mg kg⁻¹), respectively. Fine tailings had very low hydraulic conductivity with 10⁻⁴ to 10⁻⁵ cm sec⁻¹ and a small grain size of < 74 µm (Aubertin et al. 1996).

In contrast, waste rock exhibited very high hydraulic conductivity, i.e., 10^{-1} to 10^2 cm s⁻¹ and very large particle sizes ranging from sand (625 µm-2 mm) to gravel (4-32 mm) (Kossoff et al. 2014). The fine tailings were very homogenous while waste rocks exhibited heterogeneous compositions.

Plant Species

The selected plant species were white spruce (*Picea glauca*), littletree willow (*Salix arbusculoides*), trembling or quaking aspen (*Populus tremuloides*), and green or mountain alder (*Alnus viridis* subsp. *crispa*). While aspen and spruce are tree species and willow and alder occur as shrubs, any of the four species can be dominant or codominant depending upon the type of habitat (Barbour and Billings 2000). The four species are native to North America and are commonly found in the same area. The seed provenances are West Quaco (alder), Apsley (spruce), Hamtown Corner (willow) and Cambridge Narrows (aspen).

White spruce is a large coniferous tree which can be found on a variety of landforms and soil types, with many different plant associates. The tree grows best on well-drained soils and generally occurs in alluvial and riparian zones. Littletree willow is usually found along streams and rivers, or even on the floodplains. This shrub can be useful for stabilizing streambanks and for providing erosion control on disturbed sites; it grows best on wet alluvium. Trembling aspen likewise prefers a moist upland wood and can be found on high plateaus, parklands, alluvial terraces, and along watercourses. This species of aspen is the most widely distributed tree in North America, a major cover type across the continent, and common in mixed conifer forests or as a dominant species in many habitats. Green alder is a fast-growing shrub that grows well on poor soils. It may occur as an understory dominant in

open conifer or closed deciduous forests. Alder plays a role in reducing soil erosion and helps to stabilize alluvial deposits. In this experiment, we combined all four species at the initial stage of growth and observed the potential benefits of mixing species composition on the restoration processes. The fastest-growing species were aspen and willow, followed by alder. The three species are light-demanding and are considered pioneers, while spruce is a shade-tolerant and slow-growing species and is considered a mid- to late-successional species (Abrahamson 2015). The species that are listed are tolerant and adapted to poor soils and disturbed sites, and are often used for restoration and rehabilitation projects (Esser 1992; Matthews 1992; Howard 1996; Abrahamson 2015). Green alder can grow well on poor soils because of its mutual association with nitrogen-fixing actinobacteria (*Frankia* spp.) and mycorrhizae (Roy et al. 2007). This species is typically used for reforestation on infertile soils to increase soil organic matter content.

Experimental design

The experiment used a small-scale Nelder plot design (Nelder 1962) that was set-up in rectangular containers of 77 x 97 cm and 10 cm depth (with a total volume of 74.69 l) as a mesocosm under controlled greenhouse conditions. The plot is shown in Fig. 1 with outward radiating wheel rings, and the spokes connecting the center with the furthest ring. The wheel radius is calculated as $r_n = r_0 \alpha^n$, with initial radius (r_0) = 5.1 cm and constant increment (α) = 1.3. The constant increment and initial radius was determined to fit the Nelder plot on container size. Table 1 shows the wheel radius and the growing area with 16 plants per wheel, which was calculated based on the Nelder plot type A1 formula $A_n = r_n^2 \theta (\alpha - \alpha^{-1})/2$, where θ is the angle between the wheel spokes in radians and r_n = radius of the n^{th} arc in cm.

Each Nelder plot contained 16 “spokes” and accommodated 4 replicates of both monoculture treatments and 8 replicates of the two-species mixtures (see Fig. 1 and Table 1). The mixed-species treatments were planted with alternate spokes on half of the circular wheel (8 spokes). Aspen, alder, willow, and spruce were planted in pair combinations (total of 6 plots). Planting was done in both the fine tailing and waste rock materials so that the total number of experimental plots was 12. The arrangement of a Nelder-plot container is shown in Fig. 2.

The experimental unit was the individual plant. The monoculture treatment had 2 spokes replication in one plot or container with total of 6 replications in 3 combined plots, while the mixture treatment had 6 spokes replication in one container only. The plants on the border spokes and border wheel were excluded. Fig. 1 shows the arrangement of individual plants in a full Nelder cycle on each experimental plot. The overall number of experimental units was 6 spokes replication x 6 density levels x 10 species mixtures (6 mixed-species plantings + 4 monocultures) x 2 soil materials for a total of 720 experimental units.

Seeds were first sown and propagated on 1 cm diameter pellets Jiffy-7 Forestry for 10 days (Stuewe & Sons, Inc., Tangent, Oregon 97389 USA). The seedlings were then arranged in Nelder plot design, as shown in Fig. 1. Plant height was measured 90 days after planting. All plants were measured, except those on the border wheels and border spokes between the treatment blocks, as shown in Fig. 1 (within the grey area).

The plants for aboveground biomass, allometric equation and leaf area measurement were sampled from two spokes replication on each mixture treatment, and one spoke replication for monoculture treatment on each container. Total number of sampling for each species are

24 plants. The root biomass was taken for the whole mesocosm container. Leaf area was scanned and measured using WinFOLIA from Regent Instruments Inc (Québec, QC, Canada). The sampled leaf was then dried (at 70°C for 2 days) and weighed to obtain oven-dry mass for Specific Leaf Area (SLA) calculations.

Growth conditions

Greenhouse temperatures were maintained at 23°C (daytime) and 16°C (night-time), with an average relative humidity of 50%. The greenhouse has additional artificial light using HPS 600 W lamps. The experiment was set up in June 2016 for three months.

Plants were hand-watered daily in the morning (10 mm day⁻¹). Fertilizer was applied weekly using NPK 20-20-20 with the following composition: nitrate nitrogen, 5.9%; ammoniacal nitrogen, 3.9%; urea nitrogen, 10.2%; available phosphoric acid (P₂O₅), 20%; soluble phosphorus (P), 8.7%; soluble potash (K₂O), 20%; soluble potassium (K), 16.6%; iron (Fe), 0.10%; manganese (Mn), 0.05%; zinc (Zn), 0.05%; copper (Cu), 0.05%; boron (B), 0.02%; and molybdenum (Mo), 0.0005%. Fertilizer was mixed in a solution of 50 g per 100 l of water (100 ppm) and applied equally across the entire experiment, regardless of substrate. The fertilizer was applied because the mine waste is nutrient deficient.

Data Analysis

The shoot: root biomass ratio of a plant growing in monoculture or mixture reflects the relative ease with which below- and aboveground resources can be obtained. In experiments with *Dactylis glomerata* and *Plantago lanceolata* (Robinson et al. 2010), and *Picea mariana* and *Kalmia angustifolia* (Mallik et al. 2016) no difference was found in shoot: root ratio

between the mixture and isolated plants. Assuming such relation was also held in our experiment, we estimated the root biomass of species A as $(AB+AC-BC)/2$, where AB is the sum of root biomass of species A and B when combined, AC that of A plus C and BC that of B plus C, respectively. The formal formula is written as:

$$\bar{y}_a = \frac{y_{ab} + y_{ac} - y_{bc}}{2} \quad (1)$$

where y is total root biomass and the letter (a, b, c) is the species identifier. The y_{ab} indicates the total root biomass for two species (a and b) in one container, and $\bar{y}_a$ is the estimated total root biomass for one species (a) in the container.

Biomass for individual plants was estimated using the following allometric equation:

$$m = a_i h^{b_i} \quad (2)$$

where m is the individual plant biomass, h is plant height and a_i and b_i are scaling parameters for species i . The scaling parameters (a_i and b_i) were estimated from sampling data for biomass and height using a log-log linear regression model (Harja et al. 2012). While plant height can be used as biomass estimator and site quality indicator, it is also easier to measure for small plants in short period of observation time.

Effects of density on plant height were analyzed using non-linear regression applying the Holliday equation (Holliday 1960; Willey and Heath 1969) and modified to incorporate the species mixing treatments as follows:

$$w = 1/(\sum \alpha_n i_n + \beta \rho + \gamma \rho^2) \quad (3)$$

where w is individual plant height, ρ is plant density of a specific species, and α , β , and γ are the model parameters. The modified constant α_n is a parameter for each n neighboring treatment and i_n is a dummy variable for identifying the treatment data set (1 = the selected treatment, 0 = everything else). The model allows the α parameter to be fitted among the group of treatments while retaining similar β and γ estimates. The α_n can be interpreted as a measure of plant response on different neighboring species. Therefore, we propose the relative competition effect (RCE) which calculated as follows:

$$RCE = \frac{\alpha_{mono} - \alpha_{mix}}{\alpha_{mix}} \quad (4)$$

where α_{mono} is the model parameter for the monoculture and α_{mix} for the mixed planting. RCE is comparable to other measures of competition intensity when β and γ are 0 or without a planting density factor (see Weigelt and Jolliffe 2003). This value is equivalent to the yield relative ratio of the mixed system to the monoculture planting or overyielding (Ong et al. 2015). The equation assumes that differences in yield are consistent along the gradient of density following the Holliday equation.

The Holliday equation is suggested for Nelder plot data analysis and general yield density study because the estimators of its parameters are effectively unbiased and normally distributed (Gillis and Ratkowsky 1978). The original equation of Holliday is as follows:

$$w = 1/(\alpha + \beta\rho + \gamma\rho^2) \quad (6)$$

The biological interpretation of α is a measure of species genetic potential and β is a measure of environment potential, while γ shows curvature of the responses (Willey and Heath 1969; Gillis and Ratkowsky 1978).

All analyses were conducted using R software version 3.5.1 (R Core Team 2018). We used the standard library for linear and nonlinear models and the lmerTest package library for mixed models.

Results

Allometric equations

The allometric regression parameters and adjusted R-squared are significant for all species (Table 2). The allometric equation with plant height can predict about 70-75% variation of aboveground plant biomass according to the R-squared results. Since the equation was fitted with a log-log linear model, the a parameter was back-transformed from the original output ($\log(a)$) and corrected with mean squared error of the regression. This allometric parameter was later used for estimating the total above ground biomass for the whole plants.

Total biomass and growth rates

Total biomass growth on waste rock was higher than on fine tailings (Fig. 3 and Fig. 4). The fast-growing species (willow and aspen) had the highest total biomass on both materials (Fig. 3). Willow and alder had higher aboveground biomass on waste rock, while aspen and spruce had higher aboveground biomass on fine tailings.

Interactions between species also influenced early biomass growth. Combining both fast-growing species (aspen and willow) led to the greatest biomass growth on both substrates (Fig. 4), but of all species tested, only aspen increased biomass growth in mixtures. Willow and alder had higher aboveground biomass on waste rock, while aspen and spruce had higher aboveground biomass on fine tailings. The species shown to have different growth performance on each substrate materials. Differences between materials are also reflected in shoot: root ratios. But since species root biomass was estimated from total root biomass in a Nelder container with mixtures of species, we cannot interpret correlation with the substrate treatment.

Parameters of Holliday equation

Regression parameters of the Holliday equation are shown in table 3 and 4 for plants that are grown in waste rock, and in fine tailings, respectively. The curve plots are shown in Fig. 5 and Fig. 6 , respectively. The α parameter is significant which shows a constant deviance in yields between all the treatments for all species on waste rock and fine tailings. The significance of β and γ parameters varied between species, showing a high variation in magnitude or slope and the curvature on the correlation of density and plant height. The highest β is shown for alder on waste rock material, and the smallest for willow. In the same time, willow showed to have high degree variation of data with its high RSE and non-significant β and γ . The non-significant β and γ are also shown for alder and spruce on fine tailings. The β and γ for spruce and willow on fine tailings had signs opposite of what was expected.

Relative Competition Effects

Aspen is the most competitive species on waste rock as shown by Relative Competition Effect (RCE) table (Table 5). It had better yield of about 60% ($RCE = 0.6$) more when it is planted in mixtures with alder, 49% ($RCE = 0.49$) and 47% ($RCE = 0.47$), when co-planted with spruce and willow, respectively. In the same time its neighbors' losses the yields of about 5% ($RCE = -0.05$), 26% ($RCE = -0.26$) and 13% ($RCE = -0.13$), respectively. Spruce, in opposite, is always suppressed in mixture with a lost yield of about 6% ($RCE = -0.06$), 26% ($RCE = -0.26$), and 8% ($RCE = -0.08$) when it is co-planted with alder, aspen and willow, respectively.

Fast-growing species seem to dominate in competitions with slow-growing species in waste rock. Except for alder and willow, where the slow-growing species (alder) is shown to be more competitive than the fast-growing species (willow). Alder had 32% ($RCE = 0.32$) greater yield when mixed with willow, while willow lost 5% ($RCE = 0.05$). In contrast, alder and spruce have reduced yields in mixtures for both species, compared to their monocultures (Table 5).

The plants were mostly suppressed when grown in mixtures on fine tailings as shown in Table 5. Aspen had the lowest losses when it was mixed with spruce ($RCE = -0.71$), which is opposite to the response that is observed when both species are planted in waste rock. The only plants that showed benefits was aspen when it was mixed with willow ($RCE = 0.06$), and willow when it was mixed with spruce ($RCE = 0.06$). In general, we could not find any mixture that gave advantages for both species in a paired combination on any of the soil materials.

Specific Leaf Area (SLA)

Density gradient and species had significant effects on SLA ($P < 0.01$), as shown on Table 6. The interaction of density gradient and species also had significant effects on SLA ($P < 0.05$), while the addition of soil material factors to this interaction was not significant. The soil material itself had a significant effect on SLA ($P < 0.05$). The SLA on fine tailings was lower than that on waste rock soil material for most of the species (Fig. 7). SLA and density were positively correlated for most species combinations except for alder and spruce in fine tailings (Fig. 7).

Discussion

Substrate effects on tree growth

Overall plant growth in waste rock was better than in fine tailings, which could be due to the physical characteristics of the material. The fine grain size of mine tailings results in very high water retention and very low hydraulic conductivity (Aubertin et al. 1996), which are unfavourable conditions for plant species, generally. The measured fine tailings moisture content in the field site is about 20-30% at depth 15 cm. This quantity of water corresponds to the permanent wilting point for fine-grained material, such as clay or silty-clay, which have similar grain sizes in mine tailings. In contrast, waste rock has very low water retention and very high hydraulic conductivity, which is also an unfavourable condition for plant growth, but the waste rock offered a better environment than did fine tailings when it was subject to daily watering and weekly fertilization. In addition, some contaminants in fine tailings could limit growth because of their phytotoxicity.

Indeed, fine tailings contained contaminants such as aluminum, iron, magnesium, arsenic and cyanide. Although some native vegetations may be adapted to this tailing conditions (Abdul-Wahab and Marikar 2012). Despite phytotoxicity, plant tissues have shown translocation of metals from roots to the upper parts of the plants (Abdul-Wahab and Marikar 2012). Some plants are known to have adaptability and resistance to soil contaminants, either through stress avoidance or stress tolerance (Punz and Sieghardt 1993).

Indeed, species of poplar (*Populus* spp.) and willow (*Salix* spp.) are known heavy metal accumulators in phytoremediation practices (Fischerová et al. 2006; Hassinen et al. 2009; Mehes-Smith and Nkongolo 2015; Salam et al. 2016). Observations on aspen on metal-contaminated sites showed a high level of mixoploidy in the plants. These mitotic abnormalities have been shown to exert no adverse effects on plant growth and survival (Hassinen et al. 2009; Mehes-Smith and Nkongolo 2015). In some phytoremediation experiments, the growth of willow was also demonstrated to be unaffected by heavy metal contaminants (Fischerová et al. 2006; Salam et al. 2016). Alder's status is less known as a heavy metal accumulator, but it has resistance to organic and inorganic soil contaminants. It is often used for phytostabilization and as nurse species in restoration practices (Roy et al. 2007; Lalancette et al. 2019). White spruce, in contrast, is quite sensitive and less tolerant of heavy metal contamination. An experiment with spruce on heavy metal-contaminated soil showed about a 20 to 25% reduction in growth (Dixon and Buschena 1988; Nosko et al. 1988). Thus, growth reduction of white spruce on fine tailings, as shown in our experiment, could be affected by a combination of water stress and phytotoxicity.

Another plant trait characteristic that may differentiate species metabolic strategies in a stressed environment is the shoot: root ratio. The shoot: root ratios for aspen were

respectively 2.95 and 3.23 on waste rock and fine tailings, which were still in the range 2-4 for normal conditions (Peng and Dang 2003). Willow had respective shoot: root ratios of 2.51 and 1.75 on waste rock and fine tailings, which were lower than the normal 2.5-5 for willow (Dušek and Květ 2006). Alder showed respective shoot: root ratios of 4.53 and 2.80 on waste rock and fine tailings, which were far higher than the normal ratio 1 – 1.5 for alder (Lorenc-Plucińska et al. 2013). The shoot: root ratios for spruce were respectively 0.79 and 1.64 on waste rock and fine tailings, which were slightly lower than the normal 2 – 4 (Peng and Dang 2003). Thus, shoot: root ratios were either higher or lower than normal range, similarly for waste rock and fine tailings. This could be due to the similar treatments of daily watering and weekly fertilization on both waste rock and fine tailings, or limitation on the number of samples from our observation. However, there were some differences in shoot: root ratios within species groups in response to different tailings. The differences were also shown in the mixtures of species combinations, possibly due to different metabolic strategies of each species in tailing materials and interactions with neighbours.

While the species root biomass was estimated from a mixture plantation with an assumption of additivity, the conclusion may not be accurate. In this experiment we tried to use a “Minirhizotron” method for belowground observation using transparent tubes inside the container. But there were only few of the roots visible from the tubes and no conclusions could be drawn. This could be due to waste rock characteristic with big particles which leaving too many air spaces around the tubes. The root growth was also shorter than expected from the tube depth on fine tailing substrates. On the same time, it was hard to separate roots of individual plants from the mesocosm container.

Mixture effects

The Relative Competition Effect (RCE) measured relative advantages between species mixtures. We found that a mixture does not have advantages for combined pairs of species. Most of the plants grow better in monoculture, except for aspen in waste rock substrate. Trembling aspen is shown as the most competitive species in waste rock. While aspen is a fast-growing species, not all faster growing species got advantages when it planted in mixture. Here we cannot find a plant trait or specific factor correlated with advantages or disadvantages in mixture planting.

Since the plants were very small, and light was not limiting at early growth stages, belowground competition seems to be the most significant factor in this experiment. Another factor such allelopathy is less likely found in the literature for any of the species in this study. This experiment was an early-stage evaluation and might not depict the whole suite of interactions that are likely to occur between species at later stages in the greenhouse or in the field. The outcome could be different as the plants grew larger and started to shade one another, exerting both below- and aboveground interaction effects.

Density gradient effects on growth and SLA

The effect of the gradient of density on mixtures of species was consistent in all treatments. The regression fit of Holliday equation was significant for all groups. The equation intercept (α) is also significant for all species combinations, whether on waste rock or on fine tailings. Significant alpha parameters for all species mixtures on both substrates suggest the neighboring species influenced biomass growth across all conditions.

Interesting effect of planting density were shown on spruce and willow in fine tailings where it has greater growth yield at higher planting density (Fig. 6). This was unusual, as we

394 expected higher competition with increased density. This positive effect of density can be
395 explained by the Allee effect (Courchamp et al. 1999), resulting in a net positive effect
396 between facilitation and competition.

397 Another possibility of greater plant growth in denser plantings is the “elongation” effect
398 because of competition for light. Since the plants were very small, we assumed that the light
399 resources might not be limiting in this 3-month experiment. Analysis of the allometric
400 correlation between biomass and height did not reveal a significant effect of density for any
401 species (data not shown).

402 Most species are known to decrease their SLA as water stress increases (Marron et al.
403 2003; Liu et al. 2016). The deciduous aspen, willow and alder had similar SLA adaptations
404 to water stress (Hennessey et al. 1985; Splunder et al. 1996). Here, we found that SLA was
405 lower in fine tailings compared to that in waste rock for most species, except spruce (Fig. 7).
406 Considering the general SLA adaptation for water stress as mentioned above, plants on fine
407 tailings were shown to have higher water stress compared with those on waste rock. White
408 spruce exhibited greater SLA under higher water stress. This response was not surprising
409 given that this conifer was the most drought-tolerant species among those studied in our
410 experiment, and as has been reported elsewhere (Van den Driessche 1991; Abrahamson
411 2015; Moran et al. 2017).

412 Here we also found a contrasting water stress between lower and higher planting densities,
413 especially for aspen and willow (Fig. 7). Most of the plants showed a decrease in water stress
414 at higher density, which could be an indication of “plant facilitation”. But this does seem to

emerge in alder on the fine tailings. Alder seems to have more sensitivity on belowground competition, as it is known as high moisture-demanding species (Matthews 1992).

The significant effect of the density gradient on SLA adaptation may indicate a facilitative effect on soil moisture stress. One possible factor relating to soil moisture conservation under higher plant densities is microclimate improvement, soil amelioration with higher root densities, or its combination. The effect of density on microclimate improvement is a known, important aspect of restoration practices (Courchamp et al. 1999; Bechara et al. 2016). We posit that the positive density effect that was observed in our experiment is correlated with microclimate improvement. Further exploration into the details of microclimate factors such as temperature, moisture and other functional traits might be required to confirm our hypothesis.

Conclusions

Plant growth was five times greater on waste rock than on fine tailings with similar treatments. Species shoot: root ratios varied from the normal ranges of typical species but showed to have similar deviation between waste rock and fine tailings. Further experiments with more precise root measurement may be required to get the real effect of the treatments.

Positive effects of density on early growth suggested that microclimate improvement played a role in accelerating the growth of the plants. Specific leaf area (SLA), as a plant trait proxy for water stress adaptation, was greatly affected by the material and gradient density. The positive correlation of SLA with density could be an indication of facilitative effects on

water stress. This finding could be further investigated for quantifying facilitative and competitive effects on planting density experiments.

We found that mixtures do not offer advantages for both species in paired combinations. Most species grew better in monoculture, but some species grew better in mixtures. The fast-growing aspen was dominant and better in mixtures on waste rock material. Other than this response, we could not find a general correlation between species-specific traits with their adaptations to different mixtures.

The relative competition effect (RCE) quantified the advantages and disadvantages of the mixture over monoculture with proportional assumptions regarding the density gradient. Given that the experiment was of limited duration, the result may not depict a complete picture of plant-plant interactions. We suggest further experiments with longer timelines to better explore the facilitative indicators, together with competition effects, to improve the prediction method in successional dynamics modelling.

Acknowledgements

We thank Marie-Eve Beaulieu, Dr. Marie R. Coyea, Francois Larochelle and Marie-Andrée Paré for their lab and greenhouse work. We Thank Dr. W.F.J. Parsons for proofreading and critical input to the article. This research was supported by a joint research program on sustainable development in the mining sector developed by Fonds de recherche du Québec – Nature et technologies (FRQNT) in collaboration with the Ministère de l'Énergie et des Ressources naturelles (MERN) of Quebec Government. We thank the mining companies Bonterra (formerly Ressources Métanor), Eldorado Gold (formerly Integra Gold)

456 and Fournier et Fils for their logistical support. NSERC Discovery Grant (DPK) and GECA
457 Environnement (SA) supports are also acknowledged.

Draft

References

- Abdul-Wahab, S., and Marikar, F. 2012. The environmental impact of gold mines: pollution by heavy metals. *Open Engineering* **2**(2). doi:10.2478/s13531-011-0052-3.
- Abrahamson, I. 2015. *Picea glauca*, white spruce. In *Fire Effects Information System*. USDA, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, Ft. Collins, CO (Producer). Available from <http://www.fs.fed.us/database/feis/> [accessed 1 July 2019].
- Aubertin, M., Bussiere, B., and Chapuis, R.P. 1996. Hydraulic conductivity of homogenized tailings from hard rock mines. *Can. Geotech. J.* **33**(3): 470–482. doi:10.1139/t96-068.
- Barbour, M.G., and Billings, W.D. (Editors). 2000. *North American Terrestrial Vegetation*. In 2nd edition. Cambridge University Press, Cambridge, UK.
- Bechara, F.C., Dickens, S.J., Farrer, E.C., Larios, L., Spotswood, E.N., Mariotte, P., and Suding, K.N. 2016. Neotropical rainforest restoration: comparing passive, plantation and nucleation approaches. *Biodivers. Conserv.* **25**(11): 2021–2034. doi:10.1007/s10531-016-1186-7.
- Bertoncello, R., Oliveira, A.A., Holl, K.D., Pansonato, M.P., and Martini, A.M.Z. 2016. Cluster planting facilitates survival but not growth in early development of restored tropical forest. *Basic Appl. Ecol.* **17**(6): 489–496. doi:10.1016/j.baae.2016.04.006.
- Bois, G., Piché, Y., Fung, M.Y.P., and Khasa, D.P. 2005. Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry. *Mycorrhiza* **15**(3): 149–158. doi:10.1007/s00572-004-0315-4.
- Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B., and Michalet, R. 2008. Facilitation in plant communities: the past, the present, and the future. *J. Ecol.* **96**(1): 18–34. doi:10.1111/j.1365-2745.2007.01295.x.
- Courchamp, F., Clutton-Brock, T., and Grenfell, B. 1999. Inverse density dependence and the Allee effect. *Trends Ecol. Evol.* **14**(10): 405–410. doi:10.1016/S0169-5347(99)01683-3.
- Dixon, R.K., and Buschena, C.A. 1988. Response of ectomycorrhizal *Pinus banksiana* and *Picea glauca* to heavy metals in soil. *Plant Soil* **105**(2): 265–271. doi:10.1007/BF02376791.

- Doran, K., Ruess, R.W., Plumley, G.F., and Wurtz, T.L. 2001. Photosynthetic responses of white spruce saplings (*Picea glauca*) to controlled density gradients of spruce and green alder (*Alnus crispa*). *Écoscience* **8**(1): 76–88. doi:10.1080/11956860.2001.11682633.
- Dušek, J., and Květ, J. 2006. Seasonal dynamics of dry weight, growth rate and root/shoot ratio in different aged seedlings of *Salix caprea*. *Biologia* **61**(4). doi:10.2478/s11756-006-0074-0.
- Esser, L.L. 1992. *Salix arbusculoides*. In Fire Effects Information System [online]. USDA Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, Ft. Collins, CO (Producer). Available from <http://www.fs.fed.us/database/feis/> [accessed 1 July 2019].
- Fischerová, Z., Tlustoš, P., Jiřina Száková, and Kornelie Šichorová. 2006. A comparison of phytoremediation capability of selected plant species for given trace elements. *Environmental Pollution* **144**(1): 93–100. doi:10.1016/j.envpol.2006.01.005.
- Gillis, P.R., and Ratkowsky, D.A. 1978. The behaviour of estimators of the parameters of various yield-density relationships. *Biometrics* **34**(2): 191. doi:10.2307/2530009.
- Harja, D., Vincent, G., Mulia, R., and van Noordwijk, M. 2012. Tree shape plasticity in relation to crown exposure. *Trees* **26**(4): 1275–1285. doi:10.1007/s00468-012-0703-x.
- Hassinen, V., Vallinkoski, V.-M., Issakainen, S., Tervahauta, A., Kärenlampi, S., and Servomaa, K. 2009. Correlation of foliar MT2b expression with Cd and Zn concentrations in hybrid aspen (*Populus tremula* × *tremuloides*) grown in contaminated soil. *Environmental Pollution* **157**(3): 922–930. doi:10.1016/j.envpol.2008.10.023.
- Hennessey, T.C., Bair, L.K., and McNew, R.W. 1985. Variation in response among three *Alnus* spp. clones to progressive water stress. *Plant and Soil* **87**(1): 135–141. doi:10.1007/BF02277654.
- Holliday, R. 1960. Plant population and crop yield. *Nature* **186**(4718): 22–4. doi:10.1038/186022b0.
- Holmgren, M., Scheffer, M., and Huston, M.A. 1997. The interplay of facilitation and competition in plant communities. *Ecology* **78**(7): 1966–1975. doi:10.1890/0012-9658(1997)078[1966:TIOFAC]2.0.CO;2.
- Howard, J.L. 1996. *Populus tremuloides*. In Fire Effects Information System [online]. USDA Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, Ft. Collins, CO (Producer). Available from <http://www.fs.fed.us/database/feis/> [accessed 1 July 2019].

- Kossoff, D., Dubbin, W.E., Alfredsson, M., Edwards, S.J., Macklin, M.G., and Hudson-Edwards, K.A. 2014. Mine tailings dams: characteristics, failure, environmental impacts, and remediation. *Appl. Geochemistry* **51**: 229–245. doi:10.1016/j.apgeochem.2014.09.010.
- Lalancette, S., Lerat, S., Roy, S., and Beaulieu, C. 2019. Fungal endophytes of *Alnus incana* ssp. *rugosa* and *Alnus alnobetula* ssp. *crispa* and their potential to tolerate heavy metals and to promote plant growth. *Mycobiology* **47**(4): 415–429. doi:10.1080/12298093.2019.1660297.
- Liu, Y., Dawson, W., Prati, D., Haeuser, E., Feng, Y., and van Kleunen, M. 2016. Does greater specific leaf area plasticity help plants to maintain a high performance when shaded? *Ann. Bot.* **118**(7): 1329–1336. doi:10.1093/aob/mcw180.
- Lorenc-Plucińska, G., Walentynowicz, M., and Niewiadomska, A. 2013. Capabilities of alders (*Alnus incana* and *A. glutinosa*) to grow in metal-contaminated soil. *Ecol. Eng.* **58**: 214–227. doi:10.1016/j.ecoleng.2013.07.002.
- Mallik, A.U., Biswas, S.R., and Collier, L.C.S. 2016. Belowground interactions between *Kalmia angustifolia* and *Picea mariana*: roles of competition, root exudates and ectomycorrhizal association. *Plant Soil* **403**(1–2): 471–483. doi:10.1007/s11104-016-2819-z.
- Markham, J., Young, I., and Renault, S. 2011. Plant facilitation on a mine tailings dump. *Restor. Ecol.* **19**(5): 569–571. doi:10.1111/j.1526-100X.2011.00801.x.
- Marron, N., Dreyer, E., Boudouresque, E., Delay, D., Petit, J.-M., Delmotte, F.M., and Brignolas, F. 2003. Impact of successive drought and re-watering cycles on growth and specific leaf area of two *Populus × canadensis* (Moench) clones, ‘Dorskamp’ and ‘Luisa Avanzo.’ *Tree Physiol.* **23**(18): 1225–1235. doi:10.1093/treephys/23.18.1225.
- Matthews, R.F. 1992. *Alnus viridis subsp. crispa*. In *Fire Effects Information System* [online]. USDA Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, Ft. Collins, CO (Producer). Available from <http://www.fs.fed.us/database/feis/> [accessed 1 July 2019].
- Mehes-Smith, M., and Nkongolo, K.K. 2015. Physiological and cytological responses of *Deschampsia cespitosa* and *Populus tremuloides* to soil metal contamination. *Water Air Soil Pollut.* **226**(4): 125. doi:10.1007/s11270-015-2382-x.
- Moran, E., Lauder, J., Musser, C., Stathos, A., and Shu, M. 2017. The genetics of drought tolerance in conifers. *New Phytol.* **216**(4): 1034–1048. doi:10.1111/nph.14774.
- Nadeau, M.B., Laur, J., and Khasa, D.P. 2018. Mycorrhizae and rhizobacteria on precambrian rocky gold mine tailings: I. Mine-adapted symbionts promote white spruce health and growth. *Front. Plant Sci.* **9**: 1267. doi:10.3389/fpls.2018.01267.

- Nadeau, M.B., Quoreshi, A., and Khasa, D.P. 2016. Ecological restoration and bioremediation of Canadian mining boreal ecosystems. *In* Microbes for Restoration of Degraded Ecosystems. *Edited by* D.J. Bagyaraj and Jamaluddin. New India Publishing Agency. pp. 259–284.
- Nelder, J.A. 1962. New kinds of systematic designs for spacing experiments. *Biometrics* **18**(3): 283. doi:10.2307/2527473.
- Nosko, P., Brassard, P., Kramer, J.R., and Kershaw, K.A. 1988. The effect of aluminum on seed germination and early seedling establishment, growth, and respiration of white spruce (*Picea glauca*). *Can. J. Bot.* **66**(11): 2305–2310. doi:10.1139/b88-313.
- Ong, C.K., Black, C.R., and Wilson, J. (Editors). 2015. Tree-Crop Interactions: agroforestry in a changing climate. *In* 2nd edition. CAB International, Wallingford, Oxfordshire, UK; Boston, MA, USA.
- Pachas, A.N.A., Shelton, H.M., Lambrides, C.J., Dalzell, S.A., Murtagh, G.J., and Hardner, C.M. 2018. Effect of tree density on competition between *Leucaena leucocephala* and *Chloris gayana* using a Nelder Wheel trial. II. Belowground interactions. *Crop Pasture Sci.* **69**(7): 733–744.
- Peng, Y.Y., and Dang, Q.-L. 2003. Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. *Forest Ecol. Manag.* **180**(1–3): 1–9. doi:10.1016/S0378-1127(02)00486-3.
- Punz, W.F., and Sieghardt, H. 1993. The response of roots of herbaceous plant species to heavy metals. *Environ. Exp. Bot.* **33**(1): 85–98. doi:10.1016/0098-8472(93)90058-N.
- R Core Team. 2018. R: a language and environment for statistical computing [online]. R Foundation for Statistical Computing, Vienna, Austria. Available from <https://www.R-project.org/> [accessed 2 July 2018].
- Robinson, D., Davidson, H., Trinder, C., and Brooker, R. 2010. Root–shoot growth responses during interspecific competition quantified using allometric modelling. *Annals of Botany* **106**(6): 921–926. doi:10.1093/aob/mcq186.
- Roy, S., Khasa, D.P., and Greer, C.W. 2007. Combining alders, frankiae, and mycorrhizae for the revegetation and remediation of contaminated ecosystems. *Can. J. Bot.* **85**(3): 237–251. doi:10.1139/B07-017.
- Salam, M.M.A., Kaipainen, E., Mohsin, M., Villa, A., Kuittinen, S., Pulkkinen, P., Pelkonen, P., Mehtätalo, L., and Pappinen, A. 2016. Effects of contaminated soil on the growth performance of young *Salix* (*Salix schwerinii* E. L. Wolf) and the potential for phytoremediation of heavy metals. *J. Environ. Manage.* **183**: 467–477. doi:10.1016/j.jenvman.2016.08.082.

- Splunder, I.V., Voeselek, L.A.C.J., Vries, X.J.A.D., Blom, C.W.P.M., and Coops, H. 1996. Morphological responses of seedlings of four species of Salicaceae to drought. *Can. J. Bot.* **74**(12): 1988–1995. doi:10.1139/b96-238.
- Taner, M.F., Trudel, P., and Perrault, G. 1986. Geochimie de la biotite associee a certains gisements d'or de Val d'Or, Malartic et Chibougamau, Quebec. *Can. Mineral* **24**(4): 761–774.
- Van de Peer, T., Verheyen, K., Ponette, Q., Setiawan, N.N., and Muys, B. 2018. Overyielding in young tree plantations is driven by local complementarity and selection effects related to shade tolerance. *J Ecol* **106**(3): 1096–1105. doi:10.1111/1365-2745.12839.
- Van den Driessche, R. 1991. Influence of container nursery regimes on drought resistance of seedlings following planting. II. Stomatal conductance, specific leaf area, and root growth capacity. *Can. J. For. Res.* **21**(5): 566–572. NRC Research Press. doi:10.1139/x91-078.
- Weigelt, A., and Jolliffe, P. 2003. Indices of plant competition. *J. Ecol.* **91**(5): 707–720. doi:10.1046/j.1365-2745.2003.00805.x.
- Willey, R.W., and Heath, S.B. 1969. The quantitative relationships between plant population and crop yield. *Edited by* N.C. Brady. Academic Press. pp. 281–321. doi:10.1016/S0065-2113(08)60100-5.
- Wurtz, T.L. 1995. An efficient design for studies of plant species interactions: an example with white spruce and alder. *In* Special Publication - No. 10. *Edited by* D.J. Mead and Cornforth, I.S. Agronomy Society of New Zealand, Lincoln University Press, Canterbury, New Zealand. pp. 51–58.

Table 1. Wheel radius and spacing area for individual plant in Nelder plot design shown in Fig. 1.

N	Radius (cm)	Spacing Area (cm ²)
0	5.1	2.7
1	6.6	4.6
2	8.6	7.7
3	11.2	13.0
4	14.5	22.1
5	18.9	37.3
6	24.6	63.0
7	32.0	106.4

Table 2. Regression fit parameters for biomass-height allometric.

Species	<i>a</i>	<i>b</i>	R ²
Alder	0.0206***	1.94***	0.687
Aspen	0.0044***	1.73***	0.733
Spruce	0.0038***	2.28***	0.746
Willow	0.0184***	1.32***	0.760

Note: Significant codes: $P < 0.001$ ‘***’

Table 3. Regression fit parameters of Holliday equation for the plants grown in waste rock.

Species	α_{AD}	α_{AP}	α_{SP}	α_{WL}	β	γ	RSE
Alder (AD)	0.28***	0.30***	0.29***	0.22***	0.16*	-0.05	1.15
Aspen (AP)	0.03***	0.05***	0.03***	0.03***	0.13***	-0.04***	2.80
Spruce (SP)	0.21***	0.27***	0.20***	0.21***	0.11***	-0.03**	0.73
Willow (WL)	0.11***	0.12***	0.07***	0.11***	0.04	-0.01	3.62

Note: The parameter index for α_n was replaced by its corresponding neighbouring species identifier. RSE is the residual standard error of the model estimation. Significant codes: $P < 0.001$ ‘***’, $P < 0.01$ ‘**’, $P < 0.05$ ‘*’

Table 4. Regression fit parameters of Holliday equation for the plant grown in fine tailing.

Species	α_{AD}	α_{AP}	α_{SP}	α_{WL}	β	γ	RSE
Alder (AD)	0.55***	0.62***	0.64***	0.80***	0.14	-0.04	0.45
Aspen (AP)	0.10***	0.09***	0.30***	0.08***	0.28***	-0.09**	2.42
Spruce (SP)	0.44***	0.45***	0.43***	0.44***	-0.05	0.01	0.44
Willow (WL)	0.65***	0.88***	0.52***	0.55***	-0.33**	0.10*	1.46

Note: The parameter index for α_n is replaced by its corresponding neighbouring species identifier. Significant codes: $P < 0.001$ ‘***’, $P < 0.01$ ‘**’, $P < 0.05$ ‘*’

Table 5. Relative competition effect (RCE) for Nelder experiment applied in waste rock and fine tailings.

	Waste Rock				Fine Tailings			
	Alder	Aspen	Spruce	Willow	Alder	Aspen	Spruce	Willow
Alder	0.00	-0.05	-0.02	0.32	0.00	-0.11	-0.13	-0.31
Aspen	0.60	0.00	0.49	0.47	-0.09	0.00	-0.71	0.06
Spruce	-0.06	-0.26	0.00	-0.08	-0.02	-0.04	0.00	-0.02
Willow	-0.05	-0.13	0.45	0.00	-0.16	-0.38	0.06	0.00

Table 6. Type III Analysis of Variance Table with Satterthwaite's method for the effect of species, density, and substrate material on Specific Leaf Area.

Source	DF	F value	Pr(>F)
Density	1	15.326	0.000
Species	3	7.0038	0.000
Material	1	4.115	0.043
Density:Species	3	3.4499	0.017
Density:Material	1	5.1531	0.024
Species:Material	3	1.1984	0.310
Density:Species:Material	3	0.113	0.953

Fig. 1. Nelder wheel plot design for tree seedling experiments, the filled and open circles are seedling positions of two different species, respectively. Number of plants per wheel is 16. The sampled plants are inside the grey area (omitting the plants on the border).

Fig. 2. The container arrangement for mixed species of alder (AD), aspen (AP), spruce (SP) and willow (WL) in waste rock and fine tailing materials.

Fig. 3. Estimated total root and shoot biomass for each species in waste rock and fine tailings.

The value above the bars is shoot: root ratio.

Fig. 4. Total shoot and root biomasses in each mesocosm container of Nelder plot experiment for combinations of alder (AD), aspen (AP), spruce (SP) and willow (WL) in waste rock and fine tailings. The value above the bars is shoot: root ratio.

Fig. 5. Regression curves of Holliday equation on plant height with gradual density and combinations of neighbours (shown by different legends) growing in waste rock.

Fig. 6 Regression curves of Holliday equation on plant height growth with gradual density and combinations of neighbours (shown by different legends) in fine tailings.

Fig. 7. Specific Leaf Area (SLA) of individual plants along the density gradient and in waste rock and fine tailings.