Effects of Spacing and Herbaceous Hydroseeding on Water Stress Exposure and Root Development of Poplars Planted in Soil-Covered Waste Rock Slopes

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Abstract

Root development is important to ensure tree survival in conditions of water stress. Despite their long-recognized role, little attention has been given to their development on waste rock slopes subject to rapid drainage. This study was conducted in an open-pit gold mine in a boreal forest. Its main objective was to establish a plantation design allowing a moderate level of competition for water resources in waste rock slopes. A hybrid poplar plantation was established in May 2013 on soil-covered waste rock slopes of 33%. The experimental design included three different tree spacings: 1x1 m, 2x2 m without herbaceous seeding, 2x2 m with herbaceous seeding and 4x4 m. The poplars responded to the increased competition resulting from the closest spacing and the herbaceous seeding by investing less energy in diameter and height growth. Trees that were subject to a higher level of competition were able to acclimatize to water stress conditions by increasing their root length density and specific root length and by reducing their above-ground biomass. This study indicates that some clones of hydrid poplars showing phenotypic plasticity in the ratio of above- and belowground growth can be adapted for the revegetation of mine sites in the short term.

Keywords: Mine reclamation. Mine revegetation. Mine waste rock. Water stress slopes. Tree. Root development.

Funding details

This work was supported by the Natural Sciences and Engineering Research Council of Canada under Grant [number RDCPJ 462610-13].

Disclosure statement

None of the authors has any financial interest or benefit in the direct application of the research described in this article.

Résumé

Le développement racinaire joue un rôle crucial dans l'alimentation hydrique des arbres et leur survie en conditions de stress hydrique, mais reste peu considéré, surtout dans un contexte de stériles miniers qui sont généralement exposés au vent et/ou en pente et retiennent peu l'eau. Cette étude se déroule dans une mine d'or à ciel ouvert en forêt boréale. L'objectif principal est de déterminer le design de plantations permettant d'avoir un niveau modéré de compétition, en particulier pour les ressources en eau possiblement limitées dans des pentes de stériles. Ainsi, une plantation de peuplier hybride a été installée en mai 2013 sur des pentes de stériles ayant une inclinaison de 33% et recouvertes de sol. Le dispositif comporte trois espacements entre les arbres (1x1, 2x2 et 4x4 m), avec un ensemencement herbacé combiné ou non à la plantation d'arbres espacés de 2x2 m. Les peupliers ont répondu à l'intensification de la compétition, résultant de la diminution de l'espacement (1x1) et de l'ensemencement herbacé (2x2h), en diminuant leur investissement dans la croissance en diamètre et en hauteur. Les arbres soumis à un niveau de compétition élevée se sont acclimatés en accroissant leur longueur racinaire par volume de sol et leur longueur par unité de masse de racine, et en réduisant leur biomasse aérienne. Cette étude indique que certains clones de peuplier hybride démontrant une plasticité phénotypique vis-à-vis du ratio développement aérien / développement souterrain peuvent être adaptés à la végétalisation des sites miniers à court terme.

1. Introduction

Mining companies have aimed at implementing revegetation programs for several years to reduce the social and environmental impacts of their operations and comply with environmental regulation. Revegetation techniques performed on mine sites' slopes use fast-growing herbaceous plants, which contribute to stabilizing the soil and protect it from erosion and ensure the cohesion of the surface soil layer (Evanylo et al., 2005; Xia, 2004; Carroll et al., 2000). When used in conjunction with herbaceous species, tree plantation can favour colonization by local species from the adjacent forests (Nichols *et al.*, 2010), thereby facilitating the remediation of the forest landscape. However, herbaceous cover may compete with planted tree seedlings and can hinder their establishment (Halofsky and McCormick, 2005; Skousen *et al.*, 2006).

The establishment of a tree cover on waste rocks- is problematic, notably due to their physical and chemical characteristics (Aubertin *et al*., 2002; Mench *et al.*, 2003). Waste rocks are characterized by a lack of essential nutrients and absence of organic matter and micro-organisms (Burger and Zipper, 2002). Waste rock slopes are subject to wind exposure, erosion and quick drainage, all of which can negatively affect tree development. Topsoil can be used on mine waste rock to provide a more fertile substrate. The type of soil is an important determinant of tree growth on sites to be restored (Zipper et al., 2011). Its use to cover waste rock improves soil productivity and soil functionalities (Tordoff et al., 2000, Sena, 2014). Due to their quick drainage and slope, waste rock slopes form a planting environment where the supply of water to trees can be very limited. Water stress occurs when the quantity of water transpired by a plant is higher than the quantity that it absorbs (Compaoré, 2011). This generally results in lower biomass production (Benomar *et al.*, 2012). Severe water stress can cause root mortality and wilting of the plant (Condit et al., 1995; Hartmann *et al.*, 2013).

Hybrid poplar is an interesting choice for the revegetation of mine sites because of its good survival rates (Czapowskyj 1978; Clark Ashby 1995) and rapid growth (Casselman et al. 2006), which are important to control erosion in slopes (Fields-Johnson *et al.*, 2014; Fortier *et al.*, 2008). Poplar is capable of developing fine roots (diameter <2 mm) in large volumes of soil in conditions of water stress (Bauhus and Messier, 1999; Casselman *et al.*, 2006). These fine roots are highly effective for absorbing water (Leuschner *et al.*, 2004). However, most hybrid poplars are known for their high water demand (Dickmann, 2001), which may impair their~~its~~ survival especially in~~during~~ the initial years of growth (Zandalinas *et al.*, 2017). Some poplar clones show strong drought tolerance (Tschaplinski and Blake 1989, Chen *et al*. 1997), while others are more drought sensitive (Pallardy and Kozlowski 1981, Brignolas *et al*. 2000, Zhang *et al.* 2004).

Root development ensures tree survival in conditions of water stress, but has been poorly considered when assessing the success of the revegetation of mine wastes (Guittonny-Larchevêque *et al.*, 2016; Guittonny-Larchevêque and Pednault, 2016). When a tree is subject to moderate water stress, it adopts a series of adaptive strategies in order to avoid and/or tolerate the stress (Siemens and Zwiazek, 2003). The most important of these mechanisms is the development of the root system to maintain water supply (Logbo *et al.*, 2013) and the reduction of water loss due to leaf transpiration (Arango-Velez *et al.*, 2013). Several studies have shown that hybrid poplars can develop various strategies to help them tolerate drought conditions, including the reduction of foliage area, leaf abscission, the production of fine roots, development of root hair, increase in the volume of substrate explored by increasing the specific root length (SRL = the ratio of root length to dry mass of fine roots in cm/g), the root length density (RLD = the ratio of root length to volume of soil in cm/cm^3), and specific root area (SRA = the ratio of the area to dry mass of fine roots in cm^2/g), the reduction of the average root diameter and the above-ground/below-ground ratio, and the closing of the stomata (Almeida-Rodriguez *et al.*, 2010; Bengough *et al.*, 2011; Desrochers *et al.,* 2007; Marino and Gross, 1988; Monclus *et al.*, 2006; Comas *et al.*, 2013; Rodrigues *et al.*, 2013).

Inter- and intra-specific competition can increase the exposure of planted trees to water stress. The trees compete with each other for light, water and minerals. A study by Benomar *et al.* (2012) showed that the reduction of the spacing between trees of two hybrid poplar clones (*P. balsamifera x trichocarpa* (BT747) and *P. maximowiczii* x *balsamifera* (MBB915)) resulted in a reduction of the above-ground biomass without affecting the depth and biomass of the roots. The spacing between trees affects the competition for nitrogen (N), an essential element for plant growth (Yan *et al.*, 2015). Competition with herbaceous plants can have a significant impact on tree growth (Bockstette *et al.*, 2017; Coll *et al.*, 2007). Several authors have observed that understory vegetation can compete with trees for the use of water, light and nutrient resources, in particular nitrogen (Bockstette *et al.*, 2017; Powell and Bork, 2004; Casselman *et al.*, 2006). Earlier studies highlighted the sensitivity of hybrid poplars to competition from herbaceous vegetation for tree growth, particularly during the initial growing seasons (Kabba *et al.*, 2011; Grenke *et al.*, 2016; Otto *et al.*, 2010;

Pinno and Belanger, 2009; Welham *et al.*, 2007). Herbaceous vegetation can decrease the height of trees as well as on their annual growth (Grenke *et al.*, 2016).

This study was conducted in an open-pit gold mine located in the Canadian boreal forest. The main objective was to evaluate the effects of moderate level of competition for water on waste rock slopes. The plantation design was conceived for conditions of limited water supply, but sufficient to ensure tree survival and balanced above-ground and below-ground growth. More specifically, we aimed at studying the degree of exposure and capacity of adaptation of one hybrid poplars to water stress planted in different densities (spacing), with or without herbaceous seeding, in a plantation on soil-covered waste rock slopes (33% slope). The study is based on the following hypotheses: i) Herbaceous competition and/or reduced spacing between trees increase the poplars' exposure to water stress (reduction of volumetric soil water content and leaf water potential); ii) The height of the poplars will be greater for trees planted with denser spacing and lower in plots where competition from herbaceous vegetation is high; iii) Herbaceous seeding and/or the highest planting density leads to higher SRLs and RLDs compared to plantations with lower levels of competition; iv) The highest plantation densities generate the lowest ratios of leaf area to root length; v) The leaf area of the trees is lower in high-density plantations and/or with herbaceous seeding.

2. Materials and method

2.1. Site description

The Canadian Malartic gold mine is located in Malartic, Quebec, Canada (48° 08′ 00″ N 78° 08′ 00″ W). The gold deposit was discovered in 1926 and exploited through the establishment of four underground mines between 1935 and 1965. Since 2011, the mine has been operated as an open-pit mine. The Canadian Malartic mine is considered one of the largest gold mines in the world, with a production rate of 55,000 t/day and a grade of approximately 1 g/t. The town of Malartic is located in the boreal zone and has a cold and moderately humid continental climate, with cold, dry winters and warm summers (Environment Canada, 2011). The growing season in this region generally runs from mid-May to early October, with a mean temperature of about 18-19°C during the warmest months (June, July and August). The mean annual temperature is 1°C and mean annual precipitation is 900 mm (Environment Canada, 2010).

The region is located inside the clay belt of Northwestern Quebec and the dominant soils are Grey Luvisols (Agriculture and Agri-Food Canada, 2016). The forest vegetation surrounding the site is mainly composed of stands that include black spruce (*Picea mariana (Mill.) Britton*), jack pine (*Pinus banksiana Lamb.)*, tamarack or eastern larch (*Larix laricina (Du Roi) mixed with* white birch (*Betula papyrifera Marsh.*) and trembling aspen (*Populus tremuloides Michx.).*

2.2. Plant material and substrate

The experimental plots were established in May 2013 on 33% slope~~s~~ of mine waste rocks covered with 50 cm of Grey Luvisol topsoil (Agriculture and Agri-Food Canada, 2010) obtained from the excavation of the pit (Table 1). The dark, organic-rich overburden soil was previously colonized by a stand of black spruce in a humid environment and was composed of O and A horizons, (uppermost 30 cm). The choice of this moderate 50-cm soil cover was based on previous experiments conducted on waste rock slopes of 10H:1V in 2011, in which all the trees survived during a particularly hot summer in 2012 (Larchevêque *et al*., 2014). The soil was stockpiled for 30 to 36 months before use in 7-m high piles with a slope of 2.5H:1V. The characteristics of the soil and waste rock did not show any toxicity (Table 1). The analyses of the soil nutrients were conducted by Lakehead University's Centre for Analytical Services (Thunder Bay, ON, Canada) on ~~sifted~~ sieved samples (2 mm mesh), that were finely crushed and oven-dried (50°C). The total nitrogen (N) and sulphur (S) content was determined using the Dumas combustion method (CNS 2000, LECO Corporation, Mississauga, ON) and the organic carbon (OC) content was determined using thermogravimetric analysis (LECO TGA, Mississauga, ON).

The trees planted on all the plots belong to a semi-exotic hybrid poplar clone (*P. maximowiczii* × *P. balsamifera* (M×B) - clone 915319). This hybrid was chosen mainly due to its adaptive response to water stress and its ability to develop a well-anchored root system (Larchevêque *et al.*, 2011a). Indeed, the comparison of two hybrid poplars and a native poplar in the study by Larchevêque *et al.* (2011b) showed that the M × B clone demonstrated the highest resistance to water stress.

The trees were produced locally by the Ministère des Ressources naturelles du Québec (MRN). Cuttings measuring 1.2 m in length were planted directly in the soil at a depth of 30 cm. The planted poplars were fertilized with mineral N and P at the plantation site: 15 g of ammonium nitrate (34.5-0-0) and 15 g of triple superphosphate (0-45-0) were applied in a slit made near the base of each tree (20 cm from the tree, at a depth of 15 cm).

2.2. Experimental design

Four treatments were applied to evaluate the effect of tree spacing and herbaceous hydroseeding. The experimental design consisted of a randomized complete block ANOVA containing 12 experimental plots: 3 replication blocks x 4 competition treatments, including spacings of 1x1 m, 4x4 m, 2x2 m without hydroseeding and 2x2 m with hydroseeding of a grass/legume mixture, with 117, 12, and 35 MxB 915319 poplars, respectively. Three plots without trees served as a control for other follow-up (soil erosion survey) (Figure 1). The plots were separated by buffer zones measuring 4 m width. The M×B trees were planted in the bottom half (last 12 metres) of the slope to improve stability (Styczen and Morgan, 1995). In the upper half, two lines of fast-growing willows (*Salix miyabeana* Seemen, clone Sx64) were planted and hydroseeding was performed in the top 2 metres to limit soil erosion and water run-off. The hydroseeding mix (100 kg/ha) was composed of the following species: *Avena sativa* L. (cultivated oats) (11%), *Festuca L.* (Fescue) (15%), *Lolium perenne* L. (English ray grass) (12%), *Lotus sp*. (Elna Lotus) (15%), *Poa pratensis* L. (Kentucky blue grass) (15%), *Sorghum bicolor* L. (sorghum bicolor Moench) (12%), *Trifolium repens L.* (white clover) (7%), *Trifolium hybridum* L. (alsike clover) (3%), and *Trifolium pratense* L. (red clover) (10%).

2.3. Measurements

Maximum height and basal diameter (diameter at the root collar) were initially measured on the trees in the spring of 2013 and repeated each year in the fall. Mortality assessments were also conducted.

To evaluate the effect of the treatments on exposure to water stress, ECH_2O-5 probes (Decagon) were placed in the plots at a depth of 5 cm from the surface of the topsoil layer. The probes were connected to EM 50 dataloggers and the data were downloaded using ECH_2O Utility software (version 1.77).

2.4.1. Water potential

The exposure of the trees to water stress was monitored every two weeks during the third growing season (June-July-August, 2015). One measurement was taken each day (on sunny days, with temperatures generally above 17°C) between 10:00 a.m. and 2:30 p.m. on mature leaves located at the top of the trees, taken from a tree growing in the middle of each plot (N=12). The measurement of water potential using a pressure chamber (Model 600, PMS Instrument Co., Albany, OR, USA) is a widely used method for studying water stress on plants (Payan and Salançon, 2002).

2.4.2. Isotope discrimination

Carbon-13 isotope discrimination is another physiological indicator of plants' long-term exposure to water stress (Farquhar et *al.,* 1989). ^{13}C accounts for just over 1% of the carbon in atmospheric CO_2. Plants exhibit a preference for ^{12}C, which is lighter, during photosynthesis. Water deficit triggers the closing of the stomata during the day and reduces exchanges of CO_2 with the atmosphere. As a result, the $^{13}C/^{12}C$ ratio established in vegetal organic material by photosynthesis increases in relation to that in atmospheric CO_2.

Long-term exposure to water stress ($^{13}C/^{12}C$ ratio in the foliage) was measured in mature leaves at the top of the trees in one tree per plot (N=12). Leaves were~~,~~ sampled during the month of August in the third growing season. The samples were dried at 60°C for 48 hours and then finely ground. The $^{13}C/^{12}C$ isotope ratio was analyzed using spectrometry at the Natural Resources Analytical Laboratory, University of Alberta, Edmonton. Following complete combustion of the samples, the carbon was converted to carbon dioxide, which was then separated by chromatography and analyzed using a Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS, Thermo Finnigan Corp, Bremen, Germany, 2003). The carbon-13 content was calculated in relation to the international reference standard for carbon isotopes, VPDB (Vienna-Pee Dee Belemnite), which has a $^{13}C/^{12}C$ isotope ratio of RPDB = 0.0112372. The raw data derived from the mass spectrometry were then referenced to the VPDB using linear regression calculated based on the results of internal standards.

2.4.3. Leaf area

During the third growing season (in the month of August), all the leaves from a tree in the middle of each plot were sampled. The total leaf surface area was measured using a planimeter (LI-3100C leaf area meter, LiCor, Lincoln, NE, USA). The samples were dried at 60°C for 48 hours in order to calculate the dry mass. The specific leaf area (SLA: total leaf area/total dry mass in cm^2/g) was then calculated.

2.4.4. Root development

The distribution of tree roots at different depths in the soil cover was studied in the summer (June-July) of the third growing season using core sampling. For the sampling, a Voronoi polygon was established around each tree (one tree at the centre of each plot, N=12, with no sampling for the control). This is the elementary space defined by the half distances between the sampled tree and its neighbours (Snowdon *et al.,* 2002). The polygon space was subdivided into four equal parts. Two squares were selected, one to the left and above the tree, and the other to the right and below it (Figure 2). Six random locations were defined for the core sampling; the target core depth was around 10 cm, up to the waste rock (4 samples for each location). The actual sampling depth was noted in order to calculate the actual volume of each core sample. One 8 cm-diameter auger was used.

The samples were stored in a cold room at 4°C pending their treatment. The roots were washed in a sieve under running water. Then, the roots of the trees were separated from those of the herbaceous plants based on differences in colour and architecture. The fine roots of the poplar were darker in colour and had a more rigid wall than the herbaceous plants.

The roots were then scanned (EPSON Expression 10000XL) and the digital images obtained were analyzed using WinRhizo software (Regent Instruments Inc., Ottawa, ON, Canada), which is commonly used to study the root morphology (Pang *et al.*, 2011). By classifying the roots in different diameter classes, the software can be used to calculate several morphological parameters, such as the total length, area and volume of the roots. Finally, the roots were oven-dried at 60°C for 48 hours and then weighed using a precision balance. For each sample, the coarse roots (> 2mm) and fine roots (<2 mm) were weighed separately.

Root development was analyzed using several parameters chosen for this study: total root dry mass, mean root diameter, specific root length (SRL = the ratio of root length to dry mass of roots in cm/g), root length density (RLD = the ratio of root length to volume of soil in cm/cm^3), root tissue density (RTD = the ratio of root dry mass to root volume in g/cm^3), specific root area (SRA = the ratio of the area to root dry mass in cm^2/g). Only fine roots (<2 mm) were considered in the calculation of these parameters.

The ratio of the total leaf area to total root length (LA/RL) per plot was calculated. LA represents the average total leaf area for a tree. RL represents the root length corresponding to the volume of soil occupied by one tree. For each sampling depth range of 10cm, an average length by soil volume (RLD) was calculated among the 6 sampling locations around the same tree. Then, the four RLDs of each depth range were added and multiplied by 0.1 and by the surface occupied by one tree. This surface changes according to the spacing between the trees ($1m^2$, $4m^2$. $16m^2$ respectively for trees spaced at 1×1, 2×2, and 4×4m).

2.4.5. Nutrient and metal concentration

Leaves were sampled during the month of August in the third growing season (one tree per plot; N=12). The samples were dried at 60°C for 48 hours and then finely ground. Following HNO3-HCl digestion, total elemental concentrations (P, K, Ca, Mg, Na, Cd, Cr, Cu, Mn, Ni, Pb and Zn) were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Lakehead University Centre for Analytical Services, Thunder Bay, ON, Canada). Total N was determined by the Dumas combustion method (Leco CNS 2000, Leco Instruments).

2.4. Statistical tests

The data were analyzed using the R software program (ver.3.1.0). A mixed effect analysis of variance (ANOVA) was conducted. Fixed effects included treatment, sample depth and random effect included block. This ANOVA design was used to test for significant differences between the treatments for the parameters studied, namely: the specific root length (SRL), root length density (RLD), root tissue density (RTD), mean root diameter, specific root area (SRA) and total root dry mass. The same type of mixed effect ANOVA was also used to analyze the following parameters: specific leaf area (SLA), the $^{13}C/^{12}C$ isotope ratio in the foliage, and the ratio of total root length to the total leaf area (RL/LA) per plot. A repeated measures ANOVA was utilized for tree height and diameter, water potential and survival.

The ANOVA assumptions (normal distribution, homoscedasticity, independence of errors) were tested. The normality of the dependent variables was tested using a histogram and normal Q-Q plot. The distribution of all variables were normal, except for diameter which was square root transformed. When effects were significant at 5%, a Tukey test was conducted, also at the 5% level.

3. Results

3.1. Growth and survival

The survival of the trees throughout the three growing seasons remained above 98% for all the treatments. At the end of the first growing season in 2013, there were no significant differences in mean height between the four treatments (Figure 3.a). In 2014, the mean height of the trees was significantly higher for the 1x1 m and 2x2 m treatments (P<.0001) compared to the 4x4 m and 2x2 mH treatments. The differences increased over time between the 2x2 mH treatment and the other treatments. In 2015 and 2016, the 2x2 mH plants had the lowest heights (P=<.0001).

With regard to the basal diameter results, significant differences were observed between the treatments starting in the second growing season. Tree diameter was significantly larger for the plots with 2x2 m and 4x4 m spacing compared to the denser spacing (1x1 m) and the 2x2 m spacing with herbaceous hydroseeding (P=<.0001) (Figure 3.b).

3.2. Exposure to water stress

–The volumetric water content (VWC) values were lower in the 1x1 m treatment from early June to the end of August in the third growing season compared to the other treatments (P=<.0001) (Figure 4a). The plots with the lowest exposure to water stress were those in the 2x2 m treatments, which had higher VWC values in relation to the other treatments. There was no significant difference between the 4x4 m and 2x2 mH treatments for the first four weeks studied starting in early July. Monthly precipitations received on the experimental site during the experiment were in the same range as monthly averages calculated from 1981 to 2010 (Figure 4b).

3.3. -Water stress indicators

The lowest values for leaf water potential were observed in the trees in the most densely planted plots (1x1 m) and on those with herbaceous hydroseeding (2x2 mH) (P<.0001) compared to the 2x2 m and 4x4 m plots (Figure 5.a). Since the statistical analysis revealed that there was no interaction between "treatment" and "measurement date," the water potential results presented in this article represent the mean of the six measurements taken over time. The water potential measurements ranged between -0.8 Mpa (4x4 m) and -1.8 Mpa (1x1 m).

The total leaf area (P = .007, F = 27.98) and the leaf dry mass (P = <.0001, F = 53.14) were significantly higher for the 1x1m treatment, then for 2x2h m, compared with the two other treatments (4x4 m and 2x2 m). The SLAs were significantly lower for the 1x1 m and 2x2 mH treatments (P=.0004, F=32.46) relative to the 4x4 m and 2x2 m treatments (Figure 5.b). The ratio of the total leaf area to the total fine-root length for each plot was greater for the 4x4 m and 2x2 m treatments compared to the 1x1 m and 2x2 mH treatments (P=<.0001).

The statistical analyses did not reveal any significant difference between the treatments with regard to the $^{13}C/^{12}C$ isotope ratio of the tree leaves (August 2015), which varied between -28.54 (4x4 m) and -29.03 (1x1 m).

3.3. Nutrient and metal concentrations

Based on the results, the different treatments had no significant effects on the concentrations of nutrients and metals in the leaves of the planted trees.

3.4. Root development

The hybrid poplars' fine roots made up 95% of the total root mass in the samples. Moreover, it was observed that the effect of the treatments is similar when all the roots (fine and woody roots) are considered together. Accordingly, only the results obtained for fine roots (<2 mm) in direct relation to their water uptake function will be presented in this article.

The effect of treatments and depth was significant for SRL and RLD, RTD, SRA and DM (dry mass) (P=<.0001). The statistical results showed an interaction between the factors of treatment and depth. For all depths of less than 30 cm, SRL, RLD, RTD and DM were higher for the more densely planted plot (1x1 m) and the plot with herbaceous hydroseeding (2x2 mH) compared to the other two treatments (Figure 6). These variables largely decreased after 30 cm of soil depth, with no more significant differences between treatments for SRL, RTD and DM. In terms of the SRA, the 1x1 m and 2x2 mH treatments also differed from the others, with SRA values much higher and largely decreasing after a soil depth of 30 cm. There is no significant difference in the mean fine-root diameter of the poplar in terms of the effect of depth and treatments. The diameter did not vary significantly between treatments or with the depth of sampling.

The ratio of the total leaf area to total root lenght (RL/LA) was higher for the plot with herbaceous hydroseeding (2x2 mH) and the more densely planted plot (1x1 m) to the other two treatments (P=<.0001).

4. Discussion

4.1. Level of exposure to water and nutritional stress and tolerance of poplars planted in waste rock slopes

The high survival rate ~~of survival~~ after four growing seasons indicates that the acclimatization capacity of the hybrid poplar *P. maximowiczii × P. balsamifera* (M×B)~~s~~ on mine waste rock slopes is high, despite a planting environment characterized by a limited water supply. There was no planting shock and the hybrid under study was able to tolerate the water deficit on the slopes. Indeed, the treatments showed an annual mean height growth of 64 and 95 $cm.year^{-1}$ during the first and second year, respectively. These growth rates are similar or superior to those observed for other hybrid poplar plantations in similar boreal conditions. For example, growth was lower than 40 $cm.year^{-1}$ for the same poplar clone in the studies conducted by Larchevêque *et al.* (2011a) and Benomar *et al.* (2012). Our results in all treatments also showed that there was no accumulation of metals, which is perhaps due to the small total concentrations of metals and/or neutral pH of the waste rocks (Guittonny-Larchevêque *et al.*, 2016). The volumetric water content of the surface soil was generally above the permanent wilting point of 20% (VWC varied between 18% and 38%), with the exception of the 1x1 m treatment, where these values were close to 20% for certain weeks. This low water content in the soil was not associated to low precipitation. According to climate data for 2015, when water stress measurements were conducted, the monthly precipitation over the growing season (from May to October) was similar to normal mean precipitation calculated for the past 30 years. Moreover, regardless of the treatment, the leaf water potential values recorded in our study were below ~~the~~ some drought threshold reported by other authors (-3,7 to - 0,7) (Guittonny-Larchevêque *et al.*, 2016; Barchet *et al.,* 2014; Larchevêque *et al.*, 2011; Ridolfi and Dreyer, 1997) a~~.~~nd triggered a response in the trees varying with the treatment.

4.2. Variation in the level of water stress exposure based on competition and response of poplars in terms of stress indicators

4.2.1. Stress exposure and water stress indicators

Our first hypothesis was partially confirmed by the results. The exposure to water stress differed depending on the treatment. Due to competition, the values for the volumetric water content of the surface soil were the lowest in the high-density plots (VWCs were close to the 20% wilting point for certain weeks). However, there was no significant difference between the 2x2 m and 4x4 m treatments. This finding can be explained by the development of an herbaceous cover in the plantation with the large spacings (4x4 m) where canopy closure occurs later than in the closest spacings. In the study conducted on the same plantation by Remaury *et al.* (2018~~2016~~), it was observed that the above-ground vegetation cover resulting from the natural colonization of species after planting was directly dependent on the planting density.

The response of the poplars to the water stress caused by competition was characterized by a decrease in the leaf water potential. In their study on different eucalyptus clones (a fast-growing species), Hakamada *et al.* (2017) similarly observed that leaf water potential was affected by planting density. Our results did not reveal any difference between treatments in the isotope ratio. It would appear that, even in the case of low water potential, the poplars kept their stomata open, thereby exhibiting anisohydric behaviour (Tardieu and Simonneau, 1998). This behaviour has also been observed in hybrid poplars by other authors (Guittonny-Larchevêque *et al*., 2016; Attia *et al.*, 2015; Larchevêque *et al*., 2011b). As a result, the planted trees maintained their physiological functions and growth despite the reduction in the soil moisture availability (Farquhar *et al.*,1989). While this anisohydric behaviour may appear beneficial in conditions of moderate water stress, it can become very risky and endanger plant survival in more severe and/or longer cases of water scarcity (McDowell *et al.*, 2008). However, poplar trees show considerable variation in terms of their drought tolerance (Hamanishi *et al.*, 2010). Several researchers have observed an isohydric effect in certain poplars (Lüttschwager *et al.*, 2016; Theroux, Rancourt *et al.*, 2015; Barchet *et al.*, 2014; Arango-Velez *et al.*, 2011; Bassman and Zwier, 1991; Ceulemans *et al.*, 1988; Gebre *et al.*, 1998). Isohydric plants are capable of maintaining a constant water potential regardless of the water conditions (Amigues *et al.*, 2006; McDowell *et al.*, 2008).

4.2.2. Tree growth

The increased intraspecific competition associated with the smaller spacing between trees resulted in a reduction in diameter growth but did not affect tree height (there were no significant differences between the three treatments (1x1, 2x2 and 4x4 m)). Height growth is generally considered to be unaffected by variations in competition (Woodruff *et al.*, 2002). However, other studies have demonstrated that tree height can increase, decrease or remain unchanged depending on planting density (Toillon *et al.*, 2013; Pinkard and Neilsen 2003; DeBell *et al.*, 1996; Nilsson, 1994). An increase in planting density results in greater competition for water, nutrient and light resources (Toillon *et al*., 2013, Benomar *et al*., 2012). Higher densities thus favour growth in height to the detriment of growth in diameter due to increased competition for light (Benomar *et al*., 2012; Brodie and DeBell, 2004). In hybrid poplars, in particular, diameter growth is generally affected before height growth in the presence of water deficit conditions (Brodie and DeBell, 2004). Consequently, the initial spacing between trees is a key factor in the development of a stand.

As expected, and in line with our second hypothesis, the presence of herbaceous plants from the outset in the 2x2 mH plantation triggered lower height and diameter growth in the poplars. This indicates that fast-growing herbaceous plants compete for resources and can delay tree development (Bockstette *et al*., 2017; Grenke *et al*., 2016; Henkel-Johnson *et al*., 2014). Other studies have also reported the negative effect of understory vegetation on the growth of hybrid poplar (Henkel-Johnson *et al*, 2016; Goehing, 2015). Poplars are known for their high water requirements (Monclus *et al*., 2006). Competition between the trees and herbaceous vegetation occurs either above-ground for light, below-ground for water and nutrients, or as combination of the two (Balandier *et al*., 2006). Thus, our results suggest a decrease in soil moisture in the 2x2 mH plantation due to competition with weeds (Pinno and Bélanger, 2009; Powell and Bork, 2004). The availability of nutrients, particularly nitrogen, can further limit the development of poplar plantations (Kabba *et al*., 2011; Coll *et al*., 2007). Accordingly, various studies have highlighted the importance of controlling herbaceous vegetation and/or adequately fertilizing the soil, particularly during the first years of growth (Coleman *et al*., 2006). On the other hand, herbaceous plants can also have positive effects, mainly due to the retention of nutrients and reduction of soil erosion in slopes (Remaury *et al*., 2018). However, there is little information available on which herbaceous plants are capable of performing ecological functions without subjecting the trees planted to competition.

4.3. Acclimatization of poplars to water stress to explain tolerance at the below-ground and above-ground levels

The intensified competition associated with the smaller tree spacing and the herbaceous seeding increased the trees' demand for limited resources, such as water, to which the poplars responded by increasing ~~their~~ root biomass. This may constitute~~s~~ a form of acclimatization to water deficit by the clone tested. Several studies have shown that tree species that tolerate drought conditions tend to allocate more resources to the development of root biomass in order to maximize the supply of water (Larchevêque *et al*., 2011b; Marino and Gross, 1998).

The fine roots of hybrid poplars are known for their high plasticity and ability to adapt to conditions of limited water and nutrient resources. We observed that the majority of fine roots were located in the first 30 cm of soil. Other studies have also observed a higher density of fine roots at the soil surface – that is, within the 0-30 cm horizon. For example, in their study of 16 hybrid poplars, Crow and Houston (2004) reported that the 0-36 cm stratum contained, on average, 81% of the total number of roots. Consistent with our third hypothesis, all the root morphological traits (SRL, RLD, SRA, DM and RTD) studied were higher in the 1x1 m and 2x2 mH treatments relative to the two other treatments, with the exception of mean diameter, which was insensitive to the treatments. The root systems of plants that are tolerant of drought conditions can optimize the contact area with the soil and increase the substrate volume explored by increasing their SRA, SRL and RLD (Comas *et al*., 2013). The result obtained for the RTD seems to contradict those obtained for the SRL and RLD. In fact, we expected to find a negative correlation between them, with lower RTDs in the treatments where there was a higher level of competition. It is likely that the trees responded to the increased competition by an increase in root life span~~s~~. In addition, concentrations of N in the tree leaves were relatively low. Trubat *et al*. (2006) have attributed the increase in the RTD to a lack of nutritional elements that often coincides with a lack of water.

Drought tolerance through an increased investment in the root system comes at a cost in terms of reduced above-ground growth. The allocation of biomass to above-ground growth was lower in the trees subject to greater stress. Similar results have been reported by other researchers studying the effect of competition on various species, such as the poplar (Toillon *et al*. 2013; Benomar *et al*., 2012), the eucalyptus (Hakamada *et al*., 2017; Stape and Binkley, 2010), and the pine (Blevins *et al*., 2005).

In line with our fifth hypothesis, the hybrid poplars in this study also exhibited a decrease in leaf area in response to stress, thereby reducing the total transpiring area in order to conserve water. The 1x1m and 2x2 mH treatments had the lowest SLA values. Indeed, changes to the SLA in water limiting conditions reflect a modulation of the leaf structure. This resistance mechanism is often used by certain poplar clones and its principal aim is to reduce transpiration losses by reducing the leaf area exposed to light and CO_2 by unit of biomass (Marron et al., 2003; Barigah *et al*., 1994). Moreover, the higher RL/LA ratio in trees subject to greater stress indicates a water acquisition capacity coupled with a simultaneous reduction in the loss of water by transpiration, which is considered by many authors to be a criterion of drought resistance.

5. Conclusion

This work contributes to selecting a hybrid poplar planting density that would allow its survival and growth at the short term (3 years) despite poplar high requirements for water. The results showed that, when facing a lack of water in the soil, the tested poplar used a combination of above-ground and below-ground strategies to acclimatizize. The root traits examined in this study show that poplars invested more energy in the development of roots in order to acquire more water resources through a larger surface area of exchange between the roots and soil per unit of root biomass. At the above-ground level, trees adopted a more conservative resource strategy, with reduced SLAs and lower biomass production. InAt the short term, the rate of survival and growth was high for all treatments, despite some low available water contents in the soil in the treatments where competition was more intense. This confirms that some clones of hydrid poplars could be well adapted to mine sites revegetation, even on drier surfaces. However, the choice of the clone may be of first importance due to the known variation of drought resistance among clones. Moreover, the survival of the anisohydric poplar tested in this study should be followed in the longer term, since higher water requirements could accompany the increase in size of the trees. Finally, since poplar is amongst the trees having the greatest water needs, it may imply that other tree species less demanding in water could possibly cope with water stress in the waste rock slopes at the short term.

Acknowledgements

This study was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Malartic Partnership. The authors would like to thank the research technician and undergraduate students who have contributed to the collection of field data, namely Avril Jobin, Charles Frenette-Vallières, Sara Gervais, Antoine Beaulieu, Maguelonne Rajot and Liam Boivin.

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Tables

Table 1: Initial soil and waste rock characteristics.

	Unit	Overburden topsoil	Waste rock	Regulatory threshold **
pH		5.9	6.7 à 9	
EC*	mS.cm-1	0.4±0.1		
OM*	%	20±3		
total **N**	%	0.6±0.3		
total **S**	%	0.3±0.05		
total **Ca**	g.kg-1	10±1	15	
avail. **K**	g.kg-1	0.1±0.005		
avail. **Mg**	g.kg-1	0.6±0.04		
avail. **Na**	mg.kg-1	22		
total **P**	g.kg-1	0.6±0.02		
avail. **Cu**	mg.kg-1	7.4±0.4		
avail. **Fe**	g.kg-1	0.3±0.03		
avail. **Mn**	mg.kg-1	75±9		
avail. **Zn**	mg.kg-1	4.5±0.4		
total **Al**	g.kg-1	14±0.8	9.5	
total **As**	mg.kg-1	6.2±1.9	5	30
total **B**	mg.kg-1	3.5±0.7		
total **Ca**	g.kg-1	10±0.7		
total **Cd**	mg.kg-1	0.24±0.09	0.2	5
total **Co**	mg.kg-1	5.1±1.9	20	50
total **Cr**	mg.kg-1	217±34	123	250
total **Cu**	mg.kg-1	50±2	25	100
total **Fe**	g.kg-1	28±1.4	24	
total **K**	g.kg-1	4.6±0.6	10	
total **Mg**	g.kg-1	14±1.4	10	
total **Mn**	mg.kg-1	404±23	372	1000
total **Mo**	mg.kg-1	3.7±0.6	6	10
total **Na**	g.kg-1	0.2±0.04	0.2	
total **Ni**	mg.kg-1	94±10	57	100
total **Pb**	mg.kg-1	76±22	31	500
total **S**	g.kg-1	3.4±0.9		
total **Sr**	mg.kg-1	101±5		
total **Ti**	g.kg-1	0.95±0.08		
total **Zn**	mg.kg-1	96±7	63	500

Mean ± standard error. SE; N = 3. except on waste rock.
All values are expressed on a dry matter basis.
* EC : electrical conductivity ; OM : organic matter ** Quebec Government (2016)

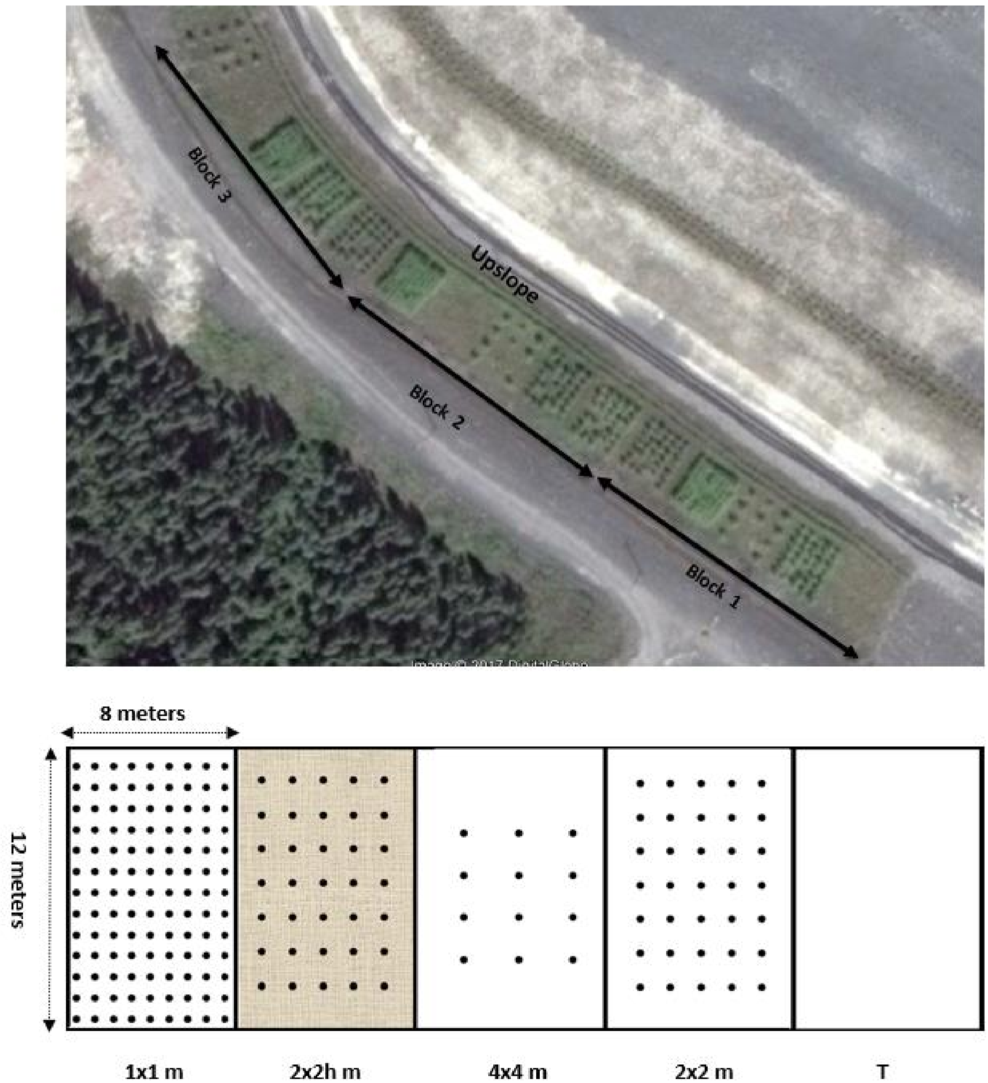


Figure. 1 Plantation and experimental design

4x4 m: Plots without hydroseeding and 4x4m tree spacing;

2x2 m: Plots without hydroseeding and 2x2m tree spacing;

1x1 m: Plots without hydroseeding and 1x1m tree spacing;

2x2h m: Plots with hydroseeding and 2x2m tree spacing;

T: Plots without hydroseeding and without trees.

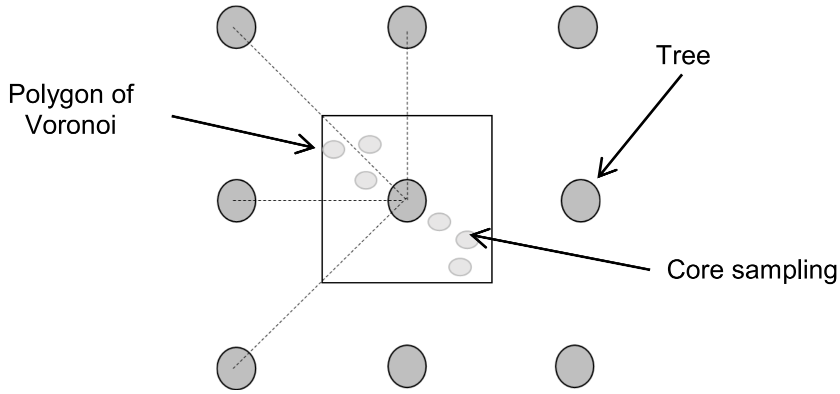


Figure. 2 Sampling method of soil cores + roots.

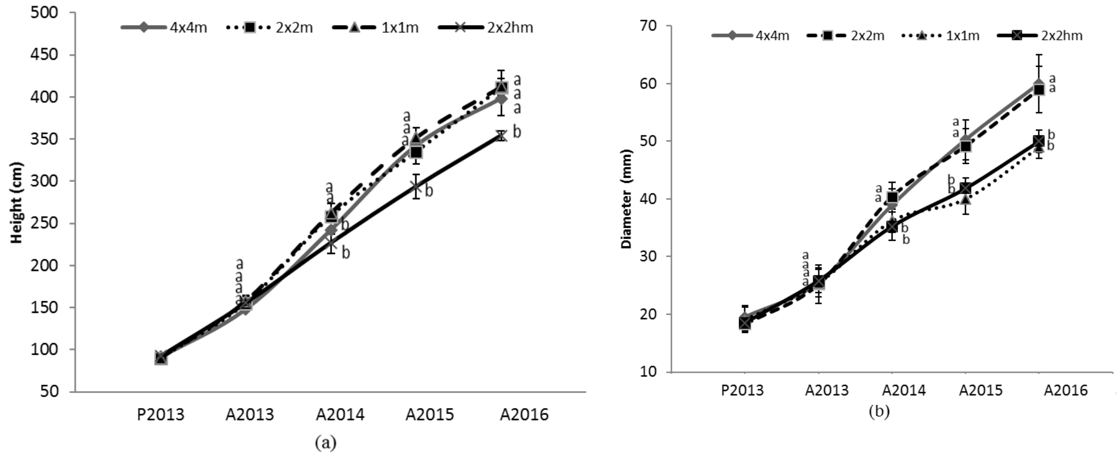
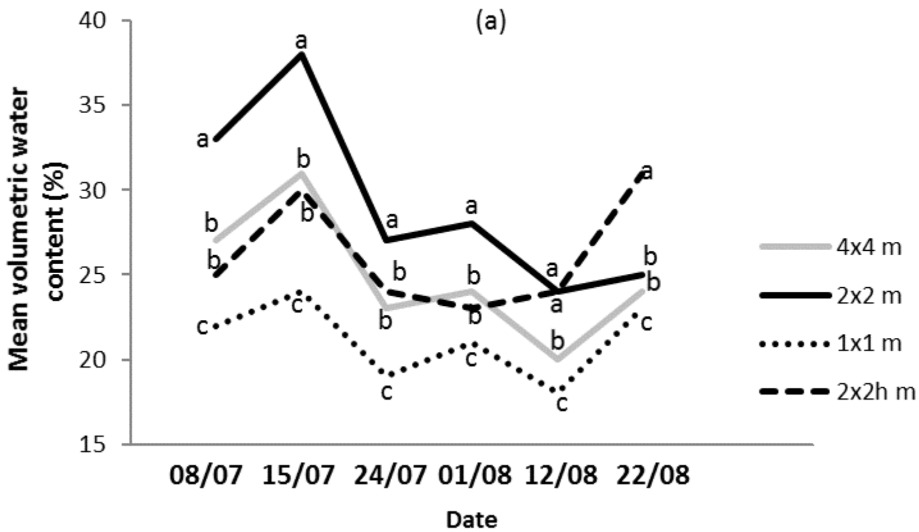


Figure. 3 Effect of treatments (spacing of trees 4x4m. 2x2m. 1x1m. and 2x2m combined with herbaceous seeding. 2x2hm) on the maximum height (cm) (a) and basal diameter (mm) (b) of the hybrid poplar stem at planting (spring 2013) and at the end of each growing season from 2013 to 2016. Mean (ES). N ⩾ 36. For each year, mean values that differ significantly at 0.05 are marked with different letters a> b).



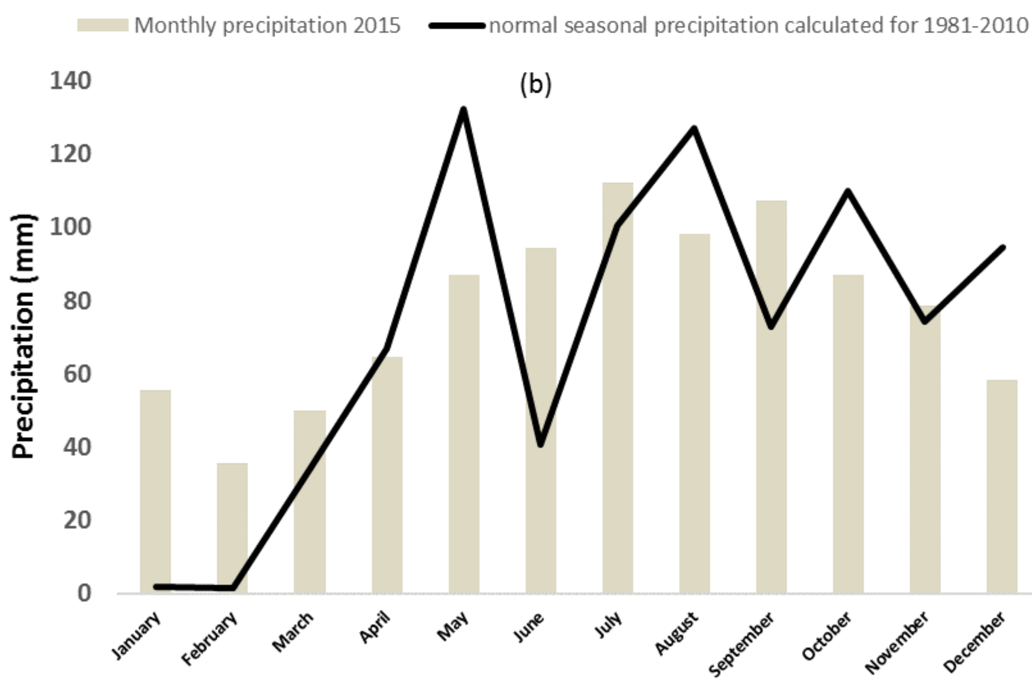


Figure. 4. (a). Effect of treatments (spacing of 4x4m. 2x2m. 1x1m. or 2x2m trees combined with herbaceous seeding. 2x2hm) on soil volumetric water contents from July to August 2015. For each year, mean values that differ significantly from 0.05 are noted with different letters (a> b> c). (b). Monthly precipitation in 2015 and normal precipitation calculated for 1981-2010.

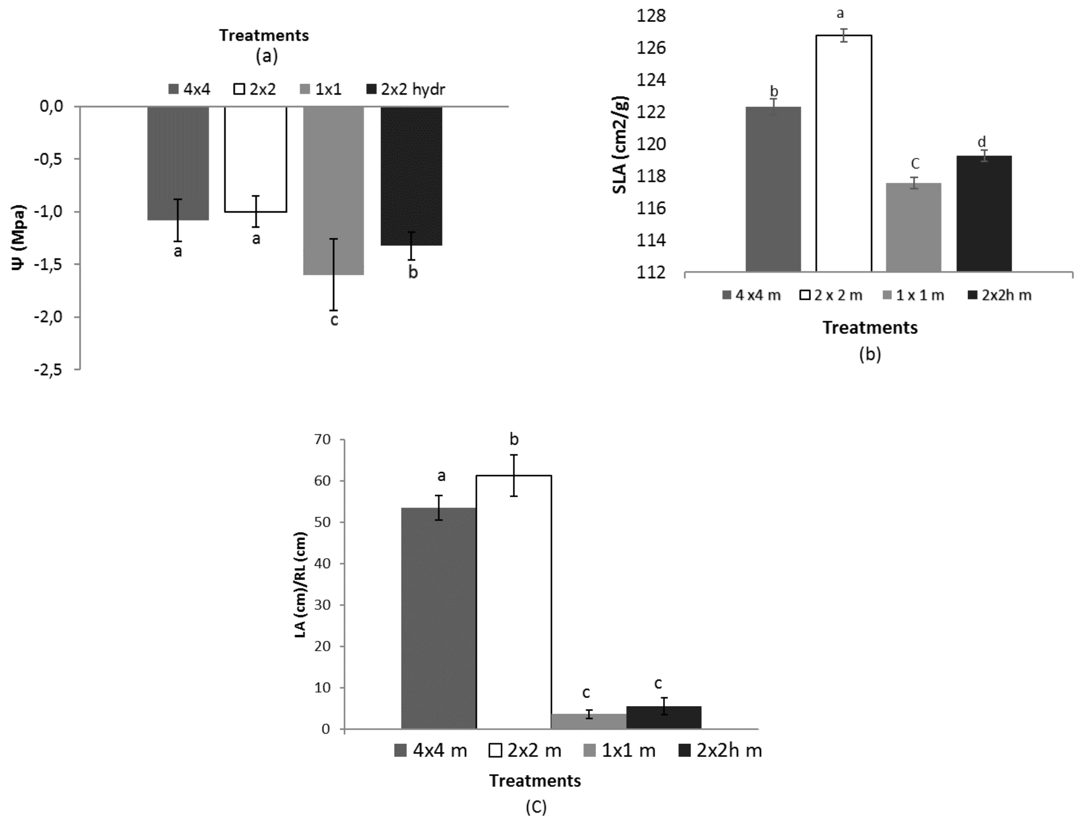


Figure 5. Effect of treatments (spacing of 4x4m. 2x2m. 1x1m. or 2x2m trees combined with herbaceous seeding. 2x2hm) on foliar water potential (a), specific leaf area (b), and ratio of total leaf area to total fine root length cm / cm (c). Means that do not differ at 0.05 are noted with the same letter (a> b> c).

Figure 6. Effect of treatments and soil depths on SRL, RLD, RTD, and dry matter for poplar fine roots

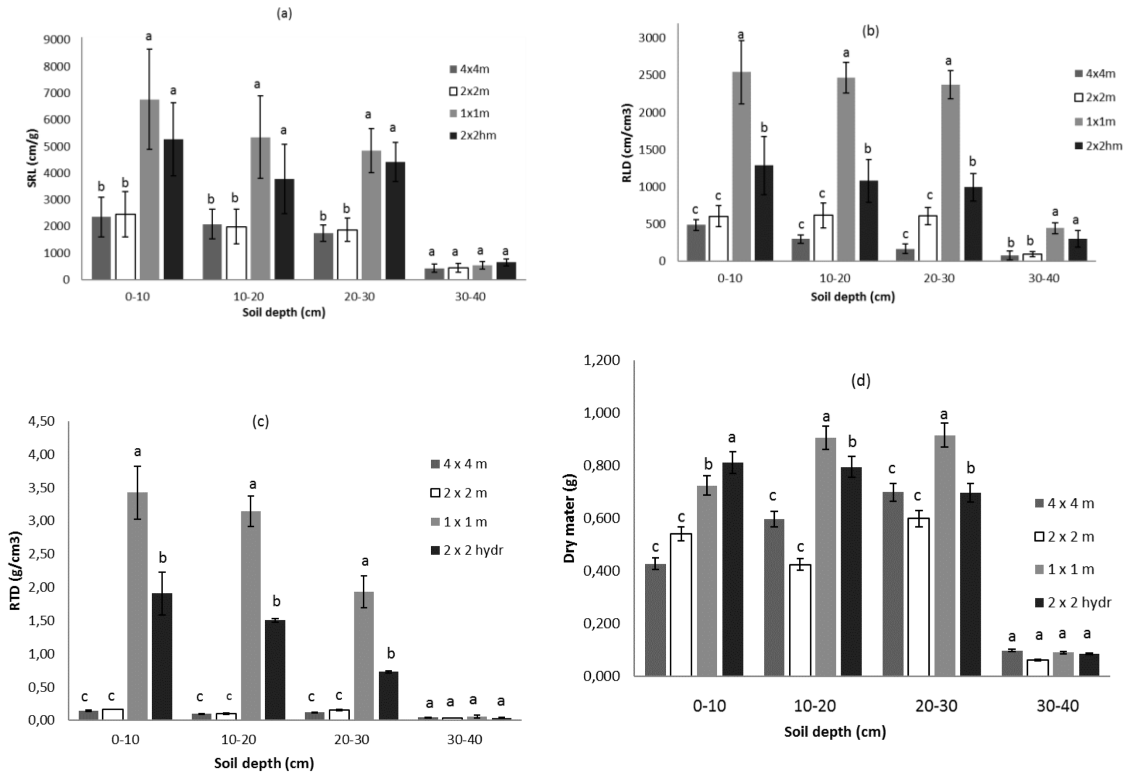


Figure 6. Effect of treatments (4x4m. 2x2m. 1x1m. or 2x2m tree spacing in combination with herbaceous seeding. 2x2hm) and soil depths (0-10, 10-20, 20-30, 30-40 cm) on SRL (a) RLD (b) RTD (c) and dry matter (d) (N = 18) for poplar fine roots. For the same depth, parameters that differ significantly at 0.05 are noted with different letters (a> b> c).