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Effect of water retaining polymers on root distribution and mycorrhization of young seedlings during summer drought

Master thesis

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Declaration

I hereby declare that I am the sole author of this work. No assistance other than permitted has been used. All quotes and concepts taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

Acknowledgments

First I would like to express my graditude to my supervisor Dr. Hans Sandén for introducing me to this experiment which yielded my master thesis. From the beginning I received a great deal of support and assistance both from him and Dr. Boris Rewald, whose expertise were crucial, especially in formulating research questions and developing methodology. Their patience and advices are highly appreciated. I would also like to thank Riccardo Siller and Josef Ruda, for great help during the setup and harvesting actions of the experiment.

Furthermore, special thanks go to my Mountain Forestry collegues: Federico Ricci, for all the fun and joy we had during our 2 years together on BOKU, and to Daniel Boehnke, for both helping me in the laboratory and supporting me all these years.

At last, but not least, I would like to thank to my family and to my Thomčica. Danke, dass du für mich da bist. Thanks are extended to all my friends in Vienna, especially to Anja and Roko (and Doni and Koko), Sven and Ivo.

To my family: Hvala vam na podršci, razumijevanju i strpljenju, znam da nije uvijek lako. Volim vas puno. Zadnje hvala je za moju mucu malu koja me je uveseljavala od svog pocetka pa do kraja, svima nam jako nedostaješ i nikada te nećemo zaboraviti.

Abstract

Facing the new challenges of climate change, regarding longer drought periods and higher temperatures, newly planted seedling has become more vulnerable to the desiccation and nutrient deficiency. Their root system is insufficiently developed and therefore prevents them from reaching deeper parts of the soil in pursuit of water and nutrients. The experiment was designed and set up to evaluate the performance of biodegradable, nutrient rich polymer, Polyter and petroleum-based hydrogel, Stockosorb, on the tree roots system and mycorrhiza development. In the experiment two-year old seedlings of four species were used: European beech (*Fagus sylvatica*), Douglas fir (*Pseudotsuga menziesii*), European larch (*Larix decidua*) and Norway spruce (*Picea abies*). The species was supplemented with three amendment treatments: control, Stockosorb and Polyter. Half of the pots were than exposed to a series of drought events. Pots were arranged in 12 blocks in randomized order. Each block had the same amount of treatments per specie (24 pots*12=288 pots). After the summer period plants were harvested and divided in two parts: polymer (roots growing under the area where Polyter/Stockosorb was placed) and outer part (roots growing in the surrounding soil). To assess the effect of drought, as well as the performance of the hydrogels, on fine roots, a size related (average diameter, length and biomass) and functional (SRA, SRL and RTD) morphological characteristics were observed, additionally with mycorrhizal colonization. Conclusively both Stockosorb and Polyter showed a positive effect on root growth only in Douglas fir and Spruce. However, their performance wasn't affected by induced drought periods, perhaps due to the fact that a moderate drought was stimulated during the experiment. Furthermore, mycorrhizal growth wasn't affected by the soil conditioners. Further research is needed to assess an optimal application of soil amendments to the roots and interspecific differences in the way trees experience water deficiency.

Kurzfassung

Die Begleiterscheinungen und neuen Herausforderungen, welche mit dem Klimawandel einhergehen, wie längere Dürreperioden und höhere Temperaturen, machen frisch gepflanzte Sämlinge/ Setzlinge anfälliger für Austrocknung und Nährstoffmangel. Ihr Wurzelsystem ist unzureichend entwickelt und hindert sie daher daran tieferliegende Bereiche des Erdreichs und damit Wasser und Nährstoffe zu erreichen. Das Experiment wurde entworfen und erstellt, um die Wirkung von nährstoffreich, biologisch abbaubare polymer, Polyter und Petroleum basierendem hydrogel, Stockosorb auf das Wurzelsystem von Bäumen sowie auf die Mykorizza Entwicklung, zu bewerten. In dem Experiment wurden zwei Jahre alte Setzlinge aus vier verschiedenen Arten verwendet: Rotbuche (*Fagus sylvatica*), Douglasie (*Pseudotsuga menziesii*), europäische Lärche (*Larix decidua*) und Fichte (*Picea abies*). Den Arten wurden drei Ergänzungsmitteln hinzugefügt: Kontrolle, Stockosorb and Polyter. Die Hälfte der Töpfe wurden daraufhin einer Reihe von Dürreperioden ausgesetzt. Die Töpfe wurden in zwölf Blöcken in unsortierter Ordnung arrangiert. Jedem Block wurde die gleiche Menge and Behandlungen je Art zugeführt (24 Töpfe*12=288 Töpfe). Nach der Sommerperiode wurden die Pflanzen geerntet und in zwei Teile aufgeteilt: Polymere (Wurzeln, gewachsen unter dem Bereich wo Polyter/Stockosorb zugeführt wurde) und der Außenteil (Wurzeln, gewachsen in dem umgebenden Erdreich). Um den Effekt der Dürre zu bewerten, sowie die Auswirkungen des Hydrogels auf feine Wurzeln wurden diese anhand größenbedingter (durchschnittlicher Durchmesser, Länge und Biomasse) und funktionaler (SRA, SRL und RTD) morphologische Merkmale analysiert. Zusätzlich wurde die Mykorhizza Besiedelung analysiert. Zusammenfassend haben Stockosorb als auch Polyter positive Effekte auf das Wurzelwachstum bei Douglasie und Fichte aufgezeigt. Allerdings wurde ihre Leistung nicht durch zugeführte Dürreperioden beeinflusst, vielleicht aufgrund der Tatsache, dass eine moderate Dürre innerhalb des Experimentes stimuliert wurde. Darüber hinaus wurde das Mykorrhiza Wachstum durch die Bodenverbesserungsmittel nicht beeinflusst. Weitere Recherche ist notwendig, um eine optimale Anwendung von Ergänzungsmitteln für die Wurzeln und interspezifische Unterschiede in der Art und Weise, wie Bäume auf Wassermängel reagieren, auszuwerten.

Table of Contents

1 Introduction

Due to their long life-spans, trees struggle to adapt to the environmental changes, therefore they are especially sensitive to climate change (Lindner et al., 2010). The effects of temperature increase and longer drought periods on forest ecosystems are being progressively analyzed and discussed. Forest ecosystem productivity and services can be considerably limited by the effects of drought and diminished water availability. Impacts of drought vary from stand level to regional forest covering several million hectares (Ciais et al., 2005; Allen et al., 2010). Direct consequences of the drought are losses in productivity and higher mortality rates. The severity and frequency of meteorological droughts such as precipitation deficit have increased in some parts of Europe, particularly in south-western and central Europe (EEA, 2016). The average annual temperature of the European land area in the last decade (2009–2018) was between 1.6 °C and 1.7 °C above the pre-industrial level, which makes it the warmest recorded decade. Furthermore, Europe has experienced several extreme heat waves since 2000 (in 2003, 2006, 2007, 2010, 2014, 2015 and 2017) (EEA, 2019). In particular, the European mountain regions, e.g. the Alps, had an increase in temperatures twice as big as the global average increase observed over the last century (Auer et al., 2007).

Newly planted trees have insufficiently developed root system which prevents them from reaching the deeper parts of the soil, while searching for nutrients and water. That makes them more vulnerable to desiccation and nutrient deficiency. Sites affected by drought can be a challenge for foresters, especially on mountainous areas, where replanting requires more complex operations including higher costs.

Austria is a country with almost half of its total area under forest cover. Commonly represented native species are Beech (*Fagus sylvatica*) in lower parts and Spruce (*Picea abies*) and Larch (*Larix decidua*) in higher elevations. Douglas fir (*Pseudotsuga menziesii*), as a nonnative drought tolerant tree, is considered as a very promising tree specie in Western and Central Europe, in terms of climate change adaptation (Eckhart, Hintsteiner, Loo, Hasenauer, & Lair, 2014).

1.1 Eco physiological response of trees to drought

Drought leads to a shortage of water in the soil, affecting the growth and transpiration of the plants. Transpiration is a driving force for pulling the water from soil to leaves, but during drought decrease in water potential occurs (Bréda, Huc, Granier & Dreyer, 2006). Water potential between the soil-root interface and leaves represents water flow in the soil plant atmosphere continuum (SPAC). When evaporation increases and soil dries, water potential in the leaf declines, resulting in stomata closure (Bréda et al. 2006). In addition, water and carbon dioxide (CO_2) flux get reduced. To prevent the internal water deficits during the drought periods it is essential to achieve more productive absorption of water from the soil. The efficiency of water uptake by trees depends on both spatial extension and density of their root system (Levitt, 1980).

1.2 Tree root systems and mycorrhizal development

The condition of the soil greatly affects the formation and development of tree roots. The roots are repeatedly adjusting to the soil nutrient availability and physical conditions. Ramified root systems can penetrate the soil towards bigger water reserves and fine roots serve for the nutrient and water absorption (Bréda et al., 2006). Physiologically they are the most active parts of root system although they constitute only a small portion of its biomass. In addition to their importance, fine roots contribute to the Carbon cycle (C cycle) due to their shorter life span (Wells & Eissenstat, 2001). Drought can change the structure of fine roots without changing the total biomass of the roots. There is a variety of root traits by which a plant can enhance its fitness under different conditions (Laughlin, 2014). Plants in stressed environments may need higher investment per length, to enable exploitation of the soil with small cost. Which is often found with species in dry conditions, to have higher SRL than species in productive environments (Tjoelker, Craine, Wedin, Reich & Tilman, 2005).

In addition to nutrient and water uptake, fine roots play an important role in symbiosis between mycorrhiza and the plant. Ectomycorrhiza forms a dense hyphae mantle covering the root tip, which serves as a most important organ for nutrient and water uptake to the host. In the following sections, we will refer to ectomycorrhiza (EM), because EM can be found on many woody plants in the temperate region. Many tree species depend on EM, in particular young trees with insufficiently developed root systems during the establishment phase. After colonizing the tree roots, EM produces an extensive network of mycelium in the soil (Smith & Read, 2008) . EM fungi can stimulate the dissolution of certain minerals to access the nutrients. With the extension of EM hyphae, roots gain bigger absorption surface and can absorb water in lower water potential conditions (Hoffland et al., 2004).

1.3 Superabsorbent polymers

Due to the drought stress effects, especially on young trees and seedlings, soil conditioners (amendments) are used to improve soil moisture condition. Hydrogels are water retaining polymers, acting as an additional water reservoir to the root system of plants. They are formed from a network of polymer chains that are crosslinked to the disable dissolution. The main characteristic of the hydrogel is the ability to absorb and retain quantities of liquids much greater than the initial weight of the material (Horie et al., 2004). Hydrogels are placed close to the root system since they swell after the plants are irrigated, due to the absorption and retention of water. During drought periods, as the soil becomes dry, hydrogel releases the stored water. Considering the fact that the hydrogel is much smaller upon planting and much bigger after watering the plants, there is an effect on soil porosity. Aeration of the soil is improved, and compaction can be reduced (Demitri, Scalera, Madaghiele, Sannino, & Maffezzoli, 2013).

Polyter is a hydro retaining fertilizer with semi-permeable membrane that allows quick absorption of water, which is then, claimed to be, slowly released (in small quantities) for the water uptake by roots. Release depends on the conditions of soil and temperature. The size of a polymer can reach 160 to 500 times of its initial size after the absorption of water. Additionally, fertilizing products (N, P, K) and trace elements (Bo, Cu, Fe, Mn, Mo, Zn) are combined within polymetric chambers of the Polyter. The role of the superabsorbent polymer is to provide water and nutrients to the plants during the drought stress, improve porosity of the soil by lowering the compaction and allowing better air circulation. Polyter claims to act as a thermal regulator for roots by reducing the soil temperature comparing to the surrounding air. The roots grow through the swollen Polyter nodules becoming an essential part of the root system (Polyter brochure).

Stockosorb is a crossed-linked potassium salt-based copolymer, that can absorb and hold a quantity of water up to 300 times of its own weigh. Additionally, it is a NK-Fertilizer 13+5 (GEFA 2017). Superabsorbent basic material is polyacrylate, a nonrenewable material prepared by polymerizing acrylic acid with a crosslinker, relying on the petroleum industry (Nnadi & Brave, 2011).

In contrast to the petroleum-based polymer, Polyter, is a cellulose-based hydrogel, claimed to be biodegradable, capable of being decomposed in 3 to 5 years and therefore environmentally acceptable, consisting of more fertilizing products compared to Stockosorb.

1.4 Aims

This study is part of a project collaboration between University of Natural Resources and Life Sciences (BOKU) and Green Legacy GmbH. The focus is given to the below ground growth and performance of two hydrogels: Polyter and Stockosorb. An experiment was designed and set up to evaluate their effect on tree root systems and mycorrhiza development of economically valuable species. Specific root traits were furthermore observed to assess the effect of drought and soil conditioners and their potential interaction.

1.5 Hypothesis

The following hypothesis are tested:

1) Soil conditioners, Polyter and Stockosorb, will have a positive effect on root growth, especially in drought treated plants.
2) Root growth, in the proximity of amendment, is higher for plants supplemented with soil conditioners compared to plants without amendments.
3) Polyter, unlike Stockosorb, has more fertilizing products and trace elements combined within the substance, which can result in better stimulation of the root growth.
4) Polyter and Stockosorb do not cause negative side effects on mycorrhizal colonization and external mycelia production.

2 Materials and methods

2.1 Plant provenances

Norway spruce (*Picea abies*) and Larch (*Larix decidua*) plant material, used in this study, origin from Austria. To be more exact, Spruce is from 4.2 area (Nördliche Randalpen – Ostteil) 900-1300m and Larch from 2.2 (Nördliche Zwischenalpen - Ostteil), 1200-1500m above sea level. Beech (*Fagus sylvatica)* and Douglas fir (*Pseudotsuga menziesii*) provenance is Germany, where Beach is from the source area 810 10 (Harz, Weser and Hessian highlands, montane stage) and Douglas fir from 853 01 (Northwest Lower Lowlands with Schleswig-Holstein).

2.2 Experiment set up

The green house where the experiments took place is in Tulln. The City is about 40 kilometers northwest of Vienna, in the Austrian state of Lower Austria. The Experiment began in April. The pots were arranged in 12 blocks in randomized order. Preparing of the soil followed. The Cambisol soil (B-horizon), collected from Wienerwald, was sieved to avoid stones and big clay particles. Sand and soil were then mixed in a ratio of 1:3. Planting started with the filling of the seven-liter pots to approximately half of their capacity. The core with an 8 cm radius and 15 cm, for Beech, or 10 cm, for conifers, in length was set in the middle of the half full pot. Using the core, a mass of 2.5g of Stockosorb or Polyter were placed in the middle of the pot (Figure 1). Two-year old seedlings of four species were used: European or common beech, Douglas fir, European larch and Norway spruce (LIECO GmbH & Co KG). Each of the species was supplemented with three amendment treatments: i) control; ii) Stockosorb, and iii) Polyter. Every block had the same amount of treatments per specie (24 pots*12=288 pots). Seedlings were furthermore dipped in the bucket containing water and soil collected under the species in the Knödelhutte research station, for mycorrhizal hyphae inoculation. Planting of seedlings followed. The rest of the pot was then filled with a mixture of soil and sand.

Figure 1 The pot with 8 cm core and Polyter placed in the middle.

To quantify EM external hyphae production in the soil, sand-filled ingrowth mesh bags were used (Wallander, Nilsson, Hagerberg, & Bååth, 2001). Bags were filled with 14g of quartz sand (7×6×6cm). Nylon mesh allowed penetration of the fungal mycelium, but not the roots. Sand prevents in-growth of saprotrophic mycelium due to the low organic matter content (Ekblad & Huss-Danell, 1995). EM fungi have a carbon source, so they can easily colonize the substrate whereas saprotrophic fungi rely on organic matter in the soil and for this reason will not colonize substrate with low organic matter content (Wallander et al., 2001). A pair of ingrowth bags were planted in every pot at the level of the hydrogel.

The pots were connected to an automatic drip irrigation that provided enough water during the establishment period (Figure 2). In the beginning we faced some difficulties with young plants. Some of them were flooded with water, therefore bamboo sticks were put under the pots, to avoid water uptake from flooded table during the drought. Due to the blockage in the drainage on the bottom of the pots, ten overflooded samples were discarded. Pots were continuously monitored.

Figure 2 Plants connected to the irrigation system.

2.3 Drought periods

In order to simulate the drought, half of the samples in all treatment regimens were withheld from irrigation. The length of drought period was chosen because it roughly corresponds to the rain pattern of the dry season in the Austria, region of Linz (ZAMG). The first drought period started on 23th of June and lasted for 2 weeks, following with the next drought period after 2 weeks of recovery. The second period lasted only one week (18.07.-24.07.). After 5 recovery days, the third one began (29.07.-05.08.). This period lasted from 16.08. to the 23.08.

2.4 Measurements during growth of the seedlings

2.4.1 Photosynthesis rate

To determine the stress-levels, CO_2 uptake was recorded to calculate the photosynthesis per cm of leaves and needles. During the first drought period, a few plants from every specie were randomly taken to measure the CO_2 uptake. A single branch was placed inside transparent tube forming a closed system, connected to the

CO_2 measuring device (Figure 3). In beech trees, the maximum of 24 leaves allowed in the chamber was counted. Using more leaves would lead to the overlapping and shading effect which in the end affects the photosynthesis. However, in conifers trees, the length of the branch was rather considered, which was around 36 cm. Transparent tube was sealed and the measurements were recorded 6 times (every 15 seconds). Difference from the first recording value and the last was used to calculate CO_2 ppm per minute, in order to ultimately determine the rate of photosynthesis per cm. If the plant is suffering from the drought, stomata will be closed, and coinciding rate of photosynthesis reduced. When the difference between the recorded values is low, it exhibits a drought effect on the plant.

Figure 3 Measuring of CO_2 concentration.

2.4.2 Soil moisture

During the last period of drought, soil water content was measured in order to prove deficiency of water within this period. TDR 100 device was used to estimate the volumetric water content. TDR is an easily transportable device that was applied in the green house in Tulln. By inserting two probes in the soil of the pot, a high voltage was injected into the cables that transport it to the probes. Velocity of the voltage between two probes was furthermore measured and associated with the soil water content. These measurements were performed twice in the last week of drought periods, after the plants have been watered the first day of the drought, and last day, to demonstrate shortage of water in the soil. All three treatments are included in the measurement.

2.5 Harvesting

At the end of the September harvesting of seedlings was done. Every tree stem was cut above the pot surface and placed in a paper bag. Samples were then transported to the drying chamber in the laboratory at BOKU University. Data was presented in Bachelor Thesis, written by Josef Ruda and Riccardo Siller, with an emphasis on aboveground growth. Extraction of the roots followed. Plastic tube with a diameter of 8 cm was inserted in the middle of the pot and extracted from the rest of the pot. Soil and roots in the tube were separated. We divided upper part with the root plug of the seedling and roots growing under the supplemented part (Polyter/Stockosorb). The part which was left in the pot was sampled as an outer part (Figure 5). Roots from every part were thoroughly washed with water through 2mm sieve and placed in a separate plastic water filled bags (Figure 6). Samples were than left in the cooling chamber. Ingrowth mesh bags were collected and taken to a freezer.

Figure 4 Polyter in the soil and after the extraction.

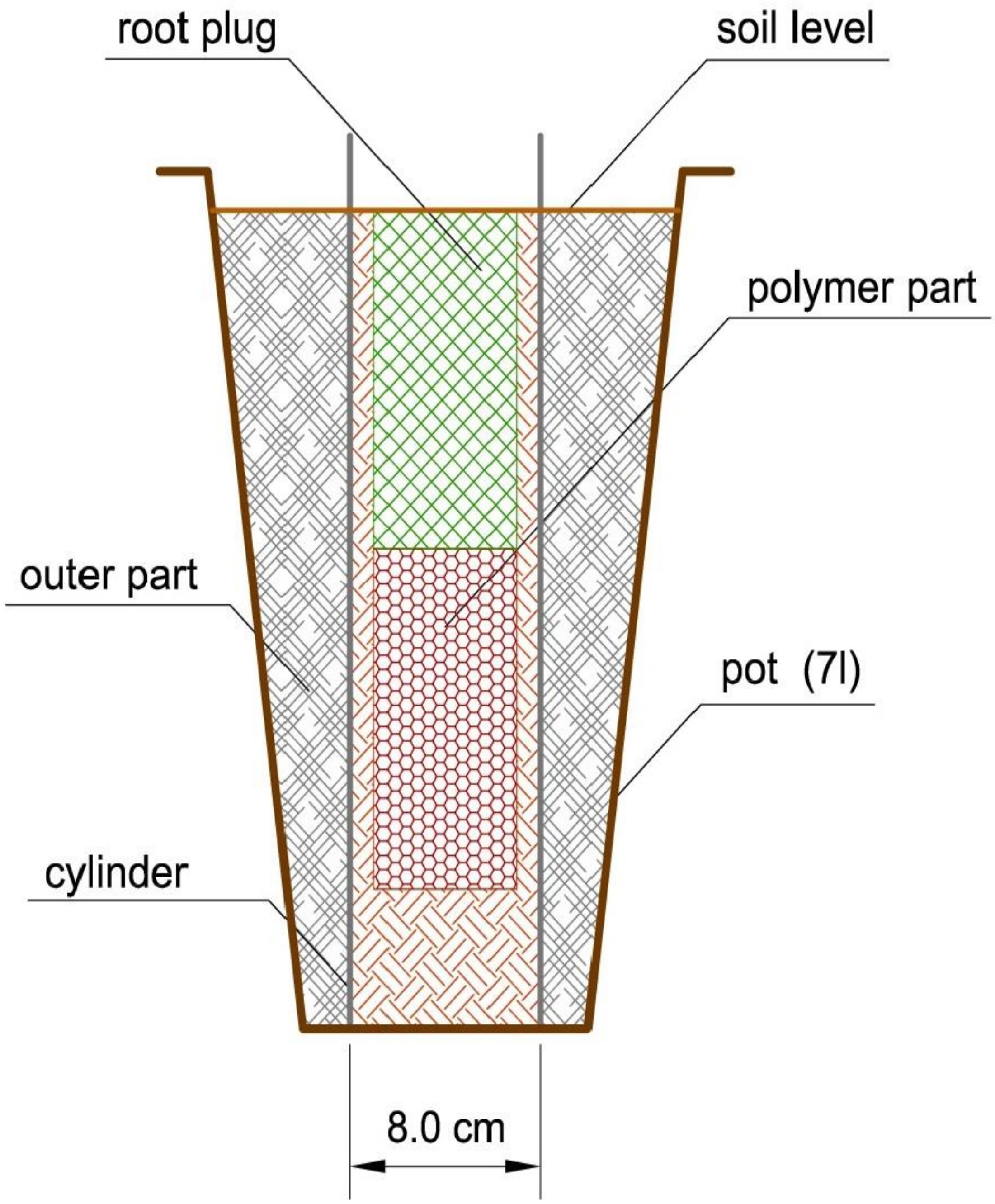

Figure 5 Schematic representation of partitioning of the soil pot (made in AutoCAD).

Figure 6 Washing of the roots through 2mm sieve.

Harvested ingrowth bags were opened in order to observe EM growth, five different categories were visually evaluated: No mycelia present (0), Infrequent hyphae present (1), Sparse mycelia present (2), Mycelia present but no aggregation of the sand particles (3), Plenty of mycelia present and some aggregation of the sand particles (4), Plenty of mycelia present and sand particles aggregated to a large extent (5) (Wallander, Göransson & Rosengren, 2004). In order to estimate mycorrhizal colonization, sampled roots from the polymer part were used. Under the microscope, tips of the roots are observed, and mycorrhiza counted (Figure 7). From the obtained data percentage of mycorrhiza (total number of root tips × 100 / total number of root tips) was calculated (Nilsen, Børja, Knutsen, & Brean, 1998) and will be referred as mycorrhiza colonization in the following text.

To acquire the data on root morphology, fine roots (roots thinner than 2 mm) were scanned and analyzed, using a digital image analysis system “WinRHIZO Pro “. Every sample from outer and polymer part was individually placed in a transparent tray, which was then filled with water to ensure the roots to spread evenly. After scanning, samples were placed in a paper bags and in the drying room for additional measurements. WinRHIZO Pro calculated average diameter (RD), surface area, length and volume of roots for every sample scanned. With measured and calculated root traits we acquired further information about root morphology. Root tissue density (RTD) was calculated as root dry mass divided by fresh root volume. Specific root length (SRL) was calculated as root length divided by root dry mass. From SRL we can determine how much root length is invested per unit of root mass (Kramer and Boyer 1995; Ryser, 2006). Furthermore, specific root area (SRA) as root surface area divided by root dry mass. Additionally, we calculated the ratio between fine root biomass and leaf biomass, considering leaf and fine roots both show short term response to stress such as drought. To examine the difference in root growth between polymer and outside part, ratio (O/P) was calculated using root dry biomass from mentioned parts. All the calculations and analyses were made separately on both polymer and outer parts.

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Figure 7 Mycorrhiza tips seen under the microscope

2.6 Statistical analyses

The data were analyzed with one-way and two-way ANOVA which were performed on dependent variable by two independent variables: i) drought treatment and ii) treatment by amendments, for each tree specie. Outer and polymer parts were analyzed separately. Prior to calculations, every outlier within the range of two standard deviations was removed from the data. Subsequently, normality with Shapiro-Wilk test was checked and assessed equality of variances with Levene's test, whereby assumptions for ANOVA were fulfilled. Some of the data were $\log_{10}$ transformed, before analysis took place, to reduce the skew. In order to test significant differences between group means ($p<0.05$) the multiple comparison of means with Tukey's HSD post hoc test for unequal number (n) with 95% confidence intervals (CI) was conducted. A non-parametric method, Kruskal-Wallis test, was performed for the measurement of volumetric water content, since assumptions for one-way ANOVA were not met. Statistical calculations and graphs were all made with R-Studio software.

3 Results

Results are presented in bar and boxplot charts. Table of p-values for every root trait and additional graphs can be found in the appendix. The following charts are the statistically significant by two-way ANOVA and therefore selected to present the results in the most interesting and representative way.

Calculations in photosynthesis per cm of leaves and needles, resulted in small differences between irrigated and non-irrigated plants, indicating a moderate effect of drought.

3.1 Soil moisture

Volumetric water content is significantly higher in the soils measured after the period of irrigation ($p<2.2e-16$), than in the soils measured after the last drought period, as is portrayed in Figure 8. With this result is demonstrated that the soil experienced deprivation of water.

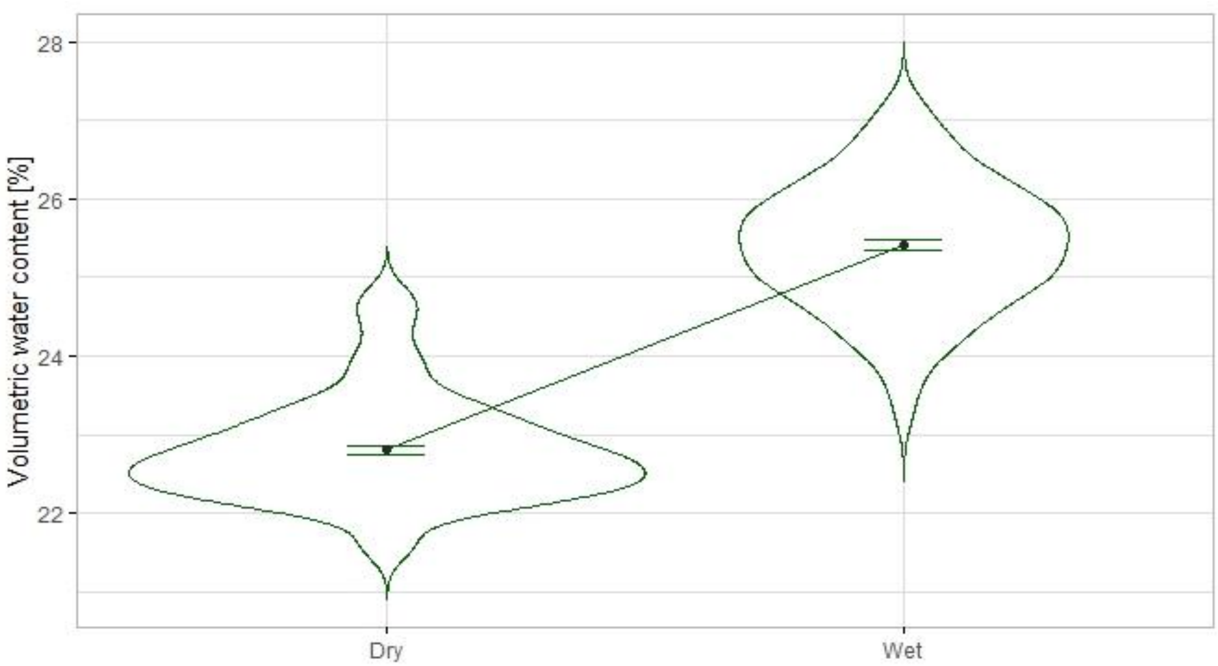

Figure 8 Volumetric water content in Dry (non-irrigated) and Wet (irrigated) soils. Given values are presented as line plot of mean +/- SE and density plot.

3.2 Root Biomass

The effect of drought on a root biomass, from the outer part, indicated more root biomass in the drought affected plants of Douglas fir (see Figure 9). In the polymer part, root biomass was considerably bigger in drought treated plants in Beech (Figure 10) and Larch (Figure 11). In Spruce an effect of treatments by amendments shows lower root biomass in control plants than in Polyter and Stockosorb (Figure 12).

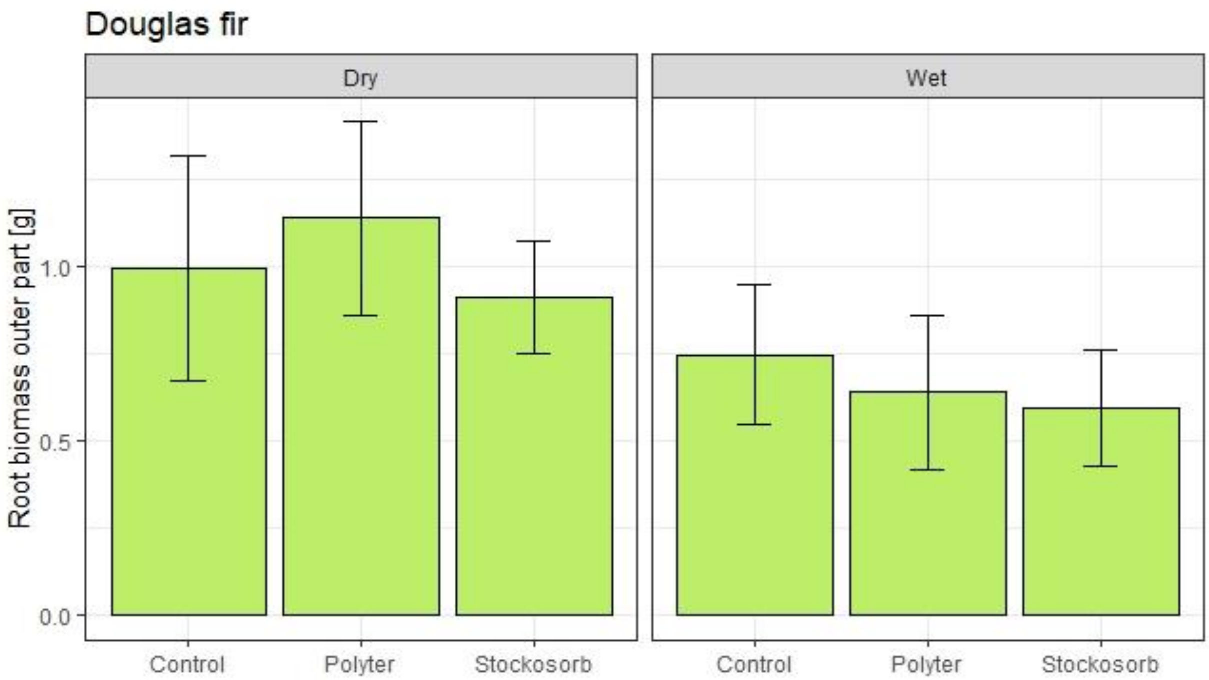

Figure 9 Root biomass from the outer part in Douglas fir. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots, and amendment treatments. Error bars= Standard error.

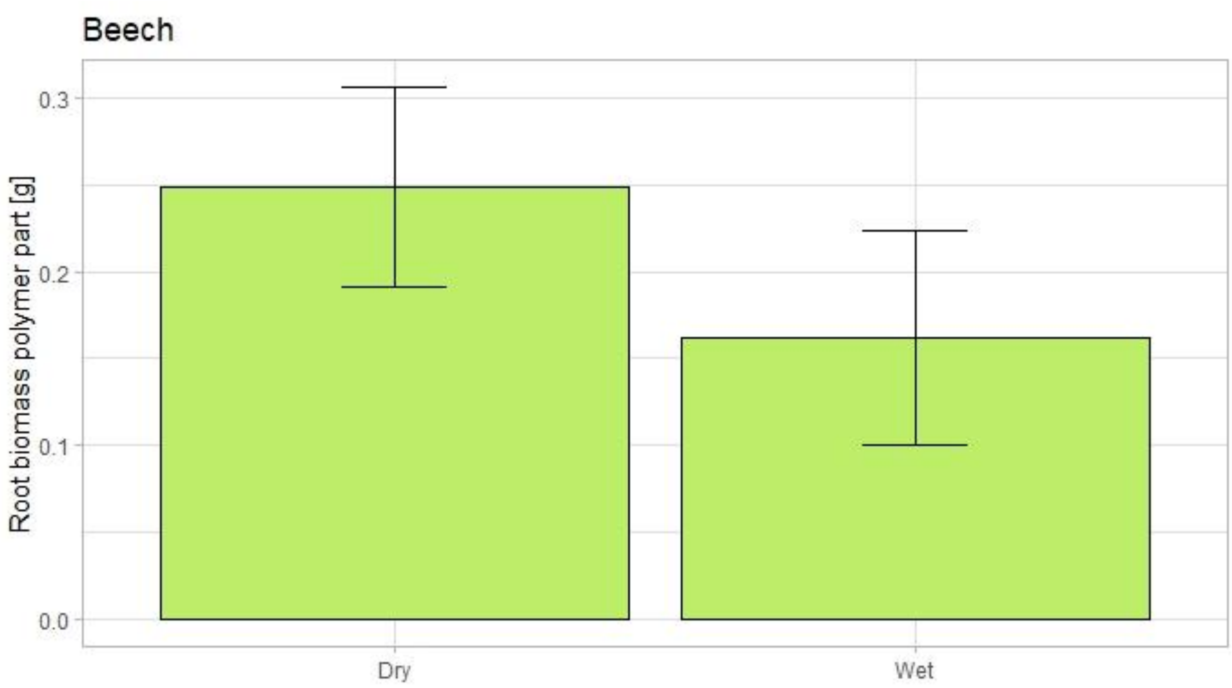

Figure 10 Root biomass from the polymer part in Beech. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots. Error bars= Standard error.

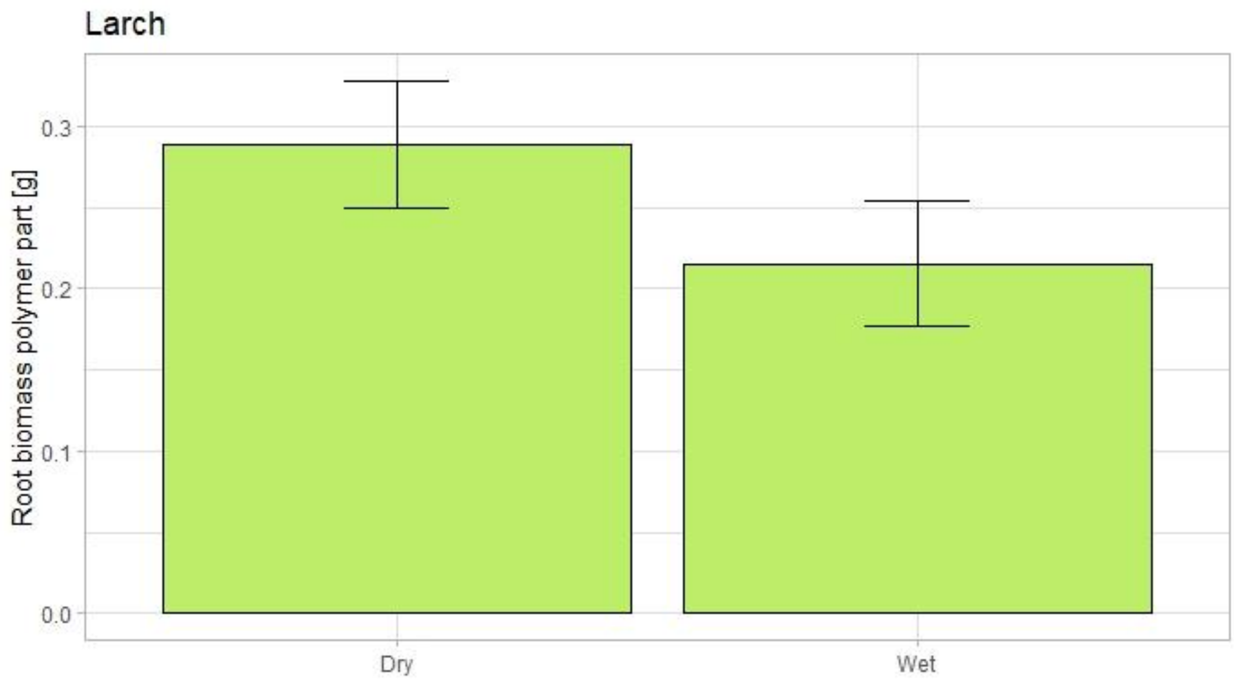

Figure 11 Root biomass from the polymer part in Larch. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots. Error bars= Standard error.

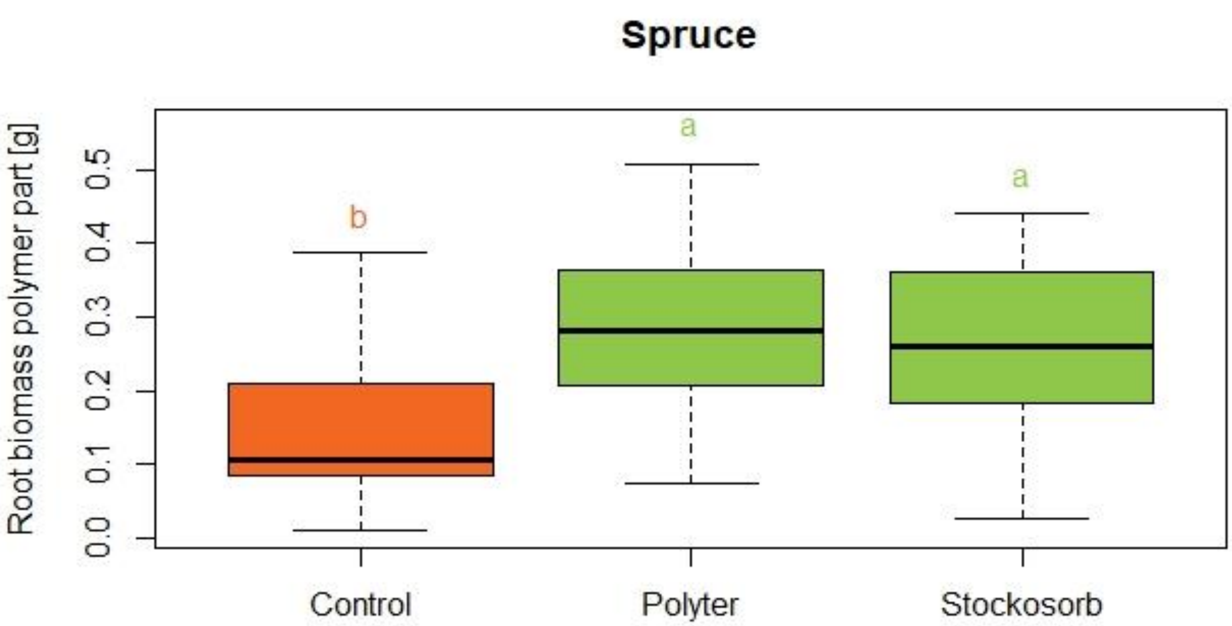

Figure 12 Difference between treatments in root biomass from polymer part in Spruce. Different letters indicate significant differences between treatments.

With the two-way ANOVA an interaction between the irrigated and non-irrigated plants was found, in a way that the treatments by an amendment affect the root biomass from the outer part in Spruce. The interaction plot portrayed in Figure 13 shows the variation in treatments by amendment, such that the control and Stockosorb show different root biomass than the Polyter according to the drought treatment. Root biomass exhibits a difference in the irrigated plants, indicating that root biomass is considerably bigger in the Stockosorb than in the control. The interaction between the drought effects and treatment by amendment on root biomass in Douglas fir was estimated from the polymer part (see Figure 14). To conclude, root biomass was higher in soils amended with Polyter and under drought stress, compared to all irrigated and control drought treated plants.

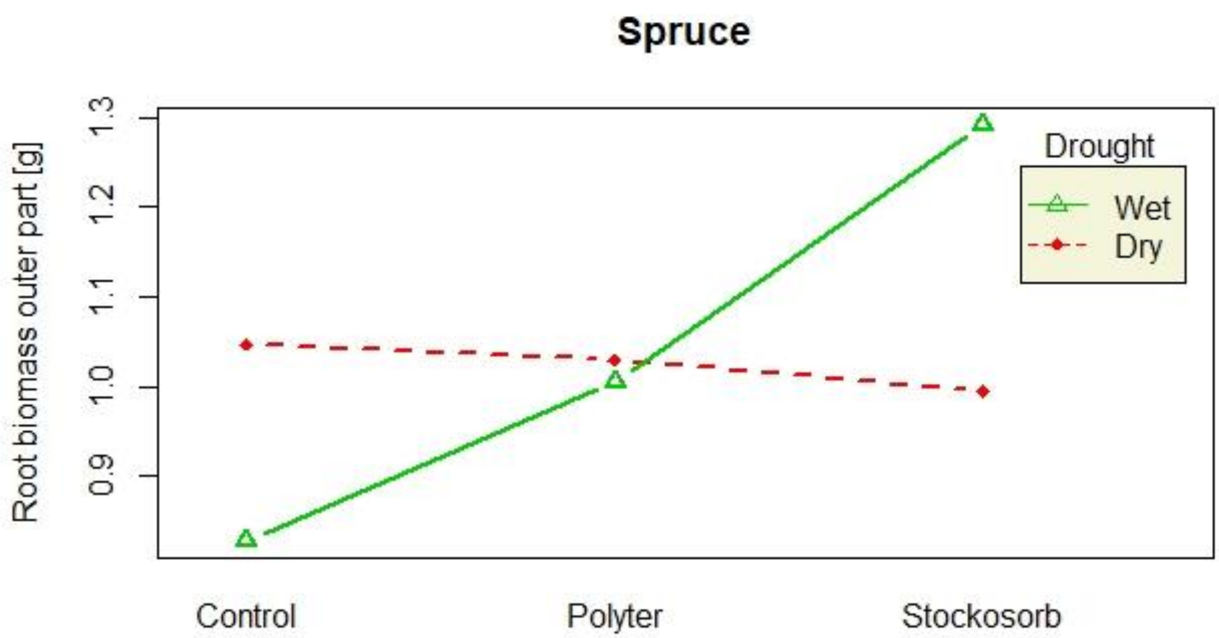

Figure 13 Root biomass in Spruce shown in means or interaction plot between treatments by amendments and irrigated (Wet) or non-irrigated (Dry) plants.

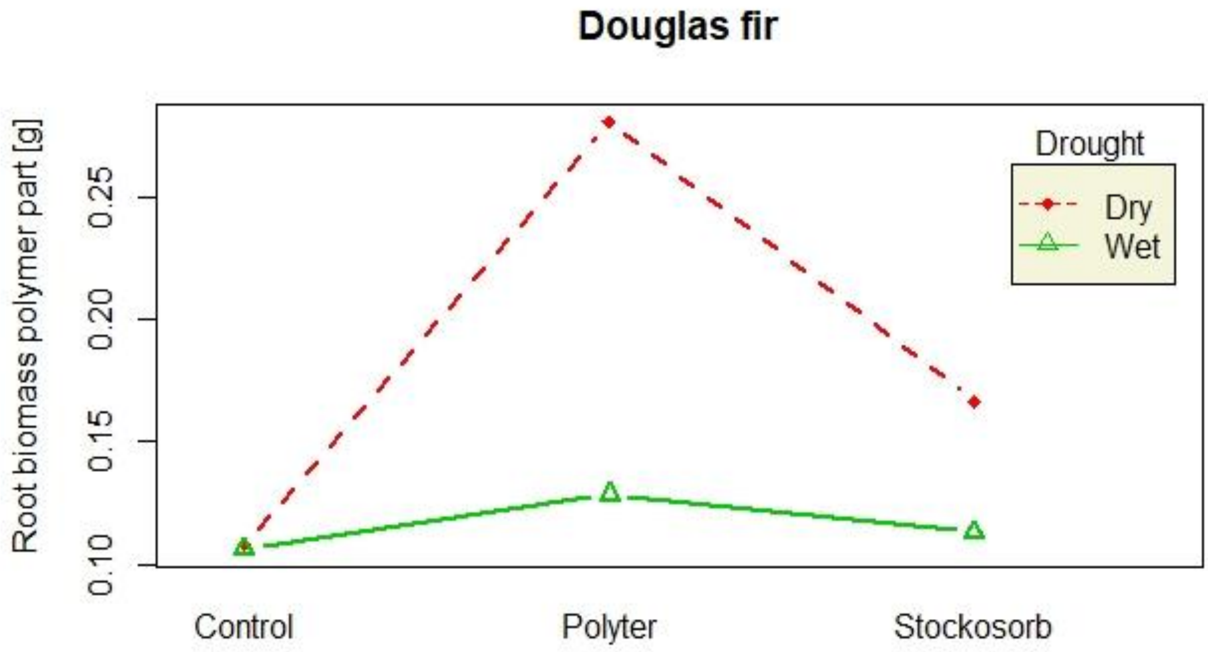

Figure 14 Root biomass in Douglas fir shown in means or interaction plot between treatments by amendments and irrigated (Wet) or non-irrigated (Dry) plants.

3.3 Root length

In the outer part of the Beech root length was bigger in irrigated plants (Figure 15). Only the Spruce showed an interaction effect of drought and amendment treatments on the root length, in the outer part (Figure 16). The effect of drought varies among treatments by amendments, such that root length is considerably bigger in irrigated plants supplemented with Stockosorb. Furthermore, irrigated plants treated with Stockosorb showed higher root length compared to wet control plants.

In the polymer part, Beech had shown differentiation when applied to various drought treatments. Root length in the polymer part was considerably bigger in the plants affected by the drought (Figure 17), unlike the root length in the outer part from Figure above (Figure 15). There was a significant distinction noticed between the treatments by amendments in Spruce in the polymer part. Variation between control and Polyter treatments indicated higher root length in Polyter than in control (Figure 18). Larch was the only species that didn't show any significant difference between drought or amendments treatments.

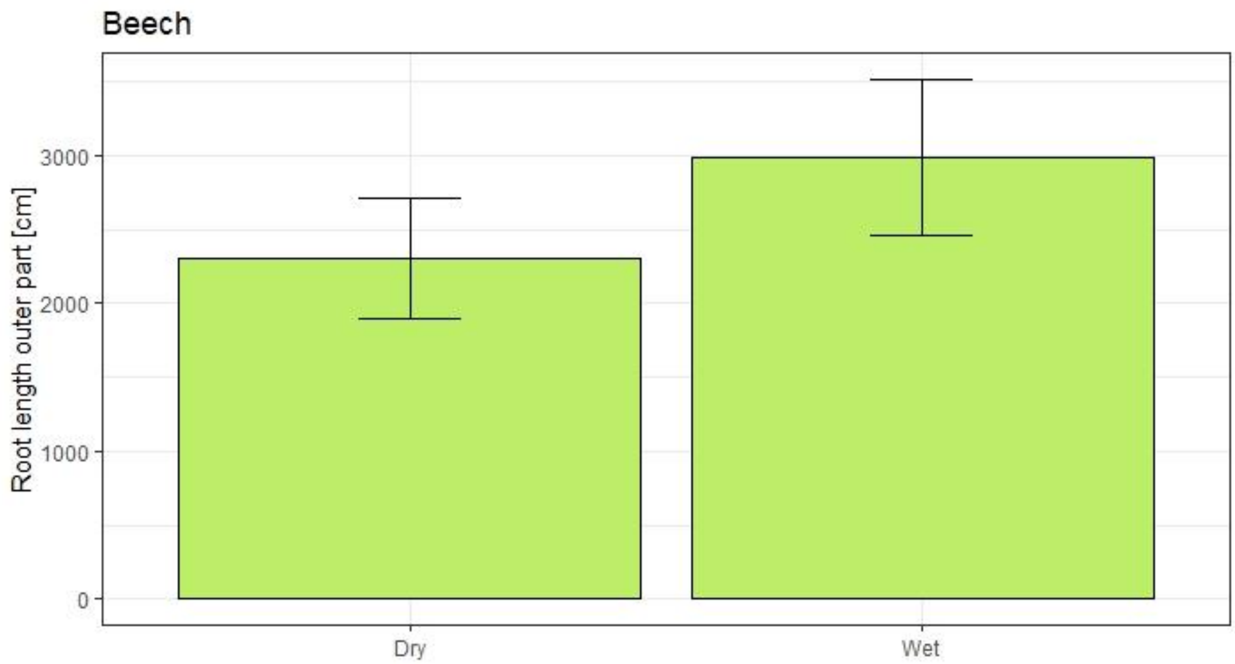

Figure 15 Root length from the outer part in Beech. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots. Error bars= Standard error.

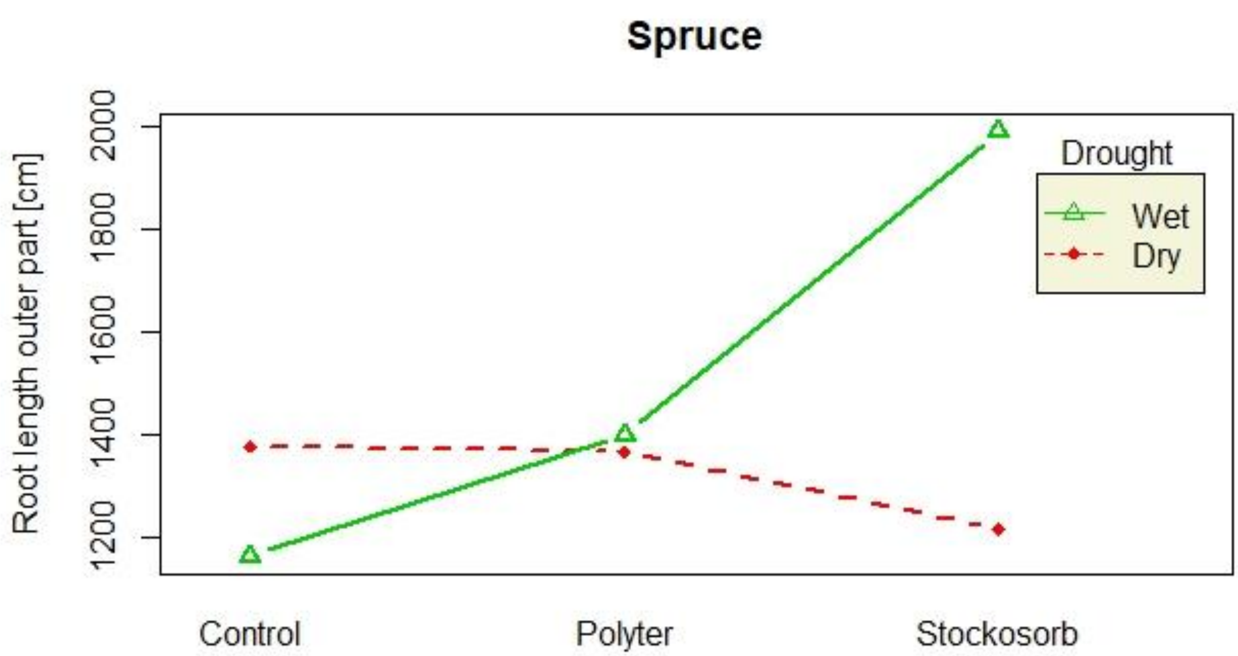

Figure 16 Root length in Spruce shown in means or interaction plot between treatments by amendments and irrigated (Wet) or non-irrigated (Dry) plants.

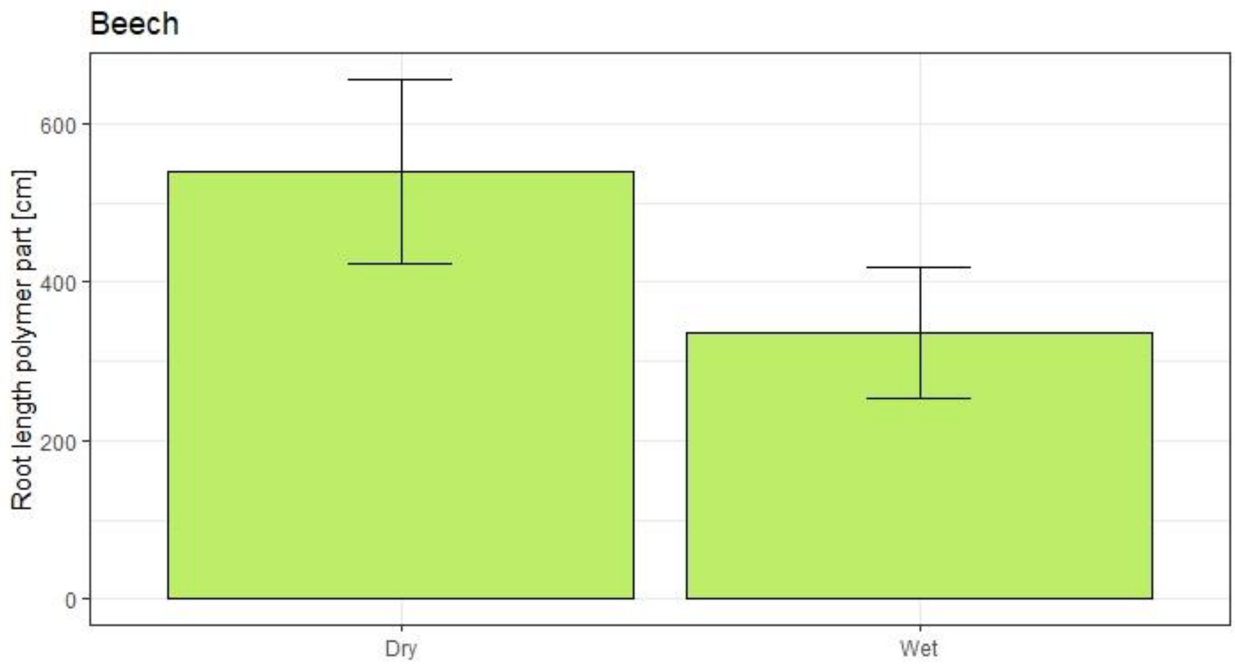

Figure 17 Root length from the polymer part in Beech. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots. Error bars= Standard error.

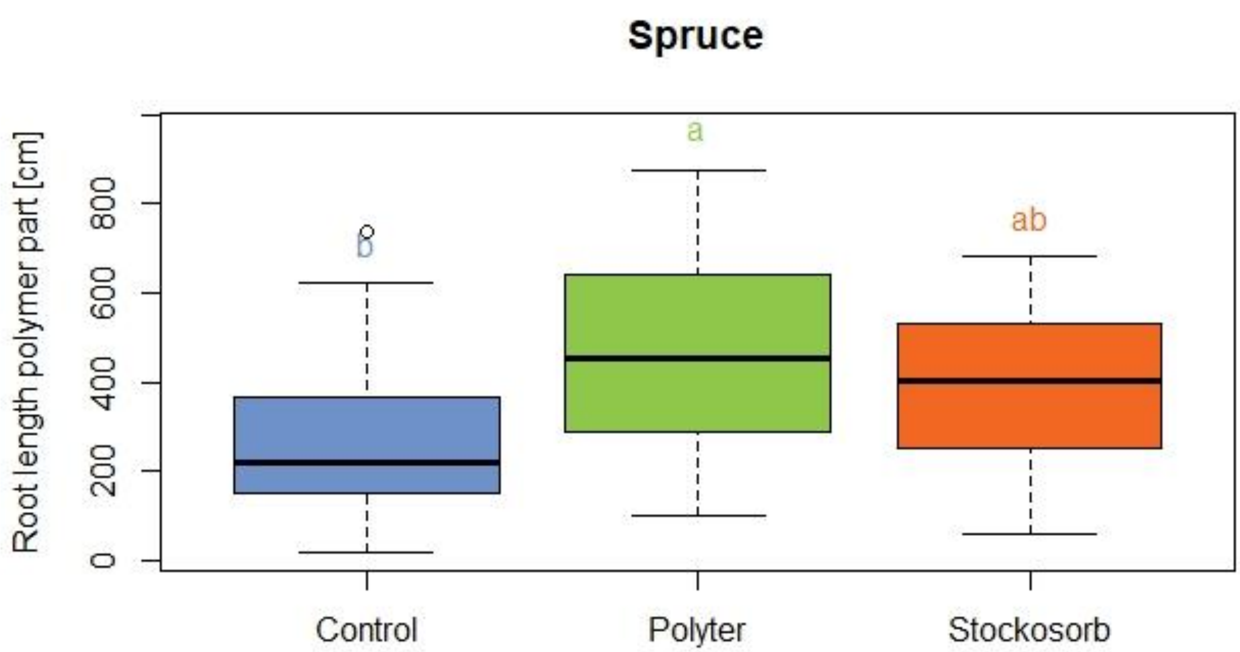

Figure 18 Difference between treatments in root length from polymer part in Spruce. Different letters indicate significant differences between treatments.

3.4 Root diameter

There was a difference found in outer part for every specie in RD between an irrigated and a non-irrigated plant, indicating lower RD for plants under drought effect (Figure 19). In the polymer part a distinction in drought treatments was shown only in Douglas fir (Figure 20) and Larch. In both species RD is higher in the irrigated pots than in the drought treated pots. Larch exhibited a differentiation between the amendment treatments, pointing out a slight rise in RD from control to Polyter (see Figure 21).

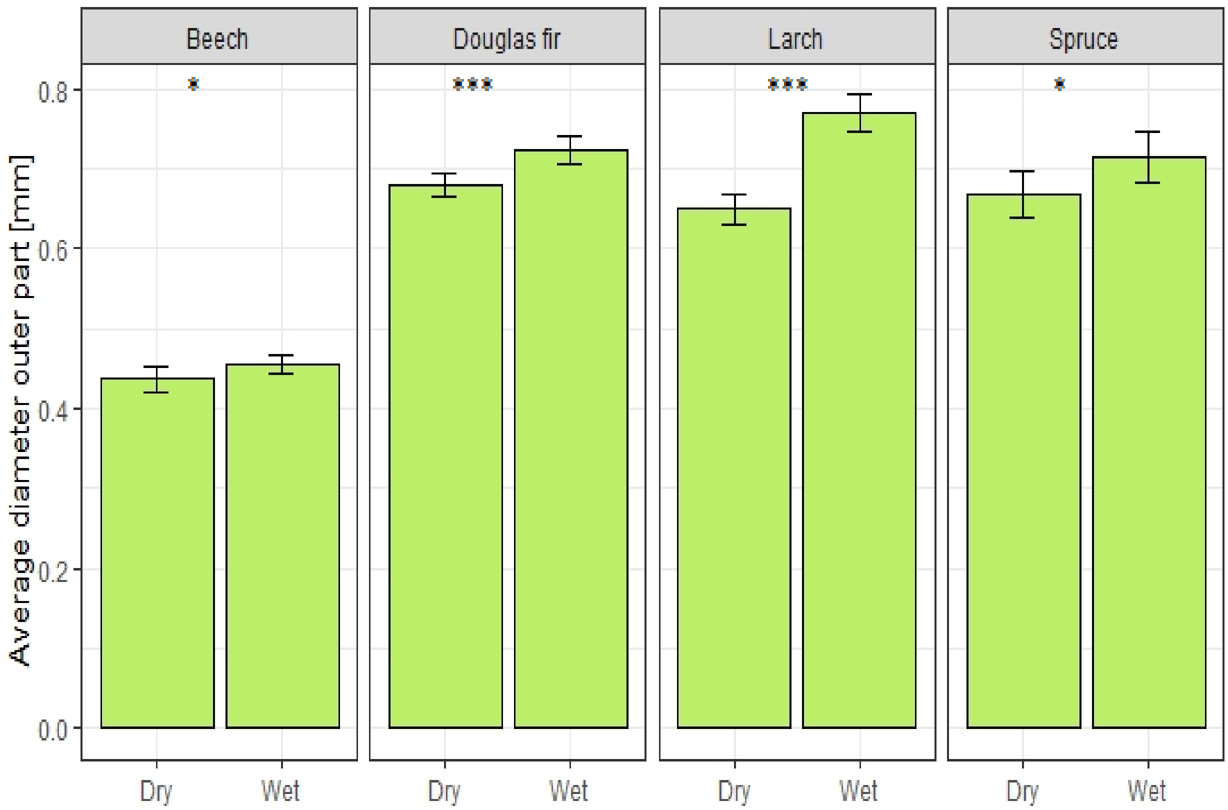

Figure 19 Mean SE +/- of Average diameter in irrigated (Wet) and non-irrigated (Dry) plants, from outer part of the pot for all four specie. The levels of significant are indicated (P<0.05; ** P<0.01; *** P<0.001).*

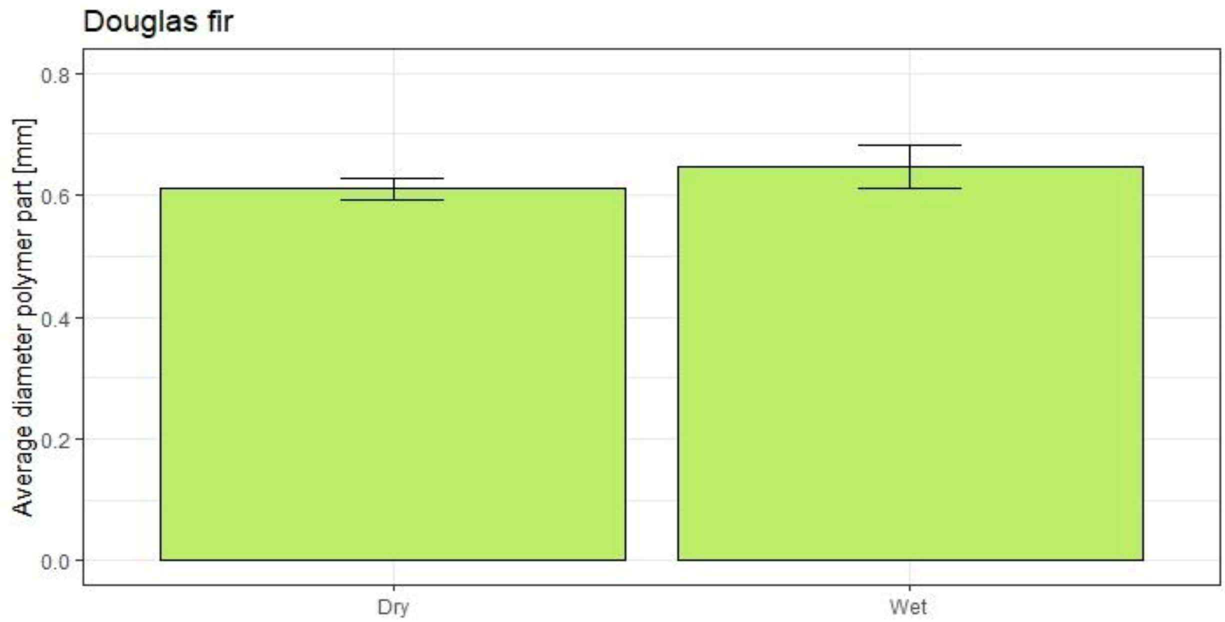

Figure 20 Average diameter from the polymer part in Douglas fir. Difference between drought treatments: irrigated (Wet) and non-irrigated (Dry) plants, calculated with Kruskal-Wallis test. Error bars= Standard error.

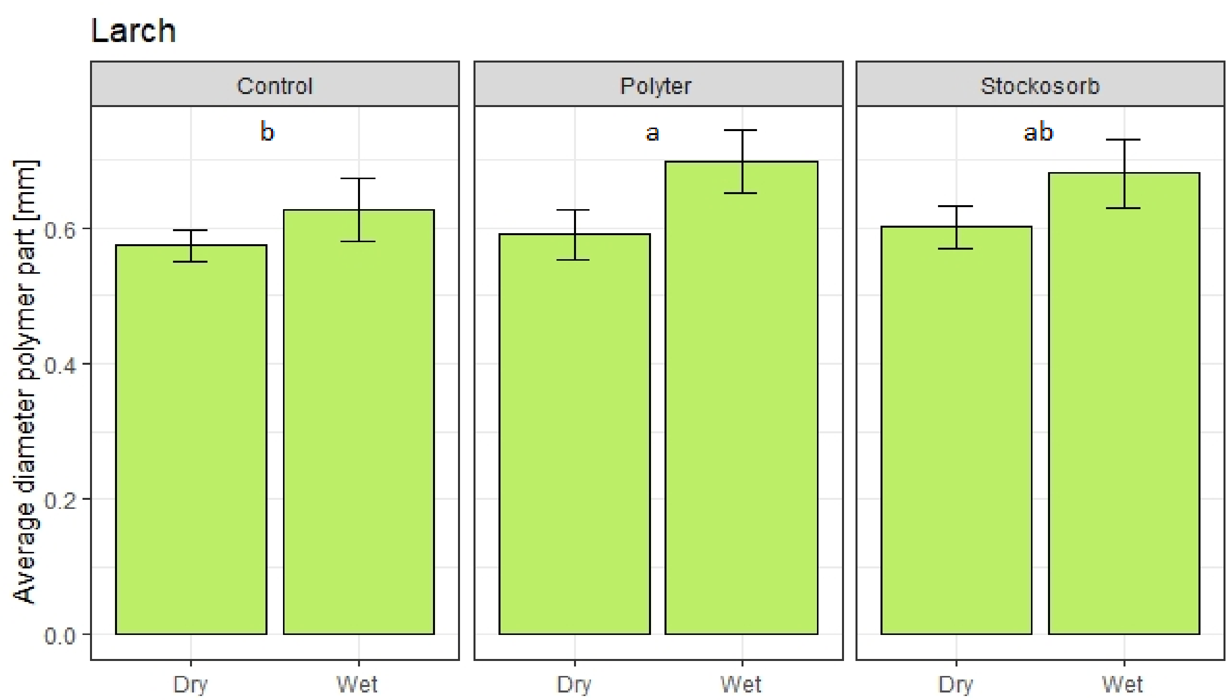

Figure 21 Average diameter in polymer part in Larch between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter). Error bars= Standard error. Different letters indicate significant differences between the treatments

Interaction between the effects of drought and amendment treatments on RD in polymer part of Spruce was statistically significant. An interaction plot indicates that plants under control treatment had lower RD under drought than plants supplemented with Stockosorb. Additionally, there was an enhancement in RD between dry and wet treatment, in plants under the control treatment (Figure 22).

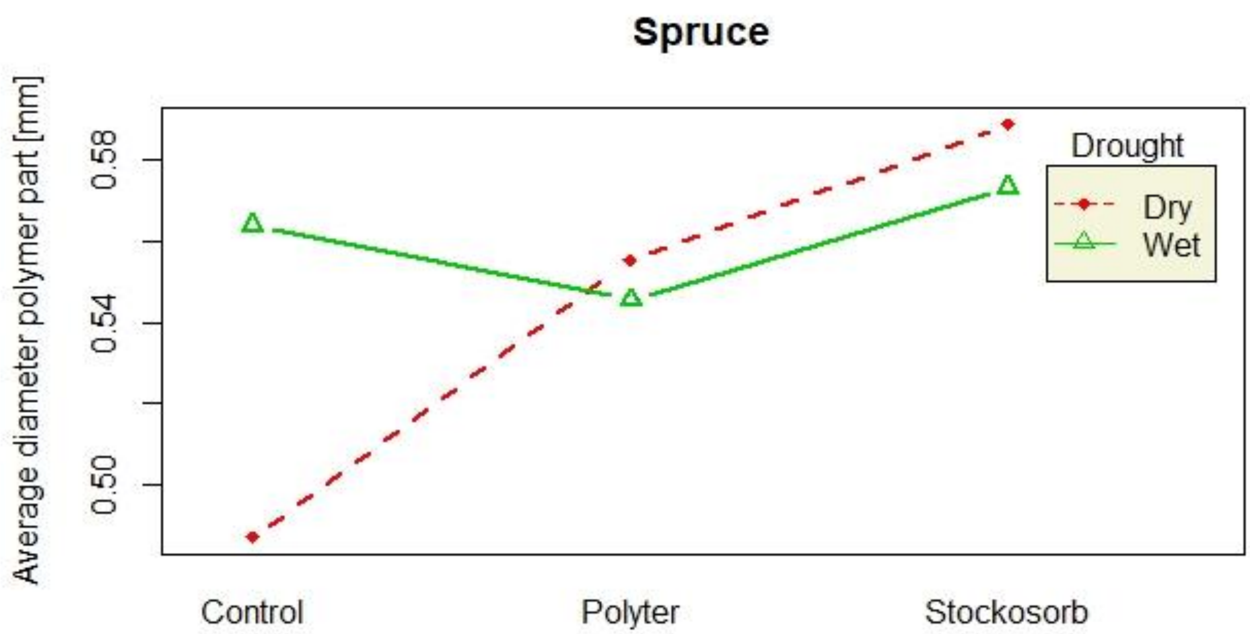

Figure 22. Average diameter in Spruce shown in means or interaction plot between treatments by amendments and irrigated (Wet) or non-irrigated (Dry) plants.

3.5 Root tissue density

Only an affected of drought treatment on RTD was notable. Meaning, there was no reaction of plants on amendments. In the outer part, Douglas fir, Larch and Spruce demonstrated higher RTD in drought affected plants (Figure 23). Furthermore, polymer part exhibits a discrepancies in all four species (Figure 24).

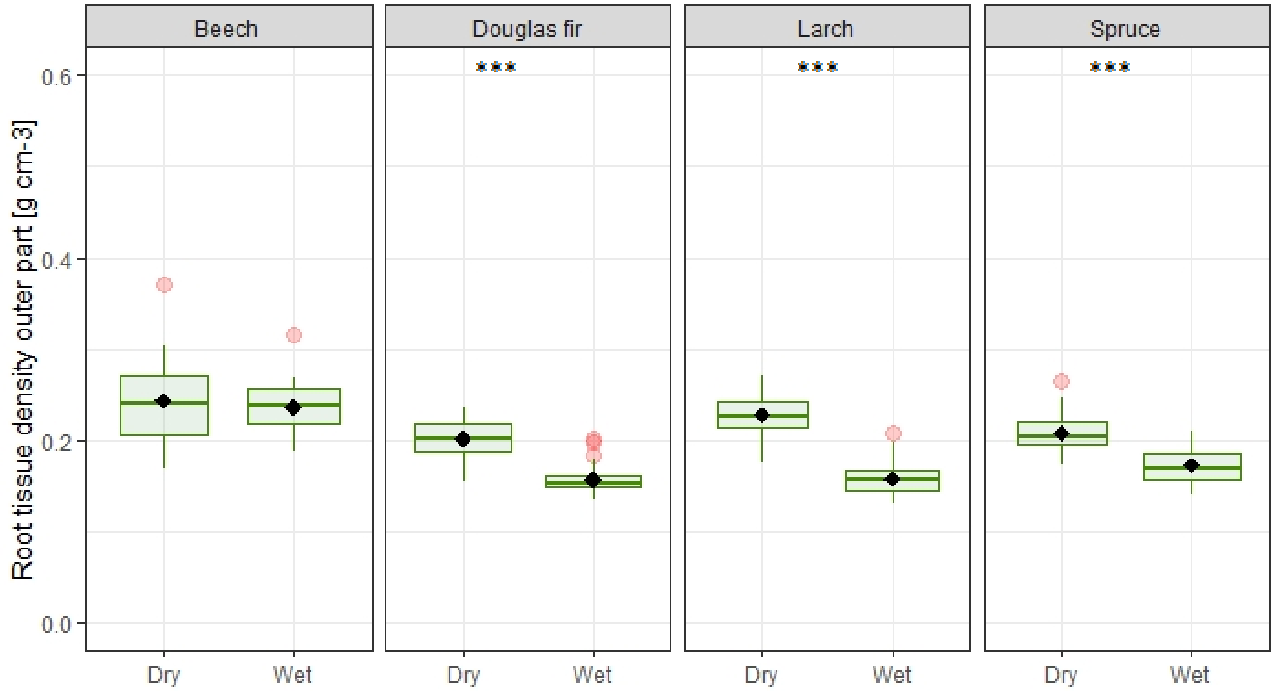

Figure 23 Means of Root tissue density from outer part in irrigated (Wet) and non-irrigated (Dry) plants for all four species. The levels of significant are indicated (P<0.05; ** P<0.01; *** P<0.001).*

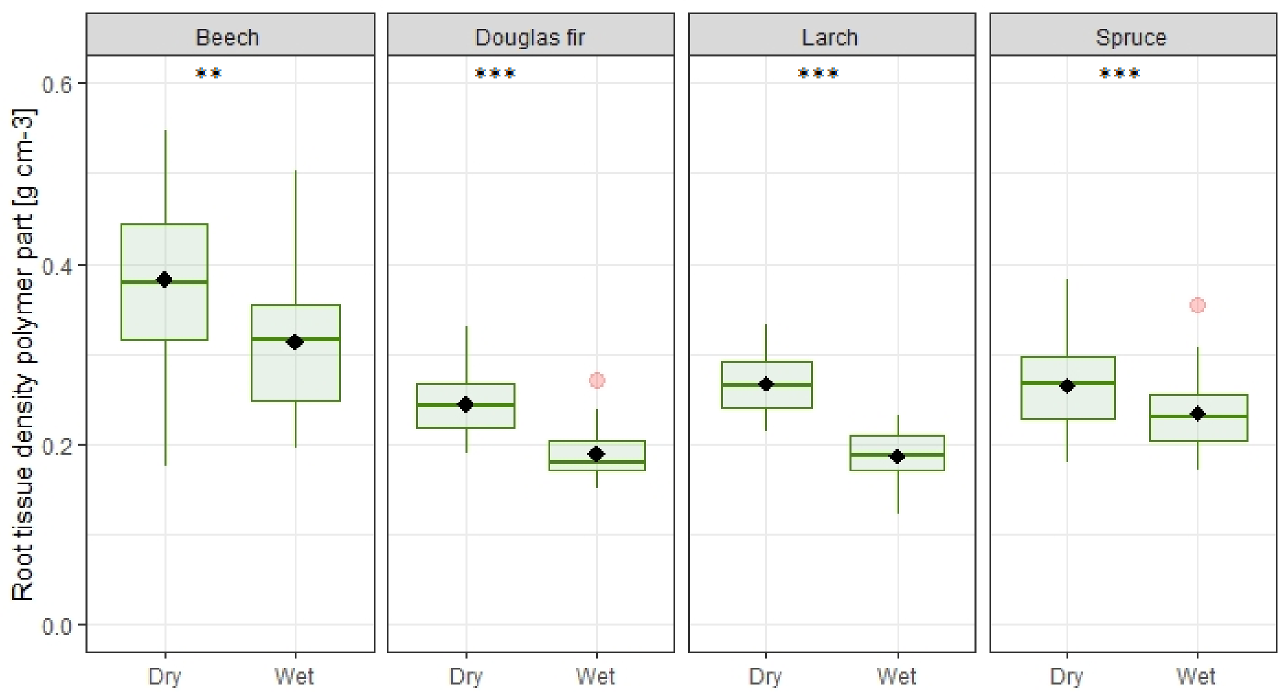

Figure 24 Means of Root tissue density from polymer part in irrigated (Wet) and non-irrigated (Dry) plants for all four species. The levels of significant are indicated (P<0.05; ** P<0.01; *** P<0.001).*

3.6 Specific root length

In the outer part of Douglas fir, difference between the drought treatments was calculated. Figure 25 shows higher SRL in irrigated plants. Rest of the results showed either no significant difference or rather a small one.

Figure 25 Specific root length (SRL) from the outer part in Douglas fir. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots. Error bars= Standard error.

3.7 Specific root area

Drought treatments highly affected SRA in all four species, unlike SRL. In the outer part, SRA was considerably lower in drought stressed plants in all species except Beech (Figure 26). Results from the polymer part are much like the ones from outer part. All testing species had considerably higher SRA in polymer part in watered plants than in drought treated plants (Figure 27). Treatments with amendments differentiated in polymer part of Spruce and Douglas fir, indicating higher SRA in control than in Stockosorb and Polyter treated plants for both species (Figure 28 and 29).

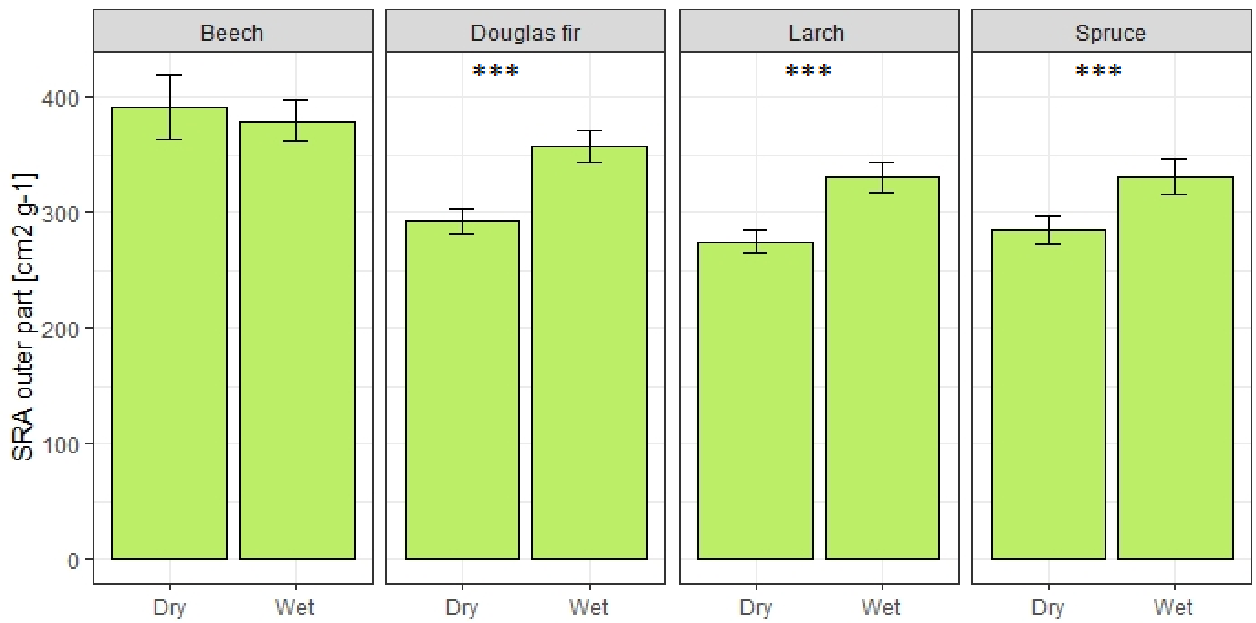

Figure 26 Mean SE +/- of Specific root area (SRA) from the outer part in irrigated (Wet) and non-irrigated (Dry) plants between all four species. The levels of significant are indicated (P<0.05; ** P<0.01; *** P<0.001).*

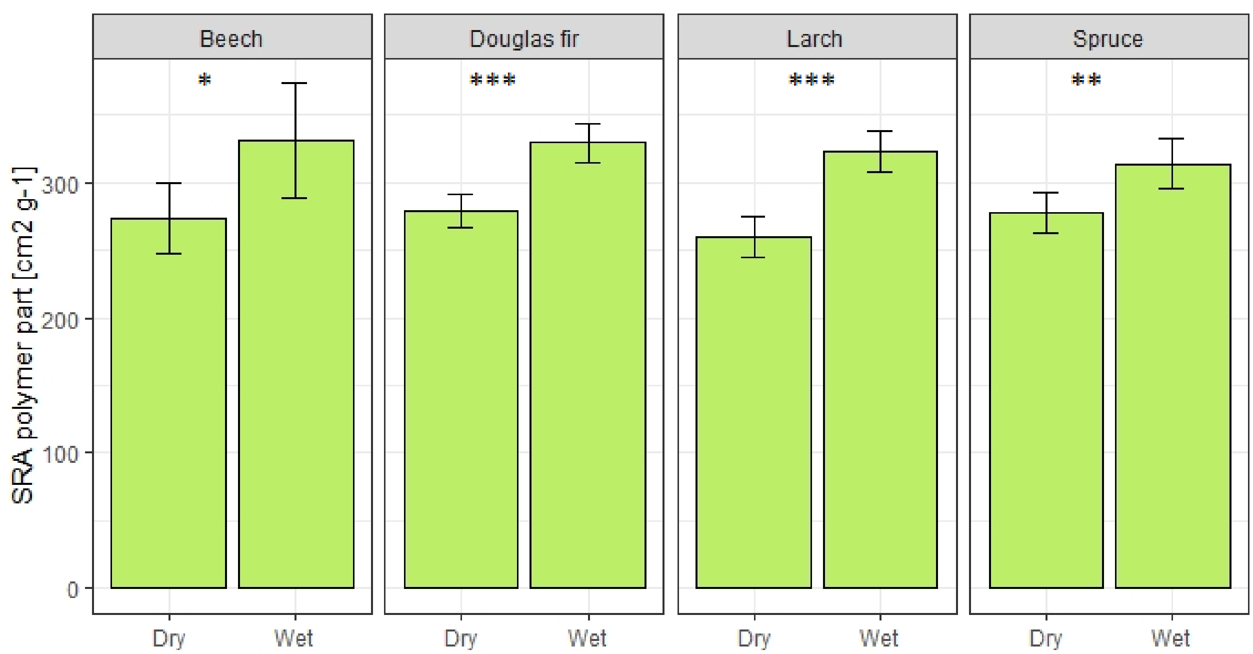

Figure 27 Mean SE +/- of Specific root area (SRA) from the polymer part in irrigated (Wet) and non-irrigated (Dry) plants for four species. The levels of significant are indicated (P<0.05; ** P<0.01; *** P<0.001).*

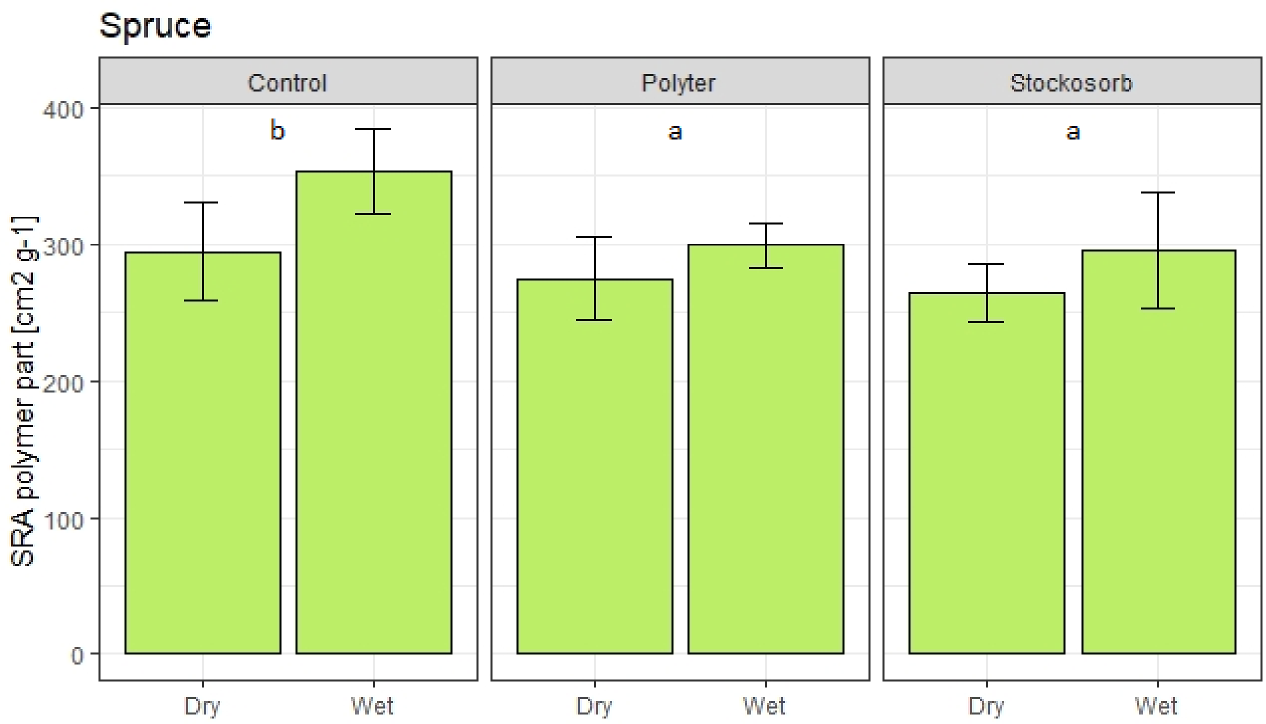

Figure 28 Mean SE +/- of Specific root area (SRA) from the polymer part in Spruce, divided into drought and amendment treatments. Different letters indicate significant differences between treatments.

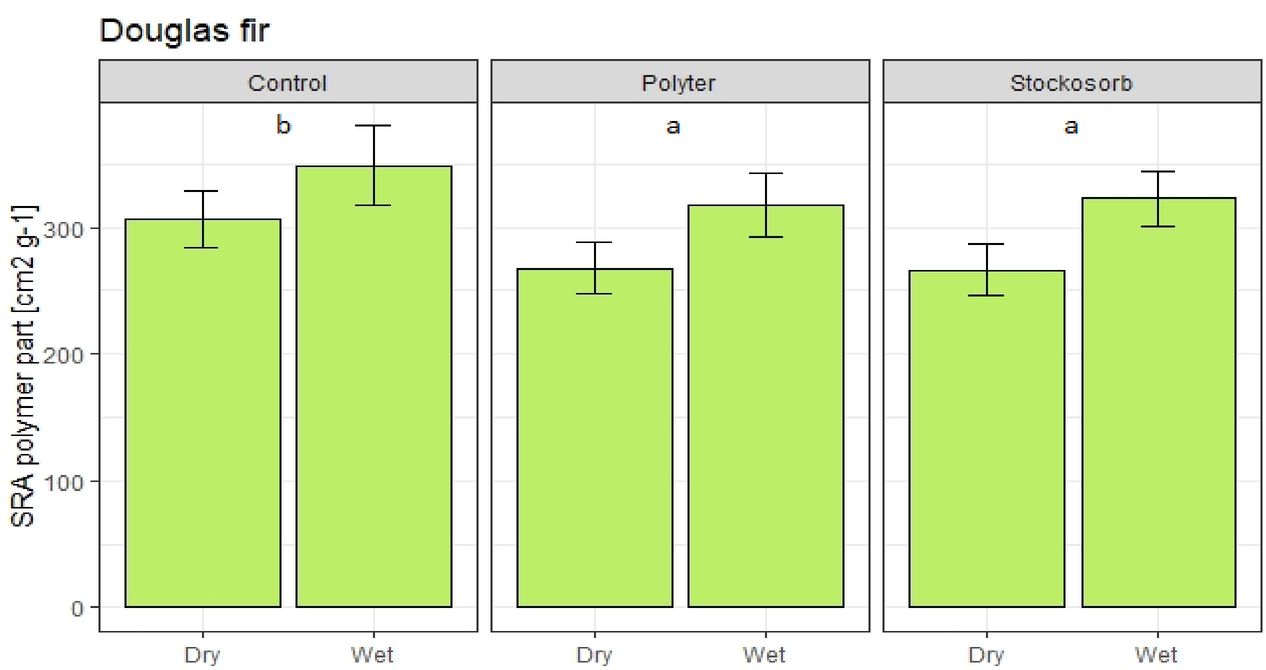

Figure 29 Mean SE +/- of Specific root area (SRA) from the polymer part in Douglas fir, divided into drought and amendment treatments. Different letters indicate significant differences between treatments.

3.8 Fine root biomass / Leaf biomass

Results found in Douglas fir demonstrated bigger ratio in drought treated plants (Figure 30). Spruce revealed a distinction in treatments by amendments, where control exhibited significantly lower ratio than Stockosorb and Polyter treatment (Figure 31).

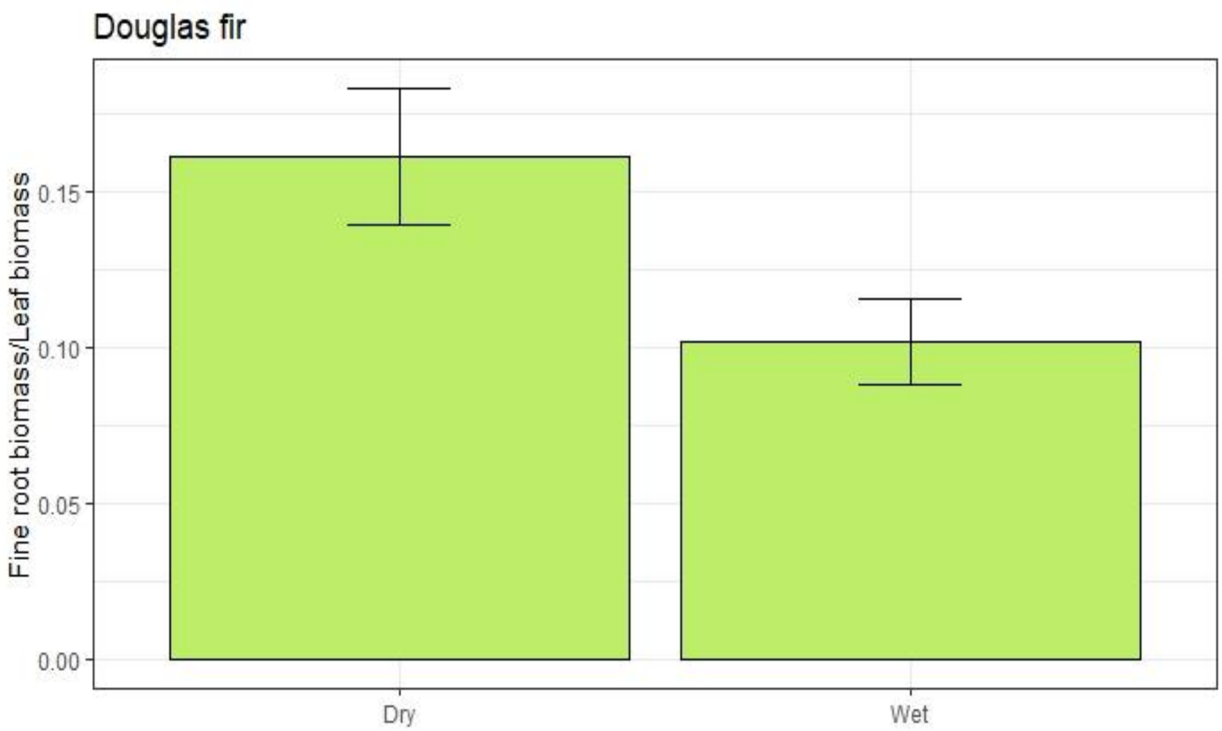

Figure 30 Fine root biomass / Leaf biomass ratio in Douglas fir. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots. Error bars= Standard error. Leaf biomass data from Ruda & Siller, 2018.

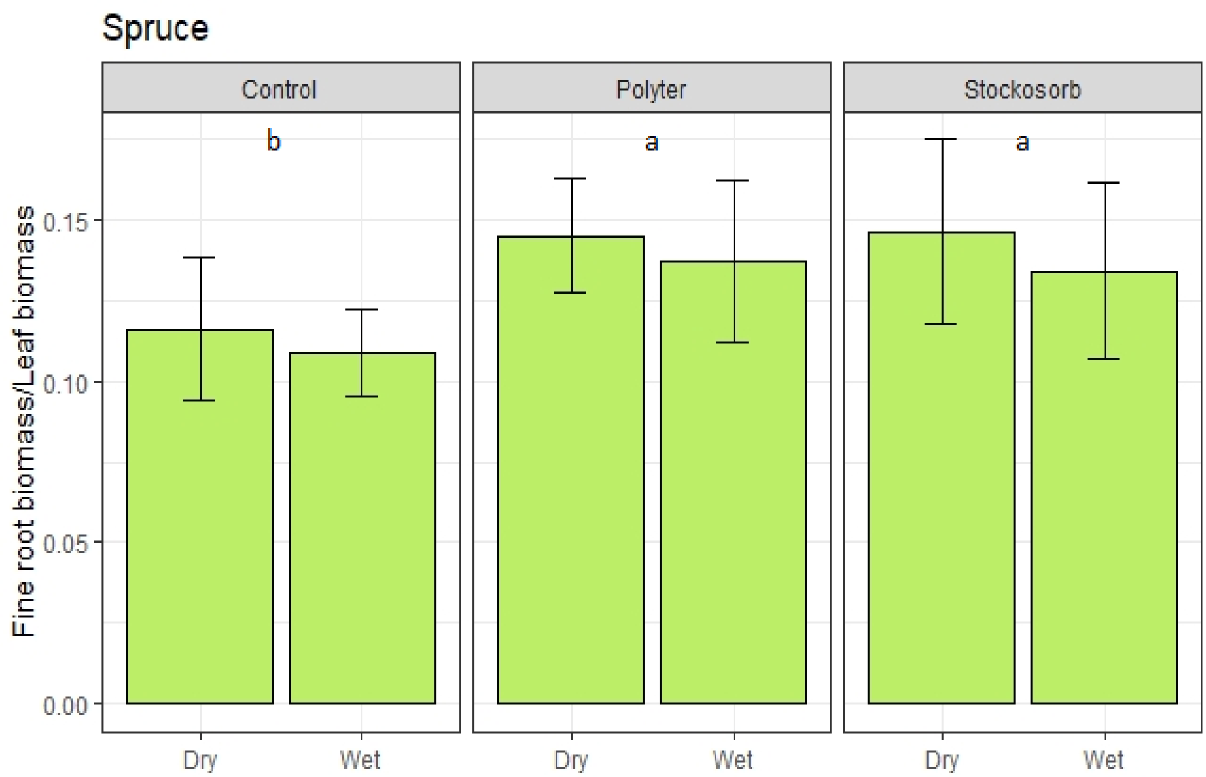

Figure 31 Mean SE +/- of Fine root biomass / Leaf biomass ratio in Spruce, between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter). Different letters indicate significant differences between treatments.
Leaf biomass data from Ruda & Siller, 2018.

3.9 Outside / Polymer ratio

Between the drought treatments, difference was found in Beech, Larch and Spruce, indicating lower O/P ratio in drought affected plants (Figure 32). The effect of an amendment treatment was found in Spruce, pointing out higher O/P ratio in control than in the Stockosorb or Polyter (Figure 33).

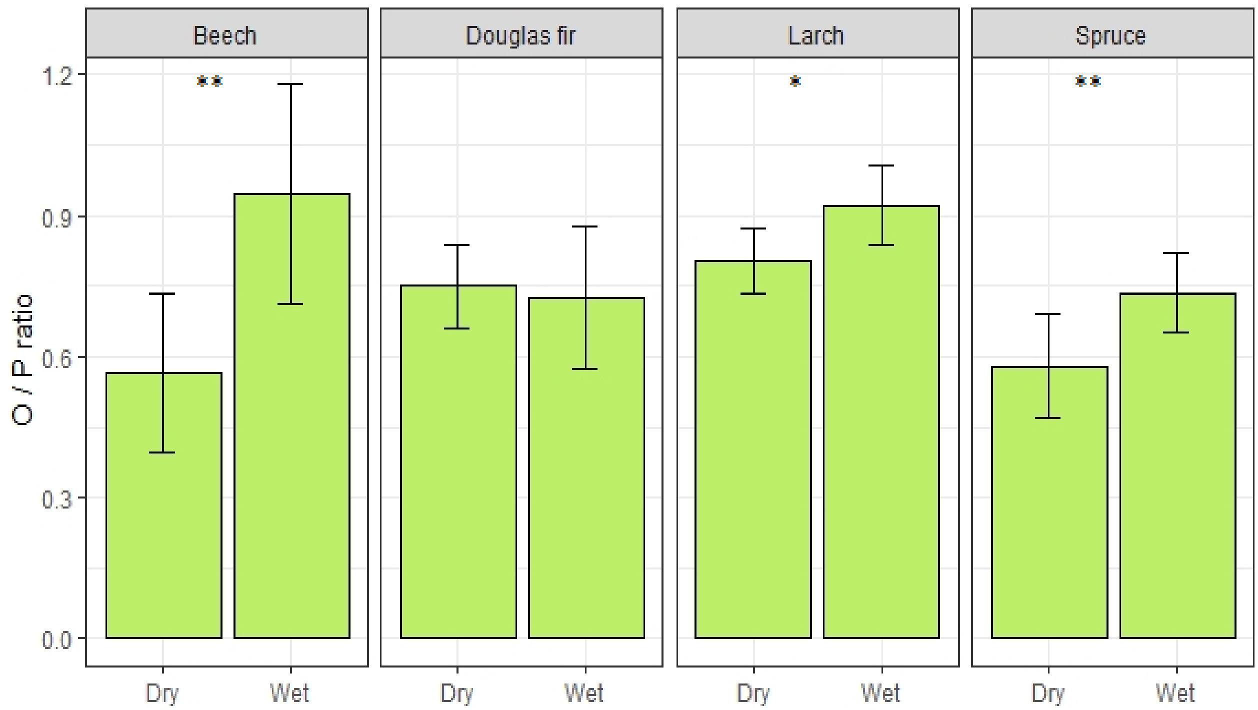

Figure 32 Mean SE +/- of Outside / Polymer ratio, in irrigated (Wet) and non-irrigated (Dry) plants for four species. The levels of significant are indicated (P<0.05; ** P<0.01; *** P<0.001).*

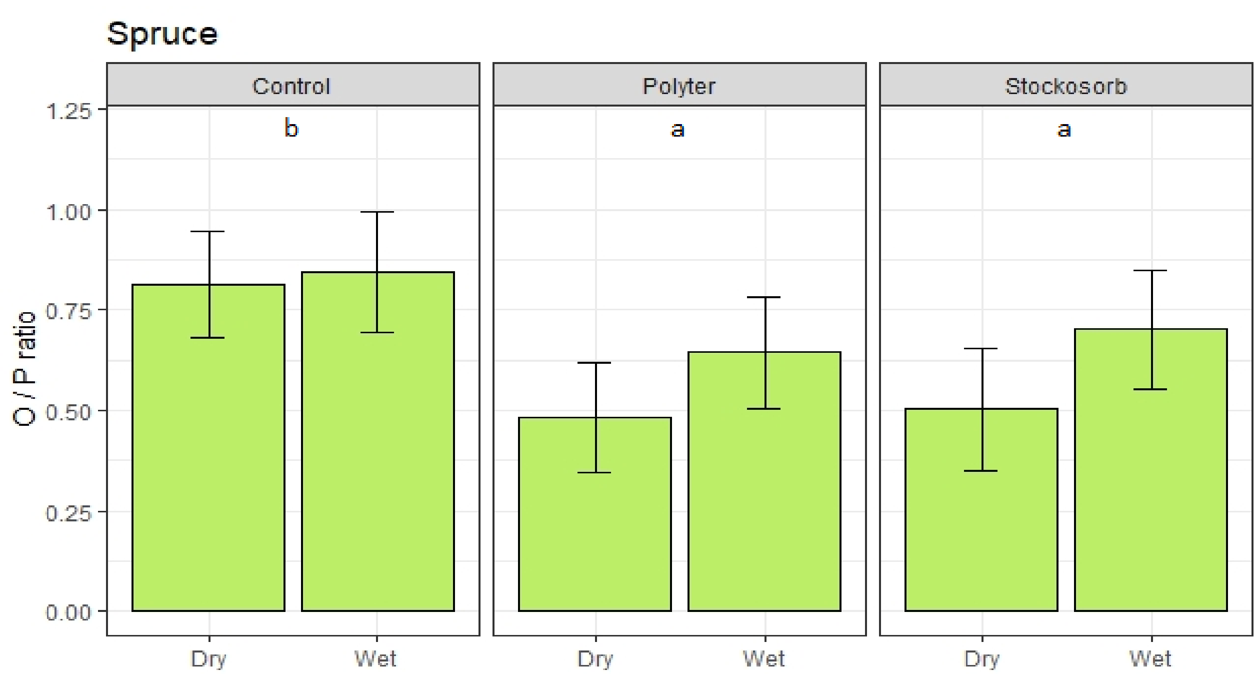

Figure 33 Differences between treatments by amendments in O/P ratio, for drought (Dry) and non-drought (Wet) treated plants of Spruce. Different letters indicate significant differences between treatments.

3.10 Mycorrhiza

Estimation of EM production in the soil from the ingrowth bags resulted in range of 0 (No mycelia present) – 1 (Infrequent hyphae present). Mycorrhiza colonization exhibited only an effect of treatments by amendments, which revealed the variation in Douglas fir. Indicating particularly higher EM colonization in plants with control and Stockosorb than in Polyter treatment (Figure 34).

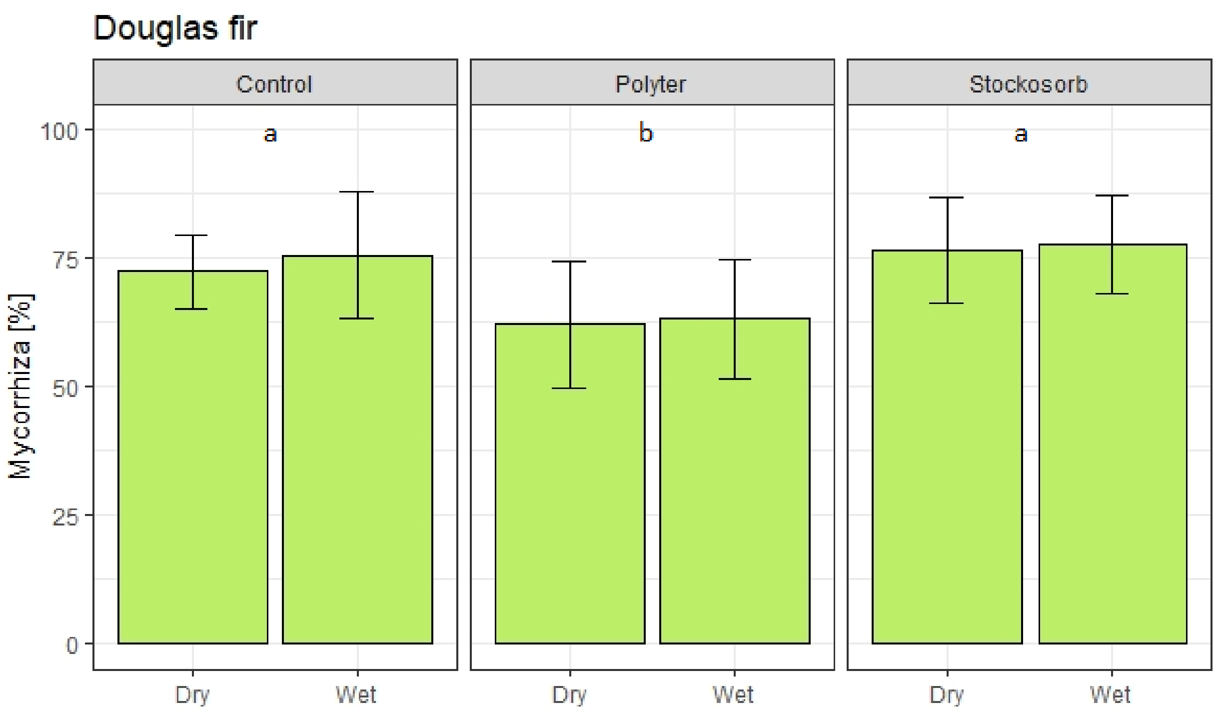

Figure 34 Mean SE +/- of mycorrhizal colonization in Douglas fir. between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter). Different letters indicate significant differences between the treatments.

4 Discussion

The experiment was performed under controlled conditions. There can be a potential limitation considering the usage of pots, where roots of seedlings can't grow horizontally, and temperature of the soil is possibly higher than in natural conditions. However, all species under all treatments were subjected to the same conditions.

The observed result in volumetric water content of the soil between dry and watered plants suggests a considerably low difference, even though a significant result was calculated. Therefore, a moderate drought resulted in lower effect on the root growth of all species and consequently on the performance of amendments.

Few studies had previously reported an increase in root biomass caused by drought (Poorter et al., 2012), which agrees with here presented results, when it comes to the drought treatment. However, soil conditioners proved to have an effect only in Douglas fir and Spruce. Roots of Douglas fir, amended with Polyter and under the drought effect, showed considerably the biggest root biomass. This may be due to the fact that, during the drought periods, roots growing in proximity of Polyter were provided with enough water and nutrients for the growth. In the outer part more roots were growing in drought affected plants, as a result of new fine roots searching for water. Despite the fact that there was no significant difference found for the treatments by amendments Figure 8 indicates more biomass in plants supplemented by Polyter. Evidently, Polyter had a positive effect on Douglas fir root biomass. Moreover, the polymer part of Spruce showed a stimulating effect on root biomass by Stockosorb and Polyter amendments (GEFA 2017, Polyter brochure). However, in the outer part of the pot a difference between irrigated control and Stockosorb was found, indicating bigger root biomass in Stockosorb. This result is quite unclear, but still suggesting a positive effect of Stockosorb.

The response of the root length to the shortage of water and nutrients had been observed in many studies. Under the drought effect, root length tends to decrease (Davies & Bacon, 2003; Manes, Vitale, Donato, Giannini, & Puppi, 2006). However, our results of root length did not show much of significant differences. In the polymer part of the pot, Spruce produced more roots in length when supplemented with nutrient rich Polyter (Philipson & Coutts, 1977). An effect of drought in this case was insignificant. Result suggests that Polyter was providing nutrients to the plant, showing a similar pattern in root biomass. In the outer part, Spruce had the same result as in root biomass, indicating a stimulation effect of Stockosorb on the root length.

Several studies have reported a decrease in diameter of fine roots during the drought periods (Fitter 1985; Baburai 2006, Cortina, Green, Baddeley, & Watson, 2008), coinciding with our results. Every specie showed a decrease of root diameter in drought treated plants, by means of the new fine roots responding to soil drying. Despite the fact that a significant difference was determined, with two-way ANOVA, between the amendment treatments in Larch, cannot be ignored an obvious impact of Polyter on RD (Figure 21). Providing enough water and nutrients resulted in higher RD, compared to the control treatment. An interaction effect was found again in Spruce. During the drought period, Spruce seedlings showed bigger average root diameter in Stockosorb and Polyter amended plants. Despite statistical difference, only between control and Stockosorb, there is a tendency for bigger RD caused by Polyter, indicating water availability provided by both soil conditioners. Result of RD correspondents with root biomass and length, where again Stockosorb and Polyter showed bigger values than the control.

Drought is known to increase RTD (Meier & Leuschner, 2008), due to the fact that water deficiency causes lignification, suberisation and narrower vessels (Steudle, 2000).

Coinciding with our result, RTD showed to be significantly higher in drought treated plants of four species. However, treatment by amendments did not show any effect on RTD of the seedlings.

Many studies identify SRL as a key root trait and most commonly measured parameter of fine roots. An increase in SRL enables the root to penetrate to deeper soil and improve the root absorption potential (Ostonen et al., 2007). This study showed no impact of drought or amendment treatment on root proliferation in deeper soil, despite the fact that SRL commonly depends on RD and RTD (Nicotra, Babicka, & Westoby, 2002), where both root traits in this study showed an effect of drought. Meier et al. (2007) stated, an exploration of deeper soil has been seen in adult trees, but not in seedlings, which can also be applied to our result. The fact that seedlings are more vulnerable to the drought is highlighted.

SRA is used as a fine root morphological parameter in much lesser extent than SRL. Unfavorable soil conditions can cause an increase in root surface area by thinner roots that have a larger SRA for a given investment of carbon (Ostonen, Lohmus & Lasn, 1999). SRA of all four species was significantly lower in drought treated plants, meaning the plants under the drought did not invest in enlarging their active root surface area both in polymer and in outer part. However, decline in SRA of polymer part in Douglas fir between the amendment treatments points out the positive effect of Stockosorb and Polyter. Lower value of SRA suggests soil conditioners were providing enough water. Spruce exhibited the same results, which also corresponds with the results in root biomass. In this study SRA showed much more significant differences than SRL. This may be due to the fact that surface area is in cm^2 and an increase or decrease can result in significant difference in statistical calculations.

Drought stressed plants generally modify their biomass allocation, meaning that proportion of the root increases (Poorter et al., 2012). By translocating carbohydrates to the roots, plant can minimize water loss by transpiration and increase the efficiency of soil exploration (Lloret, Casanovas, & Peñuelas, 1999). Fine roots / leaves biomass ratio in our study showed significant difference in Douglas fir, revealing a higher ratio in the drought treated plants, corresponding with the results of Ruda & Siller, 2018. Spruce showed bigger ratio in plants amended with Stockosorb and Polyter. Considering there was no effect of drought, we can assume that this result was obtained due to the fact that in Spruce plants both amendments were stimulating the growth of the roots, resulting in bigger root/leaf ratio. Some studies showed only a small increase of root biomass caused by allocation of carbohydrates during the moderate drought (Padilla et al., 2009, Poorter et al., 2012), which can explain the results.

Drought treated plants showed a lower O/P ratio, suggesting they were not developing the roots in the outer part due to the water deficiency. Only Spruce showed a significant difference between the amendment treatments. Ratio was higher in the control than in the Stockosorb and Polyter amended plants, indicating a stimulation of growth by soil conditioners, considering the amendments were placed in polymer part. The result is confirming our hypothesis, although for only one specie.

With the help of ingrowth bag method, we estimated external mycorrhizal growth in a range of 0 (No mycelia present) – 1 (Infrequent hyphae present), indicating no effect of amendment or drought treatment. Previous studies reported decline in mycorrhizal colonization upon the drought (Lanzac, Martin & Roldan, 1995). However, this study showed no effect of drought on EM colonization. Among all four species only Douglas fir showed variation in mycorrhizal colonization between amendment treatments. Plants supplemented with nutrient rich Polyter showed less colonization than control and Stockosorb, which may be due to the fact that EM growth can be reduced with increasing nutrient availability (Nilsson, Giesler, Bååth, & Wallander, 2005). Even though we hypothesized that Polyter will not have an impact on mycorrhiza. However, there was no effect of treatments by amendments between Beech, Larch and Spruce, which is in accordance with the hypothesis.

According to the results in size related and functional morphological characteristics of fine roots we suggest the impact of drought was moderate. All species showed a slight response to drought. Ruda & Siller,2018, reported that a superabsorbent polymers did not have an effect on above ground biomass of the plants effected by drought. From our results we can conclude that soil conditioners had a positive effect on below ground biomass, but only in Douglas fir and Spruce. Beech and Spruce showed no impact of amendments on their root growth. Results in ratio between polymer and outer part can confirm our hypothesis only in Spruce, where soil conditioners proved to stimulate root growth in their proximity.

There was no difference found between Stockosorb and Polyter effect, both above (Ruda & Siller, 2018) or below ground. Even though the Polyter was supposed to have more fertilizing products and bigger water storage, it did not differ from the Stockosorb.

5 Conclusion

Soil conditioners in this study proved to stimulate the growth of fine roots only in two species: Spruce and Douglas fir. In Spruce roots were growing substantially in the proximity of amendments. However, their performance was not affected by induced moderate drought periods. There was no clear difference between the effect of two amendments, although we hypothesized Polyter will cause better stimulation of the root growth, due to additional fertilizing products. Nevertheless, soil conditioners had no impact on mycorrhiza, indicating a positive result and enabling mycorrhizae formation. Additionally, we need to consider experiment set-up, namely the experiment was conducted in the greenhouse with seedling planted in 7 l pots for only one summer. As already suggested by Ruda & Siller, 2018, and proved by Hüttermann, et al. 1999, the amount of amendment supplemented to the roots is of great importance. Further research is needed to assess an optimal application of soil amendments and interspecific differences in the way trees experience water deficiency.

6 Literature

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7 Supplementary material

Appendix Table 1: Analyses or variance (two-way ANOVA) for root traits, from polymer and outer part.

Root biomass	Beech		Douglas fir		Larch		Spruce	
	p	o	p	o	p	o	p	o
Treatment	0.03733 *	0.03742 *	0.006032 **	0.3413825	0.1278	0.3897	0.000174 ***	0.032587 *
Drougth_treatment	0.03916 *	0.07277	0.006549 **	0.0001192 ***	0.008731 **	0.5007	0.1241	0.8374
Treatment:Drought_treatment	0.51595	0.55743	0.042880 *	0.4863029	0.989539	0.5175	0.61689	0.007419 **

Root lenght	Beech		Douglas fir		Larch		Spruce	
	p	o	p	o	p	o	p	o
Treatment	0.085269	0.04438 *	0.08935	0.17287	0.6856	0.35	0.01009 *	0.071506
Drougth_treatment	0.004653 **	0.02677 *	0.04449	0.01466	0.114	0.9534	0.43346	0.110506
Treatment:Drought_treatment	0.508162	0.92929		0.54704	0.5849	0.4018	0.31566	0.003624 **

Average diameter	Beech		Douglas fir		Larch		Spruce	
	p	o	p	o	p	o	p	o
Treatment	0.8707	0.1801	0.4176	0.2834389	0.0249 *	0.8164	0.02649 *	0.34675
Drougth_treatment	0.9673	0.02054 *	0.001929	0.0003626 ***	2.467e-06 ***	1.641e-11 ***	0.27985	0.03461 *
Treatment:Drought_treatment	0.9789	0.05151		0.9753972	0.3427	0.6188	0.02819 *	0.29352

Root tissue density	Beech		Douglas fir		Larch		Spruce	
	p	o	p	o	p	o	p	o
Treatment	0.370034	0.5792	0.6271	0.921	0.2613	0.4224	0.430051	0.6237
Drougth_treatment	0.002194 **	0.6102	2.22E-09	2.246e-16 ***	2.181e-14 ***	<2e-16 ***	0.003636 **	3.48E-09
Treatment:Drought_treatment	0.548307	0.1441	0.8314	0.1947	0.9258	0.7729	0.251663	0.2501

SRL	Beech		Douglas fir		Larch		Spruce	
	p	o	p	o	p	o	p	o
Treatment	0.82008	0.1964	0.2527	0.2796674	0.05631	0.4886	0.02405 *	0.06376
Drougth_treatment	0.08358	0.4244	0.2130	0.0007372 ***	0.02375 *	0.4982	0.16506	0.18654
Treatment:Drought_treatment	0.81892	0.2067		0.4206321	0.34947	0.5801	0.73277	0.61335

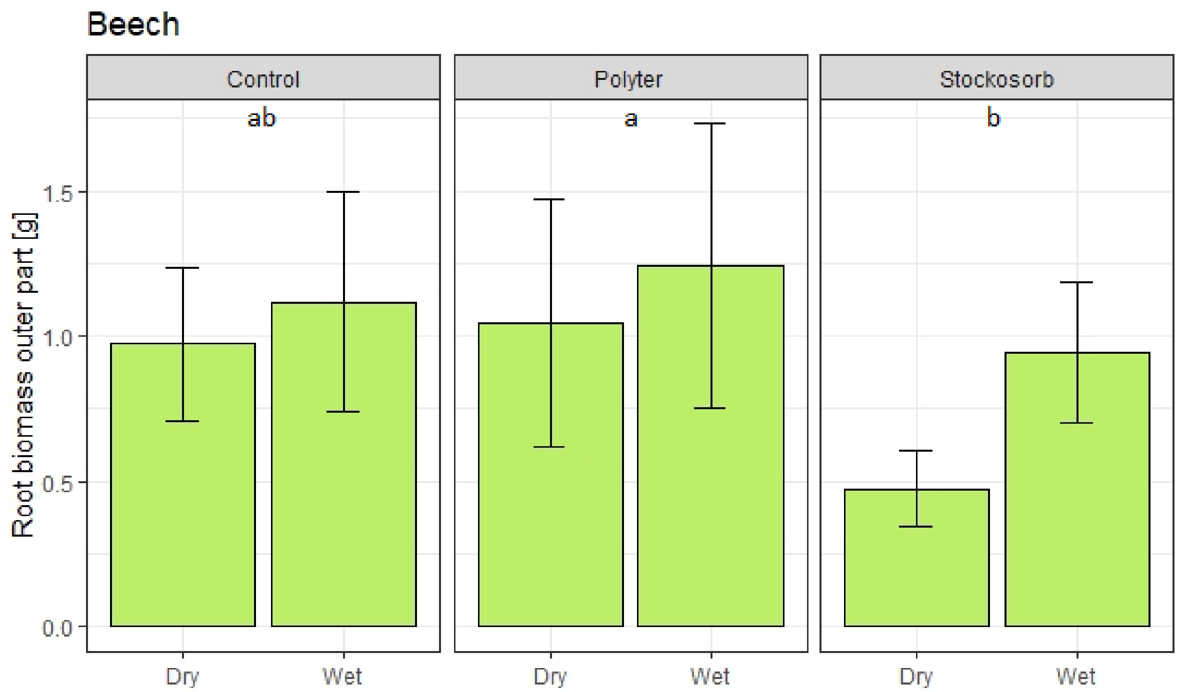
SRA	Beech		Douglas fir		Larch		Spruce	
	p	o	p	o	p	o	p	o
Treatment	0.68412	0.9	0.002441	0.5483	0.6538	0.4523	0.007897 **	0.02315*
Drougth_treatment	0.02534 *	0.4583	4.05E-07	1.41E-10	1.33E-07	1.06E-08	0.001778 **	8.66E-06
Treatment:Drought_treatment	0.71023	0.4178	0.817698	0.1664	0.3768	0.698	0.429706	0.91905

Root biomass/Leaf biomass	Beech	Douglas fir	Larch	Spruce
Treatment	0.2286	0.3503	0.6446	0.01228 *
Drougth_treatment	0.7478	1.207e-05 ***	0.198	0.2848
Treatment:Drought_treatment	0.2261	0.1456	0.953	0.96122

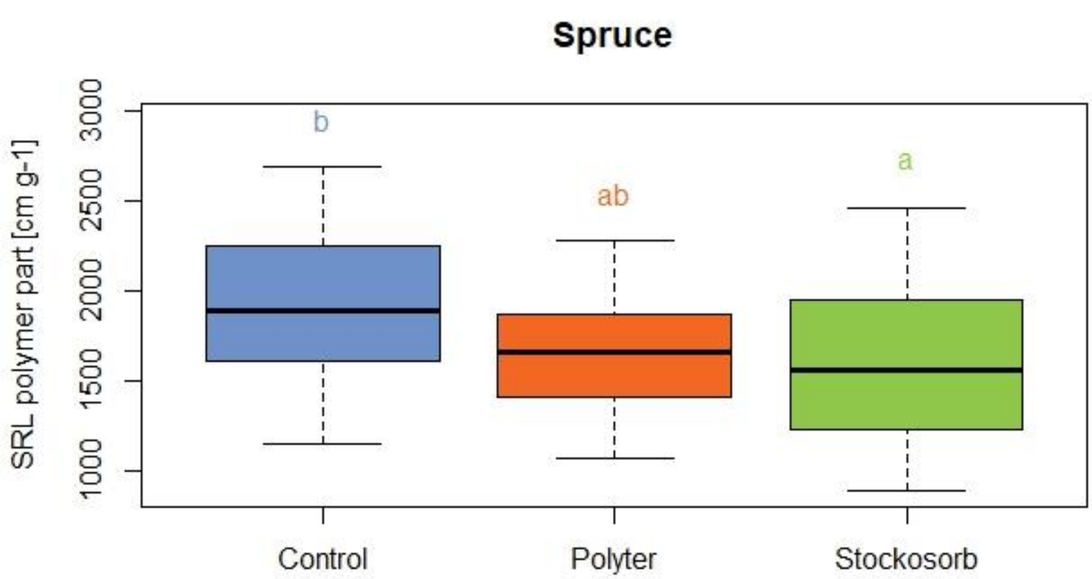
Outside / Polymer ratio	Beech	Douglas fir	Larch	Spruce
Treatment	0.11249	0.01945	0.43993	0.0001768 ***
Drougth_treatment	0.00681 **	0.7349	0.04034 *	0.0049133 **
Treatment:Drought_treatment	0.55938		0.44323	0.3389961

Mycorrhiza	Beech	Douglas fir	Larch	Spruce
Treatment	0.15788	0.009401 **	0.04977 *	0.122
Drougth_treatment	0.06464	0.6502	0.987	0.2455
Treatment:Drought_treatment	0.70901	0.96871	0.03281 *	

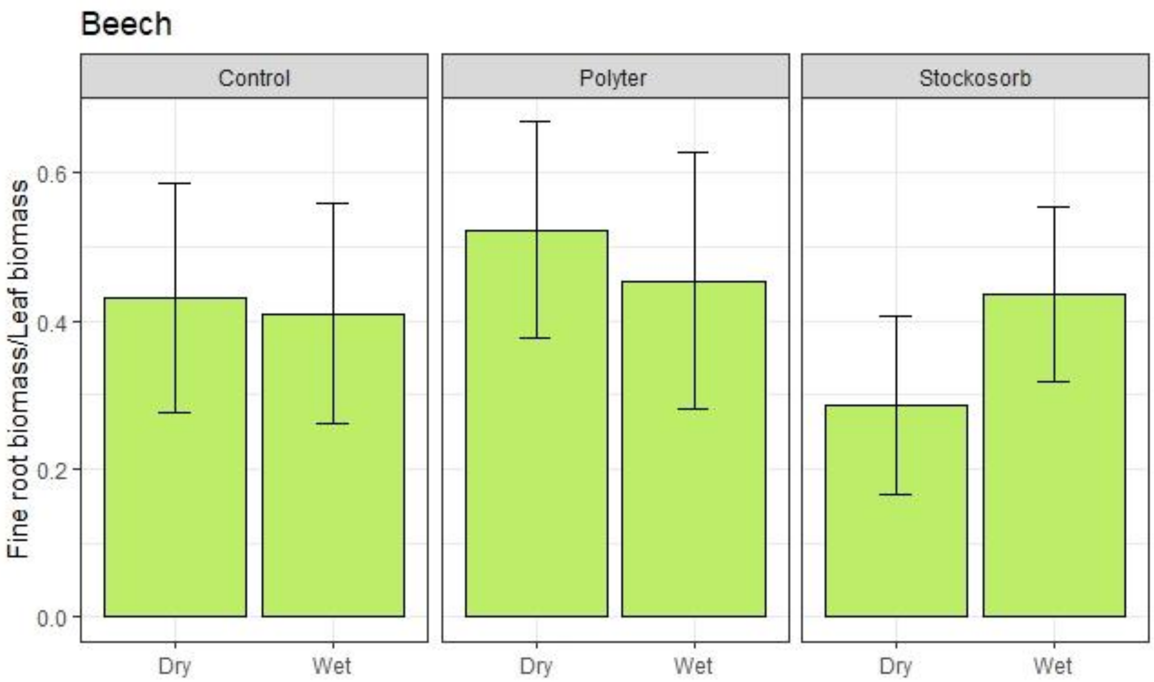
(yellow)	= significant difference (<0.05)
(orange)	= Kruskal-Wallis rank sum test
(light orange)	= nearly significant difference



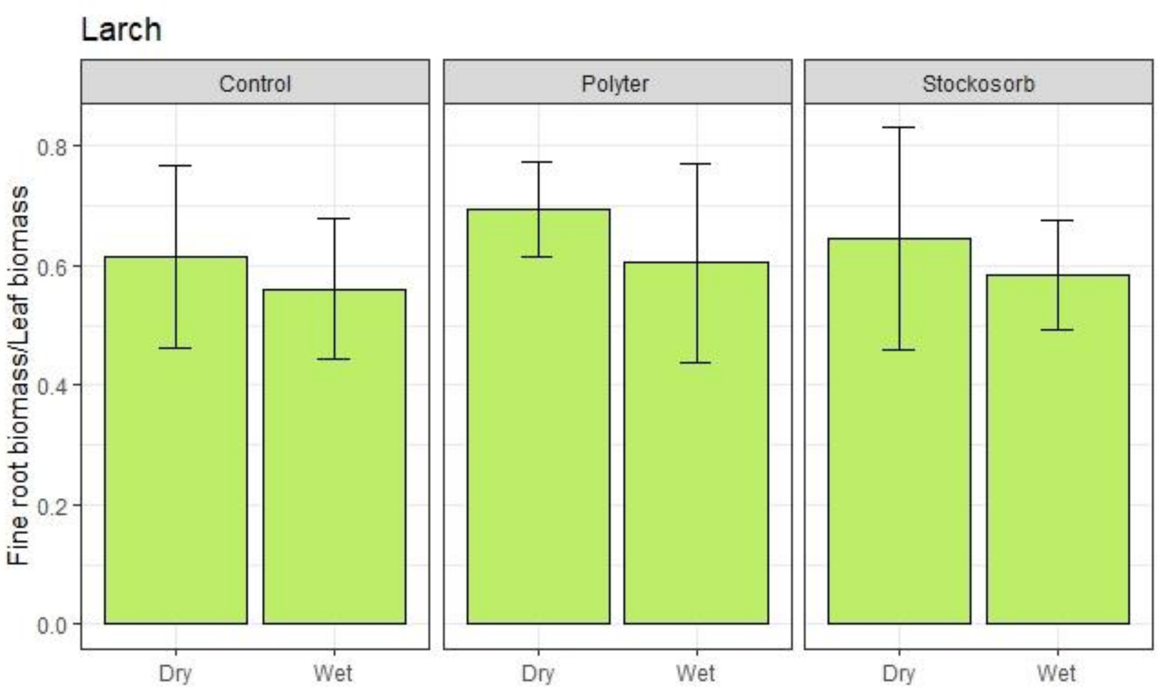
Appendix Figure 1 Root biomass from the outer part in Beech. Difference between drought treatments: Dry (non-irrigated) and Wet (irrigated) pots. Error bars= Standard error. Different letters indicate significant differences between the treatments.



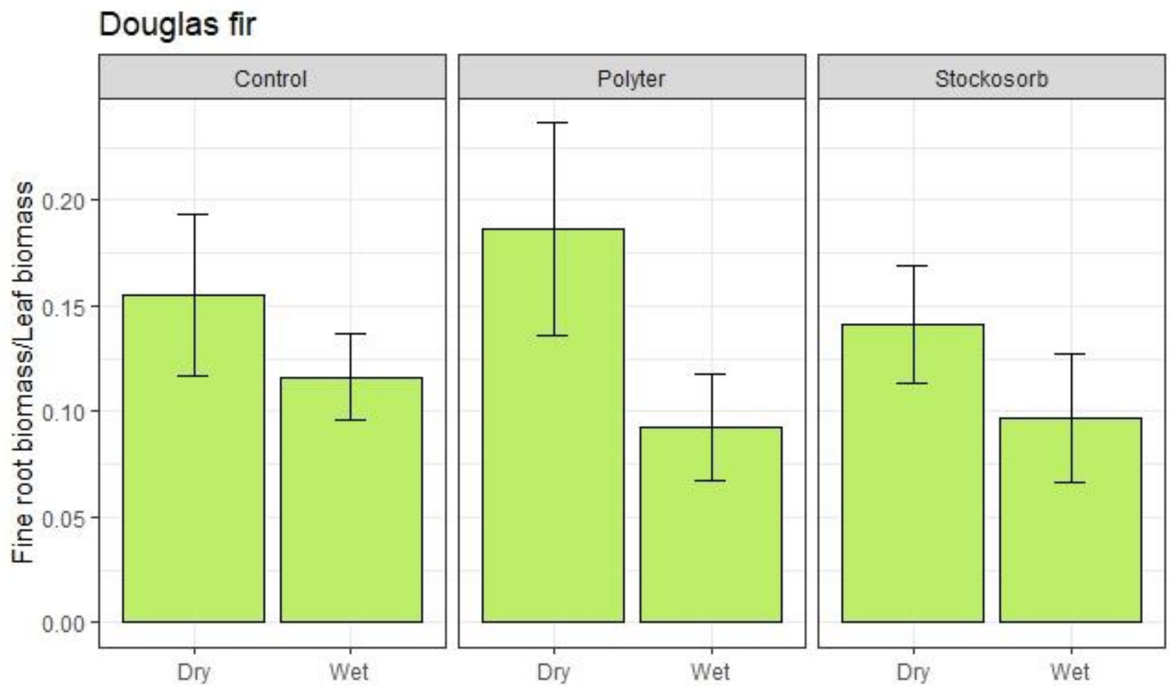
Appendix Figure 2 Specific root length (SRL) from the polymer part in Spruce. Different letters indicate significant differences between treatments.



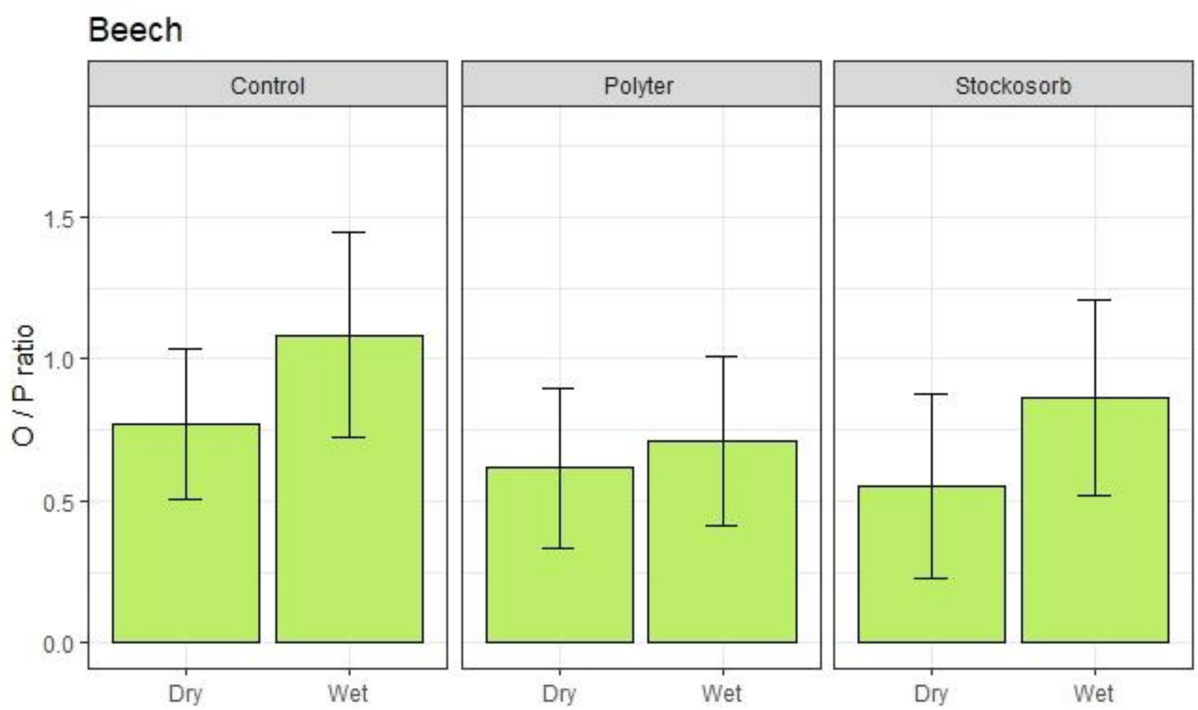
Appendix Figure 3 Mean SE +/- of Fine root biomass / Leaf biomass ratio in Beech, between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter). Leaf biomass data from Ruda & Siller, 2018.



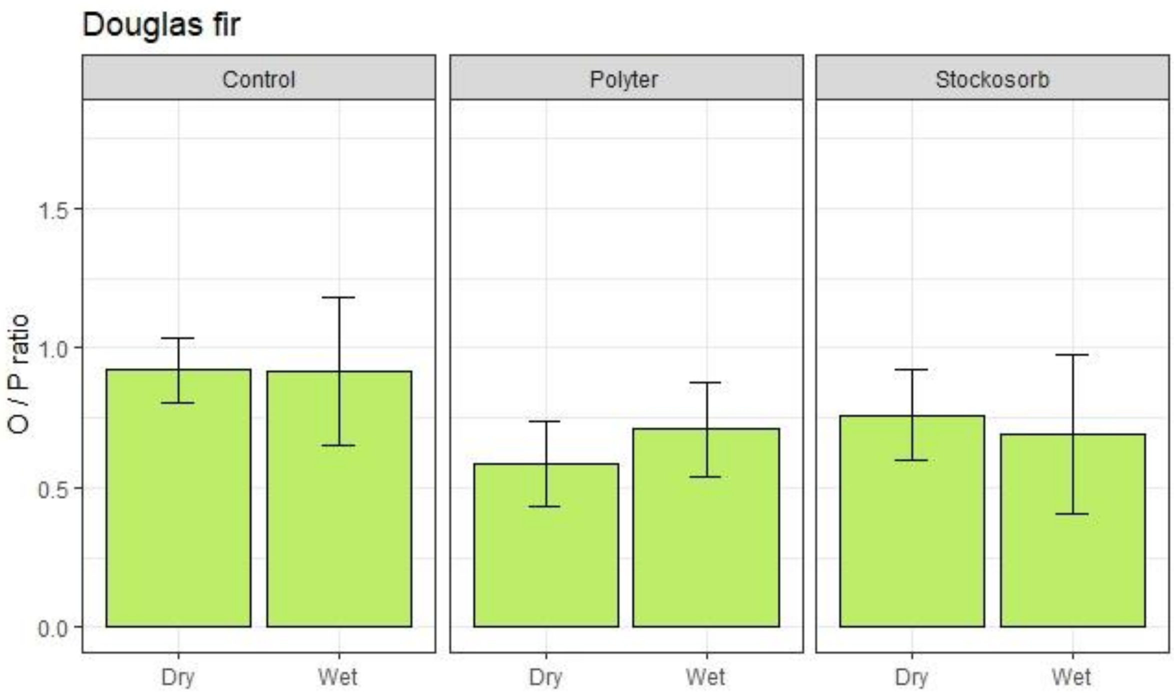
Appendix Figure 4 Mean SE +/- of Fine root biomass / Leaf biomass ratio in Larch, between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter). Leaf biomass data from Ruda & Siller, 2018.



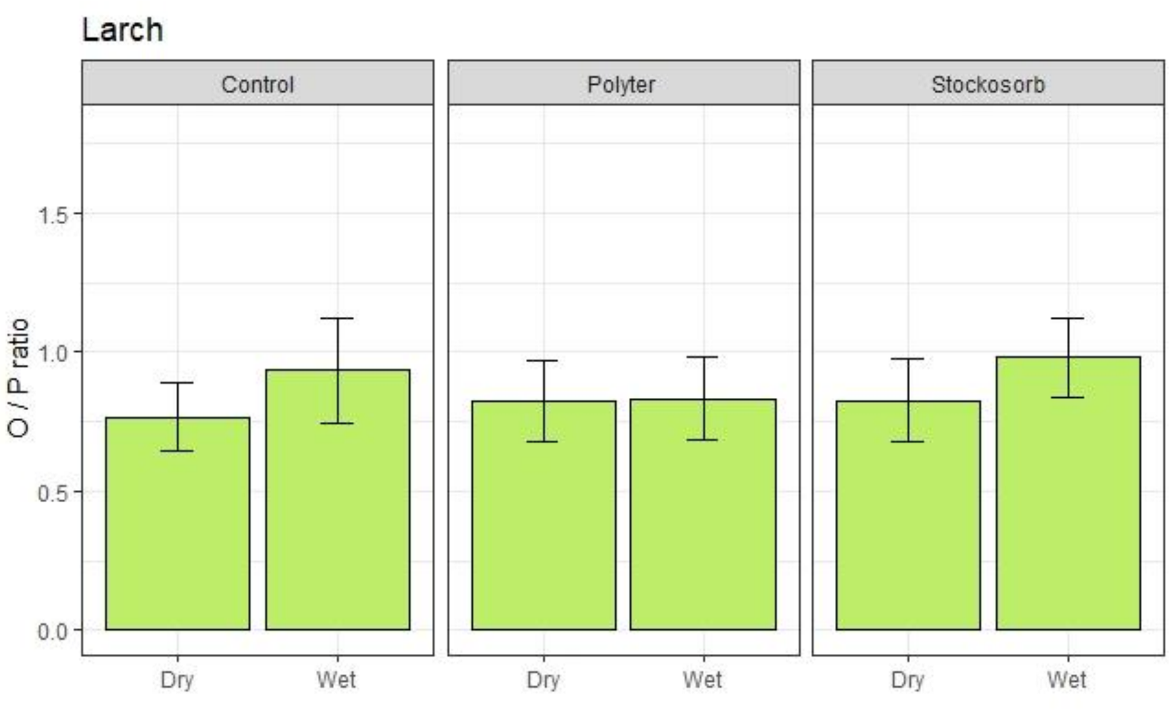
Appendix Figure 5 Mean SE +/- of Fine root biomass / Leaf biomass ratio in Douglas fir, between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter). Leaf biomass data from Ruda & Siller, 2018.



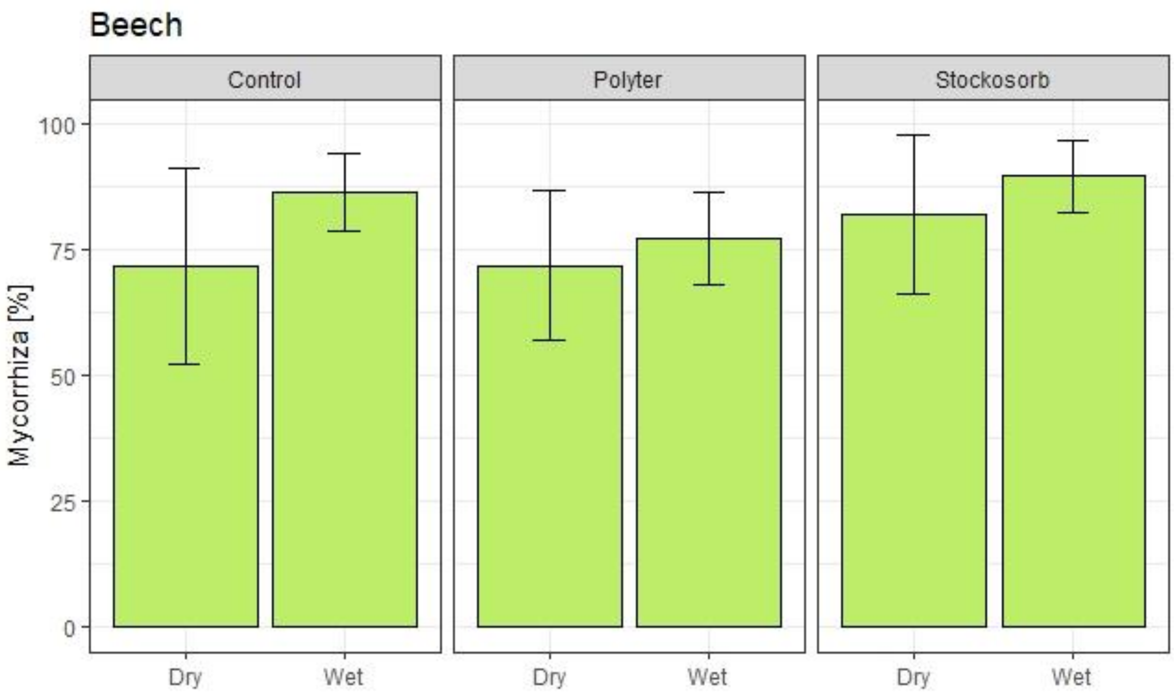
Appendix Figure 6 Differences between treatments by amendments in O/P ratio, for drought (Dry) and non-drought (Wet) treated plants of Beech.



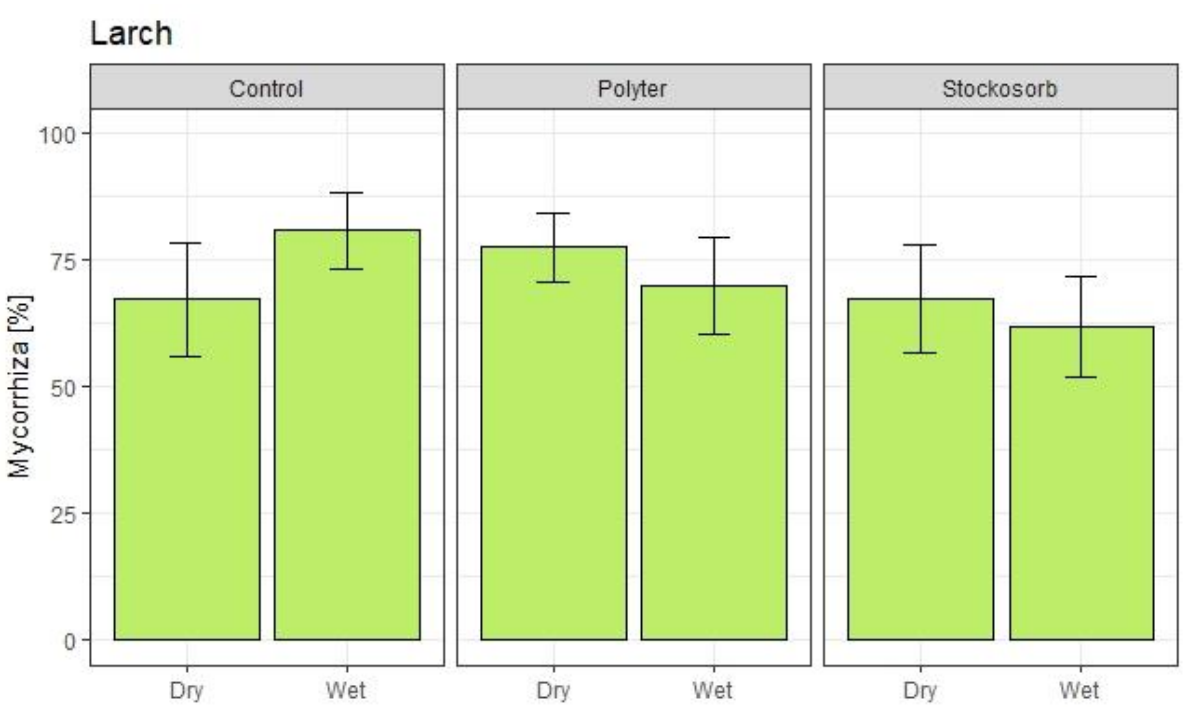
Appendix Figure 7 Differences between treatments by amendments in O/P ratio, for drought (Dry) and non-drought (Wet) treated plants of Douglas fir.



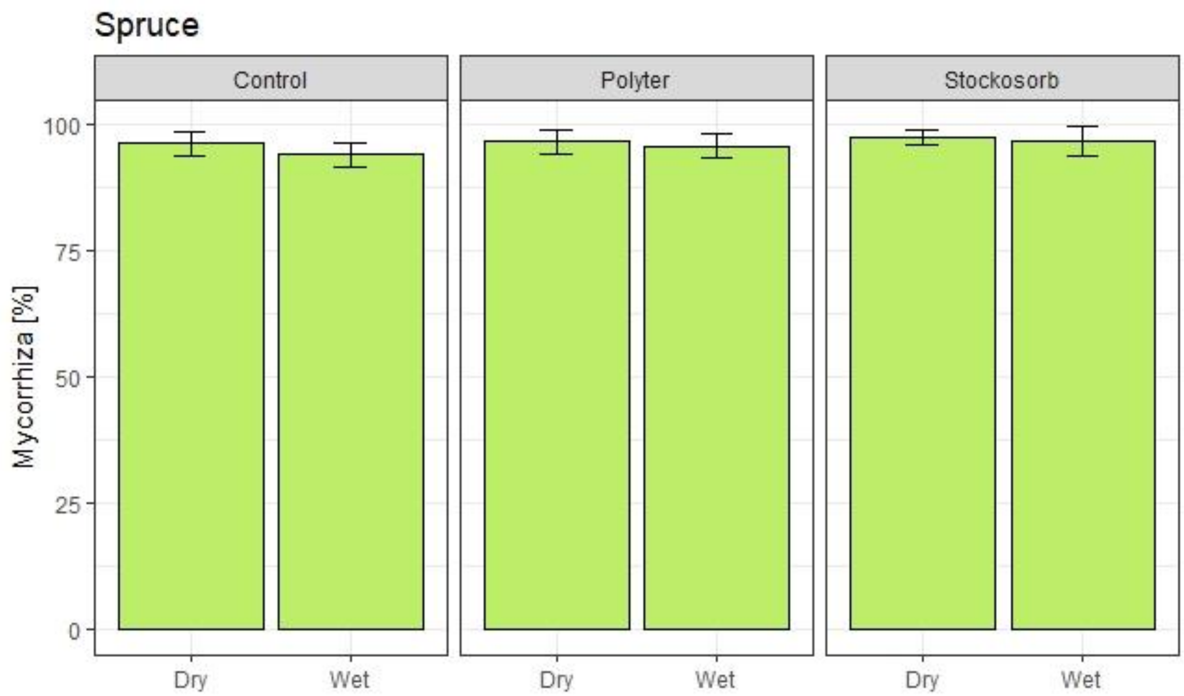
Appendix Figure 8 Differences between treatments by amendments in O/P ratio, for drought (Dry) and non-drought (Wet) treated plants of Larch.



Appendix Figure 9 Mean SE +/- of mycorrhizal colonization in Beech, between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter).



Appendix Figure 10 Mean SE +/- of mycorrhizal colonization in Larch, between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter). Statistical difference found between Control/Wet and Stockosorb/Wet.



Appendix Figure 11 Mean SE +/- of mycorrhizal colonization in Spruce, between irrigated (Wet) and non-irrigated (Dry) pots divided by amendments treatment (Control, Stockosorb and Polyter).