EFFECT OF SPECIES COMPOSITION AND SEASON CHANGE ON FINE ROOT LENTH INCREASE WITH STAND DEVELOPMENT IN NORTHWEST ONTARIO

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ABSTRACT

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Keywords: The Minirhizotron system, root length, stand composition, seasonal difference, Image collection.

The root length is an important indicator to reflect the growth characteristics of the tree, but the root length is difficult to obtain through direct measurement, and the root as a sensitive organ of plants can indirectly reflect the growth state of plants, but it is also susceptible to external factors. The root length, as an important component of the root system, it can reflect the growth state of plant by using its own length change, and studies the influence of environmental factors on the root length, which helps to improve the yield of plants better.

Season, species composition, and soil condition are all factors that affect root growth. And the mutual effect between them cannot be ignored. Past biological experiments have shown that the mixed biomass is greater than pure forest, and the seasonality affects the root length by affecting rainfall and temperature, but due to the lack of a single experiment, we can’t draw firm conclusions. Therefore, our experiment uses the season and species composition as independent dependent variables through 3 groups of repetitions. Our experiments are trying to verify two hypotheses that the fastest growing season of root is summer and root biomass in mixture stand is greater than pure forest. The tool that we used to measure root length in our experiment is Minirhizotrons. We recorded three kinds of forest stand (conifer, deciduous and mixture) repeats three times and their five-month seasonal changes. We obtained data through RootSnap software analysis and verified our conjecture through statistical analysis

This article reveal that the fastest root length increase is summer time and all species reach to the peak in August. The average of root length of all species in August is 96.11 cm and is 37.91 cm in June. This shows that the average root length increased by 1.5 times from June to August. However, there is no evidence to clear root length is longer in mixture compared to monocultures.

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ACKNOWLEDGE

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INTRODUCTION

Root is a significant vegetative organ of plants (Moore& Slocum 2017). Root not only responsible for absorbing moisture and dissolved inorganic salt soil, roots account for more than 80 percent of plants' biomass in ecosystems such as tundra and shortgrass prairies and has and will consume more than half of the photosynthate for substances and other physiological activities of plant life processes especially important (Zhishan et al 2006).

There are many sectors in root system and our emphasis is on the root length, because of the sensibility of root length and easy quantification. Based on the existing cognition, we believe that the longer the root length is, the larger the root surface area will be, and thus more biomass the root has. However, the most studies are focus on how the fine root influence the production and how to use properly method to have a higher efficient agricultural system. In the agriculture field, root length also as an important indicator that indirectly imply that most mixtures stand has higher production than monocultures (Tilman et al. 2001) stand and seasonal differences lead to differences in temperature and rainfall (Han et al 2016) so that summer time should be the most efficient season in productivity.

Our research object is the length of the root but root system is difficult to approach, it is hard to observe underground that we know much less of live roots dynamics than shoots (Johnson M 2001). A special method is required to study the distribution, dynamics and turnover rate of plant roots, which plays an important role in the carbon budget and the root water uptake model of the ecosystem (Vogt & Persson 1991). The traditional destructive methods such as soil coring, in-growth cores, whole root system excavation (Majdi 1996), while more recently Minirhizotrons have been used as a new technique. Minirhizotrons provide a nondestructive method for viewing roots and are one of the best tools for directly studying roots (Johnson el at 2001). They permit the simultaneous measurement of fine root production and disappearance, which cannot be accomplished using coring, or excavation approaches. Minirhizotron techniques have improved significantly since they were first proposed and are widely utilized to study the dynamics and functions of fine roots in agricultural and natural plant communities (Tilman et al. 2001). Talking about Minirhizotron system itself. It typically consists of a Minirhizotrons tube that has been inserted into the soil, a color micro video camera, a camera control unit to focus the camera and adjust the light levels, and a monitor for viewing images as they are collected (Taylor et al 1991).

The study of root is mainly in the aspects of distribution, dynamics and plant root. Because of the restriction of time and energy, it is difficult to carry out complete experiments on the above aspects. Therefore, our experiment will be based on the study of the root length of trees in different species composition and different seasons.

As far as the root length is concerned, first of all, it is necessary to make clear the effect of root length on plants, and the change of root length will affect the surface area of roots, while the surface area of roots can be used as a variable to control the uptake of water and nutrients. Second, trees of different species, especially hardwoods and conifers, may still have root differences in the length and shape of roots. Here is generally believed that root broad-leaved trees grow faster, and root lignin content, and conifer root because of slow growth, with its roots in the cellulose more, so this time we hope through the quantitative analysis method of Minirhizotrons root length differences due to different tree species（Yunping et al 201). Third, seasonal differences lead to differences in temperature and rainfall, and forest production (FP) is positively correlated with seasonal soil temperature (Han et al 2016). To be specific, within a temperature range where the root can grow normally, the increase of soil temperature is accompanied by the increase of forest production which contributed by root length and root number (Han et al 2016). Therefore, seasonal changes can affect the number and length of roots by changing rainfall and soil temperature.

As far as my experiment is concerned, we have set up 9 plots and 10 to 18 plots of ID, of which 10-12 plots are deciduous, 13-15 plots are conifer, 16-18 plots are mixture and 3 sites from each of the season classes (June, July, August, September and October). In addition, the experimental data are extracted by using Rootsnap software and the assumption of normal and homogeneity of the data is checked before the statistical analysis is carried out. The conclusion is obtained by statistical analysis when the data meets the test standard. We have two hypotheses that summer time is the fastest season of root length growth and mixture stand has higher root length than monocultures. And the purpose of this article is going to confirm these two hypotheses and try to provide a reasonable explanation if the hypotheses are incorrect.

EXPERIMENTAL DESIGN

Study area description

The study was conducted in the boreal forest north of Lake Superior and west of Lake Nipigon in the Upper English River Forest Region approximately 150 km north of Thunder Bay, Ontario between 49º27' N to 49º38' N and 89º29' W to 89º54' W. The closest meteorological station is located in Thunder Bay, Ontario (48º 22' N, 89º 19' W, 199 m elevation). The study area has a moderately dry, cool climate with short summers. The average annual precipitation for Thunder Bay (1971-2000) is 712 mm and the average annual temperature is 2.5 ºC (Environment Canada 2005). Topographic features were shaped by the retreat of the Laurentide Ice Sheet approximately ten millennia ago.

The natural stand-initiating disturbance of the area is predominately stand-replacing crown fire, which is the most common stand-replacing mechanism in the boreal forest (Johnson 1996). The study region represents the transition between the longer fire cycles of the eastern and the shorter fire cycles of the western Canadian boreal forest. While the fire cycle of the region is unknown, it is likely to be between 75 to 100 yrs, which were respectively estimated for central Saskatchewan and northwestern Quebec (Bergeron 2010). The forest of this region is characterized by a mosaic of stands dominated by P. tremuloides, P. banksiana, P. mariana, paper birch (Betula papyrifera Marsh.), and balsam fir (Abies balsamea (L.)) in various proportion on upland mesic sites (Rowe and Halliday 1972).

To limit site variability, sites were allocated on flat mid-slope positions, with no slope exceeding 5%, on well drained glacial moraines greater than 50 cm in thickness, which is the prevailing site type in the region. In the field, site condition was determined by topographic characteristics, and soil profile determined from a soil pit dug in the center of the plot.

Sampling strategy

To facilitate testing the influence of different seasons and species composition on fine root length, a total of 9 spatially interspersed sites were randomly sampled from the dominant mesic site type in the young boreal forest, 3 sites from each of the season classes, i.e., June, July and Autumn since fire with varying overstory tree species diversity from pure trembling aspen (Populus tremuloides Michx.) and jack pine (Pinus banksiana Lamb.) to their various levels of mixtures. Following the definitions for single- and mixed-species stands in the forest resource inventory, criteria for stand selection were that single-species-dominated stands would contain ≥ 80% stand basal area of a single species, while in evenly mixed-species stands, none of the component species would have ≥ 80% stand basal area. All selected stands established naturally after stand-replacing crown fire and developed without silvicultural treatments. Sample stands were allocated several kilometers apart from each other, by selecting them from different road accesses using forest resource inventory maps and stratified random sampling, to minimize neighborhood influences and unknown environmental influences that may be spatially correlated among sample stands. To limit site variability, all selected stands were >1 ha in area, fully stocked, visually homogeneous in structure and composition, and were located on relatively flat, upland, mid-slope position. To help ensure that tree species composition was the only significant source of variation among stands, all stands were selected on mesic sites using an ecological classification approach (Taylor 2000), by allocating all sites on mid-slope positions of well-drained glacial moraines > 50 cm in thickness. Soil moisture regime class was confirmed by examination of a soil profile, dug to the parent material, within each selected stand. Similarity of sites were further validated by soil physical and chemical properties, that is, concentrations of total nitrogen and total carbon, cation exchange capacity and soil texture composition of the mineral soil at a depth of 30–55 cm.

Field measurements

Four minirhizotron tubes (6.4 cm diameter; 60 cm length) that can sample large soil volume were randomly installed in each of the 9 plots during the month of June 2014. The location of each installed core was marked by a steel rod for future identification. To minimize soil compaction at the tube-soil interface and give good soil tube contact, we used auger to drill a hole with 45° and similar diameter as minirhizotron tube. Carefully insert the minirhizotron tubes to prevent damage or smearing on the outside of tubes which cannot be easily cleaned or rectified without removing the affected tubes. An indexing handle that physically locks the camera in a precise position relative to the minirhizotron tube facilitates repeated observations, but only works if the tube is stable relative to the soil. So, to prevent minirhizotron from rotation, we will secure tubes by driving a rod into the soil adjacent to the top of the minirhizotron tube and securing the tube and rod together using a zip tie. To prevent light disturbance, a black tape will cover the tube above soil surface. After the soil recovered from disturbance, we will use root-scanner to scan the root and upload the image to tablet biweekly for two growing seasons. The image will be brought back to lab and analyzed by RootSnap software (CID Bioscience Inc., Camas, WA, USA) to observe fine root dynamic and morphological traits. Each scan generates a near 360- degree image (21.59 * 19.56cm). The reason why I added mixed forest stand in experiment because the vast majority of studies in forests have focused on above-ground responses to differences in tree species diversity, while systematic analyses of the effects of biodiversity on root systems are virtually non-existent (Pretzsch et al 2015). Moreover, most of the research on the relationship between the species composition and biomass production are carried out by model experiment. In an article that study on the influence of broad-leaved tree species diversity to the root biomass and morphology, the differences in tree species diversity affected neither stand fine root biomass nor vertical root distribution patterns. Fine root morphology showed marked distinctions between species, but these root morphological differences did not lead to significant differences in fine root surface area or root tip number on a stand area basis. Moreover, differences in species composition of the stands did not alter fine root morphology of the species (Catharina et al 2009). In another hand, some ecologists believe that different species have different energy and nutrient requirements, so mixed forests can make greater use of environmental resources. Here are hypotheses have to be confirmed in experiment, the root length is peaked in summer and the root length is longer in mixed forest stand than pure forest stand.

To explore the influence of mixed tree species on growth dynamics, yield, resilience and stability is the hot spot of forest management. It is hoped that through the study of root system, we can find more efficient management methods to develop forest.

Statistical Analysis

For our experiment, as the variables are measured from a sample at different plots and seasons of time, the measurement of the dependent variable is repeated. Using a standard ANOVA in this case is not appropriate because it fails to model the correlation between the repeated measures: The data violate the ANOVA assumption of independence (Zulfiqar 2016). Then we consider using repeated measure ANOVA, but we have to check the assumption of normality and homogeneity before we use it. Because of both meet for the analysis thus repeated measures ANOVA was used in the measurement of repeated dependent variables.

RESULT

Based on the Figure 1 and 2, we found the increase from June to July was the largest and the increase from July to August decreased compared to June to July but the total root length reached a maximum. Then, followed by a slight decrease from August to September. And from September to October root length increased slightly. However, there is no clear evidence to explain the relationship between species composition and longer. Therefore, we have taken the average of data in the same species composition for 5 months to make Figure 3. In Figure 3, we can see the root length of broad-leaved leaves is 80.742 cm that higher than conifer's 74.49 cm and mixture's 62.43 cm.

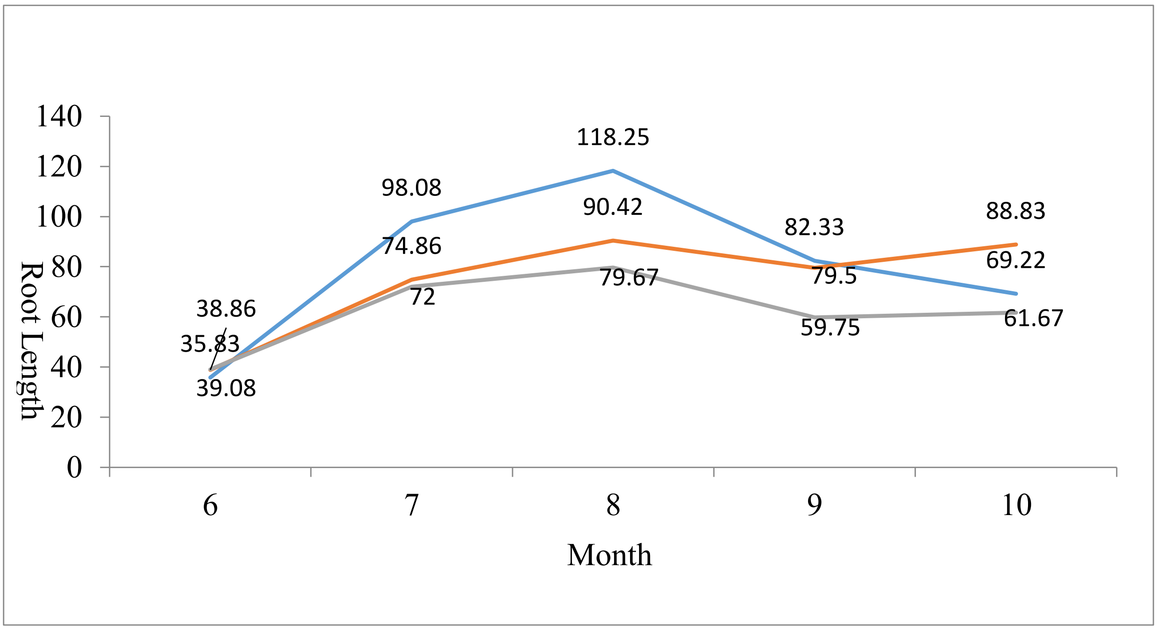

Figure 1. Root length of different species composition in different seasons

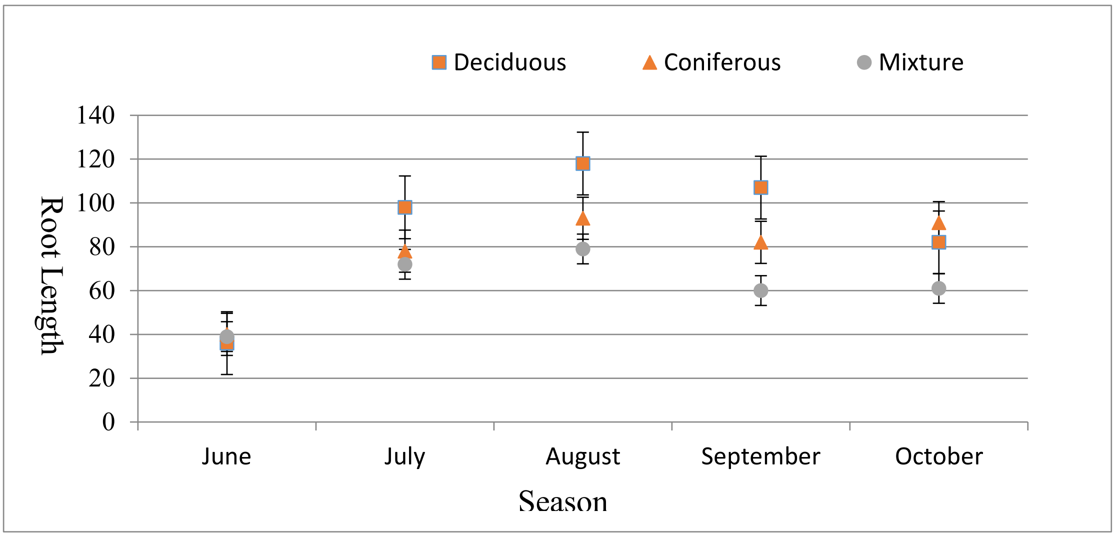

Figure 2. Average root length in different species composition in different season

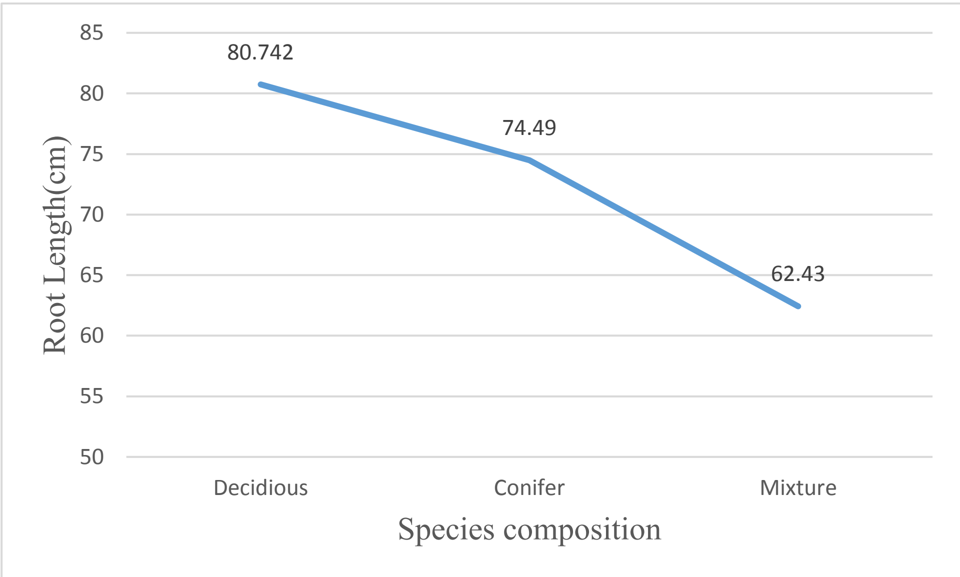

Figure 3. Average root length changes in 5 months with the different tree species

The results of repeated-measure ANOVA showed that total fine root length was not significantly affected by stand type (Table 6). However, total fine root length differed significantly with sampling date which peaked in the August (Table 6; Fig. 4).

Table 1. Effects of species composition and sampling season on fine root length

Source	d.f.	MS	P
Between subject			
Stand composition	2	9946	0.711
Error	6	27561	
Within subject			
Sampling season	4	17274	6.69E-05
Stand composition x Sampling season	8	1581	0.523
Sampling error	24	1732	

Listed are source of variation (Source), degree of freedom (d.f.), mean sum of squares (MS) and significance (P).

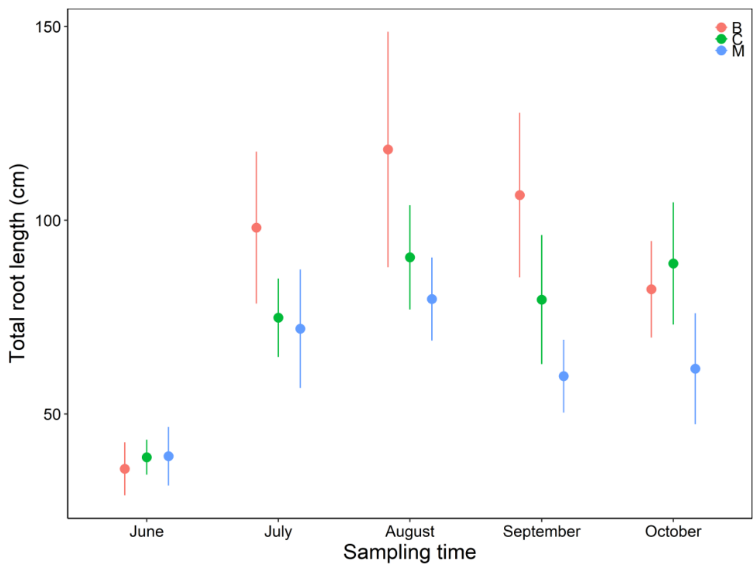

Figure 4.Total root length (within 21.59 * 19.56cm area) in different stand compositions at different sampling seasons (B: broadleaf; C: conifer; M: mixed forest).

DISCUSSION

Minirhizotrons technology-related problems in the actual operation

While the benefits of minirhizotrons are now widely recognized, they are not as frequently utilized as some other methods. In my experiment, I didn't need to consider the installation and image acquisition of the minirhizotrons system because the preliminary work had been completed. However, the analysis of the data involves the extraction of the time and difficulty associated with the root data of video images in a reasonable time and is also the limitation of the use of Minirhizotrons. Manual image analysis is a time-intensive process that typically requires every root segment in each minirhizotron to be digitized for length. This can represent a substantial time investment and research cost; analysis times per minirhizotron tube often range from 30 min to 8 h per sampling period in their experience (Crocker et al 2003).

Although some computers software can help us analysis images, like CI-600 which can quickly and easily calculate parameters including root length, area, volume, diameter & branching angle. However, in terms of my data processing, we need to use the PhotoShop in the image preprocessing, especially mark the shape of the root in color by Photoshop method, and then separate these marked sections from the image to form a new picture alone. And then through the computer analysis to calculate the length of root. Therefore, in the process of pre-treatment of images, whether there is a standard to determine the root is particularly important.

However, there are two basic types of roots, woody and nonwoody (Sherry R 1992). Nonwoody roots are found mostly in the upper few inches of soil. The primary function of these roots is to absorb water and nutrients. In addition, some deciduous trees such as ash, have extensions called root hairs which increase root surface area and these hairs are hard to find on images. Woody roots are large lateral roots which form near the base of root and they are obviously on images. The primary purpose of these roots is support and anchorage for the tree. They also provide water and mineral transport as well as carbohydrate storage (Sherry R 1992).

The number of roots is positively related to the age of trees in a certain range, the larger age of the selected forests the more complex and large root they will have， to facilitate the development of a unified standard to analyze the root of the images, we chose the sample of stands in age of 8 years old.

Comparison of our experimental data with published literature

From Figure 1 above we found that root length decreased from August to September which made us confused, so we compared our results to the published literature.

First of all, because fertilization will reduce the root length of most tree species (Mark 2018), we consider that from August to September with the advent of autumn, the increasing of forest littered leaf leads to an increase in soil fertility that causes the situation in our experiments, but this is very quickly denied by us because the condition of September to October and August to September are similar, but the result of the two is inconsistent. Secondly, our experimental plots have strict tree species accounting requirements and therefore cannot explain the situation in coniferous forests in this reason as well. Therefore, we attributed the reasons for this result from August to September is the length of live roots increased to a peak (August) during the establishment period, and slowly declined (September) after the overlapping of adjacent tree roots (Mark 2018). However, this is just our assumption for the reasons after this situation appeared, and it needs further verification.

CONCLUSION

From the result tables and statistical analysis, the conclusion we can find are as following:

We tested the effect of stand type and sampling date on total fine root length (cm) using repeated-measures ANOVA and only diameter less than 2mm will be counted. Total fine root length was not significantly affected by stand type. However, total fine root length differed significantly with sampling date which peaked in the August.

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