Water mapping in Scots pine stands after thinning

Michalina Grabowska

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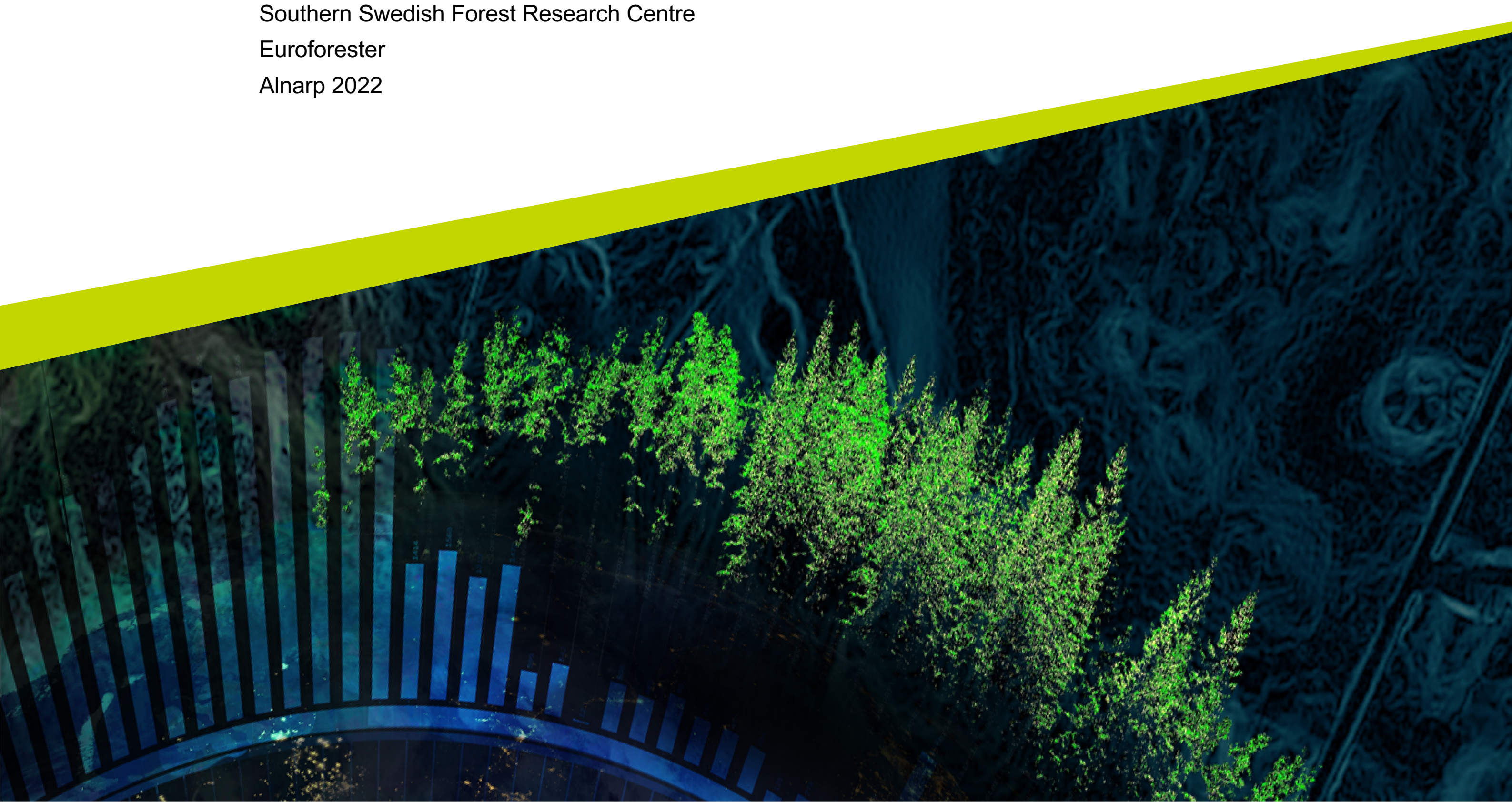

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Michalina Grabowska

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Abstract

Climate change is an ongoing issue in various fields, including forestry. More frequent and severe droughts have been already noted around the world, also in Europe. Visible reduction in available water in forests has adverse consequences such as increased tree mortality, higher vulnerability and smaller growth. Appropriate silviculture treatment can improve forest conditions. Proper thinning practices can reduce belowground competition for water.

In the current study, water with a detectable isotope (deuterium, ^{2}H) was applied in Scots pine stands with different intensities of thinning in order to investigate water route and horizontal root systems structure. Based on the amount of the label in the sapwood cores of trees, water uptake was examined. In addition the sapwood area of each analysed tree was calculated. Knowing the exact location of trees, the water route on the plot was mapped.

The results showed a strong correlation between the place of water application and the distance of a tree. Belowground roots overlap was proven and reached 3,5 trees /m^2. In heavily thinned plots labelled trees uptook the biggest amount of ^{2}H. There was no correlation between the sapwood area of a tree and the amount of uptaken label. Visual presentation of trees distribution showed belowground avoidance of competition – root systems are not developed uniformly around tree stem.

Although there are some limitations of this study such as the short time between thinning and experiment establishment, the results obtained gave a valuable insight into the root system and connected with other research, including examination of root development after thinning in a few years, can be helpful and useful in adjusting thinning practice in face of lack of water in the forest.

Keywords: Scots pine, *Pinus sylvestris*, drought, thinning, root system, water uptake, heavy water

Preface

My full appreciation and sincerest thanks go to my main supervisor Emma Holmström and co-supervisor Amanda de Castro Segtowich for their support, scientific guidance and invaluable help every time I needed it throughout the whole time of my Master thesis research.

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Abbreviations

DBH	Diameter at breast height
GNP	Gross national product
IPCC	The Intergovernmental Panel on Climate Change
IRMS	Isotope-ratio mass spectrometry
PCT	Precommercial thinning
SFA	Swedish Forest Agency
SFI	Swedish Forest Industries Federation
SoEF	State of Europe's Forests

1. Introduction

1.1. Trees and climate change

Consequences of climate change on the Earth are set to impact various sectors, including forestry. In line with forecasts, increasing intensity of warming is expected as well as more frequent extreme events such as droughts (e.g. heat waves in 2003 and 2018) (Buras et al. 2020). The future IPCC scenario states that during the 21st century 1.5-2.0°C warming will be exceeded if the emission of CO_2 and other greenhouse gas is not reduced (IPCC 2021). According to another projection, the average temperature of surface is expected to raise 4.8 °C in northern Europe (IPCC 2013 see Subramanian et al. 2015). Even though during the 20th century in Fennoscandia the amount of precipitation increased, climate models show that increase during wintertime, whereas summer is projected to be drier (Seftigen et al. 2012). More frequent droughts and less precipitation have been already noted recently, not only in Sweden but globally (Adams et al. 2011).

Because of changing weather conditions, the competition for water among trees in the forest appears to be more visible and important (Albert et al. 2015). It has already resulted in lower forest productivity, higher mortality of trees and sometimes can lead to vegetation die-off (Sohn et al. 2016). Higher temperatures and droughts foster also insects and phytopathogens outbreaks and strengthen the adverse effect of the pine mistletoe (*Viscum album* ssp. *austriacum* (Wiesb.)), how it was shown in Swiss valleys (Giuggiola et al. 2013). Some of the negative effects of climate change can, at least partly, be reduced by adapting forest management (Blennow 2012). It is important especially in countries like Sweden where forestry plays a relatively important role in the national economy (Keskitalo et al. 2016).

1.2. Water management in the forest

Water uptake and transport in the plant are regulated by above- and belowground mechanisms as well as underground properties of the root system e.g. its depth and distribution. Soil features, such as the content of water, water potential and conductivity and soil water aeration and temperature are equally important for the process of uptake and fluxes of water (Pallardy, 2007).

Water transport in the plant relates to soil-plant-atmosphere continuum (SPAC) (McElrone et al. 2013; Steudle 2002). The simplistic water route in the plant is shown in the figure (Fig. 1): soil→root cells→xylem→leaf cells→stomata→air (Feddes & Dam 2005). The direction of water movement depends on and is determined by the different water potentials in various plant tissues and soil (Landsberg & Sands 2011).

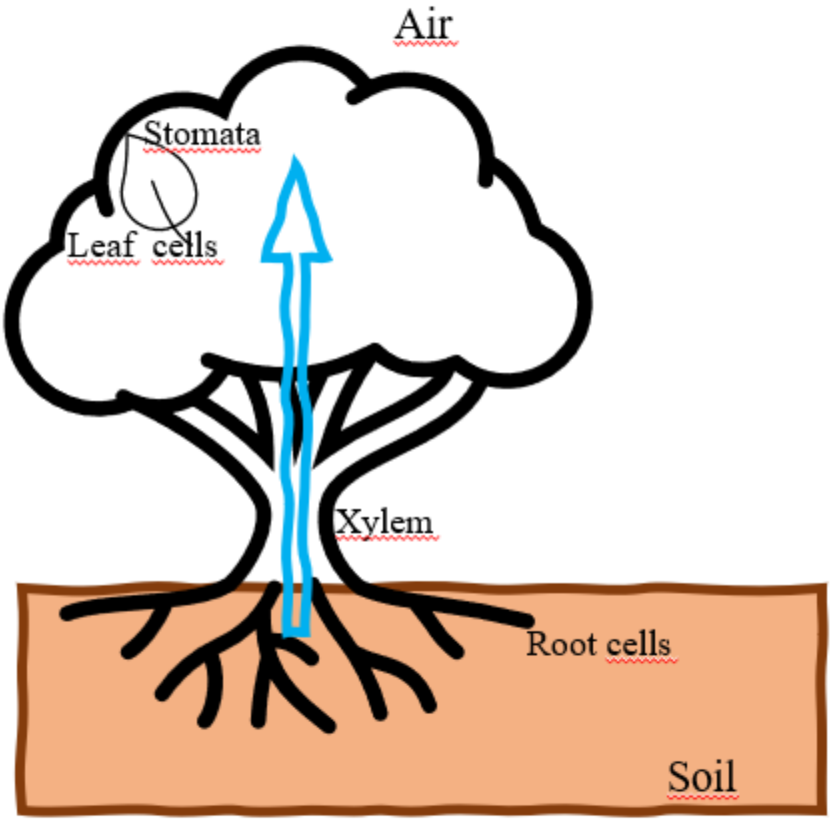


Figure 1. Simplistic water route in the plant (Based on the concept of Feddes & Dam 2005).

In general the whole amount of water collected by land plants is uptaken from the soil by their root systems. The efficiency of roots water uptake is dependent on differences in water potential between soil and roots which in turn depend on the amount of water lost due to the transpiration process and moisture condition in the soil (Landsberg & Sands 2011). The most permeable component of the root system are fine roots, which can be covered with roots hair increasing the surface absorbing water. In woody plants (such as trees) the roots create bark, however, water can be still retrieved (McElrone et al. 2013).

Water from the soil is absorbed by roots through the osmosis effect. The surface of roots is like a semipermeable membrane. Water is able to move through that membrane in two directions, whereas solutes can go into the roots and are held back inside, resulting in low osmotic potential. Since the osmotic potential in the soil is higher, water penetrates roots. The pressure in the roots enables to carry up the water to a height of 10-20 m. However pumping the water by the roots is not crucial for water uptake, but the sucking action by the leaves, which occurs when they lose water to the atmosphere (Feddes & Dam 2005).

Before reaching the xylem tissues, which are specialized in water transport, absorbed water firstly has to cross epidermis cortex and endodermis cells traveling through pathways along apoplast and (or) through the symplast. Once water gets xylem tissues, which are tracheids and vessels forming sapwood, movement over a long distance in the stem is easy (McElrone et al. 2013). During the transpiration process, trees lose water through stomata – open pores on the leaves' surface.

1.3. Sapwood

In a tree wood, two types of wood can be distinguished: heartwood which is the inner part of the wood and outward sapwood. Heartwood consists of dead cells and fulfils only the mechanical functions, providing structural support for a tree.

Sapwood, in turn, contains both dead and alive cells and its main role is: resources conduction through the whole plant, mechanical support and food reserves storage. The first two functions are fulfilled by tracheids in softwoods (conifers) and vessels in hardwoods (angiosperms). These ones, when mature, are dead cells of sapwood and do not have a protoplast. The mechanical support is possible thanks to lignified walls of these cells. Both tracheids and vessels have pits in their cell walls, which allow for water (including minerals) to flow between cells. Vessels in addition have open both ends so individual elements are connected, forming continuous ”pipe”, which simplifies water transport. The third function occurs by means of ray and axial parenchyma cells, where food reserves are stored primarily in the form of starch (Bamber 1977), which is fundamental reserve material in plants (Lee et al. 2018).

The starch grains, which were produced from sugar from the photosynthesis process and then transported to the phloem tissues, can get back to the soluble form of sugar and be used by the plant during the intense growth period. They are stored in stems, roots and seeds (Lee et al., 2018). The amount of starch varies a lot between and within species. The ray and axial parenchyma cells are alive (Bamber 1977).

1.4. Scots pine

1.4.1. General information about species

Scots pine (*Pinus sylvestris* L.) is one of the most widely distributed conifers in the world (del Río et al. 2017) and one of the most important tree species in commercial forests in Eurasia. It is a light-demanding, pioneer tree which grows well on a wide range of soil types. Scots pine is considered to be adapted to dry conditions (Taeger et al. 2013) and is more resistant to droughts when compare with Norway spruce, European beech, oak and Douglas fir (Albert et al. 2015). The wax layer on the epidermic and imbedded stomata can be considered as a protection against drought (Pretzsch et al. 2019). Scots pine strategy against droughts stress is called avoidance due to the mechanism of closing their stomata under adverse conditions and deep root system in addition (Zang et al. 2011). When there is a deficit in soil water, mature trees are able to close their stomata adequately to avoid considerable xylem embolism (Irvine et al. 1998) and regulate the transpiration process when drought is in the early stage (Pretzsch et al. 2019).

However in the face of climate change, Scots pine is already suffering because of more frequent and intensive droughts and a reduced number of Scots pine trees is noticed, especially decline in marginal areas of its distribution is noticed (del Río et al. 2017). As a tree typically planted on poor sites, the negative drought effects there is even more adverse. Ten out of 25 cases of die-off events in Europe were related to Scots pine forests (Seidel et al. 2016, Allen et al. 2010). The native provenance of trees is also significant due to differences in drought resilience (Taeger et al. 2013). Choosing proper tree provenances, and hence individuals which are better adapted to drier conditions, when the new forest is being established matters considering future climate change. For example pine seedlings from the Mediterranean area cope better with drought at a moderate level than seedlings from more continental climates (Seidel et al. 2016).

1.4.2. Scots pine in Sweden

Forests in Europe occupy an area of 227 million ha (35 % of the total land area of Europe, excluding Russia). Sweden is in second place, after Finland, if it goes to the proportion of forest area in the country, with a forest cover of nearly 69 % of total land use (SoEF 2020); 57 % of that is productive forest land. Forest products constitute 3 % of the Swedish GNP and 10 % of the export value (Keskitalo et al. 2016), placing Sweden in third place among the world's leading exporters of pulp, sawn and paper timber (SFI 2019).

Biomass production in Swedish forests is based on two native species: Norway spruce *Picea Abies* and Scots pine *Pinus sylvestris*, which constitute respectively 40 % and 39 % of the standing volume (SFA 2020).

The typical silviculture system applied to Scots pine in Sweden is clearcutting followed by artificial regeneration, usually planting. Natural regeneration using seed trees or under the shelter is also implemented, however, the percentage of naturally regenerated area decreased from 40 % in 2000 year to about 10 % in 2018 (SFA 2018 see Lula et al. 2021).

The next silvicultural activity is precommercial thinning, conducted when the stand is 5 to 10 years old and achieves height 2-3 meters, traditionally reducing the stand density to 1500-3500 stems/ha (Fahlvik 2015). The purpose is to regulate density and species composition. However, in pine stands the PCT is sometimes delayed, as a measure to reduce the effects of browsing, but then more complicated thinnings are needed in the future (SFA 2020). The aforementioned operations together with site properties are essential to establish new stands (Lula et al. 2021).

The next common operation in commercial forests is thinning. The total surface area which is thinned annually is the largest from among all forest treatments (Valinger et al. 2018). As concerns Sweden, it is approximately 380 000 hectares per year (every year about 1% of Swedish forests is thinned). The traditional thinning objectives are: removing undesirable trees, reducing competition between trees and promoting the growth of the best individuals. As a result, trees retained to the end of the rotation are of better quality and higher dimension, the rotation length can be shortened and the forest owner can get an income before final felling (Lagergren et al. 2008). Typically in Swedish forestry about 30% of the basal area is removed in one to three thinning with intervals of 10-40 years and with a tendency to thin from above, which means that the dominant trees are cut (Egnell 2000).

The rotation age depends on the species, site and location. In the Forestry Act there are recommended minimum lengths between two final fellings, and so for Scots pine it is between 45 years on more productive areas in the southern Sweden, to 100 years in the northern part of the country (SFA 2020).

1.5. Thinning relevance

Trees in the forest, like any other organism, compete with each other for resources such as sunlight, nutrients and water (Kocher & Harris 2007). The competition can be above- or belowground, inter- and intraspecies.

In accordance with thinning objectives, appropriate thinning operations play a significant role in forest management in terms of competition. Thinning is a tool to control to some extent resources in the forest stands and affect the allocation of resources to individuals (Albaugh et al. 2017). Reducing the competition for resources above and belowground positively affect the growth of remaining trees. It is possible because of lower transpiration and hence water consumption and interception and more available nutrients (del Río et al. 2017).

Since thinning is a great tool to manage forest stand, much research on its effect has been done already; in Scots pine stands for over one century. The majority of the recent thinning experiments in Scots pine stand have been focused on its effect on the growth and yield of trees, but there is still a need for new studies covering a broader range of ecosystem services. Nevertheless, ecological aspects of thinning have been already examined as well, including e.g. nutrient cycle, understory vegetation or litterfall (del Río et al. 2017).

The positive effect of thinning as regards mitigating the extreme effects of drought has recently been reported for several species, including Scots pine, although the results vary according to species, sites, and thinning regimes (Sohn et al. 2016; Ammer 2016). According to another study, the reduction in growth, as well as trees mortality caused by lack of water, is correlated with stand density (Martínez-Vilalta et al. 2011). Results from the study in the Mediterranean on the short-term effect of thinning considering tree use of water also showed improved tree growth conditions and reduced evapotranspiration.

1.6. Research questions

This thesis is made within a long-term experiment on water stress and interactions with thinnings in Scots pine stands. The aim of the research is to investigate how water resources and water use is affected by different thinning regimes, both in the short- and long-term.

In my study I used a water mapping method with irrigation of heavy water (deuterium oxide, $^{2}H_2O$) to explore the initial structure of tree roots belowground.

I examined the correlations of below- and aboveground stand structures, such as basal area, sapwood area and root structure. In my research I investigated:

1. Belowground root overlap (trees per m^2);
2. The relative uptake of heavy water in the trees depending on distance to the application zone;
3. Differences in treatments regarding label uptake reatained basal area and sapwood area;
4. Spatial distribution of trees in each plot, indicating labelled trees.

2. Material and methods

2.1. Thinning experiment

A thinning experiment in Scots pine was established in 2020, in one stand in Siljanfors, Dalarna County and in Jädraås, Gävleborg county in the central part of Sweden (geographic coordinates: 60º53’N, 14º24´E, 60°51’N, 16°24'E) (Figure 2).

Figure 2. Location of the experiment.

The mean annual precipitation for these locations is 674 mm, the mean temperature is 3.3ºC. For the summer months (June-August) the mean precipitation is 55 mm and the mean temperature is 14.0°C. The snow coverage is on average 150 days per year. Predominated soil type is various types of the moraine with granite, diabase, porphyry and clayey slate. The most common rocks are sandstones.

Basic information about both stands is shown in the table (Table 1).

Table 1. Initial data about experimental area (SLU); dominant height as an average dominant height for all plots in a given location; site index estimated on the dominant height at the given age.

Characteristic \ Location	Siljanfors	Jädraås
Height above sea level [m]	300	205
Regenration method	Planting	Planting
Establishment year	1980	1984
Site index	26 (at 40 years)	28 (at 37 years)
Dominant height [m]	15.1	15.7

The experiment is composed of four blocks, in every block four plots about 0.1 hectare in size. On every plot within one block four different thinning treatments were conducted. The treatments were:

1. thinning from below with 30% of intensity;
2. thinning from below with 70% of intensity (heavy thinning);
3. thinning from above with 30% of intensity;
4. no thinning (a control plot).

Thinnings were conducted in February 2021. All trees within the treatment plots were numbered and calipered prior to the thinnings.

2.2. Heavy water labelling

The two blocks in Siljansfors were used for the water mapping study. Water mapping has been done by tracking the water uptake by roots, using a detectable hydrogen isotope. The methodology and analysis were performed according to the protocol from recently published studies using the same technique (Henriksson et al. 2021, Lutter et al. 2021).

Heavy water (^2H_2O, D_2O) – deuterium oxide – is the form of water in which instead of protium isotope (^1H), there is deuterium (^2H or D, known as heavy hydrogen), the second stable hydrogen isotope (PubChem n.d.). The presence of ^{2}H can be detected by using the method of isotope-ratio mass spectrometry (IRMS). Implementation of the isotope to the soil allows observing the rotation of water in the growing plants (in this case – trees). If the trees absorb the ^{2}H isotope it can be found in the sapwood of the last year's growth.

2.2.1. Application of heavy water

At the beginning of the growing season, June 2021, the heavy water was applied in one application zone per treatment, in total application zones. The distribution of the application zones was made systematically and each of them was a circular plot with 0.66 m radius to get a 1 m^2 big area with a buffer zone measuring 10 cm on radius (Figure 3).

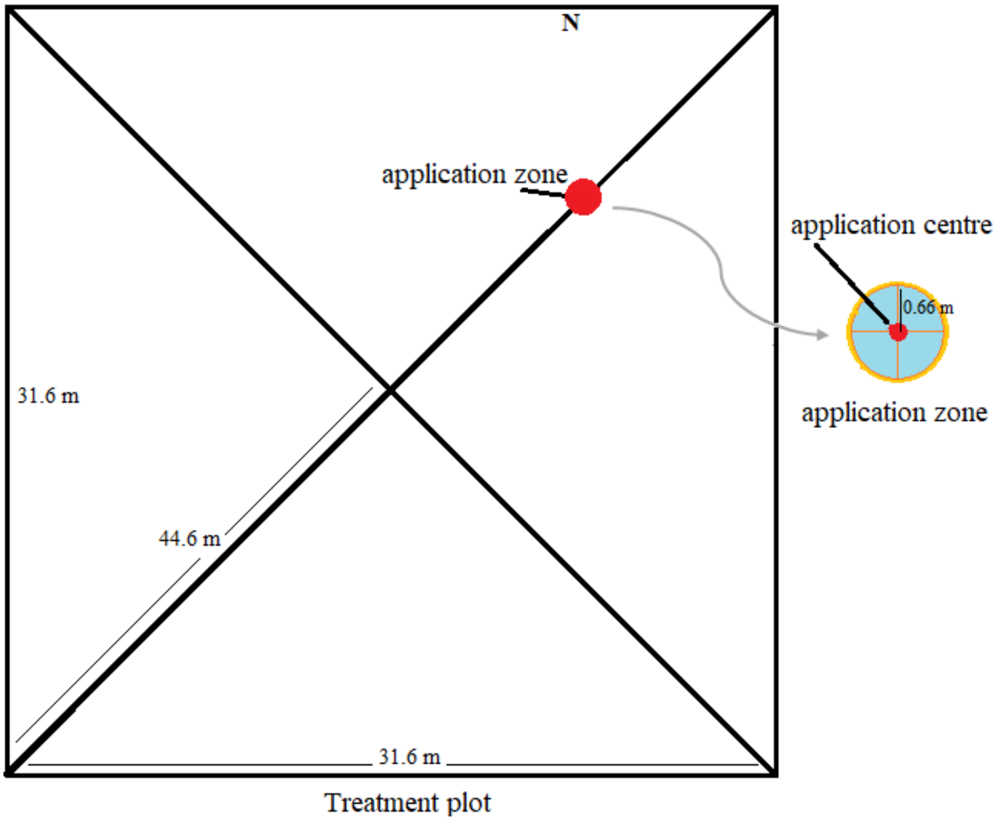


Figure 3. Location of the application zone on the plot.

All the above-ground vegetation was removed from the application zone, to avoid uptake the heavy water by mosses, grasses, berries and other plants. In addition all rocks, bark, branches, etc. were taken away, to enable the applied water to seep into the soil.

Each application zone was watered with 15 litres of tap water mixed with 500 ml of the heavy water (^2H_2O, 99.9 atom% pure; Cambridge Isotope Laboratories, Andover, MA, USA). It was done with traditional watering cans, using a circular frame to mark the border, in order to supply every part of the area with an equal amount of water. It was important to not get stuck in any place aiming to supply an equal amount of label to any part of the application zone. The watered plots were thereafter covered by plastic in case of rain and to avoid evaporation. The plastic was removed after four days.

2.2.2. Collection of wood samples

After the growing season (mid-September) the wood samples were collected. On each treatment plot all trees within the 8 m radius from the application centre were selected and the exact distance (with an accuracy of ± 5 cm) to the application centre was measured together with the diameter at breast height (DBH, 1.3 m above ground). In addition the angle of every tree was noted down using a compass.

From each tree, four sapwood cores were taken, using 8 mm big hole puncher and a hummer, roughly 30 cm above the ground. If the diameter of the tree was small (less than 11.5 cm), only three samples were collected, to avoid a negative impact on the tree.

2.2.3. Separating the sample tissue

The whole procedure of separating the wood sample to the analysis of the isotope concentration followed the protocols described in the previous studies with the heavy water (Henriksson et al. 2021, Lutter et al. 2021).

From each wood core the outer ring (last year's growth ring) was carefully separated using the scalpel under the microscope (Figure 4). All four (or three) samples from one tree were pooled together and dried in the oven at the temperature 65°C for 48 hours.

Figure 4. Sapwood core; A) sample before cutting; B) separation the outher ring from the bark layer; C) separated outer ring.

The next step was milling samples until getting the texture of powder. 0.35 mg of milled wood from each sample was analysed using the isotope ratio mass spectroscopy (EA-IRMS, Flash EA 2000 and DeltaV, Bremen, Germany) at the Stable Isotope Laboratory at Swedish University of Agricultural Sciences in Umeå. Only samples from trees within the distance 6 m were analysed, in total 104 trees.

The spectroscopy provides three values, describing mass fraction of hydrogen, ratio of the deuterium isotope to the naturally abundant first isotope and the calculated fraction of the deuterium isotope:

- ωH = mass fraction of H [g H per g dry mass],
- δ^2H = ^{2}H/^{1}H ratio expressed on the VSMOW-SLAPscale [‰],
- F_H = isotopic amount fraction [^{2}H/(^{1}H + ^{2}H)],

where:
ωH – mass fraction of hydrogen, H – hydrogen, g – gram, ^{2}H – deuterium, ^{1}H – protium, δ^2H – hydrogen isotope ratio, VSMOW-SLAP – Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation, F_H – fraction of the isotope

F_H was calculated from δ^2H using R_{ref} = ^{2}H/^{1}H = 0.00015576 (Reference: IAEA-TECDOC-825, 1995, Reference and intercomparison materials for stable isotopes of light elements). $F = 1 / (1 + 1 / (R_{ref}*(1 + \delta\ (‰)/1000)))$.

The percentage of F_H , denoted as atom%, is hereafter used in this study to represent the magnitude of the label (as followed by the protocol from Henriksson et al. (2021) and Lutter et al. (2021)).

Since a certain amount of the ^{2}H is present in nature, its natural abundance had to be determined so that the excess of ^{2}H from its natural abundance could be specified. In the previous research the natural abundance of ^{2}H was 0,0141 atom% (Henriksson et al. 2021, Lutter et al. 2021). In this study the natural abundance of ^{2}H was established as 0,0145 atom% (which is -70 isotopic ratio expressed using the VSMOW-SLAP scale). That value is set up considering data from the GRIMSO precipitation station which was the closest to the study area. Hence all trees with the atom% higher than 0,0145 and δ^2H > -70 were considered to uptake the label and were thereafter classified as “labelled trees”.

2.3. Sapwood area

To analyse the sapwood area, sample trees from all four blocks, two blocks in Siljansfors and two in Jädraås, were used.

2.3.1. Sampling

On each of 16 plots one sample tree was cut in autumn 2020, before thinning. From these trees, discs have been sampled at DBH height (1.3 m).

With the aim to measure the sapwood area, iodine (povidone-iodine; Jodopax vet) was applied on the discs. The iodine test is an easy and rapid, but still effective method to detect starch. Therefore, since starch is present in the sapwood and at the same time absent in the heartwood, the iodine-starch test was done.

A few minutes after the application of iodine, the sapwood became colored (Figure 5). Then the discs were scanned and the images were analysed in the ImageJ software, obtaining the size of sapwood.

Figure 5. Tree discs sampled at height of DBH (1.3 m) with colored sapwood area.

2.3.2. Calculation

The correlation between tree diameter and sapwood area was positive (correlation value 0.952; a p-value <0.01 in ANOVA test indicates significant correlation). R^2 value for the linear regression was calculated and amounted 0.9057 (Figure 5). Based on this the equation for the sapwood area was derived: $y = 13{,}388x - 71{,}69$. Using that equation and tree diameters measured previously, the sapwood area of every tree used in the heavy water experiment was calculated.

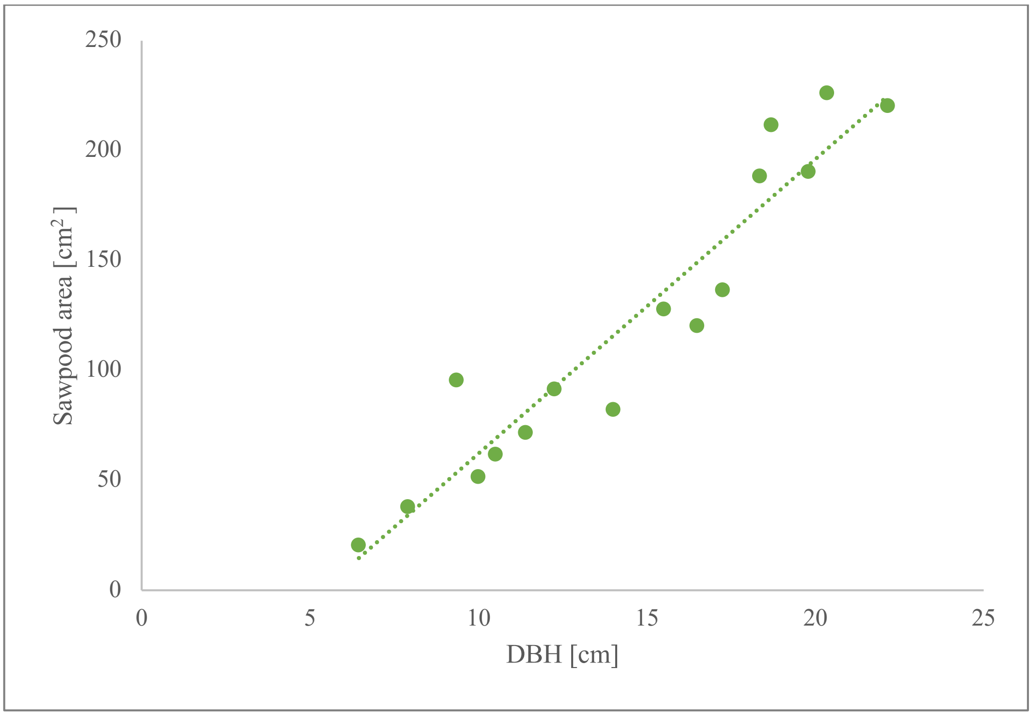


Figure 6. . Correlation between tree diameter [cm] and sapwood area [cm^2].

2.4. Data analyses

The belowground root overlap was calculated as the sum of labelled trees divided by the area of the application zone (1m^2).

All analysed trees were collated to their distance from the application centre. Any dependency on the distance to the application zone for the magnitude of the atom% was tested for each tree within 6 meters, using an ANOVA.

The distance dependency was also evaluated for distance classes of 1 m, only for labelled trees. The average of atom% uptaken per tree for each distance class was calculated. Further on, the proportion of labelled trees from all trees in a given distance (in 0.5 m intervals) was illustrated using the measurements of distance for each tree.

Treatment differences were thereafter described using plot-level data the average of uptaken atom% per labelled tree in every plot and the proportion of labelled trees in different treatments.

The sapwood area of every selected tree was calculated using the above sapwood function, derived from the harvested trees (Figure 6). The treatment differences in the sapwood area and basal area were thereafter visually presented.

The position of each tree was determined utilizing an angle between trees and their distance to the application centre. From this data all trees in the 8 metres radius watering plot were mapped in the Plotly Chart Studio.

3. Results

3.1. Belowground root overlap

The number of trees in control plots that uptoook the label were 3 trees/m^2 in block 1 and 4 trees/m^2 in block 2, referring to the belowground root overlapping.

Table 2. Belowground root overlap in each plot.

Treatment	Number of labelled trees per plot	
	Block 1	Block 2
Control	3	4
Thinning from above	5	5
Thinning from below	5	4
Heavy thinning	3	2

3.2. Distance

In total 104 selected trees from eight treatment plots were sampled and analysed using the IRMS. 31 of the trees were regarded as labelled trees which had an isotope proportion higher than the threshold for natural abundance, corresponding to 30 % of the sampled trees (Figure 7; Appendix 1).

For labelled trees the amount of uptaken ^2H decreased exponentially with the increase in distance, what indicates a lower density of roots with increasing distance from the tree (Henriksson et al. 2021). Almost all trees within a distance of 2 meters uptook water (88%). The farthest tree which uptook water was noted within a distance of 4.45 m. The closest tree which did not uptook the water was located 1.55 m from the label source. The mean distance from the label source to the labelled tree was 2.13 m.

A p-value<0.01 (0.00001739) with a confidence interval of 0.05 indicates that distance is a statistically significant predictor of uptaken atom%.

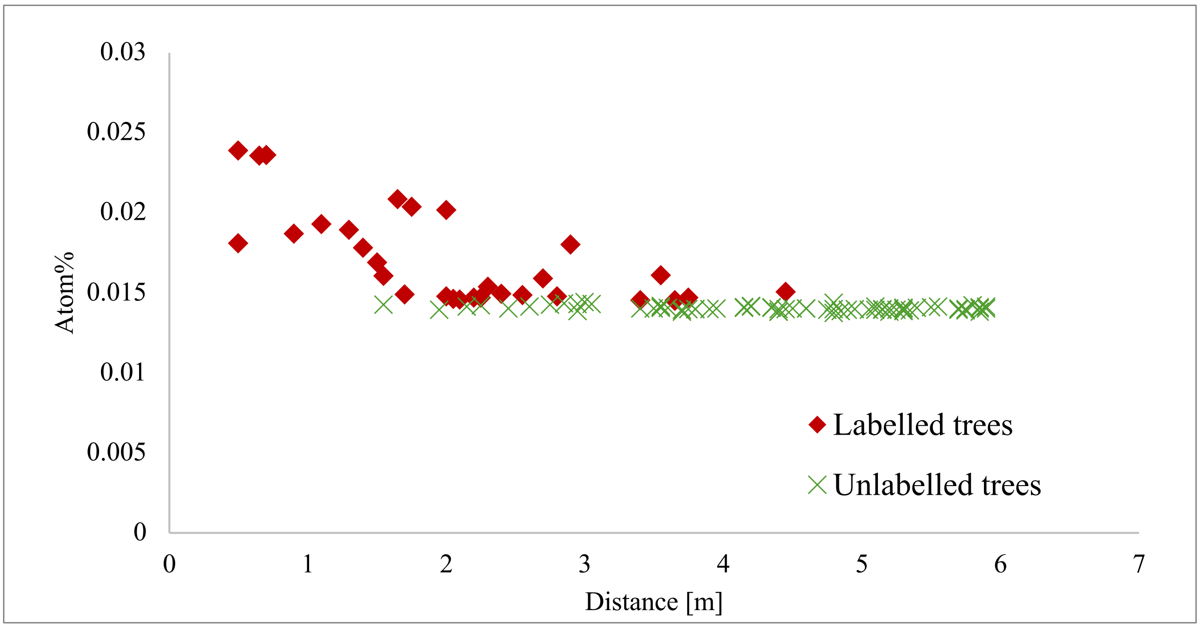


Figure 7. Distance of trees [m] in relation to the amount of uptaken label [atom%].

The average tree growing in the distance 0-1 m from the label source uptook 1.2-fold more ^{2}H than the average tree within the distance between 1-2 m and 1.5-fold more than the average tree growing 4-5 m from the label source (Figure 8). Simultaneously the highest proportion of labelled trees is between 2-3 m (38% of 31 labelled trees).

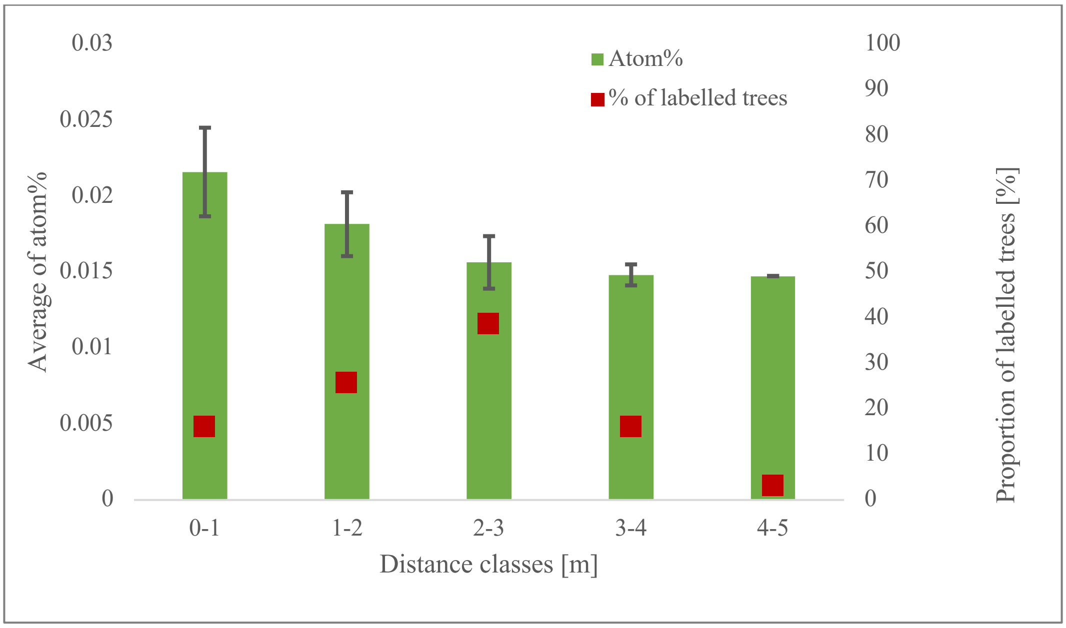


Figure 8. Distance classes of trees [1 m intervals] related to the average label uptaken per tree [atom%] and proportion of labelled trees [%].

Within a distance 0.5-1.5 m from the watering spot all present trees uptook water are considered as labelled trees. From trees distant by 1.5-2.5 m about 71% of individuals uptook water. Between 2.5 and 3.0 m from the label application 44% of trees uptook water. Exactly one-quarter of trees within distance 3.0-4.0 m uptook water and only 8% of trees distant by 4.0-4.5 m. There were no trees that uptook water further than 4.5 m from the label application. There was no tree within a distance 0.0-0.5 m (Figure 9).

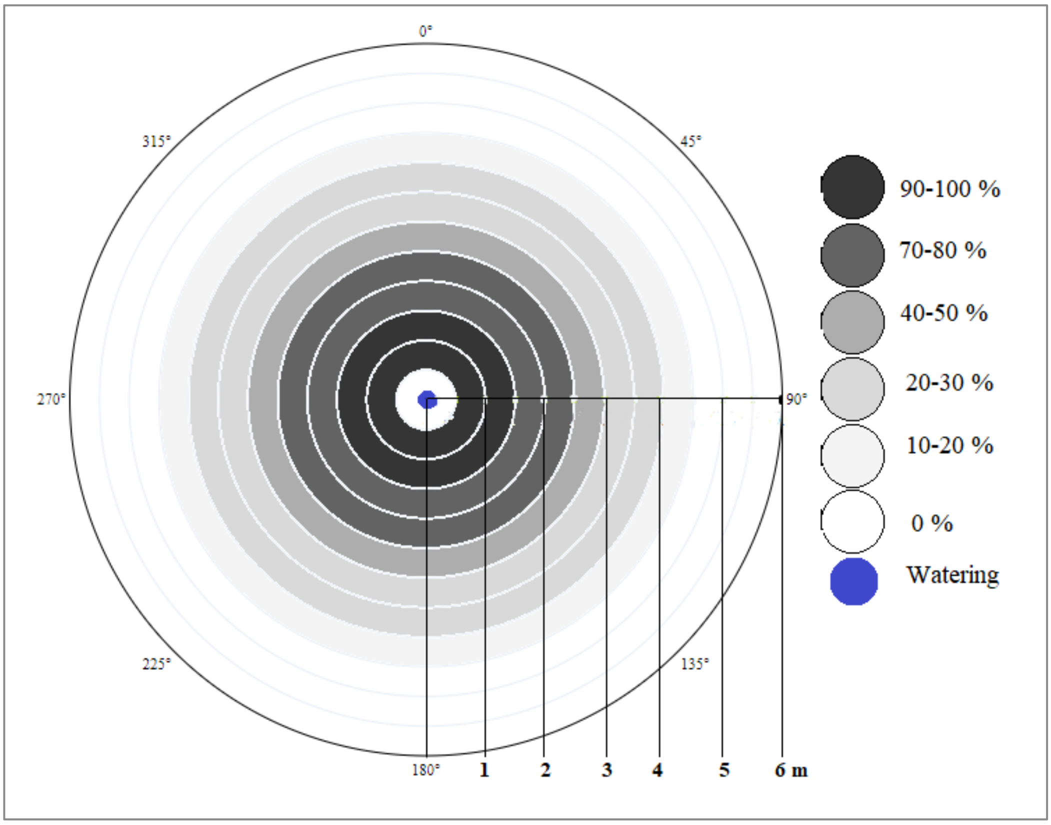


Figure 9. Proportion of labbeled trees in the given distance (intervals 0.5 m).

3.3. Treatment

The average amount of uptaken label per labelled tree in different treatments is the highest in the plot with heavy thinning in block 2 (0.0194 atom%) and the lowest in block 1 with thinning from below (0.0158 atom%) (Figure 10).

The percentage of labelled trees in different treatments indicates that in block 1 with thinning from below the proportion of labelled trees was the highest. At the same time it was a plot with the lowest average amount of atom%. However, on average the most trees uptook water in a plot with heavy thinning, where the competition was reduced to the greatest extent (Figure 11).

The fewest trees were labelled in the control plot in block 2, with the highest competition.

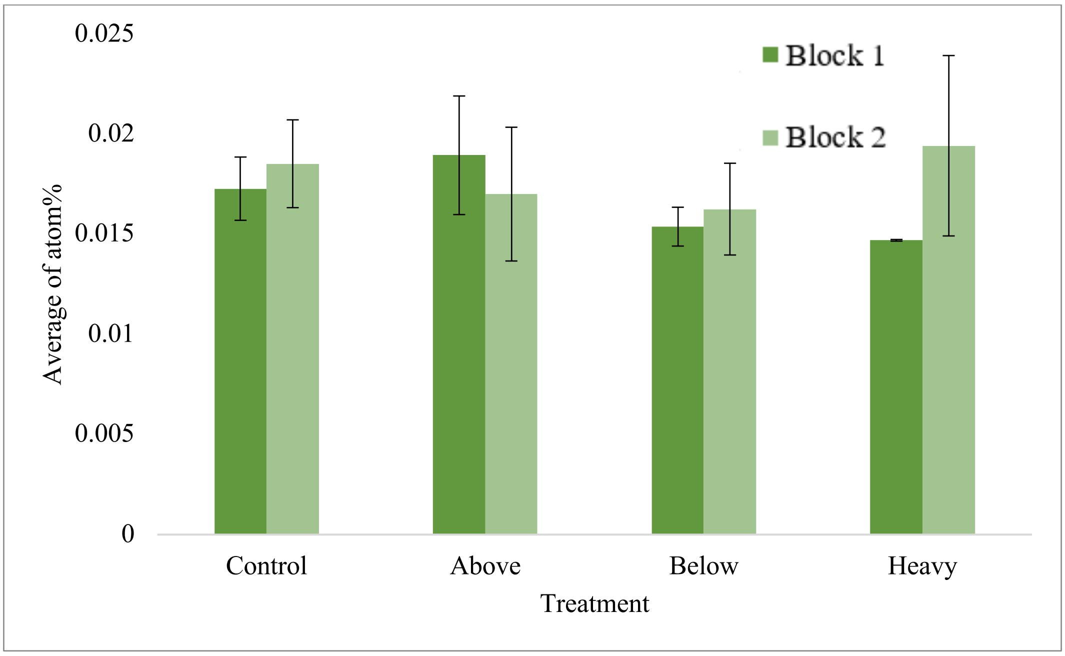


Figure 10. Average of uptaken label per labelled tree in different treatments.

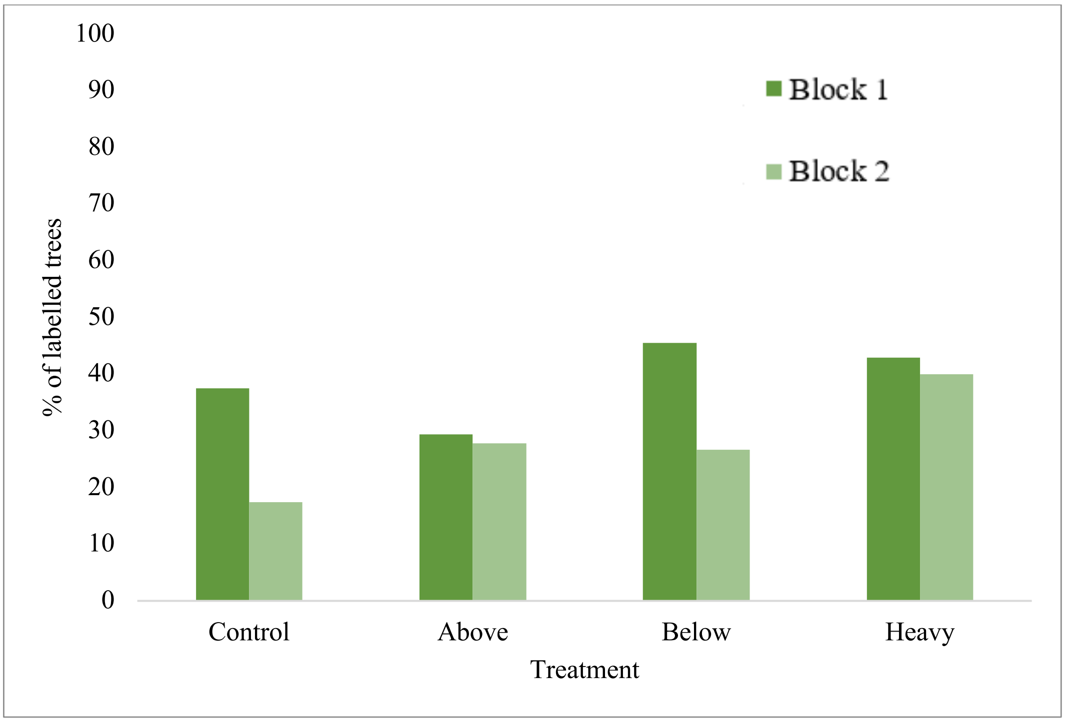


Figure 11. Proportion [%] of labelled trees in a given treatment.

3.4. Sapwood area

There was no correlation between the sapwood area of a given tree and the amount of label uptaken by labelled tree (a p-value>0.1 (0.244857)). What is more, a labelled tree with almost the smaller sapwood area uptook one of the biggest amounts of ^{2}H (0,024 atom%) whereas a tree with the biggest sapwood area uptook 1.6-fold less label (Figure 12).

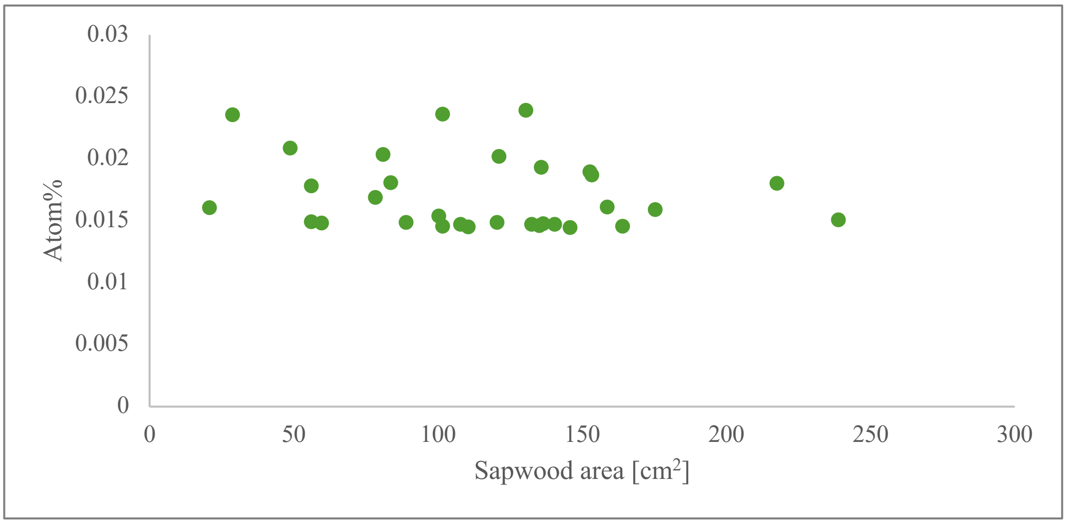


Figure 12. Tree sapwood area [cm^2] of labelled trees in relation to the amount of uptaken label [atom%].

Looking at the sum of atom% and sapwood area on the plot level, it can be seen that the differences between plots are relatively similar. The exception is control treatment, but in the control plot in block 1 there were just a few big trees analysed (Figure 13 and 14).

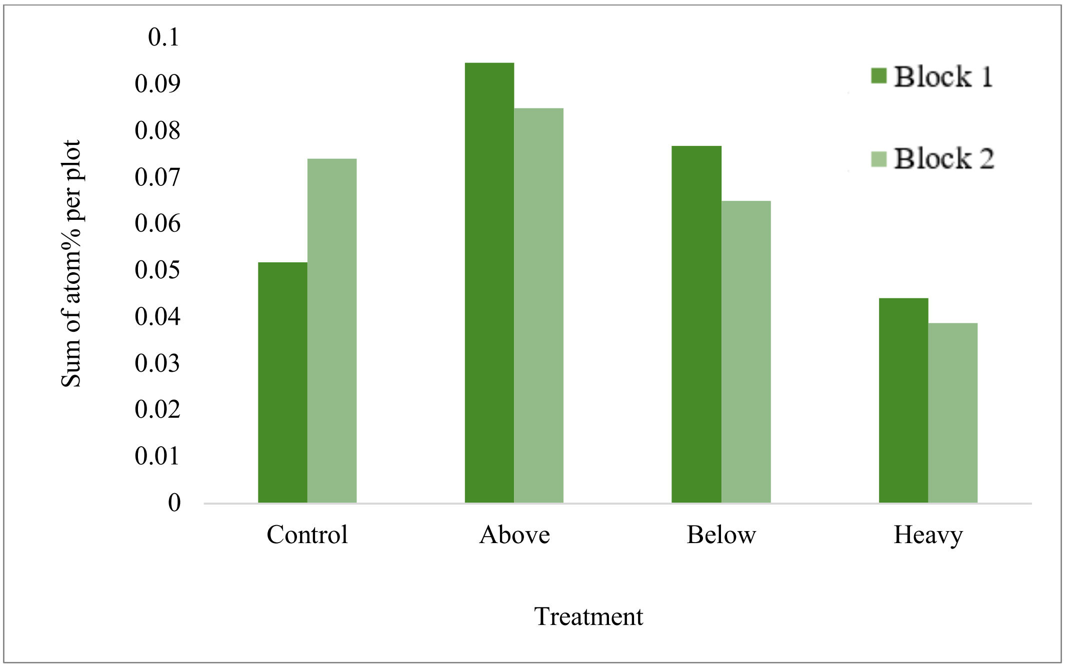


Figure 13. Sum of uptaken deuterium per plot in different treatments.

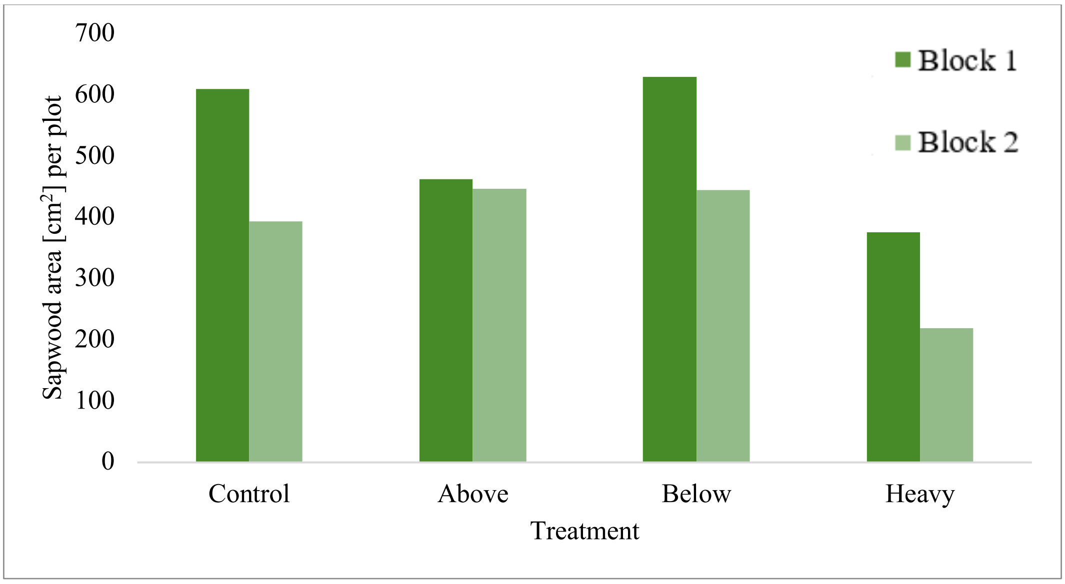


Figure 14. Sum of sapwood area [cm^2] per plot in different treatments.

3.5. Basal area in treatments

The regression model showed a moderate level of correlation between the basal area of a given plot and the sum of uptaken atom% in that plot (R^2= 0.5955, p-value= 0.025) (Figure 15).

It is possible to see that in the heavy thinned stand where the basal area is the lowest, the sum of the uptaken label is also the lowest. The biggest amount of label was uptaken in one of the control plots with the highest basal area.

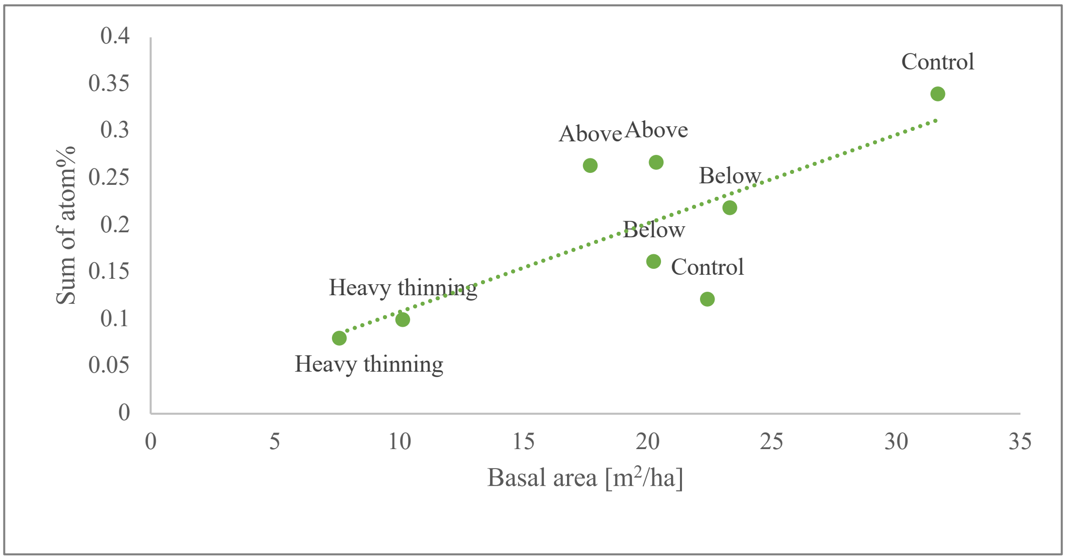


Figure 15. Basal area [m^2/ha] of the stand related to the sum of label uptaken [sum of atom%].

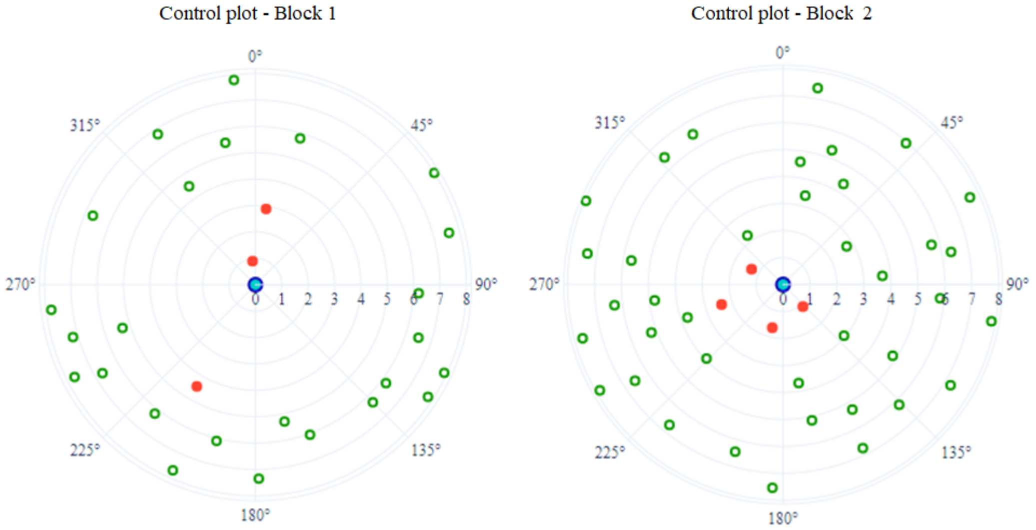
3.6. Water mapping

The distribution of labelled and unlabelled trees is not equal around the application zone. It appears that trees avoid belowground competition, expanding their roots in the direction where the other trees do not.

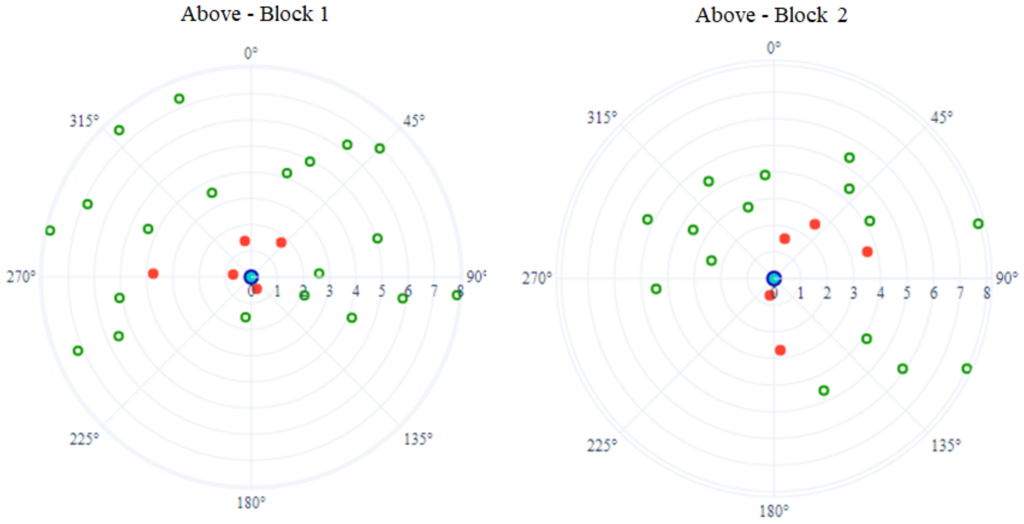
However, even if there are no competing trees along the trajectory, if the distance is too big, trees' roots do not reach the application zone, as is in the heavy thinned plots (Figure 16 d).

In the control plot of block 1 there are not many trees in 6 meters radius, which may influence the results (Figure 16 a).

a)


Control plot - Block 1
Control plot - Block 2
0°
45°
90°
135°
180°
225°
270°
315°
0
1
2
3
4
5
6
7
8

b)


Above - Block 1
Above - Block 2
0°
45°
90°
135°
180°
225°
270°
315°
0
1
2
3
4
5
6
7
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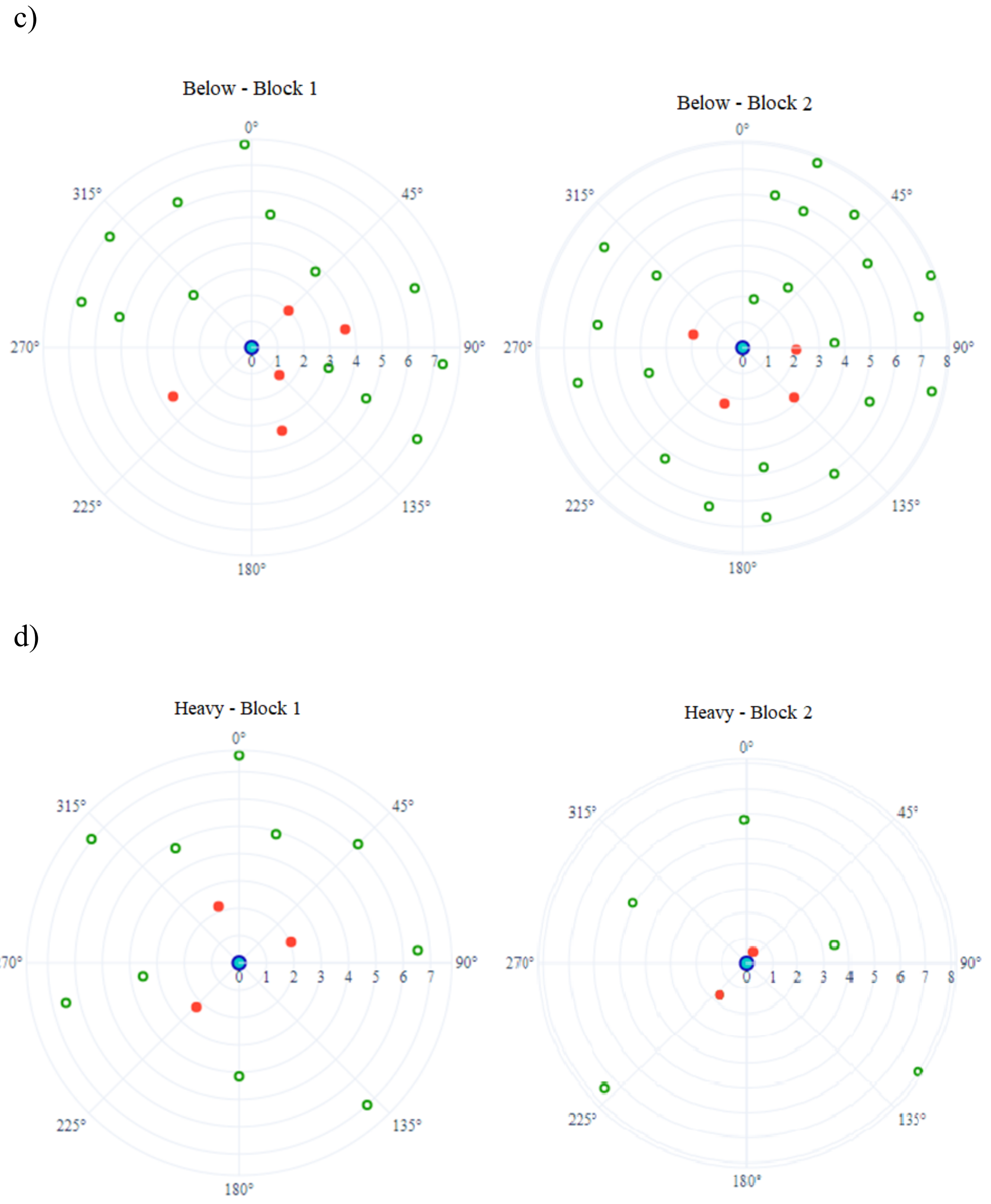

Figure 16. Distribution of all trees within 8 m radius from the appliaction centre in each plot: a) control plots b) plots with thinning from above c) plots with thinning from below d) plots with heavy thinning. Red full dots – tree with uptaken label; green circles – trees without label; blue dots – application zone.

4. Discussion

In the current study an approach using deuterium, the stable hydrogen isotope, was applied to investigate the horizontal range of root system in Scots pine stands with different thinning intensities. Better understanding belowground competition for water is beneficial in the face of climate change and caused by this reduction of available water in the soil.

4.1. Stable isotopes in research

Uptaking resources from the soil by root systems is much less understood than, for example, the process of transpiration because of difficulties to get access to roots underground (Steudle 2002). Therefore using stable isotopes instead of invasive methods consisting in penetration soil, such as direct digging or excavation, is a useful tool in the research of plants ecology and general ecohydrology. Digging and excavation require more labour input and allow only for a look into roots architecture and placement (De Deurwaerder et al. 2020). Sometimes the root biomass is specified, but the reason why it is not the best determinant of resources uptake is that large. and therefore heavier, roots usually do not absorb water, fine roots do not absorb water from dry soils. In addition, it is difficult to diversify various species roots (Mazzacavallo & Kulmatiski 2015).

Detection of naturally present isotopes as well as artificially implementation were already used in different studies concerning plant roots for many years. For instance, in 1966 radioiodine was applied to the soil in a longleaf pine plantation to investigate root extension in this species (Ferrill & Woods 1966). When it comes to hydrogen, the formerly used radioactive tritium ^{3}H was replaced by deuterium (Wullschleger 1998), which is a stable isotope with no ecological objection. Another advantage of deuterium is that even very small amounts of that isotope are detectable (Beyer 2016).

Deuterium has been used to analyse a vertical range of the root system, which is possible when is applied to different layers of soil (Beyer 2016) or when the differences in ^{2}H in groundwater at different depths are examined. In another study the component of deuterium isotopes in xylem water was analysed to identify the source of water in the plant (Xu 2011). The current Study presented refers to the horizontal extension of roots which is less frequent than investigation vertical root structure.

4.2. Belowground root overlap

Lutter et al. (2021) in their study found that in Scots pine monocultures on average 3.5 tree/m^2 upotook the label, which is exactly the same number as the average of control plots in current research. Differently, Henriksson et al. (2021) got the belowground root overlap of 8 trees/m^2.

The root system area of a given tree seems to not expand as a regular circle area around that tree but is unevenly distributed depending on resource (in this case water) availability (Lutter et al. 2021) and avoidance of other roots (Schenk 2006). Both root systems of trees and soil resources are not distributed regularly in the soil (George & Marschner 1996). Kalliokoski et al. (2011) indicated a highly asymmetric spread of root system in Scots pine trees.

4.3. Distance

The maximum distance between labelled tree and the application centre is very similar to the results from the previous research with the heavy water (Henriksson et al. 2021, Lutter et al. 2021). In the literature it can be found that the maximum lenght of roots for vital Scots pine tree is between 4.5 m to 17.1 m in 14- and 52-years old stand respectively. Scots pine develops bigger horizontal root system on poorer soils than on better and bigger on sandy soils than on rocky soils (Skilling n.d.).

Taking into account different tree species, the maximum distance between tree roots of species such as dogwood, hickory and red cedar was 9.7 m, 16.6 m and 10.1 m respectively, whereas the highest amount of uptaken label was noticed within 1.9 m, 3.7 m and 2.9 m (Brown & Woods 1968). In the longleaf pine plantation distance from labelling point to the farthest tree which uptook the radioactive isotope was 7.6 m if the label was applied on the surface (if applied to deeper layers of soil, then the distance was bigger) and the closest tree which did not uptake the label was between 3.0 to 4.5 m from label applying (Ferrill & Woods 1966). In lodgepole pine stands the maximum distance which reached roots was about 5.2 m in dominant trees and 4.3 m for codominant individuals (Bishop, 1962).

Abovementioned assymetry of root system can justify the “gap” in the uptaken label within 2 meters. Results from different research also show some exceptions within the distance where all the other trees were labelled (Henriksson et al. 2021, Lutter et al. 2021).

4.4. Treatment

With regard to differences between treatments, it can be stated that since thinning was conducted just a few months before the establishment of the heavy water experiment, it is too short period to notice relevant results. The “thinning shock” a short time after thinning is a commonly noted phenomenon. It can result in e.g. decline in the growth of remaining trees even though the amount of available water increased and the efficiency of photosynthesis improved (Park et al. 2018). Simonin et al. (2006) considered the “thinning shock” as a short-term effect. In their research water conductance from soil to leaf in ponderosa pine was reduced after the first spring after treatment, but higher one and two years after thinning in thinned plots than in unthinned. Therefore focusing on the differences in water uptake by trees in different treatments (intensity of thinning) in the same year when thinning was conducted may not provide enough evidence for treatment effect or thinning response.

According to Park et al. (2018), a significant increase in tree water use was noted three years after thinning, whereas in the first two years the differences in using water were not meaningful. In the same study the water availability increased only in the plot with high intensity of thinning (40%).

Considering root systems' ability to adjust to the accessibility of water and nutrients in soil (Feddes et al. 2001), outcomes of the heavy water experiment in a few years, when the study will be repeated, may show the response of roots in plots with different thinning intensities.

4.5. Sapwood area and basal area

Lack of correlation between sapwood area and amount of atom% detected in labelled tree possibly can be caused by the distance and the position of an individual tree and hence distribution of its roots. Similarly, research on Douglas fir roots showed a weak correlation between DBH and horizontal root system diameter (Kuiper et al. 1990). Day et al. (2010) suggessted strong correlation between the diameter of trunks of urban trees and roots spread, however, authors underlined irregular roots distribution, which can affect that correlation.

Therefore it can be said that not the tree DBH or sapwood area of the tree, which is highly related to DBH, but rather roots placement under the ground affect the process of uptaking soil resources. Nevertheless on the plot level total sum of atom% related to total sapwood area in a plot (sum of sapwood area of all analyzed trees) and plot basal area showed a certain pattern.

4.6. Study limitations

There is always a trade-off when it comes to expensive methods for analysing data. What we can gain in precision (with the label being present or not) we will definitely pay with a high cost for the isotope, field work and spectrometry analysis. The water mapping was only possible to do in two of the total four blocks. The limited number of replicates is a weakness in the study but may be compensated with the strength of a balanced, randomized experimental design.

In one of the control plots there were rather few trees nearby the application zone, which may be possibly explained by the removal of trees from other tree species. This is a standard procedure for long-term experiments but should anyway be taken into account in this study. This could be the case in this part of the treatment.

Another shortcoming of this study may be a lack of attention to the soil structure and possible differences in its properties between plots. The amount of resources which is accessible to plant roots is undoubtedly dependent on soil features (Marschner & Rengel 2012). Especially soil water is spatially and temporally heterogeneous (Schenk 2006). Since soil is a very complex structure it cannot be ruled out that it affected the heavy water retrieving to the ground at the beginning and uptake by trees later. Similarly, the terrain can vary between plots and even a steep slope could have an impact on heavy water movement.

Moreover, there is no guarantee that the presence of the label is equal in each spot around the stem and water can move in a preferred route in sapwood, what was also pointed out by Lutter et al. (2021). Therefore even if there were collected four sapwood cores from each tree, there is still a possibility that any of them contained the label.

4.7. Future

4.7.1. Thinning as an useful tool

The positive effect of thinning on the mitigation of extreme effects of drought has recently been reported for several species, including Scots pine, although the results vary according to species, sites, and thinning regimes (Sohn et al., 2016; Ammer, 2016).

Results of the study in the Mediterranean on the short-term effect of thinning in pine stands, considering tree use of water, shown improved tree growth condition and reduced evapotranspiration (del Campo et al. 2014). Similarly, the research in the Scots pine stand on the xeric site suggested the possibility of mitigation drought impact thanks to thinning, because of the increase in the ratio of leaf area to sapwood and reduction in competition for water.

4.7.2. Implementation of research results

Results of this study, combined with finding from other research in differently thinned Scots pine stands considering water (and other resources) uptake in a long-term may lead to developing new thinning guidelines. It can be valuable in view of climate change, more frequent droughts and generally decline in water availability in the forest and enable management of water in the most efficient way.

4.8. Conclusion

1. The extension of root systems is not radially regular. Trees to some extent avoid belowground competition if possible and develop their roots in directions where are favourable soil conditions. Nevertheless, the root systems of some trees do overlap.
2. Distance of growing tree to the application zone influences the amount of uptaken label. Trees closer to the application zone uptook relatively more water than further individuals.
3. The sapwood area on the individual level is not correlated with the amount of uptaken label per individual labelled tree. However, a relationship between the sapwood area of all analysed trees on the plot or plot basal area and the sum of uptaken deuterium is visible on the plot level.

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Appendix 1

Supplementary table - all 104 analysed trees with their properties.

Block	Treatment	Plot no.	Tree ID	Distance	DBH	Atom%	Labelled	Sapwood area [cm^2]
Block 1	Control plot	11	45	0,90	168,0	0,01871	Yes	153,23
		11	44	2,90	216,0	0,01805	Yes	217,49
		11	46	4,45	232,0	0,01508	Yes	238,91
		11	36	4,50	192,5	0,01403	No	186,03
		11	35	5,30	170,0	0,01389	No	155,91
		11	77	5,30	174,5	0,01409	No	161,93
		11	38	5,50	176,5	0,01412	No	164,61
		11	43	5,80	258,0	0,01423	No	273,72
	Thinning from below	12	62	1,50	112,0	0,01692	Yes	78,26
		12	61	2,00	155,5	0,01481	Yes	136,49
		12	51	3,00	244,5	0,01443	No	255,65
		12	101	3,05	176,0	0,01431	No	163,94
		12	99	3,40	136,0	0,01456	Yes	110,39
		12	46	3,55	172,0	0,01613	Yes	158,58
		12	102	3,65	162,5	0,01450	Yes	145,87
		12	59	3,80	150,0	0,01399	No	129,13
		12	100	4,80	174,5	0,01441	No	161,93
		12	54	5,15	106,5	0,01397	No	70,89
		12	8	5,20	159,5	0,01403	No	141,85
	Heavy thinning	13	100	2,05	154,5	0,01464	Yes	135,15
		13	58	2,20	152,5	0,01473	Yes	132,48
		13	61	2,25	134,0	0,01473	Yes	107,71
		13	62	3,55	96,5	0,01413	No	57,50
		13	97	4,15	190,5	0,01411	No	183,35
		13	48	4,80	133,0	0,01378	No	106,37
		13	50	4,90	134,0	0,01396	No	107,71

Block	Treatment	Plot no.	Tree ID	Distance	DBH	Atom%	Labelled	Sapwood area [cm^2]
Block 1	Thinning from above	14	58	0,50	116,0	0,01812	Yes	83,61
		14	57	0,70	129,5	0,02365	Yes	101,68
		14	55	1,40	95,5	0,01784	Yes	56,17
		14	59	1,55	56,0	0,01430	No	3,28
		14	100	1,75	114,0	0,02040	Yes	80,93
		14	98	2,15	149,5	0,01417	No	128,46
		14	99	2,60	126,5	0,01416	No	97,67
		14	54	3,55	111,0	0,01408	No	76,92
		14	47	3,75	158,5	0,01474	Yes	140,51
		14	97	4,15	97,0	0,01406	No	58,17
		14	102	4,20	56,5	0,01416	No	3,95
		14	48	4,35	127,0	0,01409	No	98,34
		14	103	4,95	181,0	0,01399	No	170,63
		14	113	5,05	152,5	0,01397	No	132,48
		14	9	5,10	100,0	0,01414	No	62,19
		14	14	5,55	104,0	0,01416	No	67,55
		14	115	5,85	134,0	0,01411	No	107,71
Block 2	Heavy thinning	21	64	0,50	151,0	0,02393	Yes	130,47
		21	50	1,70	120,0	0,01492	Yes	88,97
		21	61	3,50	137,5	0,01404	No	112,40
		21	10	5,10	155,0	0,01394	No	135,82
		21	53	5,75	171,5	0,01402	No	157,91
	Thinning from below	22	60	1,95	104,5	0,01397	No	68,21
		22	48	2,00	144,0	0,02020	Yes	121,10
		22	100	2,10	176,0	0,01461	Yes	163,94
		22	62	2,30	128,5	0,01541	Yes	100,35
		22	99	2,80	98,0	0,01482	Yes	59,51
		22	59	2,95	183,5	0,01434	No	173,98
		22	101	3,60	97,0	0,01409	No	58,17
		22	46	3,80	166,5	0,01399	No	151,22
		22	49	4,40	186,5	0,01401	No	178,00
		22	67	4,75	162,0	0,01395	No	145,20
		22	44	5,30	157,5	0,01404	No	139,17
		22	98	5,40	183,0	0,01409	No	173,31
		22	10	5,75	86,5	0,01390	No	44,12
		22	58	5,85	167,5	0,01383	No	152,56
		22	103	5,90	146,5	0,01405	No	124,44

Block	Treatment	Plot no.	Tree ID	Distance	DBH	Atom%	Labelled	Sapwood area [cm2]
Block 2	Thinning from above	23	131	0,65	75,0	0,02359	Yes	28,72
		23	146	1,55	69,0	0,01606	Yes	20,69
		23	132	2,45	85,0	0,01405	No	42,11
		23	145	2,55	143,5	0,01488	Yes	120,43
		23	129	2,70	184,5	0,01594	Yes	175,32
		23	143	2,85	82,0	0,01435	No	38,09
		23	133	3,55	64,0	0,01420	No	13,99
		23	147	3,65	129,5	0,01458	Yes	101,68
		23	142	3,90	159,5	0,01400	No	141,85
		23	149	4,15	162,0	0,01412	No	145,20
		23	176	4,20	127,5	0,01421	No	99,01
		23	138	4,40	78,5	0,01386	No	33,41
		23	177	4,40	129,5	0,01386	No	101,68
		23	134	4,45	83,5	0,01399	No	40,10
		23	128	4,60	181,0	0,01404	No	170,63
		23	136	5,25	95,5	0,01398	No	56,17
		23	178	5,35	110,5	0,01391	No	76,25
		23	150	5,90	203,0	0,01415	No	200,09
	Control plot	24	51	1,10	155,0	0,01932	Yes	135,82
		24	53	1,30	167,5	0,01896	Yes	152,56
		24	52	1,65	90,0	0,02089	Yes	48,80
		24	37	2,25	83,5	0,01424	No	40,10
		24	54	2,40	95,5	0,01494	Yes	56,17
		24	50	2,75	104,5	0,01429	No	68,21
		24	90	2,95	126,5	0,01390	No	97,67
		24	39	3,40	168,0	0,01405	No	153,23
		24	49	3,70	115,5	0,01395	No	82,94
		24	88	3,70	208,0	0,01383	No	206,78
		24	56	3,75	145,0	0,01402	No	122,44
		24	55	3,95	120,0	0,01404	No	88,97
		24	42	4,35	92,0	0,01412	No	51,48
		24	40	4,60	135,0	0,01403	No	109,05
		24	35	4,80	156,0	0,01398	No	137,16
		24	91	4,85	166,5	0,01389	No	151,22
		24	86	5,15	149,0	0,01401	No	127,79
		24	57	5,20	141,5	0,01386	No	117,75
		24	41	5,30	156,0	0,01391	No	137,16
		24	87	5,30	121,5	0,01408	No	90,97
		24	36	5,70	121,0	0,01403	No	90,30
		24	48	5,70	216,0	0,01397	No	217,49
		24	92	5,85	107,0	0,01405	No	71,56