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Similarities and differences in biomass allocation to xylem and needles in branches of Scot's pine trees from two sites differing in soil development

By

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ABSTRACT

The ability of a species to survive and adapt to adverse environmental conditions is determined by the vulnerability of its xylem to embolism and the availability of carbon resources. The vulnerability to embolism is complex, and species-specific within the trait of tree hydraulic architecture. Drought limits long-distance water transportation of the water due to reduced leaf-to-soil water potential. In general, low soil water availability can reduce growth and be highly dangerous to plant survival. Furthermore, biomass partitioning, carbon acquisition, and carbon resource utilization have great importance in adaptation and acclimation studies. The phenotypic plasticity of the xylem or the photosynthetic apparatus may play an important role in the adaptation of pioneer isohydric species like *Pinus sylvestris* to new/ harsh environments. How plants modify the biomass allocation to the different soil developmental areas, like wet and dry conditions affecting this coordination between biomass allocation to the xylem and needles of the branches, has been poorly investigated. In this experimental study, mature Scots pines from 2 different environmental conditions that are only a few hundred meters apart in the Italian Dolomites, were evaluated for their xylem anatomical hydraulic traits and biomass resources. One of the study areas is a mature mixed forest with high water availability, and the other is a pure pine forest growing on a landslide (1814) with low water availability due to the soil conditions. In this study, we selected the topmost apical branches 150 cm from the apex and selected several sampling positions at different distances from the stem apex in two groups of trees. We measured the annual Ring area, the xylem hydraulic diameter (Dh) and leaf (needle) biomass (LM), the branch biomass (BM), leaf mass (LM), and leaf mass area (LMA). The experimental results show that pines in the landslide area have significantly reduced growth but with contrasting xylem anatomical results for hydraulic diameter, which show smaller conduits in the first 50cm from the branch apex and become larger afterward. Needle biomass in the two areas is not statistically different, with the same pattern of cumulated mass along the branch axis. Low-water-availability pines have the same stomatal density as high-water-availability pines but have higher leaf mass per area (LMA) due to a higher number of smaller, denser, and heavier needles. Scots pine is an early isohydric species that likely closes stomata to avoid excessive transpiration at the cost of reduced growth.

Keywords: *Pinus sylvestris*. L, Different soil conditions, Xylem anatomy, Biomass allocation, Leaf area.

RIASSUNTO

La capacità di una specie di sopravvivere e adattarsi a condizioni ambientali avverse è determinata dalla vulnerabilità del suo xilema all'embolia e dalla disponibilità di risorse di carbonio. La vulnerabilità all'embolia è un tratto complesso e specifico della specie dell'architettura idraulica degli alberi. La siccità limita il trasporto idrico a lunga distanza dell'acqua a causa delle differenze nel potenziale idrico nelle foglie e nel suolo. In generale, la scarsa disponibilità di acqua nel suolo può ridurre la crescita ed essere altamente pericolosa per la sopravvivenza delle piante. Inoltre, la ripartizione della biomassa, l'acquisizione di carbonio e l'utilizzo delle risorse di carbonio hanno una grande importanza negli studi di adattamento e acclimatazione. La plasticità fenotipica dello xilema o dell'apparato fotosintetico può svolgere un ruolo importante nell'adattamento di specie isoidriche pioniere come *Pinus sylvestris* ad ambienti nuovi/duri. Il modo in cui le piante modificano l'assegnazione della biomassa alle diverse aree di sviluppo del suolo, come le condizioni umide e secche, che influenzano questo coordinamento tra l'allocazione della biomassa allo xilema e gli aghi dei rami, è stato scarsamente studiato. In questo studio sperimentale, i pini silvestri maturi provenienti da 2 diverse condizioni ambientali che si trovano a poche centinaia di metri di distanza nelle Dolomiti italiane sono stati valutati per i loro tratti anatomici idraulici xilematici e le risorse di biomassa. Una delle aree di studio è un bosco misto maturo con elevata disponibilità idrica, l'altra è una pineta pura cresciuta su una frana (1814) con scarsa disponibilità idrica dovuta alle condizioni del suolo. In questo studio, abbiamo selezionato i rami apicali più alti a 150 cm dall'apice e selezionato diverse posizioni di campionamento a diverse distanze dall'apice del fusto in due gruppi di alberi. Abbiamo misurato l'area dell'anello annuale, il diametro idraulico dello xilema (Dh) e la biomassa fogliare (ago) (LM), la biomassa del ramo (BM), la massa fogliare (LM) e l'area di massa fogliare (LMA). I risultati sperimentali mostrano che i pini nell'area della frana hanno una crescita significativamente ridotta, ma con risultati anatomici xilematici contrastanti per il diametro idraulico, che mostrano condotti più piccoli nei primi 50 cm dall'apice del ramo e diventano più grandi in seguito. La biomassa dell'ago nelle due aree non è statisticamente diversa, con lo stesso modello di massa accumulata lungo l'asse del ramo. I pini a bassa disponibilità di acqua hanno la stessa densità stomatica dei pini ad alta disponibilità di acqua, ma hanno una massa fogliare per area (LMA) maggiore a causa di un numero maggiore di aghi più piccoli, più densi e più pesanti. Il pino silvestre è una specie isoidrica precoce che probabilmente chiude gli stomi per evitare un'eccessiva traspirazione a scapito di una crescita ridotta.

Parole chiave: *Pinus sylvestris*. L, Differenti condizioni del suolo, Anatomia xilematica, Allocazione della biomassa, Area fogliare.

1. INTRODUCTION

Human activities impact and influence the dynamics of terrestrial ecosystems, among other things, through anthropogenic climate change and changes in land use (Vitousek et al., 1997). Climate change encompasses global warming; as a result of global warming, the level of CO_2 and other greenhouse gases in the atmosphere rises along with the mean annual temperature (Sean et al., 2009) (Figure 1). As the temperature rises, plants are exposed to various environmental challenges (Okoro et al., 2019). Trees are particularly sensitive to climate change as they are relatively long-lived compared to other organisms and have a limited adaptive capacity to respond to rapid environmental change (Lindner et al., 2010). The impact of climate on tree development is most noticeable since temperature limits tree growth, and the growth of the pines is determined by temperature precipitation and sunshine duration (Petrov et al., 2015). Due to climate change, droughts, and heat waves are occurring more frequently and intensely worldwide.

Huming et al., (2022) stated abiotic stressors such as drought, salinity, and extreme temperatures. These stressors greatly limit plant distribution and alter plant growth and development. Drought is one of the environmental factors that have a significant effect on plant growth (Mahmoud et al., 2021). Drought also causes stomatal closure, which reduces transpiration (Silva et al., 2009). High temperatures triggered an increase in transpiration rate in plants, particularly under a water-stressed environment (Sadok et al., 2020).

Gas exchange mainly occurs through the stomata; water evaporating from the mesophyll cells diffuses into the atmosphere as water vapor, as CO_2 diffuses from the atmosphere into the mesophyll, where it is fixed by photosynthesis. In Plants, water is transported through xylem conduits under negative water potential due to forces of adhesion and cohesion (Tyree & Zimmermann, 2002). This negative water potential is created at the canopy level in leaves, where transpired water is needed to fuel gas exchange and photosynthesis. The water lost must be replaced and absorbed from roots to sustain more leaf transpiration (Kiorapostolou et al., 2020). The water flow rate throughout the root and xylem vessels is determined by the root pressure and transpiration rate (Elizamar et al., 2009).

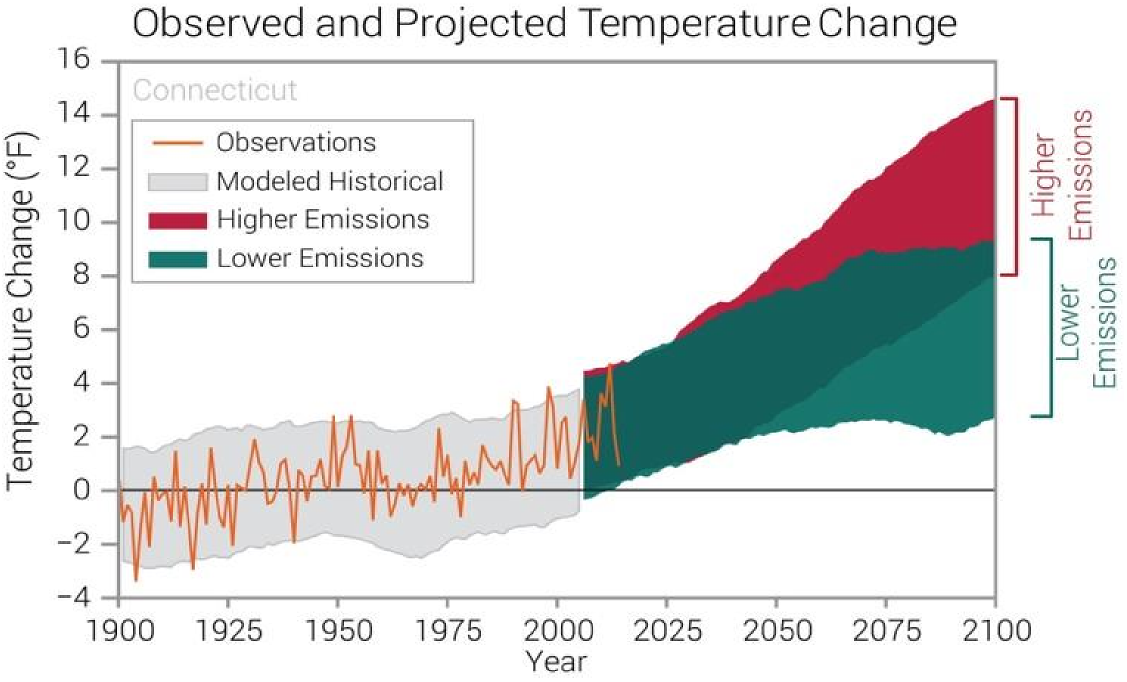

Figure 1: Source: NOAA State Summaries, 2019.

Temperature changes were observed and projected in Connecticut's air temperature (compared to the 1901-1960 average). Changes projected for 2006-2100 are based on global climate models for two possible futures: one in which greenhouse gas emissions continue to rise (higher emissions) and another in which greenhouse gas emissions rise at a slower rate (lower emissions) (lower emissions). The orange line depicts Connecticut temperatures. The shading represents the annual temperature range from the set of models. Unprecedented warming is expected to continue into the twenty-first century. Less warming is expected with lower emissions (the coldest years are about 3°F warmer than the long-term average; green shading) and more warming with higher emissions (the hottest years are about 10°F warmer than the hottest year in the historical record; red shading).

An increase in transpiration rate improves mineral element absorption and translocation in the xylem (Mengel & Kirkby, 2001). According to Darcy's law ($F = \Delta\Psi \times K$), the xylem sap flow rate (FX) is directionally proportional to the differences of water potential between leaves and roots ($\Delta\Psi = \Psi_{LEAF} - \Psi_{SOIL}$) and to the total xylem conductance (K) (Tyree & Ewers, 1991). Reduced water availability and differences in water potential between leaf-soil affect the long-distance transportation of water in plants and drought limits the long-distance transportation of water. Under drought stress, the tree's xylem and phloem efficiencies are essential to their survival, even when producing larger tracheids (Kiorapostolou et al., 2020). This might also make the xylem more vulnerable to xylem embolism formation. With the advent of the water regime, a highly conductive and efficient xylem is required to keep the rate of photosynthesis high (Tyree, 2003).

The ability of anatomical structure of the xylem facilitates the safe and effective upward water transport of the tree. Fibres, tracheid elements, and parenchyma cells are the three main cell types present in the mature xylem in the plant's vascular tissue (Stupianek et al., 2021). The parenchyma plays a crucial role in the storing and transporting of non-structural carbohydrates. When the xylem vessels are dead at maturity, and the parenchyma is alive (Morris et al., 2016; Hoch et al., 2003). The vessels impact the plant's hydraulic performance and have increased the diameter from tip to base (Olson et al., 2020). The hydraulic behavior of the entire transportation system is modulated by the diameter or size of the xylem hydraulic conduits (Petit et al., 2010). Wide and large conductive elements have the advantage of increased transport capacity and efficiency because, according to Hagen-Poiseuille law, the flow scales at the fourth power of the conduit diameter (Tyree & Zimmermann, 2002; Sperry et al., 2005). Small changes in xylem conduit diameter may result in great increases in the overall conductivity and efficiency of the system.

With increasing distance from the stem tip, the diameter of the xylem conducting conduits increases predictably. Even though widening the conduits aids in reducing the increased hydraulic resistance (Olson et al., 2020). Transverse conduits in the stem are bigger in the outer rings and smaller close to the pith. With the gradual increase in height, the axial conduits widen from the stem apex to the same tree ring, which helps to stabilize the hydraulic path resistance (Anfodillo et al., 2013). As a tree's height increases, more conduits are required to connect the absorbing roots to the transpiring leaves along the longer hydraulic path (Prendin et al., 2018). The diameter of vascular conduits grows larger as they approach the stem base. It has been proposed that the profile is an efficient anatomical feature for reducing hydraulic resistance as the tree grows taller. However, the mechanisms that control the cell diameter along the plant still need to be completely understood (Anfodillo et al., 2012).

The ability of trees to transport water and nutrients from the roots to the leaves decreases when soil moisture availability is low and there is a high vapor pressure deficit (atmospheric drought) (Gurien et al., 2020). It's also affecting the physiological process for necessary growth; due to low water availability, cavitation may occur inside the single conductive elements. Air bubbles may seed and spread to adjacent conduits, thus decreasing the total hydraulic conductance and water transport (Cohard, 2006). By maintaining a positive carbon balance and a transportation system that is not embolism-affected, it is possible to adapt to scarce water resources. The so-called "safety vs. efficiency" tradeoff was not observed across different species or environments, even though plants should benefit from a water transport system that is effective enough to support transpiration and safe from potential emboli formation (Gleason et al., 2016). Vulnerability to embolism was reported to be strongly related to vessel/ tracheids size (Pittermann et al., 2006; Hacke et al., 2006) and pit properties (Becker et al., 2003; Lens et al., 2011; Lazzarin et al., 2016).

Tree survival is based on maintaining a positive carbon balance, the difference between carbon intake through photosynthesis and carbon utilized for all physiological functions such as growth and respiration (Kiorapostolou et al., 2018). Phenotypic plasticity and acclimation might be achieved not only by changes in the hydraulic structures. Photosynthetic biomass and carbon allocation are essential in endurance and survival during stressful conditions because, depending on the balance of carbon acquired and used, plants start to utilize C reserves (McDowell et al., 2008, 2011; Sala et al., 2010).

The tree's survival depends on preserving a positive carbon balance (Kiorapostolou et al., 2018). which differs from the amount of carbon assimilated through photosynthesis and the amount of carbon used for all physiological processes and metabolism in trees, such as transpiration, growth, and allocation (Kotowska et al., 2020). The continuous depletion of stored carbon may lead to carbon starvation, strictly bonded with hydraulic failure (inability to deliver water to stomata for transpiration), causing plant death (McDowell et al., 2020). One major differentiation between species is given by their stomata regulation. Pine, which is a relatively isohydric species, closes its stomata early in the day and supposedly protects the hydraulic transport system from embolism, but at the cost of possible usage of stored carbon, which may lead to carbon starvation in case of prolonged drought (McDowell et al., 2008).

According to recent studies, the xylem in drier environments is safe and resistant. The same species in environments with more water availability have larger conduits but are more susceptible to embolism (Pfautsch et al., 2016; Schuldt et al., 2016; Larter et al., 2017). Anatomical analysis of tree cores shows that the production of xylem cells and their size at the trunk base is strongly reduced in dry environments (Castagneri et al., 2020). On the other hand, some studies using various sampling approaches reported increased efficiency and larger conduits under drier conditions (Petit et al., 2016; Guérin et al., 2018a; Kiorapostolou & Petit 2018).

Moreover, other specific studies on conifers and isohydric species displayed no xylem plasticity towards either safety or efficiency under drought (Sancho et al., 2017, Petit & Zambonini et al., 2022). Commonly, punctual anatomical analysis (fixed for age or internode) does not consider the axial widening pattern of the hydraulic structures. Multiple studies have reported and confirmed that xylem conduits increase from the tips of branches in the crown to the base of the trunk and that this conduit widening is the primary cause of anatomical variation within a single tree (Anfodillo et al., 2013; Olson et al., 2014; Lazzarin et al., 2016).

In both the xylem and phloem, the hydraulic diameter increased from the stem apex to the base. The phloem transports the sugar solution produced by photosynthesis to all living tissues, which is required for cell respiration and growth (Petit et al., 2014). The development of the hydraulic and mechanical properties was similar and had a positive relationship with one another. By maintaining the biomass allocation to the leaves and adjusting their growth rate and xylem production to maintain xylem conductance, trees in different climates adjust their functional balance between water transport and leaf transpiration (Petit et al., 2018). Water makes up 80-95% of the fresh biomass of the plant body and plays an important role in numerous physiological processes, including plant growth, development, and metabolism (Brodersen et al., 2019).

Functional and structural traits, such as conduit diameter, have been shown to reflect allometric relationships, which usually follow the power scaling functions of Equation 1 Y = a * Xb. In this equation, X and Y represent the functional and structural traits, while a and b are the allometric constant and the scaling exponent, respectively. It was proposed in the theory of optimality principles (West et al., 1999) that these trait relationships should converge towards a common scaling exponent, which exhibits the fundamental balance between structure and function in the view of a stable and positive carbon balance (Anfodillo et al., 2016). Eventual differences in the allometric constant "a" could be explained by different species strategies or changes in the absolute proportion of traits within the same species in different environments (Weiner, 2004). (e.g., needle biomass over branch biomass).

According to Leyre Corcuera et al., (2011), the *Pinus sylvestris* population from mesic origins demonstrated a high responsiveness and adaptation to drought circumstances, as well as a high wood density and growth. This research is being conducted in two study areas in the Italian Dolomites. Both study areas are only a few hundred meters apart and have similar sun exposure, air temperature, and rainfall/snowfall patterns. The main difference is due to a landslide in 1814, on which Scots pine (*Pinus sylvestris* L.) started to colonize after a few decades. The soil in the landslide study area has a low organic matter horizon and limited plant water retention and availability.

Problem statement

It is hypothesized that Scot's pine trees successfully coexist in different soil developmental areas like dry and wet soils. We are analyzing for similarities and differences in their anatomical structures, especially in the xylem conduits-hydraulic diameter in apical parts of Scot's pine trees and also how they are allocating the carbon, differences in biomass allocation led to morphological changes in the trees, when trees may produce effective and low C cost and anatomical adjustments in the xylem close to the treetop, where most of the hydraulic resistance is concentrated.

Based on the work of Kiorapostolou & Petit et al., 2018 & 2020. Suggested that anatomical analyses in the central understanding of the selected trees synthesized, stored, and allocate biomass even under drought conditions. And produces more conductive xylem to compensate for the drought-related hydraulic limitation to the gas exchange. Moreover, if trees produce fewer, larger tracheids, they are more vulnerable to xylem embolism. Beyond this, will biomass allocation be the same in the trees living in good water retention sites and changes in their morphological and anatomical xylem characteristics?

Objectives

The general objective of this research is to examine the differences and similarities in functional xylem anatomy and biomass allocation patterns of Scot's pine species present in the two different soil developmental areas, with specific objectives:

1. To analyze the similarities and differences in the xylem anatomy, particularly in the ring area and the hydraulic diameter of the xylem conduits of Scot's pine trees in different areas.
2. To quantify the biomass allocation of the branch and needles from the distance from the apex.
3. To determine differences or modifications in xylem anatomical patterns along the main axis.

2. MATERIALS AND METHODS

2.1 Study Area

San Vito di Cadore is a small town and commune in the province of Belluno in the Italian region of Veneto, nestling at the foot of majestic peaks in a broad green valley in the heart of the Belluno Dolomites. Behind it is Antelao, in front of Pelmo, both included in the list of UNESCO World Heritage. Situated above 1.011 meters above sea level, latitude and longitude is 46º27’05.1’N 12º12’55.8’E. (Fig 1). A mature mixed forest serves as the control stand, and the other stand, which is growing on an 1814 landslide, is entirely made up of *Pinus sylvestris* (L.) trees. The Koppen-Geiger classification is Dfb, with an average yearly temperature of 3°C and 1885mm of precipitation. The landslide soil has reduced water availability and organic matter. The area enriched meadows, coniferous and broad-leaved mixed forests, dominated by the presence of forest tree species Norway spruce, larch, Scots pine, and silver fir.

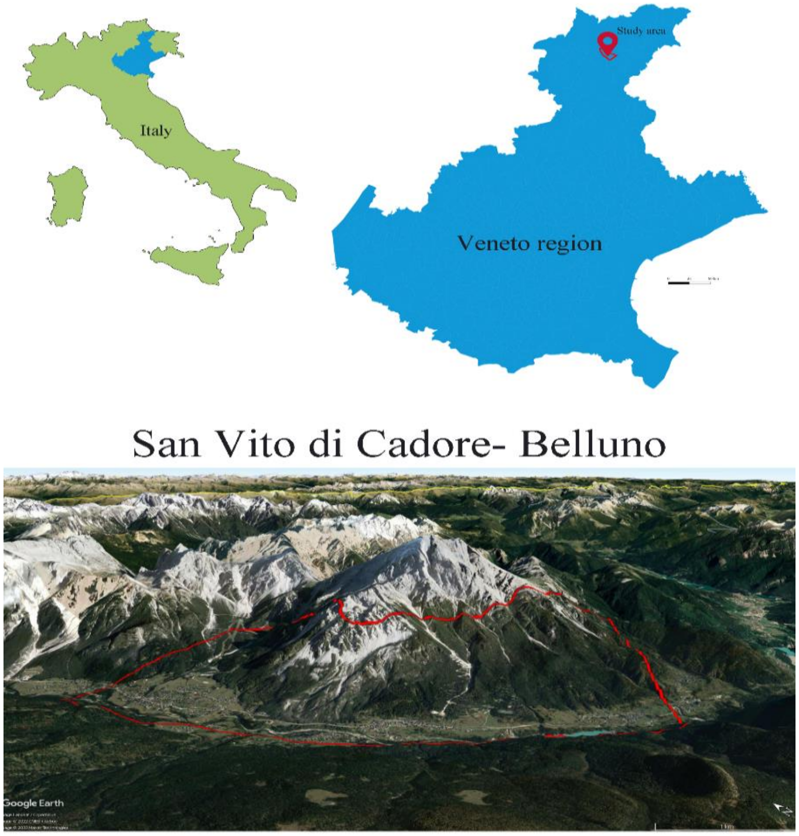

Fig 2.1 The map of the San Vito di Cadore, Belluno, Italy.

2.2 Species description

The most widespread species of the genus Pinus, the Scots pine (*Pinus sylvestris* L.) present from the subalpine zone through the boreal, temperate, and subtropical climate zones in a significant portion of the northern hemisphere (Milberg et al., 2015). It grows to an average height of 23–27 m, a diameter of 50–80 cm, and an age of 200–300 years. The needles are embedded stomata, and thick-walled epidermis with a wax coating that protects the needle from water loss, and these two features help the needles adapt to cold and drought (Krakau et al., 2013). From an economic point of view, wood is one of the important softwoods and is easily workable, having an excellent strength-to-weight ratio. In addition to being used for furniture, pulp, and paper, it is primarily used as building and construction timber. Due to its tolerance for poor soils, it is commonly used for land reclamation and to bind loose sands. It is frequently used as a Christmas tree in America. Because it has a long lifespan and typically grows in unfavorable environments where even little changes in temperature or moisture can significantly impact its growth (Durrant et al., 2016). The widespread Scots pine (*Pinus sylvestris* L.) is a colonizing gravel species and recently formed limestone-dolomite debris. The transition from the pine forest to the spruce forest is somewhat hampered by the aridity of the soil that defines the slopes of the left bank of the Boite, even though this succession sometimes happens inexorably at lower altitudes.

2.3 Sample collection

The samples were collected in April 2022 in San Vito di Cadore, Italy. The study area was divided into two sites called wet and dry areas, classified based on the morphological characteristics of tree species. In these two sites, the trees are well-grown in the wet region and reduced growth in the dry area. In each site, five trees are taken into account, and cut one branch at each tree. The branches were chosen as they are apical and facing southward and range in length from 50 cm to 150 cm. The samples from the wet sites were labeled as W1, W2, W3, W4, and W5. In Dry areas, D1, D2, D3, D4, and D5, respectively (Fig 1.2). We removed and weighted a 2cm disc from each sample for anatomical analysis, at each internode or sampling position, it was assigned its length and calendar year. Since the annual increments of low water availability pines are limited and not easily recognizable by internodes, for these trees, the annual elongation increment (ΔL) and corresponding distance from the apex (DFA) at the year of formation was estimated by the difference in the number of rings in the xylem between two sampling points.

The needles are separated from the branches. The leaf (needle) of almost 25 needles of each segment was measured with a precision balance calibrated to the nearest 0.1 g (Acculab ALC-1100.2), needles were scanned with an EPSON GT-20000 scanner (Seiko Epson Corporation), and the needles were analyzed with the aid of IMAGEJ software. Afterward, the needles are dried for 48 hours at 60 °C in an oven. In the branch, a sampling point was selected at approximately 1cm from the base of each internode, and their distance from Apex (DFA) was measured. The stem segments were extracted at each sampling point and were then conserved for anatomical analysis. After drying for 24 hours at 72º C in an oven, the dry material was weighed to obtain the remaining branch (wood) and needle biomass. The leaf area, wood biomass, and needle biomass were measured down the apex, and their sums were taken from the apex as they were cumulated to each sampling point.

Fig 2.2. (a) Wet site: Soil with good water retention and availability. (b) Dry site: Low soil water availability.

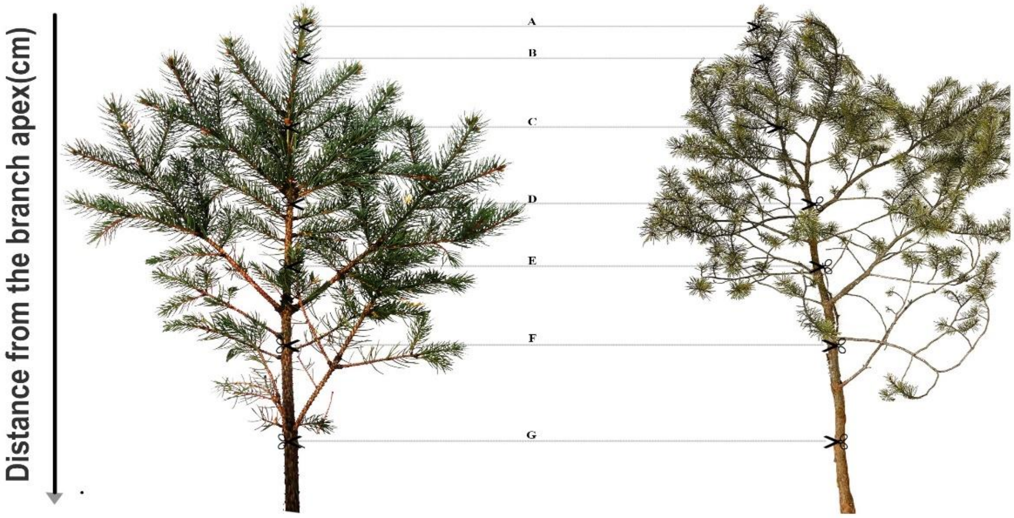

Fig 2.3 A Scheme of twig selection and Data Construction

(A, B, C, D, E, F, G – Samples are pointed on the branches.)

In April 2022, samples were soaked in water and about 50% ethanol solution to soften them and prepare them for cutting. The water and alcohol solutions were constantly changed to prevent the growth of molds in the samples. Samples were cut using a rotary microtome LEICA RM 2245 (Fig 2.4) (Leica Biosystems, Nussloch, Germany) at 10-15µm. The transverse micro section was stained with the solution of safranin (1 and 0.5% in distilled water, respectively) and Astra blue. Stained sections were rinsed with water, 50% ethanol solution, and then 99% ethanol; the samples were dried with absorbing paper and fixed with Eukitt (Bio Optica, Milan, Italy) on permeant slides. Sections were placed in the oven at 60°C for 12 hours for drying. To obtain clear microscopic images, the microscopic slides were thoroughly cleaned of dried Eukitt by scraping them with old blades and wiping them with a clean lens towel.

Fig 2.4 Rotary microtome used for sample preparation (Leica RM 2245, Leica Biosystems, Nussloch, Germany).

Fig 2.5 Axio Scan 7. (Zeiss, Jena, Germany) High-performance Slide Scanner for Fluorescence, Brightfield, and Polarization Scanner.

2.4 Stomatal analysis

The needles from both dry and wet samples are collected, the ventral side of the fresh needles applying wax along the lengths give some time to fix and the tapes are used to take the impression of the stomata, and the tapes are pasted on the microscopic slides, after some time the tapes are removed. The microscopic slides are scanned with the help of Axio Scan 7. (Zeiss, Jena, Germany), with pixels of 2.892982781 resolution per micron. The microscopic slides are analyzed with the help of ImageJ software.

2.5 Xylem Anatomy

The scanned images obtain through Axio Scan 7. (Zeiss, Jena, Germany) High-performance Slide Scanner for Fluorescence, Brightfield, and Polarization with a resolution of 2.27 resolution per micrometer and a 100x magnification (Fig. 2.5). The picture analysis was conducted using ROXASn3.0.1 (von Arx & Carrer., 2014). a program that analyzes images and assesses several xylem properties. The images obtained for each slide were then analyzed with Ifranview 4.58 (Irfan Skilijan) to convert them to jpg files. Xylem anatomical analysis was performed on a wedge of known angle (α) (70º-120º) centers at the pith for each image and later analyzed using ROXAS 3.0.1 (von Arx and Carrer 2014), totaling 90 images of different segments run with Roxas as a batch run. After running the samples in batch mode, each was manually edited on ROXAS. The procedure was carried out to improve the results' quality and get rid of any picture flaws (von Arx et al., 2016). Broken cells, split cells, connected cells, numerous borders, outlier borders and cells, xylem pits, and resin ducts were specifically corrected. After a thorough examination, annual rings are manually drawn to assist in the anatomical characterization of interannual differences in the samples. At the ring level, Roxas automatically measured the following quantities (i) Ring Area, RA (μm^2); (ii) Mean hydraulic diameter, *Dh* (µm), which was calculated by ($Dh = \sum d5/\sum d4$, where d is the conduit diameter); (iii) Theoretical hydraulic conductivity, *Kh* (kg.m^{-1}s^{-1}MPa^{-1}) which was calculated according to Hagen-Poiseuille (Tyree & Ewere, 1991)

$$Kh = \frac{\pi \times \sum d4}{128 \times n \times l}$$

Where *d* and *l* are, respectively, the diameter and length of a given vessel, n is the water, and (iv) specific hydraulic conductivity *Ks* ($Kg\ m^{-1} S^{-1} MPa^{-1} g^{-1}$) was calculated by dividing *Kh* by xylem cross-sectional area.

Table 2.1 Acronym and description of variables used in this study

Variables	**Description**	**Function**	**Units**	**Reference**
RA	Ring Area	Growth	(μm^2)	
DFA	Distance from Apex	Descriptive	(cm)	
Dh	Hydraulic Diameter	Hydraulic efficiency	(µm)	(Kolb and sperry 1999)
LA	Leaf Area	Metabolic Functions	(cm^2)	
BM	Branch Biomass	Growth	(g)	
LMA	Leaf Mass Area	Growth	(gcm^2)	

Statistical analysis

Statistical tests were performed for differences in the trees from the two different water availability sites for several allometric scaling relationships. We used linear mixed-effects models that were fitted using maximum likelihood (REML). For statical analysis, the package lme4 (Bates et al., 2015) of the software R (R Core Team., 2022) was used. Our data were first log10 transformed to account for the assumptions of normality and homoscedasticity (Zar, 1999). Using the tree ID as a random factor in all initial models, we investigated the fixed effects of the treatment (TE/CO) and distance from the apex (DA), as well as their interaction for each target attribute. The maximum likelihood approach was used to select the ideal model based on the Akaike Information Criterion (AIC) (Zuur et al., 2009).

3. RESULTS

The Akaike information criterion is followed by all model results that are provided here (AIC: Burnham & Anderson, 2002). One with an attraction and the other without is one of the two models created for this experiment based on subject interest. The models are examined for patterns of biomass distribution and anatomical variations between species found in wet and dry environments. The distance from the apex (*DFA*) was the fixed factor for the models comparing the anatomical differences between the species in two distinct areas. The ring area (*RA*), leaf area (*LA*), and conduit diameter (*Dh*) were the dependent variables. The distance from the apex (*DFA*), cumulative needle biomass (*LM*), and branch biomass (*BM*) were the fixed effects in the model created for biomass allocation.

3.1 Anatomical comparisons

3.1.1 The ring area along with the branch axis (RA-DFA)

The Ring area, referred to as a plant anatomical indicator of growth, was noticed to increase from the apex down the branch. Figure 3.1 shows that the ring area increases, in dry and wet sites, increasing the distance from the apex. Eventually, the ring area grew as the distance from the apex increased. Compared to the dry environment, wet trees grow more quickly and have larger rings, and grow fast. The trees in the dry areas are trees are smaller, grow more slowly, and have more rings, although the rings are very small. In both circumstances, the ring area steadily increases with the distance from the apex.

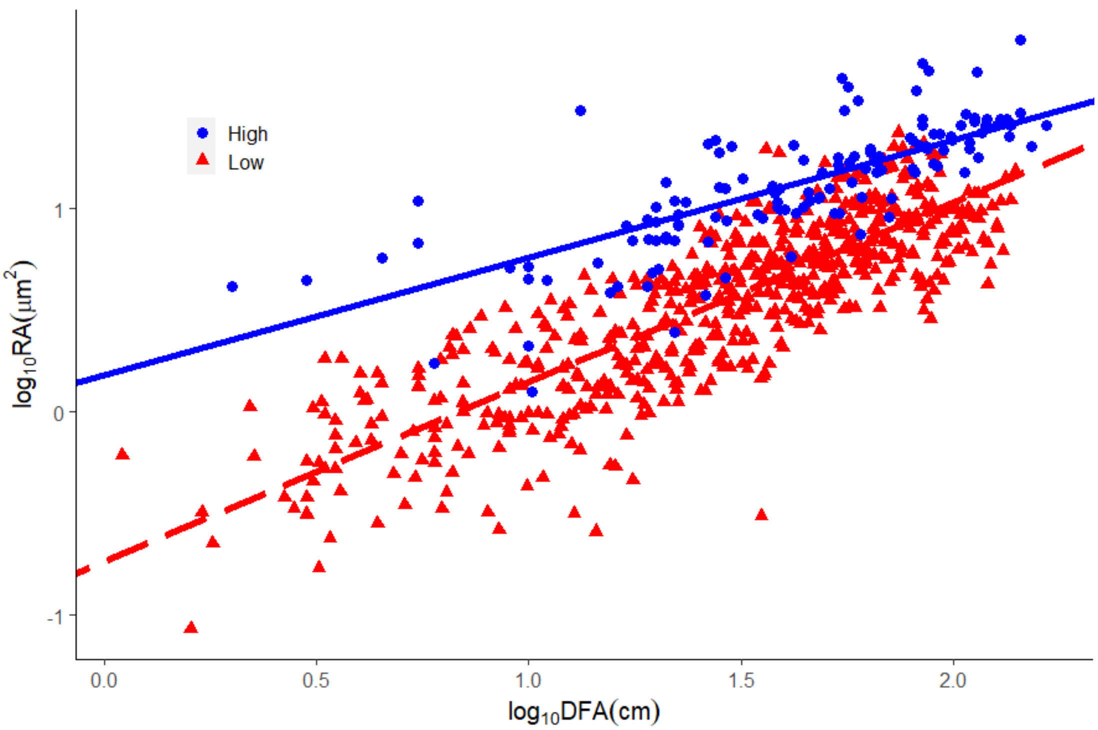

Fig 3.1 Relationship between Ring Area RA(μm^2) and Distance from the Apex DFA (cm).

Variation in ring area (RA) with the distance from the branch apex (DFA) in High water availability (filled blue circles) and Low water availability (filled red triangles). Fitting lines (continuous for High, long dash for Low) are according to the models in Table 3.1.

3.1.2 Hydraulic diameter along with Distance from the apex

Ring data for xylem anatomy time frame from 2021 to 1954 in trees from the landfall site and from 2021 to 2014 in trees from the high-water availability site. The graphic uses data to show how the hydraulic diameter and the distance from the apex in *Pinus sylvestris* trees growing in dry and wet areas relate to one another. The anatomical data from each ring are then assigned to the corresponding DFA at the year of formation. Hydraulically weighted average conduit diameter (Dh) for both areas shows a clear pattern of increase along the axial gradient from tip to branch base. In the initially wet sites, trees have bigger conduits than the dry area trees. When moved down, the dry samples have bigger conduits than the wet samples. The statistical models show significant differences in both y-intercept and slope (Table 3.1), with pines from the "low" area increasing their conduit diameter with an isometric scaling of the b exponent of 0.2. For "high" trees, the conduit widening along DFA follows a gentler increase from apex to branch base with a scaling exponent of 0.13.

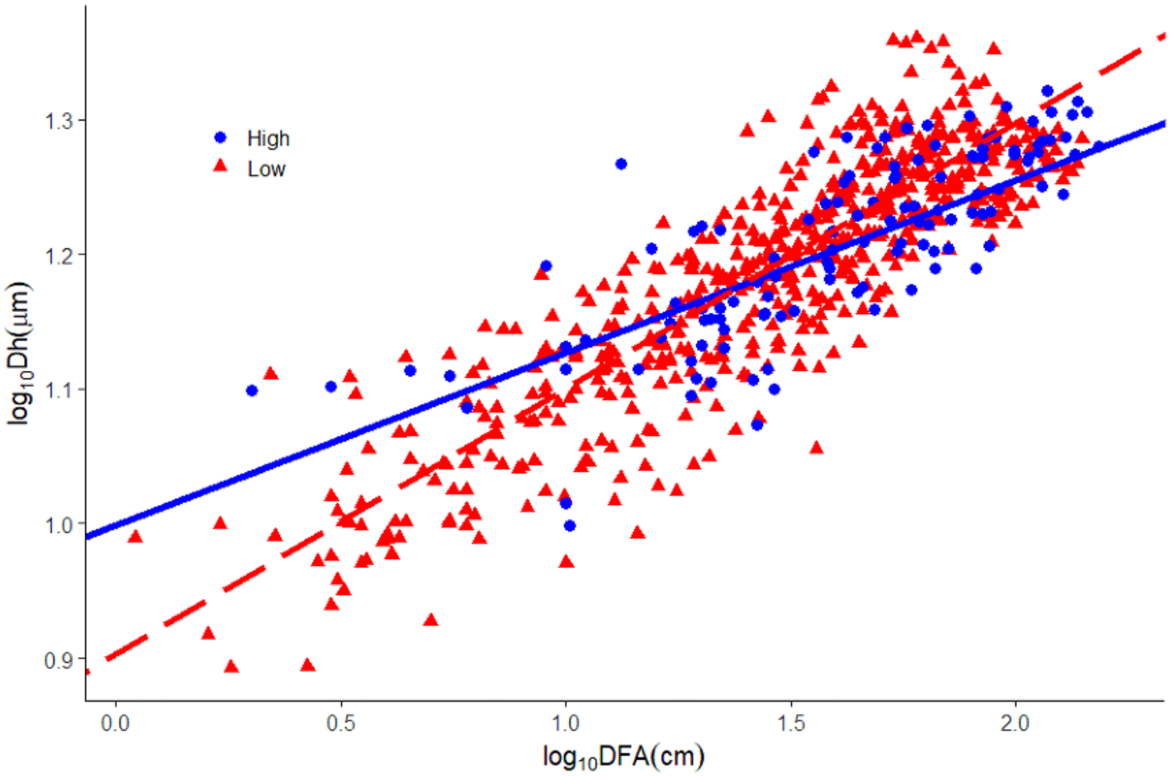

Fig 3.2 Hydraulic Diameter Dh (μm) and the Distance from the Apex DFA (cm).

Table 3.1 Variation in conduit hydraulic diameter (Dh) with the distance from the branch apex (DFA) in High water availability (filled blue circles) and Low water availability (filled red triangles). Fitting lines (continuous for High, long dash for Low) are according to the models in Table 3.1.

3.1.3 Comparison of hydraulic diameter in wet and dry areas

Larger or smaller conduits could influence the water flow throughout the plant, and sometimes even minimal changes in the hydraulic diameter of a conduit can result in significant variations in flow. The results in Figure 3.3 are important in this case. Comparing the trees between the dry and the wet regions, as commonly done in ecological studies, the trees in this dry area have smaller conduits. In the wet area, trees have large conduits.

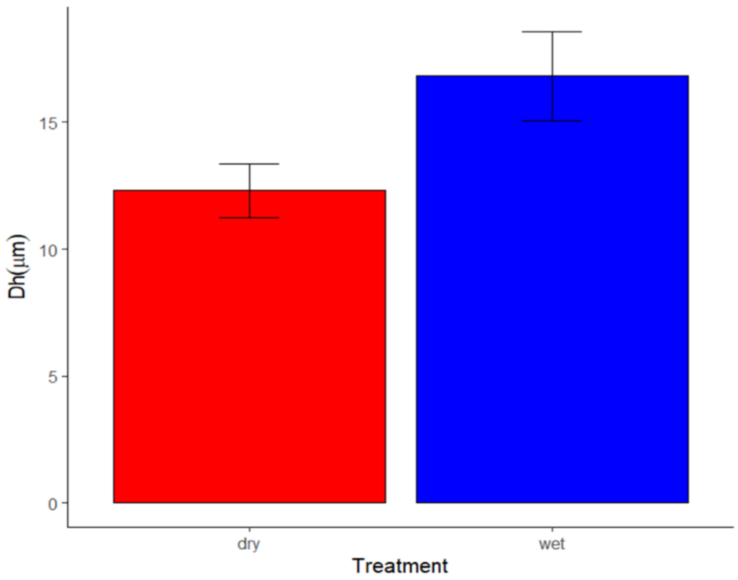

Fig 3.3 Hydraulic diameter Dh (µm) and Treatments of Wet and Dry area at the age of 3 years.

3.1.4 The average distance from the apex dry and wet areas of trees

Figure 3.4 depicts the three-year average growth of the trees based on their distance from the apex. The tree in the dry region has expanded by almost 7 cm during the past three years. The wet area trees, on the other hand, have risen 60 cm in the last three years The growth of the wet area trees is approximately nine times greater than the growth of the tree in the dry region.

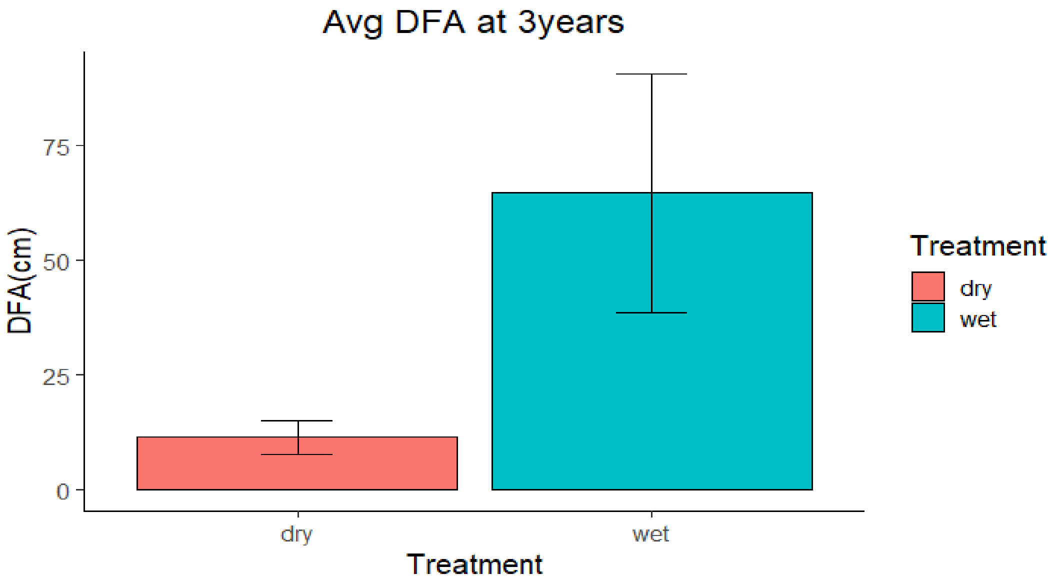

Fig 3.4 Relationship between the Average Distance from the Apex DFA (cm) in the last three years in both Wet and dry Regions. (Welch Two Sample t-test)

3.2 Carbon allocation

3.2.1 Needle biomass allocation and distance from the apex (Leaf biomass – DFA)

Along with DFA, the total leaf mass increased, accumulating from the branch apex along the main branch axis (LM) (G. Petit et al. 2022). Figure 3.5 displays data from both dry and wet locations, with needle biomass being significant. The stable increase in needle biomass is similar and consistent for both study sites, with no statistically significant differences in slope or intercept in the models (Table 3.2).

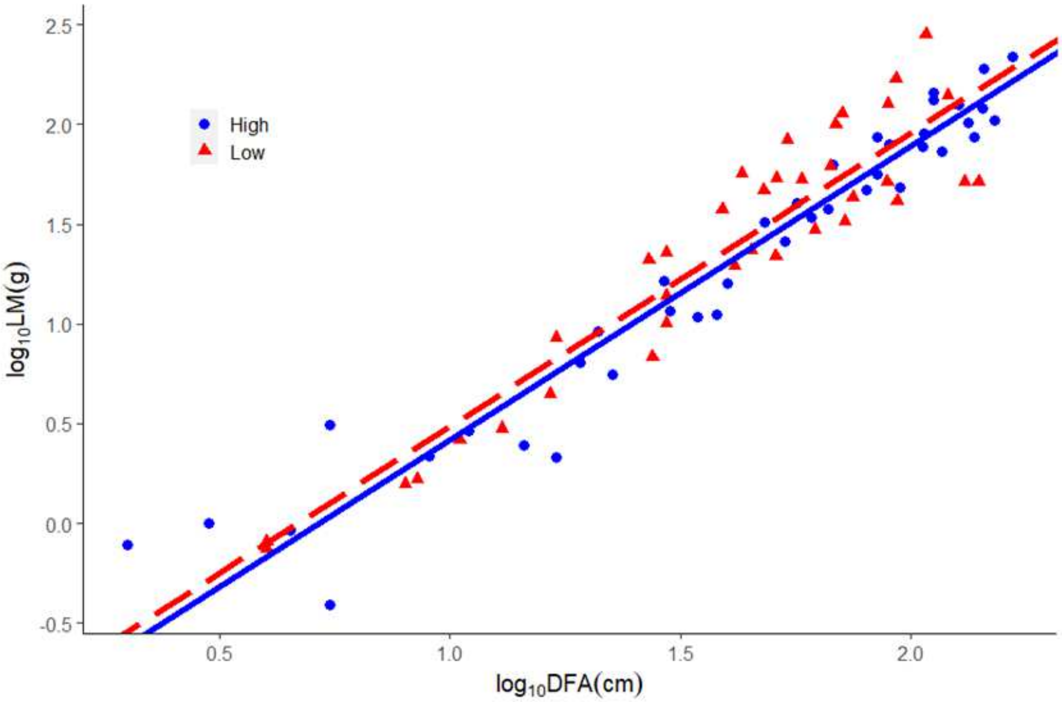

Fig 3.5 The relationship between the Leaf Biomass LM (g) and Distance from the Apex DFA (cm).

Table 3.2 Variation in needle biomass (LM) with the distance from the branch apex (DFA) in High water availability (filled blue circles) and Low water availability (filled red triangles). Fitting lines (continuous for High, long dash for Low) are according to the models in Table 3.2.

3.2.2 Branch biomass allocation from distance from the apex.

There is a significant difference for both the y-intercept and slope of the branch woody biomass (BM) that was cumulatively calculated as a sum starting from the branch apex along the axis (DFA) (Figure 3.7, Table 3.2). However, the biomass of the branches increases with distance from the apex. At the apex, trees from the wet region have greater branch biomass than those from the dry region. Moving downwards from the top, the results are inverted, with the dry region trees having greater biomass than the wet area trees, as the distance from the apex increases, the biomass also increases gradually. Trees from the two study areas show similar allometric scaling, increasing BM over DFA.

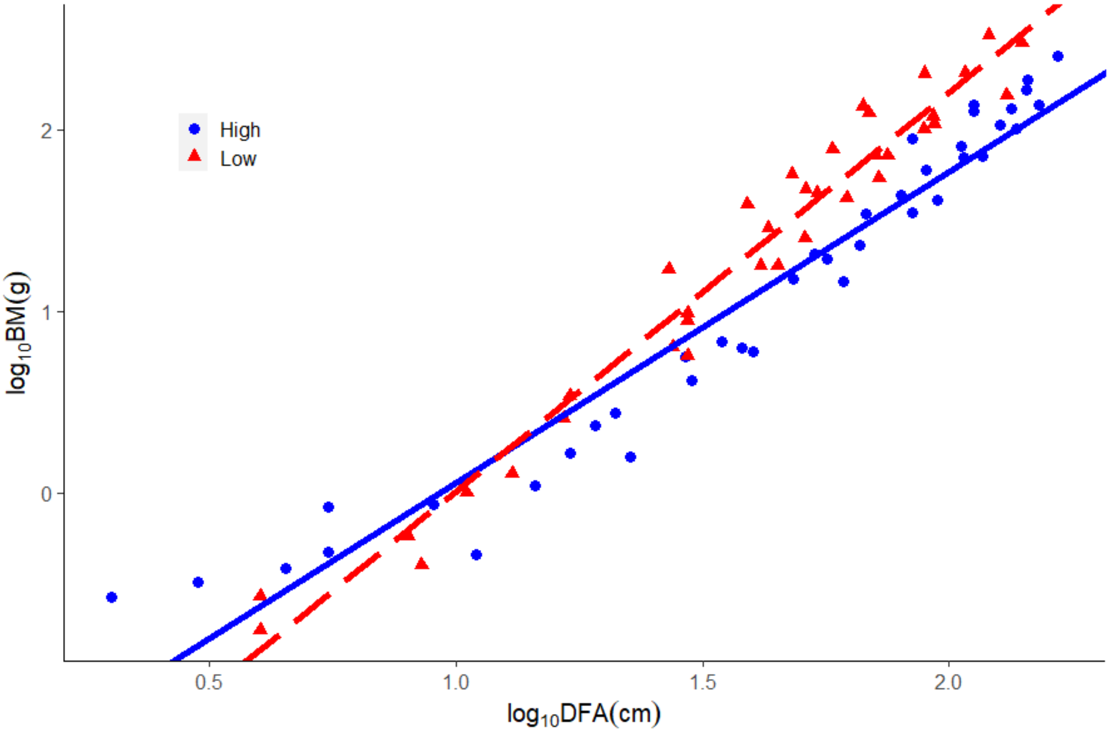

Fig 3.7 The Branch biomass BM (g) and Distance from the Apex DFA (cm).

Table 3.2 Variation in branch wood biomass (BM) with the distance from the branch apex (DFA) in High water availability (filled blue circles) and Low water availability (filled red triangles). Fitting lines (continuous for High, long dash for Low) are according to the models in Table 3.2.

3.2.3 Branch Biomass and Leaf Biomass

The biomass of the branch and needle of the trees is clearly shown in Figure 3.8 to be increasing from the apex to the base. In both dry and wet locations, it was also tested the allometric relationship expressing the biomass partitioning of branch biomass (BM) supported by needle biomass (LM) and the results of the model show statistical differences between the two study areas. According to models with a higher y-intercept and a lower slope, the branch biomass accumulated in trees from the low water availability site showed higher values at the apical-most points of the branches and lower values at the branch base when compared to the "high" site (Table 3.2). Usually, the biomass of wood is always greater than the needles.

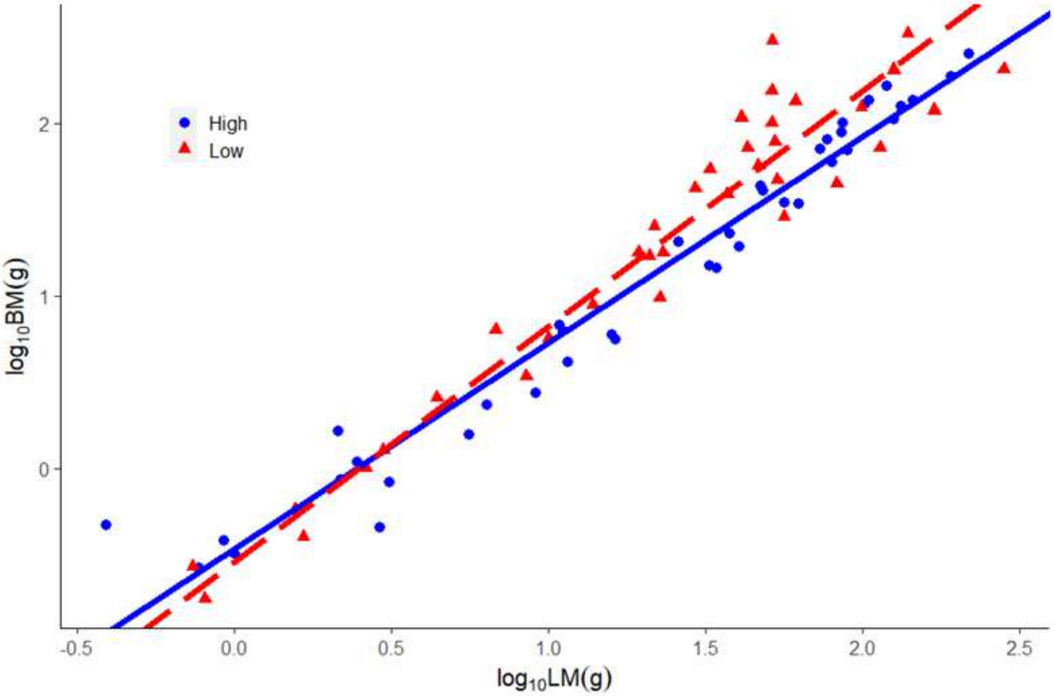

Fig 3.7 Branch biomass BM (g) and Leaf Biomass LM (g).

Table 3.2 Variation in cumulated branch wood biomass (BM) with the cumulated leaves biomass (LM) in High water availability (filled blue circles) and Low water availability (filled red triangles). Fitting lines (continuous for High, long dash for Low) are according to the models in Table 3.2.

3.2.4 Leaf Mass and Leaf Area

The leaf mass per area (LMA) of the trees from the "low" site and the trees from the "high" site differs significantly. Low water availability trees display higher LMA (Figure 3.8) due to an increased number of smaller, heavier needles. The trees are grown in dry locations to have more leaf mass area than the plants in wet areas.

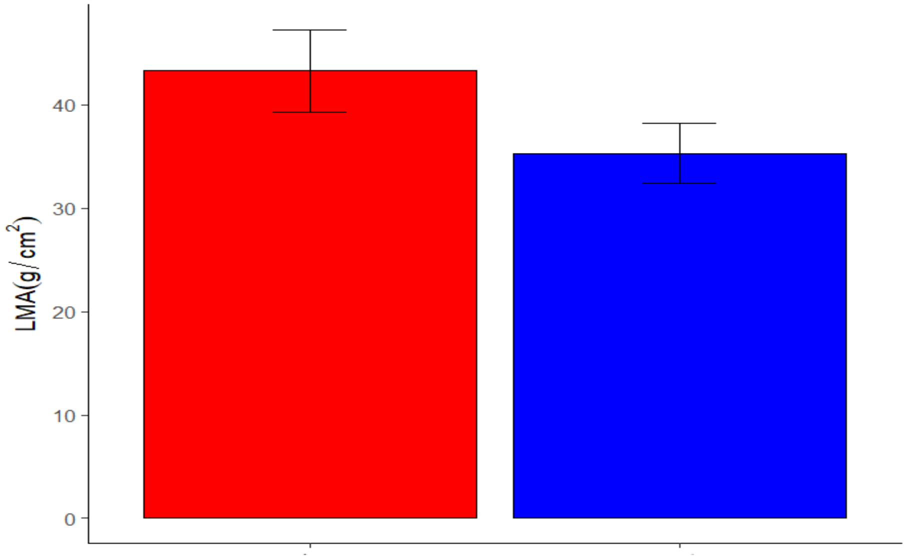

Fig 3.8 Relationship between Leaf Mass Area LMA x Leaf Area (g/cm^2) in dry and wet region trees.

Table 3.2 Differences in leaf mass per area (LMA) in High water availability (blue) and Low water availability (red). The Welch Two Sample t-test t = 3.5913, df = 7.3071, p-value = 0.008226**

3.2.5 Stomatal analysis

Figure 3.10 shows that the stomatal density of both dry and wet areas did not show significant statistical differences.

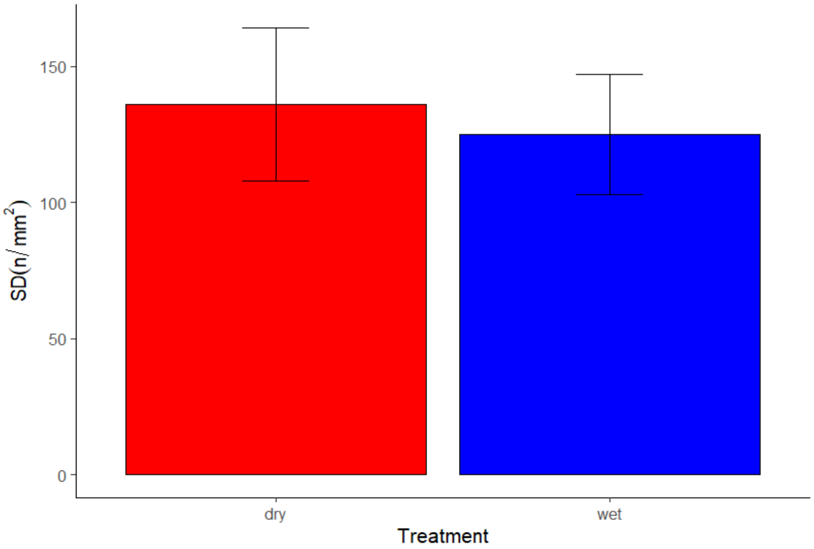

Fig 3.9 The stomatal density SD(n/mm^2) of the needles in both wet and Dry areas.

Table 3.2 Differences in stomatal density (SD) in High water availability (blue) and Low water availability (red). The Welch Two Sample t-test t = 3.5913, df = 7.3071, p-value = 0.5091.

Table 3.1 Results of the optimal linear mixed-effect models predicting the effects of log10DFA and Site on (A) log10Dh, (B) log10RA Tree ID was used as a random factor in all models. Asterisks indicate different estimates with significance at ** = p < .05 or at * = p < .1.

	(A) log10(Dh)		(B) log10(RA)	
Predictors	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	1	<0.001	0.18	0.054
DFA [log10]	0.13	<0.001	0.58	<0.001
Site [Low]	-0.1	<0.001**	-0.92	<0.001**
DFA [log10] * Site [Low]	0.07	<0.001**	0.3	<0.001**
Random Effects				
σ2	0		0.05	
τ00	0.00 Tree_ID		0.01 Tree_ID	
ICC	0.21		0.17	
N	10 Tree_ID		10 Tree_ID	
Observations	700		731	
Marginal R2 / Conditional R2	0.746 / 0.798		0.753 / 0.794	

Table 3.2 Results of the optimal linear mixed-effect models predicting the effects of log10DFA and Site on (A) log10BM, (B) log10LM .(C) is the model log10BMvsLM. Tree ID was used as a random factor in all models. Asterisks indicate different estimates with significance at p < .05 or at p < .1.

	log$_{10}$(BM)		**log$_{10}$(LM)**		**log$_{10}$(BM+LM)**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	-1.66	**<0.001**	-1.06	**<0.001**	-0.47	**<0.001**
DFA [log$_{10}$]	1.71	**<0.001**	1.47	**<0.001**		
Site [Low]	-0.53	**0.003****	**0.003****	0.55	-0.07	0.58
DFA [log$_{10}$] * Site [Low]	0.49	**<0.001****				
LM [log$_{10}$]	**1.2**	**<0.001**				
LM [log$_{10}$] * Site [Low]	**0.17**	**0.002****				
Random Effects						
σ2	0.04		0.02		0.03	
τ00	0.01 Tree_ID		0.03 Tree_ID		0.03 Tree_ID	
ICC	0.14		0.53		0.51	
N	10 Tree_ID		10 Tree_ID		10 Tree_ID	
Observations	76		76		76	
Marginal R2 / Conditional R2	0.949 / 0.956		0.904 / 0.955		0.940 / 0.970	

4. DISCUSSION

In this study, we analyzed the xylem anatomy and carbon allocation on branches of Scot pine trees in two different water availability sites due to a landslide that created different soil conditions. The pioneer species *Pinus sylvestris*, which is isohydric, dominated and colonized temperate continental Europe (Pretzsch et al., 2014). The objective of the study was to test if successful acclimation to low water availability conditions could be achieved utilizing phenotypic plasticity traits. Power scaling relationships between the two populations were tested to determine whether plasticity was brought about by a change in the absolute proportions of the allometric constant (a) or scaling exponent (b). These relationships were checked for (i) biomass allocation patterns for branches and needles and (ii) differences or modifications in xylem anatomical patterns along the main axis.

Xylem anatomy

The anatomy of the xylem, the ring area, often known as the plant anatomical indicator of growth, increased from the apex down the branch (Prendin et al., 2018). The annual allocation into xylem biomass in both declining and non-declining trees exhibited an axial scaling relationship between the xylem ring area (RA) and the distance from the stem apex (DFA) increased (Kiorapostolou & Petit, 2018). Similarly, our anatomical analyses of the ring area at the apex of the branches in two different environments, such as wet and dry, show that the ring area is the increased distance from the apex. Interestingly the ring areas are significant in both environmental conditions. The trees on the wet site have a larger ring area than the dry area (Fig 4.1). It has been established that trees grow more quickly and have larger rings in wetter areas. In dry regions, the trees have small ring areas and the number of rings is higher due to the soil drought conditions, as tree height (and the corresponding hydraulic limits) cannot decline, at least not without apical dieback.

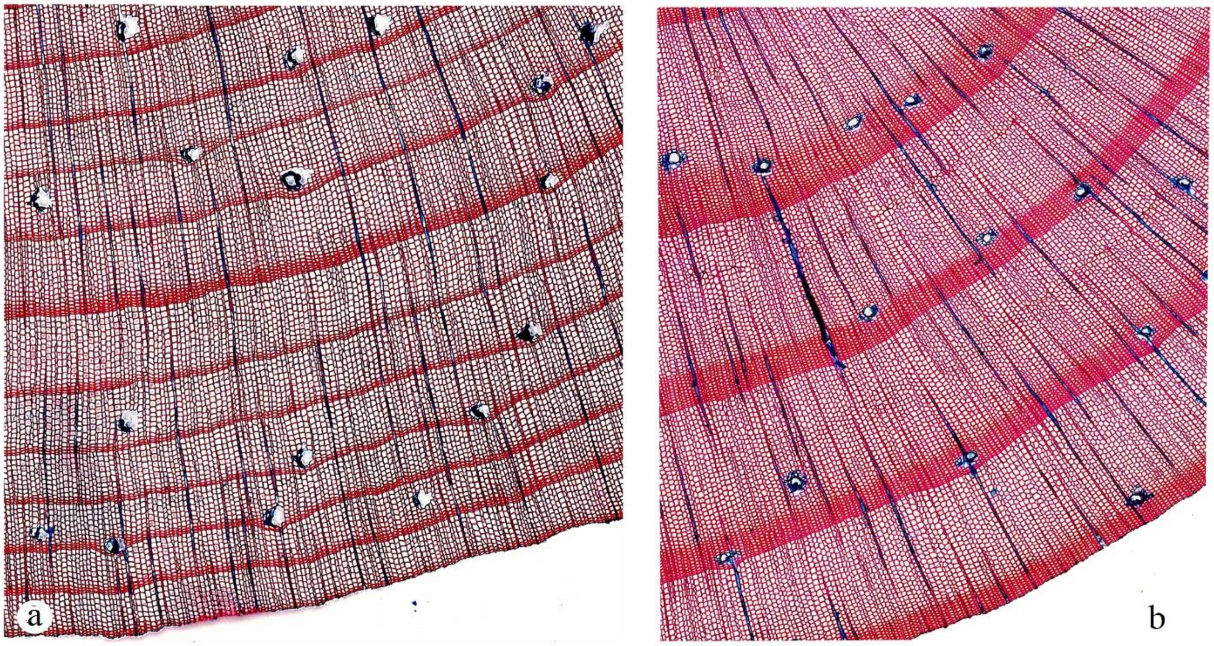

Fig 4.1. a) Anatomy Sample from Wet site (Distance from the Apex = 50 cm). b) Anatomy Sample from Dry site (Distance from the Apex = 50 cm).

There is a strong possibility that the observed anatomical changes at the apical stem in taller trees greatly influence the available carbon and hydraulic economy in trees (Olson et al., 2014). Additionally, they align with a previous study that found that tree height increased with an increase in conduit diameter at the stem apex. Petit & Anfodillo (2009), accounting for the stem apex, represent the hydraulic bottleneck of the entire xylem architecture because a significant portion of the total hydraulic resistance is constrained there due to the axial widening of xylem conduits. The branch's xylem conduits are growing along with it (Anfodillo et al., 2006; Petit et al., 2011).

The hydraulic diameter of the conduits is significant in dry and wet regions initially, wet trees have larger conduits than dry trees due to the distance from the apex; additionally, as DFA increases, dry trees have larger conduits than wet trees. Over the last three years, the tree in the dry area has grown by almost 7 cm. In contrast, the height of the trees in the wet area has increased by 60 cm over the past three years. Several factors influence tree growth. In this case, hydraulic efficiency is greater in the wet region trees than in the dry region. The simplest way to ensure full compensation in taller trees is to simply produce wider conduits at the top of the xylem path length, the first vascular elements below the apex, and further below, continuing to increase conduit diameter in following the usual widening pattern. This is consistent with a recent study on ash trees that shows that in drier environments, leaf-specific conductivity is maintained in drier environments. At the same time, the carbon allocation to the current year xylem is decreased by producing fewer but wider vessels (Petit et al., 2016).

Our results exhibit hydraulically weighted average conduit diameter (Dh) followed by a conduit widening power scaling exponent of b=0.13 for high water availability pine trees and b=0.2 for the low water availability pine trees. These results are coherent with others found in the literature, which usually show a scaling exponent (b) between 0.1 and 0.3. There is a strong conservativism of the conduit-widening trait. It is found to be ontogenetically stable (Prendin & Petit et al, 2018) and consistent in different environmental conditions (Anfodillo et al., 2013; Olson et al., 2014). In the scaling equation, our results show not only a different slope but also a significantly different intercept (which corresponds to "a" in the power scaling equation). The two fitting lines show that the xylem conduit diameter in control trees is larger for the first 50 cm of the branch, then it converges to the values of the trees from the landslide area and then gradually decreases from there.

Given that the conductance of a conduit scales with the fourth power of its diameter (Hagen-Poiseuille law, Tyree and Ewers 1991) and that the vulnerability to embolism is correlated with the conduit size (Hacke et al., 2006), in this experiment, the pines in low water availability exhibit two completely different responses within less than two meters of the branch apex. In the apical-most portion of the branch, where the tissues also experience higher resistances and pressures, these modifications demonstrate a lower vulnerability (lower (Tyree & Sperry, 1989)). The differences along the branch in conduit hydraulic diameter and thus, xylem vulnerability to embolism open serious data interpretation problems for many studies that fix the sampling for age/internode or collect only one sample per branch. For example, in this study, fixing the sampling at just one specific distance from the branch apex would have created biased results toward an increased/decreased vulnerability depending on the position of the sampling. To understand short- and long-term drought responses or acclimation strategies to low water availability, it is necessary to design a sampling procedure that takes considers the rigid axial variation of xylem traits. Our results, in accordance with Lechthaler et al., (2019) and Kiorapostolou et al., (2019) clearly show that neglecting the distance from the apex of the stem/branch can potentially lead to a not correct interpretation of results. Plants growing in dry environments, which on average have smaller shoot elongation yearly, if sampled for one fixed age, will wrongly exhibit smaller conduits.

Carbon allocation

The branch biomass is associated with a given leaf area in the analyzed top branches (Petit et al., 2022). According to our findings, the branch biomass of the trees in dry regions has a higher density and mass than the trees in the wet areas, which is closely related to the work of (Petit et al., 2016). Slow-growing trees invested less carbon than fast growing in the main branch axis' xylem vascular structures, as evidenced by their slower branch elongation rate and smaller ring area. A core principle of plant hydraulics holds that hydraulic efficiency and safety must be traded off (Schumann et al., 2019). Initially, scientists believed that since hydraulic conductivity increases with conduit diameter by the fourth power, the vessel's diameter should be the source of the safety-efficiency trade-off (Tyree & Sperry, 1989; Nardini et al., 2017; Plavcova et al., 2016).

The functional economic characteristic that characterizes a plant's overall biochemical efficiency and energy balance is its leaf area (Niinemets et al., 2007). At the level of individual plants, the amount of leaf area supported per plant is less variable between species than the amount of leaf biomass, in line with earlier studies Duursma et al., 2016. It was also discovered that the species with the highest leaf biomass also had the highest leaf mass per area. Smaller LMA species typically have a fast metabolism or high rates of photosynthesis and respiration per unit leaf dry mass, despite their low needle biomass (Poorter et al., 2009) (DE la Riva E.G et al., 2016). and high decomposition rate. The BM vs. LA relationship can describe the maintenance cost of branch living biomass for a given leaf area. Indeed, the evaluated scaling relationship was equal, which means that BM and LA vary in the same proportions (Petit et al., 2022). Similar findings were made in this study, where it was discovered that species of trees with a dry climate have higher LMA initiation rates than those with a wet region. Trees allocated relatively more biomass to leaves, more leaves are needed to compensate for the negative effects of lower stomatal conductance and prolonged stomatal closure on the seasonal carbon balance (Kiorapostolou & Petit, 2020).

5. CONCLUSION

This experimental study enhances the knowledge of the rigidity of the conduit widening pattern along the tree's main axis. Furthermore, a consistent biomass allocation pattern and stomal density suggest that many of the trait’s allometric scaling are constant and rigid even under harsh growing conditions. Small anatomical changes can greatly influence the ability of an isohydric species like *Pinus sylvestris* to acclimate and colonize new environments. Scot pines from the landslide area likely operate close to their survival limit. Since pine species were reported to suffer both from hydraulic failure and carbon starvation (McDowell et al., 2008c; Sevanto et al., 2014), in a changing climate with more frequent and intense droughts, these pines will likely face increased dangers.

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