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XXXVIII cycle

Plant root development: from cell to ecosystem level

Doctoral Dissertation of:
Alberto Danieli

Supervisor: **Prof. Antonio Montagnoli**

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Contents

Chapter IV – Functional Wood and Root traits in Mediterranean successional sequence for understanding plant response to climate change and land use change

Abstract: Climate is profoundly reshaping plant growth and ecosystem functioning. However, much of our current knowledge relies on aboveground traits, leaving belowground responses underexplored. This dissertation addresses this gap by integrating analyses at multiple biological scales—cellular, individual, and ecosystem levels—to investigate how roots respond to environmental stresses and management practices.

At the cellular level, experiments on *Populus nigra* L. revealed contrasting responses of the vascular cambium under combined heat and drought stress. Root cambium maintained higher activity and showed recovery capacity after rewatering, while stem cambium exhibited impaired resilience, underscoring organ-specific plasticity in secondary growth. At the individual level, biochar was tested as a sustainable alternative to peat in nursery growing media. Results demonstrated comparable root morphology to peat substrates, but improved leaf water status, highlighting biochar's dual role in promoting plant performance and reducing the environmental footprint of horticulture. At the ecosystem level, studies on *Pistacia lentiscus* across the Mediterranean ecosystem illustrated how competition, secondary succession, and climate interact to shape root and shoot growth dynamics. Fine root and leaf trait analyses further revealed both convergent and divergent strategies in resource acquisition and stress tolerance.

Together, these findings demonstrate that plant resilience cannot be fully understood by focusing on a single organ or scale. Instead, integrating above- and belowground perspectives reveals differences that critically impact growth, survival, and carbon allocation. By linking cellular processes, organ-specific strategies, and ecosystem dynamics, this thesis advances a holistic framework for predicting plant and ecosystem responses to global change. Such an approach is essential for improving forest management, guiding peat-free horticultural practices, and enhancing our knowledge to preserve ecosystem services in a warming and resource-limited world.

Chapter I: General Introduction

1.1 Root systems and the significance of fine roots in environmental change scenarios:

Plants, in contrast to mobile organisms, are unable to relocate from adverse conditions; however, they are far from passive entities. Instead, they are highly dynamic and capable of perceiving and responding to environmental signals with remarkable sensitivity (Kumar, 2020). Through a combination of movement, morphological adjustments, and physiological responses, plants continuously interact with their surroundings (Liu et al., 2019; Mawphlang & Kharshiing 2017).

A central aspect of this adaptive capacity is developmental plasticity, allowing plants to sense environmental cues and convert them into growth and developmental choices (Nicotra et al., 2010; Malamy, 2005). Consequently, they can strategically position their organs to maximize access to critical resources such as light, water, and nutrients (Grime et al., 1986; Freschet et al., 2018). This sophisticated ability not only makes plants highly resilient but also excellent indicators of environmental shifts.

Roots are particularly significant among organs due to their multifunctional roles at both the individual plant and ecosystem levels. In plants, roots facilitate water and nutrient absorption (Malamy, 2005; Montagnoli et al., 2019), provide anchorage (Nicoll & Ray, 1996), and serve as storage organs for non-structural carbohydrates (Fitter, 2002). At the ecosystem scale, roots affect soil chemical and physical properties (Gill & Jackson, 2000; Jin et al., 2017), play a crucial part in nutrient cycling, interact with soil microorganisms, and form mutualistic relationships with mycorrhizae that improve resource acquisition (Cudlin et al., 2007). Additionally, roots help maintain soil stability and regulate hydrology, thereby reducing erosion, landslides, and avalanches (Reubens et al., 2007). Due to these diverse functions, root systems are considered essential drivers of plant health and the overall functioning of the ecosystem.

The concept of root system architecture (RSA) has gained increasing significance in understanding the interactions between roots and their environment. RSA refers to the three-dimensional configuration of the root system, which results from the interplay between endogenous developmental processes (e.g. hormones Baesso et al., 2018, 2020) and exogenous factors such as soil texture, nutrient distribution, water availability, salinity, and biotic interactions (Grime et al., 1986; Szymanowska-Pułka, 2013). The architecture of roots directly influences their efficiency in resource acquisition, thereby determining a plant's competitive capacity and survival prospects. Despite its critical importance, RSA remains considerably less studied than aboveground traits, such as leaves, primarily owing to methodological challenges associated with accessing and quantifying root systems (Canadell & Rodà, 1991; Lynch, 1995). This knowledge gap, coupled with the multifaceted functions of roots, underscores their significance as a key subject for investigating both biological processes and environmental interactions.

Within the root system, a clear demarcation exists between coarse and fine roots, distinguished by their diameter and functions. Coarse roots (> 2 mm) primarily serve to provide mechanical support and facilitate the transport of water and nutrients to the shoot, whereas fine roots (< 2 mm) are responsible for the uptake of water, oxygen, and nutrients, often through associations with mycorrhizal fungi (Silver & Miya, 2001; Cusack et al., 2009; Keplin & Hüttl, 2001; Knorr et al., 2005; Tobin et al., 2007). Despite constituting a relatively small biomass fraction, fine roots play a disproportionately large role in ecosystem processes. Specifically, fine roots generally account for less than 5% of total tree biomass; however, their production may constitute a significant proportion of annual net primary productivity across various ecosystems (McClaugherty et al., 1982; Joslin & Henderson, 1987; Montagnoli et al., 2012). Furthermore, their rapid turnover has the potential to contribute up to 33% of the annual net primary production (McClaugherty et al., 1982; Vogt et al., 1995). Owing to their simplified anatomical structure, fine roots represent the most dynamic and responsive component of the root system, exhibiting swift reactions to environmental variations such as nutrient availability, water stress, and soil temperature (Helmisaari et al., 2002). This sensitivity, combined with their high turnover rate and vital role in resource acquisition, underscores the importance of fine roots in elucidating plant adaptation mechanisms and ecosystem functioning, particularly in the context of environmental change (Mausolf et al., 2018; Brunner et al., 2019).

1.2 Vascular cambium and response to environmental changes:

The vascular cambium (VC) is a bifacial lateral meristem that extends continuously from the roots, through the stem, and into the branches, ensuring growth and connectivity throughout the entire plant body. By producing secondary xylem and phloem, it is essential for both mechanical stability and the long-distance transport of water, nutrients, and assimilates (Plomion et al., 2001; Tyree & Zimmermann, 2002; Fonti & Jansen, 2012; Cuny et al., 2015). While phloem distributes the products of photosynthesis, xylem contributes to hydraulic efficiency, structural support, and carbon fixation. Through xylogenesis, the vascular cambium continuously converts photosynthates into wood, making it one of the largest terrestrial carbon sinks. Forest ecosystems, which cover about one-third of the Earth’s land surface, are estimated to absorb between 2–3 billion tons of carbon annually (Pan et al., 2011). Much of this carbon is stored in secondary xylem, highlighting the cambium’s pivotal role in linking plant growth with the global carbon cycle (Plomion et al., 2001; De Micco et al., 2019).

Cambial activity and differentiation are regulated by a combination of endogenous factors—such as hormones, signaling pathways, and gene expression (Aloni, 1980; Baesso et al., 2018, 2020)—and exogenous factors, including temperature, nutrient availability, and water supply (Montagnoli et al., 2019; De Micco et al., 2019). Environmental drivers exert a significant influence on the dynamics of xylogenesis: for instance, water deficits can impair cell enlargement, which depends on cell turgor, while temperature affects cell wall deposition, which requires adequate carbohydrates and metabolic energy (Steppe et al., 2015; Schiestl-Aalto et al., 2015).

In temperate regions, cambial activity generally follows a seasonal cycle, starting in spring and halting during winter dormancy. Early in the season, xylem tends to form large conduits (earlywood) that optimize water transport, whereas later in the season, it produces smaller, thicker-walled cells (latewood), thereby enhancing mechanical strength (Creber, 1977; Chave et al., 2009). The alternation between periods of activity and dormancy within the vascular cambium results in the formation of distinct annual growth rings (Schweingruber, 2006). These tree rings, visible as concentric bands of earlywood and latewood, offer a permanent record of cambial dynamics and are extensively utilized as indicators of historical environmental conditions (Cuny & Rathgeber, 2016). Their anatomical and chemical characteristics may reflect the constraints experienced during xylogenesis, with cell enlargement being particularly sensitive to water availability and other climatic factors (Cuny & Rathgeber, 2016). Importantly, drought can disrupt normal growth patterns, occasionally inducing the formation of latewood-like cells even in spring, thereby complicating the delineation of annual boundaries (De Micco et al., 2019). Mediterranean species often face intense summer droughts that can temporarily stop the activity of the vascular cambium. This leads to a bimodal growth pattern, with peaks in spring and autumn (Montserrat-Martí et al., 2009; Battipaglia et al., 2010; Olano et al., 2015; Vieira et al., 2015).

By modifying the timing and rate of cambial activity, climate variability directly influences tree growth, wood characteristics, and the forest's ability to sequester carbon (Babst et al., 2014; Cuny et al., 2015).

1.3 Peatlands and sustainable alternatives in agriculture:

Peatlands are ecosystems formed by the long-term accumulation of partially decomposed plant material under waterlogged and oxygen-poor conditions, mainly in the boreal and subarctic regions of the Northern Hemisphere. Despite covering only about 3–4% of the Earth' s surface, they store nearly 30% of the world's soil carbon—making them one of the largest terrestrial carbon reservoirs (Leifeld and Menichetti, 2018). Estimates suggest that peatlands contain between 500 and 1, 000 Gt of carbon, exerting a strong cooling influence on the global climate by removing CO_2 from the atmosphere (Nichols and Peteet, 2019; Yu et al., 2021). Their ability to accumulate and conserve carbon is linked to their particular environmental conditions: low pH, low nutrient availability, and oxygen limitation created by constant water saturation, which slows down decomposition processes (van Breemen, 1995).

However, human activities have profoundly altered these ecosystems. Globally, around 12–15% of peatlands have already been degraded, mainly through drainage for agriculture, forestry, infrastructure, and peat mining (Urák et al., 2017). In Europe, exploitation has been particularly intense: it is estimated that about 60% of the original peatland area has been transformed, with more than 90% in Central Europe affected by agriculture, forestry, or extraction (Joosten, 1997; Joosten, 2010; Swindles et al., 2019).

The consequences of peatland degradation are severe: increased greenhouse gas emissions, subsidence, pollution of surface and groundwater, biodiversity loss, and, in some cases, catastrophic fires (Dawson et al., 2010; Fraixedas et al., 2017; Ziegler et al., 2021). It is estimated that drained peatlands alone contribute at least 4% of total anthropogenic global emissions each year (UNEP, 2022).

A primary factor contributing to the exploitation of peatlands is the horticultural utilization of Sphagnum peat moss, which has become the raw material for growth media due to its well-balanced physicochemical properties, stability, homogeneity, and relatively low cost (Blievernicht et al., 2013; Gruda, 2019). However, this extraction process is environmentally unsustainable. Peat accumulates at an extremely slow rate, and its removal results in the destruction of fragile ecosystems and the loss of their carbon sequestration capacity (Belyea and Malmer, 2004; Leifeld and Menichetti, 2018; Alexandrov et al., 2020). Although peat harvested for horticultural purposes constitutes only 0.5% of the global peatland area (Clarke and Rieley, 2010), its impact on climate change is disproportionately significant. For instance, in the European Union, peat extraction and its use in horticulture led to the emission of 12 million tonnes of CO_2 in 2019 (UN Climate Change, 2021).

As a result, the horticultural sector faces rising pressure to adopt sustainable practices. In addition to initiatives such as water conservation techniques and the use of environmentally friendly materials (Evans et al., 2010; Beaulieu et al., 2022), the development of peat-free or peat-reduced growing media has become an essential priority. Alternative substrates derived from renewable organic waste streams are increasingly recognized as both a formidable challenge and a significant opportunity, given their potential to decrease dependence on peat while supporting principles of a circular economy (Chong, 1999; Tsakaldimi and Ganatsas, 2016).

1.4 Climate change issue and future perspectives:

Climate change is one of the most urgent global issues today. Recent IPCC reports indicate that from 2011 to 2020, the average surface temperature was approximately 1.1 °C higher than during pre-industrial times, with forecasts suggesting this increase could surpass 1.5 °C within this century (IPCC 2021, 2023). This gradual warming is accompanied by a rise in the frequency and intensity of extreme weather events—such as heatwaves, droughts, storms, and wildfires—which are projected to persist (Spinoni et al., 2018; Clarke et al., 2022). These shifts are already exerting significant pressure on forests worldwide, threatening their capacity to serve as carbon sinks and provide vital ecosystem services (Pan et al., 2011; Babst et al., 2019; Salomón et al., 2022). Particularly, extreme heat and water shortages can greatly diminish photosynthesis, hinder biomass growth, and raise the risk of tree mortality (Ciais et al., 2005; Teskey et al., 2015; López et al., 2022). The situation worsens when heat and drought occur simultaneously, as their combined effects are often more damaging than their individual impacts (Vile et al., 2012; Hu et al., 2015).

Recent events highlight this vulnerability: the hot, dry summers of 2018 and 2022 in Central Europe caused widespread early leaf drop, lowered forest carbon absorption, and increased mortality risk among many tree species (Arend et al., 2022; Bastos et al., 2020; Buras et al., 2020; van der Woude et al., 2023). Klasse and colleagues (Klesse et al., 2024), through long-term projections under moderate climate change scenarios, suggest that beech trees (*Fagus sylvatica* L.) will experience reduced growth across most of their habitat, with declines of 12–18% in northwestern Central Europe and 11–21% in the Mediterranean region.

The Mediterranean Basin, recognized as a global climate change hotspot (Giorgi, 2006; Giorgi & Lionello, 2008), has already exceeded the global average. Droughts there are expected to become more frequent and prolonged (Piñol et al., 1998; Medrano et al., 2009; Vicente-Serrano et al., 2010), making the region particularly vulnerable, with forests and shrublands increasingly threatened by water shortages and nutrient deficits. Understanding plant responses to these stresses is therefore a key focus in global change studies. Plants develop various morphological and physiological adjustments to face stressful conditions, helping them sustain resource uptake and survival (Nicotra et al., 2010; Freschet et al., 2018; Pérez-Ramos et al., 2019). Evaluating these adaptive responses is essential for predicting ecosystem resilience and understanding how forests will operate under future climate scenarios.

1.5 Aims of the research

Although these topics seem different, they are closely linked through their roles in the global carbon cycle and terrestrial carbon storage. Fine roots, although they make up a small portion of total plant biomass, are highly active—they undergo rapid turnover and contribute to forest soil carbon stocks by releasing exudates or decomposing dead root tissue. The vascular cambium controls secondary growth and wood formation, serving as a key carbon sink by incorporating assimilates into long-lived biomass. Understanding how these systems respond to environmental stresses is crucial for predicting ecosystem responses to ongoing climate and land-use changes.

This research has three interconnected goals:

(i) To investigate how heat and drought together affect the vascular cambium of poplar (*Populus nigra* L), a species sensitive to water stress. By examining xylogenesis under different water conditions, the study aims to clarify how cambial activity and wood formation respond when multiple stresses occur together and after the rewatering phases.

(ii) To address the urgent need to reduce peatland exploitation by exploring biochar—produced via pyrolysis or gasification of raw organic materials—as a sustainable alternative to peat in nursery growing media. This strategy not only aims to reduce greenhouse gas emissions from peat extraction but also promotes recycling of organic residues.

(iii) To study *Pistacia lentiscus*, a Mediterranean shrub well-suited for examining the impacts of climate and land-use change on plant growth. By analyzing both fine root dynamics and annual rings in stems and coarse roots, the research seeks to understand how environmental pressures influence plant development in one of the world's most climate-sensitive regions.

Through comparing root with stem responses, our goal is to better understand the mechanisms that regulate plant growth and stress responses. This approach improves our ability to predict ecosystem changes under environmental stress and supports the development of more sustainable management practices.

Chapter II: Effect of Heat, Drought, and their interplay on Stem and Root Cambium cell activity in Populus nigra L.

Abstract: The increasing frequency of extreme weather events (EWEs) under current climate change scenarios is reshaping plant growth as an adaptive strategy to environmental stress. Since the vascular cambium plays a pivotal role in secondary growth and is strongly influenced by external drivers, this study investigated how drought, elevated temperature, and their combination affect cambial activity and the development of derivative cells in both roots and stems of poplar (*Populus nigra* L.). We further considered the role of stress duration and intensity, as these factors may also modulate growth dynamics, and assessed the capacity of plants to recover after rewatering. Our results showed that cambial cells displayed a certain degree of resistance during the early stages of drought, with recovery after rewatering occurring mainly in plants not subjected to the combined drought and heat stress. By contrast, post-cambial cells and secondary wall-forming cells exhibited early alterations compared to the control, indicating a potential influence of drought and heat on processes of cell enlargement and lignification. In addition, the phloem, also produced by cambial activity and essential for assimilate transport, appeared less sensitive to applied stresses in stems, while roots responded differently, particularly under prolonged stress. In several cases, plants exposed to high temperatures were unable to fully recover after rewatering, highlighting a stronger vulnerability of belowground phloem tissues. Overall, these findings demonstrate that drought, heat, and their duration exert distinct and interactive effects on cambial activity, with notable differences between roots and stems. This underscores the importance of integrating belowground tissues into studies of cambial dynamics and plant responses to extreme climatic events.

2.1 INTRODUCTION

Vascular cambium (VC), which plays a crucial role in radial growth, is a bifacial lateral meristem composed of secondary meristematic cells that differentiate into secondary xylem and phloem. These are the key tissues responsible for transporting water, nutrients, and sap between roots and shoots (Plomion et al. 2001). While phloem facilitates the transport of assimilates, xylem is fundamental for the mechanical and hydraulic properties of plants (Tyree and Zimmermann 2002) and becomes crucial for water transpiration and carbon fixation through photosynthesis (Fonti and Jansen 2012, Cuny et al. 2015).

In temperate forests, cambial activity follows a seasonal pattern. During winter, it becomes inactive but resumes in spring, producing new xylem cells inward and new phloem cells outward.

Unlike phloem cells, which remain alive after differentiating from the vascular cambium (VC) until they are pushed against the bark during plant development (Sankar et al., 2014; Serra et al., 2022), xylem formation (hereafter referred to as xylogenesis) involves a structured process with four distinct stages, ultimately leading to programmed cell death. First, a cambial mother cell divides mitotically, producing two daughter cells. Then, the newly formed xylem cell expands radially. This marks the start of the cell wall lignification process. Ultimately, once lignification ends, the cell dies, fulfilling its role in water transport. It is widely accepted that the activity and differentiation of the vascular cambium (VC) are influenced by various internal factors—such as genes, transcription factors, hormones, and signaling molecules (Aloni 1980, Baesso et al. 2018; 2020)—as well as external factors like temperature, nutrient levels, and water availability (Denne 1971, Hsiao 1973, Montagnoli et al 2019, De Micco et al. 2019). In fact, during xylogenesis, environmental factors can influence the formation of secondary xylem, particularly vessels and fibers, affecting water transport rates, wood quality, and quantity (Balducci et al., 2013, 2015). They also impact phenological events like flowering, leaf abscission (Peñuelas et al., 2002), and fine root elongation (Montagnoli et al., 2012, 2014). For example, the cell enlargement phase is a turgor-driven process strongly affected by water availability (Pantin et al., 2012; Steppe et al., 2015; De Micco et al., 2019). Yet, assimilates are required to build a thick secondary cell wall, and heat is necessary to sustain metabolic reactions (Schiestl-Aalto et al., 2015).

Begum and colleagues (Begum et al., 2007) conducted an experiment in which cambial activity was reactivated in deciduous hardwood hybrid poplar (*P. sieboldii* × *P. grandidentata*) after four weeks of localized temperature application. They observed that temperature influenced the onset of cambial activity.

Consequently, the number and the activity of cambial cells reflect the environmental conditions in which the plants grow. For instance, in harsh environments such as the Mediterranean region, plants are exposed to extreme droughts and high temperatures. During these events, the vascular cambium (VC) reduces its growth significantly, potentially stopping altogether, which can lead to Intra Annual Density Fluctuations (IADFs) (Campelo et al. 2007; De Luis et al. 2007; De Micco et al. 2007).

Sadly, global change continues to be a matter of serious concern. The global surface temperature rose by approximately 1.1 °C between 2011 and 2020 in comparison to the period 1850–1900, and it is projected to surpass 1.5 °C during the 21st century (IPCC 2023). As human-induced climate change alters the fundamental patterns of plant growth, numerous studies in recent decades have investigated its effects on forests and plant organisms, aiming to elucidate their adaptive responses to the rapidly changing environment (Shah & Paulsen 2003, Daniels et al., 2011; Montagnoli et al. 2016, 2019; Haynes et al., 2014). It is broadly acknowledged that current climate change has led to an increase in both the frequency and intensity of various Extreme Weather Events (EWEs) (IPCC, 2014; Melillo et al., 2014; Donat et al., 2013). By definition, EWEs are rare phenomena occurring at specific locations and times of year, typically at or beyond the 10th or 90th percentile of a probability distribution based on historical data. Unlike the gradual progression of long-term climate change, EWEs occur suddenly, are directly observable, and impact specific geographic areas and periods within the year (IPCC 2022). Events such as heatwaves, flooding, droughts, windstorms, extreme rainfall, and wildfires pose significant risks to plant adaptation, as they are key components of terrestrial ecosystems (Jentsch & Beierkuhnlein 2008, Ummenhofer & Meehl 2017).

Among these, heatwaves and droughts are currently being widely studied for their effects on plants (Ciais et al., 2005; López et al., 2022). Heatwaves, for instance, can impair the rate of photosynthesis, intensify oxidative stress, promote leaf abscission, reduce leaf growth, hinder overall tree development, and consequently alter biomass allocation (Teskey et al., 2015). On the other hand, dry environments affect the lumen area of cells, leading to cavitation and, in turn, changes in hydraulic conductivity (Olano et al., 2014; Martin-Benito et al., 2017; McDowell et al., 2008). The situation exacerbates when heat and drought occur simultaneously, as they often do. These stresses may act synergistically when combined, affecting plant performance (Vile et al., 2012; Hu et al., 2015; Zscheischler et al., 2014). Treating such extreme events as independent drivers can underestimate the likelihood and effects of their combined occurrence. Additionally, assessing the severity of their impact on ecosystems requires considering both the duration and intensity of dry and hot periods (von Buttlar et al., 2018).

Approximately one-third of the Earth's terrestrial surface is covered by forests (FAO 2020). Through the process of xylogenesis, wood cells serve as a repository for atmospheric CO 2 (Plomion et al., 2001). Between 1990 and 2007, Pan and colleagues (Pan et al., 2011) estimated that forests had the capacity to sequester approximately 2. 4 ± 0. 4 billion tons of carbon annually. Indeed, forest ecosystems are recognized as some of the largest carbon sink structures globally (Du et al., 2025).

While the ongoing debate regarding whether plant growth is primarily driven by photosynthesis (the carbon source) or by the ability of meristems to convert carbon into biomass (the carbon sink) remains unresolved (Körner, 2015), it is unequivocal that the vascular cambium of woody plants plays a critical role in global biomass production (De Micco et al., 2019).

Since ancient times, at least 460,000 years ago (Barham et al., 2023), wooden artifacts have been utilized by humans as instruments in the development of civilizations. Even in contemporary times, wood remains vital, playing an essential role in diverse sectors of the economy, including construction timber, pulp and paper manufacturing, energy production, and chemical industries (D' Orsi et al., 2025; Girona et al., 2023). Beyond these functions, forests are also important as biodiversity hotspots (IUCN, 2017; Augustynczik et al., 2020) and serve as venues for recreation and possess cultural significance (Pintilii, 2022; Oleyar et al., 2008).

Regrettably, recent decades have seen numerous studies documenting forest damage caused by multiple droughts and heat events worldwide (Frei et al., 2022; Klesse et al., 2024), leading to increasing tree mortality rates (Choat et al., 2018; Schuldt et al., 2020; Senf et al., 2020). As a matter of concern, forest dieback represents a widespread and increasingly alarming phenomenon (Allen et al., 2015).

These vital functions of forests within our society, as highlighted above, underscore the urgent need to implement measures aimed at their protection and the preservation of their indispensable roles.

In this context, the objectives of our research were: (i) to investigate how vascular cambium (VC) activity, as well as its capacity for recovery, is affected by varying levels of drought severity; (ii) to assess the combined impacts of heat and drought on vascular cambium (VC) activity and cambial derivatives during stress conditions; (iii) to explore whether and how vascular cambium (VC) activity differs between root and stem tissues under stress conditions.

2.2 MATERIAL AND METHODS

2.2.1 Plant material and growth chamber conditions

The study was carried out between April and July 2024 in the Laboratory of Applied and Environmental Botany at the University of Insubria, Varese, Italy. In late October 2023, two-year-old dormant Populus nigra L. seedlings, grown from seed, were sourced from a commercial nursery (Società Agricola Forbici SS, Desenzano, Italy; coordinates: 45°28'13.8"N, 10°30'31.0"E). The seedlings were supplied in trays with individual cells measuring 60×60×150 mm. The nursery location has a mean annual temperature of 13.4°C, with January and July being the coldest and warmest months, respectively, and an annual precipitation total of 1045 mm. Upon delivery, the seedlings exhibited an average stem diameter of 0.36 ± 0.03 cm and an average height of 53.7 ± 4.3 cm.

In February 2024, the seedlings were transplanted into 6.2 L pots (dimensions: 175×175×250 mm) containing a soil mix composed of agricultural loamy-sandy soil (pH 7.9, 2.4% organic carbon), perlite, and peat in a 3:1:1 ratio. Prior to potting, the root systems were carefully rinsed with tap water to remove existing soil and checked for signs of mechanical damage, rot, or insect infestation. To distinguish between old and new roots formed during the experiment, each root system was enclosed in a soft acrylic mesh cylinder (55 mm in diameter, 135 mm in height, 10 mm grid size), following the method described by Montagnoli (Montagnoli et al., 2014). The cylinders were positioned centrally in the pots and filled with the soil mixture, after which the remaining space in the pots was filled with the same substrate.

The potted seedlings were maintained in controlled-environment chambers with a 16-hour light / 8-hour dark photoperiod, light intensity of 400 $\mu mol\ m^{-2}\ s^{-1}$ at canopy level (Valoya RX325HV Solray 385), day/night temperatures of 25/20°C, relative humidity between 50–65%, and ambient CO_2 concentration of 400 $\mu mol\ mol^{-1}$. Temperature and humidity were continuously monitored using data loggers (Elitech RC-5, ThermElc TE-02) at 30-minute intervals. Soil temperature was manually recorded at 20 cm depth near the tap root of 10 randomly selected seedlings using a soil probe thermometer (CheckTemp1, Hanna Instruments). The seedlings were allowed a 2-month acclimation period to exit dormancy (i.e., until new leaves were fully expanded) and adapt to the controlled growth conditions. Watering was carried out every two days to maintain field capacity in the well-watered treatments and the rewatering phases, and no fertilizers were applied during the experiment.

2.2.2 Experimental setup

The experiment followed a randomized block design incorporating three factors: temperature regime, watering regime, and time. At the start of the experiment (April 2024), seedlings were split between two climate-controlled rooms set to simulate two temperature conditions: optimal (OT; 25/20°C day/night) and elevated (HT; 35/30°C day/night). For each temperature treatment, a subset of seedlings underwent progressive water deprivation through three distinct drought stages—moderate (MD, 16 days at 52% of field capacity), severe (SD, 23 days at 36% of field capacity), and extreme (ED, 40 days at 22% of field capacity). These stages were designed to obtain gradual soil drying, simulating natural drought progression. Following each drought period, half of the water-deprived plants were rewatered to restore optimal soil moisture. In this matter, sampling was conducted after the recovery phases, once observations indicated that plants had reached full leaf expansion (10 days for MDr and SDr, and 40 days for EDr).

Soil water potential (Ψsoil) was regularly monitored using gypsum block sensors (Delmhorst KS-D1 Digital Soil Moisture Tester), placed at a depth of 8 cm in the center of each pot, with six sensors per treatment.

A total of 225 seedlings were sampled across seven time points, including an initial sampling at the experiment's onset and one for each combination of temperature and watering treatment (see Figure 2). Control plants under both temperature regimes were kept at optimal soil moisture throughout. Pot weights were measured regularly to calculate water loss and replenish it to maintain consistent soil moisture.

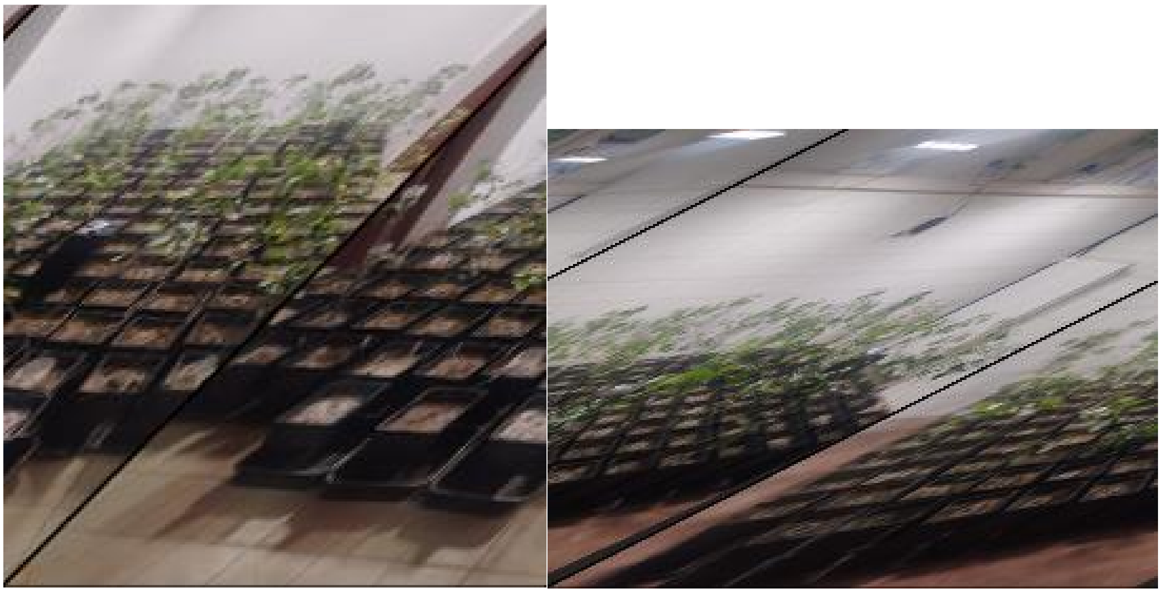

Figure 1: Growth chambers at optimal (20–25 °C, left) and high (30–35 °C, right) temperature.

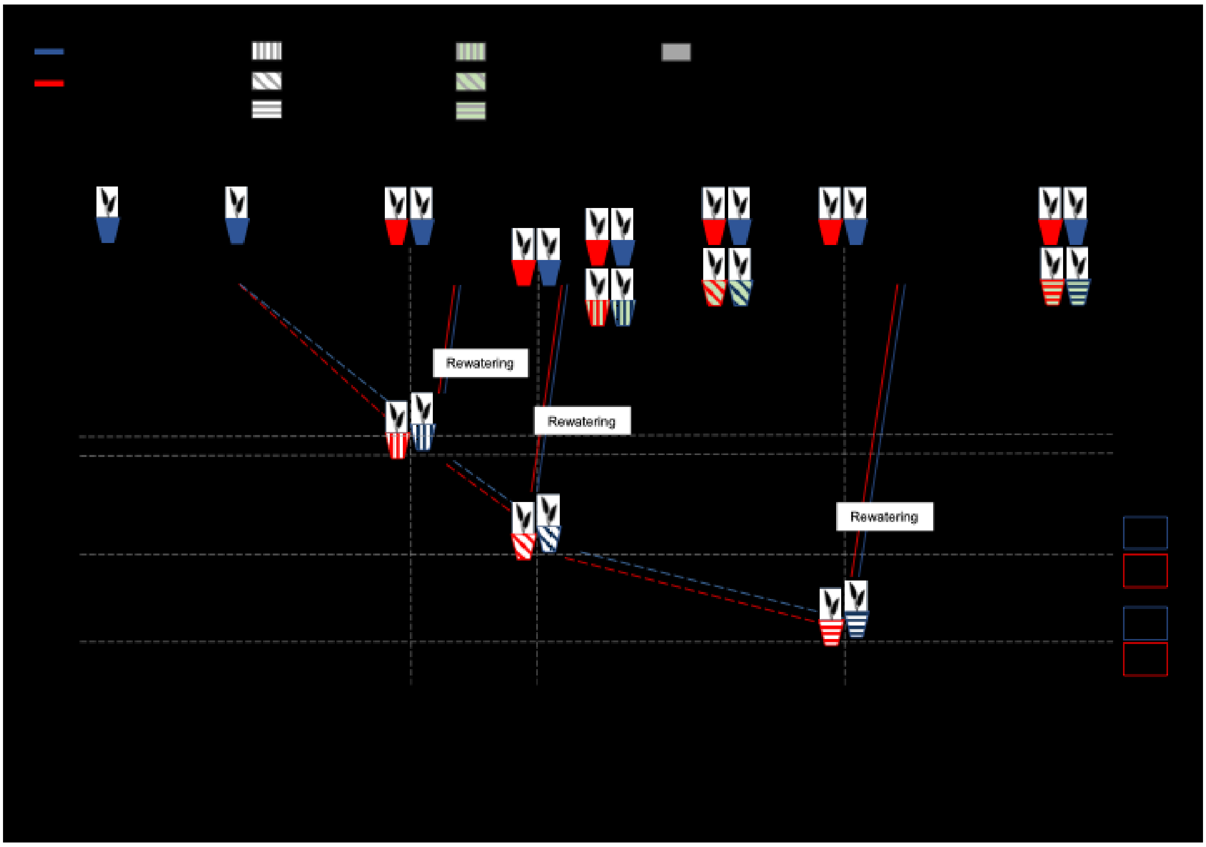

Figure 2: Experimental design. The figure shows the different time points and the relative soil water content (Mpa) and soil water potential (%). The x-axis represents the seven different sample points, starting from day 0 (in brackets)

2.2.3 Stem and root anatomical analysis

For each time points, the samples were collected using a garden shears approximately 2 cm above and below the collar, respectively for the stem and the root side. The samples were stored in F.A.A solution (38% formaldehyde, glacial acetic acid, 50% ethanol—5/5/90 by volume) for one week. Then, samples were progressively dehydrated through a graded ethanol series (70%, 90%, 95%, and 100%), cleared with Bio-Clear (D-limonene), and embedded in paraffin using a Leica TP1020-1 tissue processor (Leica, Nussloch, Germany). Transverse sections (9 µm thick) were prepared with a semi-automatic rotary microtome RM 2245 (Leica, Nussloch, Germany), and subsequently stained with a water-based solution of safranin (0.04%) and astra blue (0.15%) (Van der Werf et al. 2003). Microscopic examination was carried out using a Nikon Eclipse 800 light microscope, and digital images were captured with a DS-Fi1 camera equipped with NIS-Elements BR 3 imaging software (Melville, NY, USA). The anatomical sections were evaluated to differentiate cambial and differentiating xylem and phloem cells based on morphological features such as cell size and shape (De Micco et al. 2016, Čufar et al. 2008, de Luis et al. 2011). Specifically, cambial cells (CC) were identified by their radially flattened shape (Figure 3). Cells undergoing radial enlargement, with only primary walls and lacking birefringence under polarized light, were classified as post-cambial (PC) cells (Balzano et al., 2018; Rossi et al., 2006). Cells depositing secondary wall material were classified as developing secondary wall (SW) cells, while those with fully lignified walls were identified as mature cells (MC). Finally, non-collapsed phloem (NCP) cells were also counted (Fig. 3).

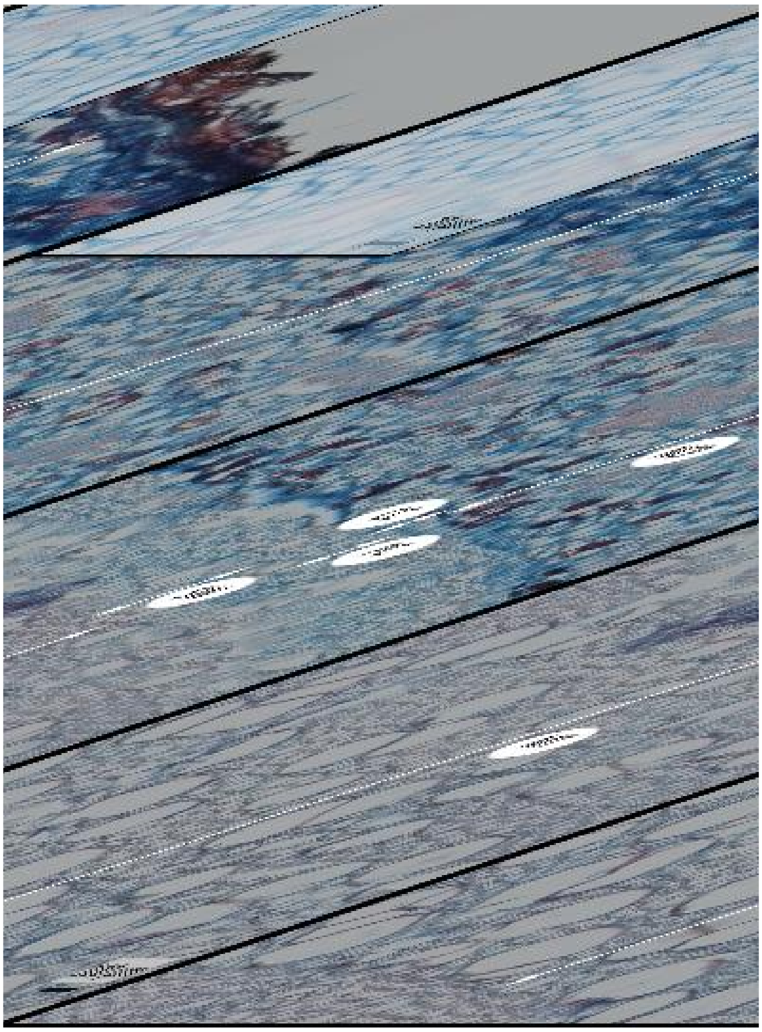

Figure 3: The inner figure shows a detailed view of flat-shaped cambial cells, while the whole figure displays a cross-section of Populus nigra, illustrating the measured tissue regions. From the stem periphery toward the inner xylem, the following cell types can be distinguished: non-collapsed phloem (NCP), cambial cells (CC), post-cambial cells (PC), secondary wall-forming cells (SW), and mature cells with fully lignified secondary walls (MT).

The radial thickness of each anatomical zone described above was measured using the image analysis software ZEN Blue (Zeiss). Measurements were taken perpendicular to quantify the extent of each developmental stage.

2.2.4 Statistical analysis

The normality of residuals was assessed for each dependent variable by visually inspecting histograms and Q–Q plots, and by applying the Shapiro–Wilk test. To evaluate the effects of temperature regime, water availability over time, and tissue type (stem or root) on cambial and cambial derivatives three-way ANOVA models were applied. Besides, post hoc comparisons were performed using the cld() function from the emmeans package, with Bonferroni adjustment for multiple testing ($\alpha = 0.05$). Treatments not sharing the same letter are significantly different at the $p < 0.05$ level.

To further explore relationships between soil water content and response variables, linear regression models were fitted using water content as the independent variable and the mean values of selected anatomical features (e.g., cambial cells thickness) as dependent variables. These analyses were supported by visualizations such as boxplots to illustrate treatment differences.

All statistical analyses were performed in R (version 2024.12.1).

2.3 RESULTS:

2.3.1 Cambial Cells:

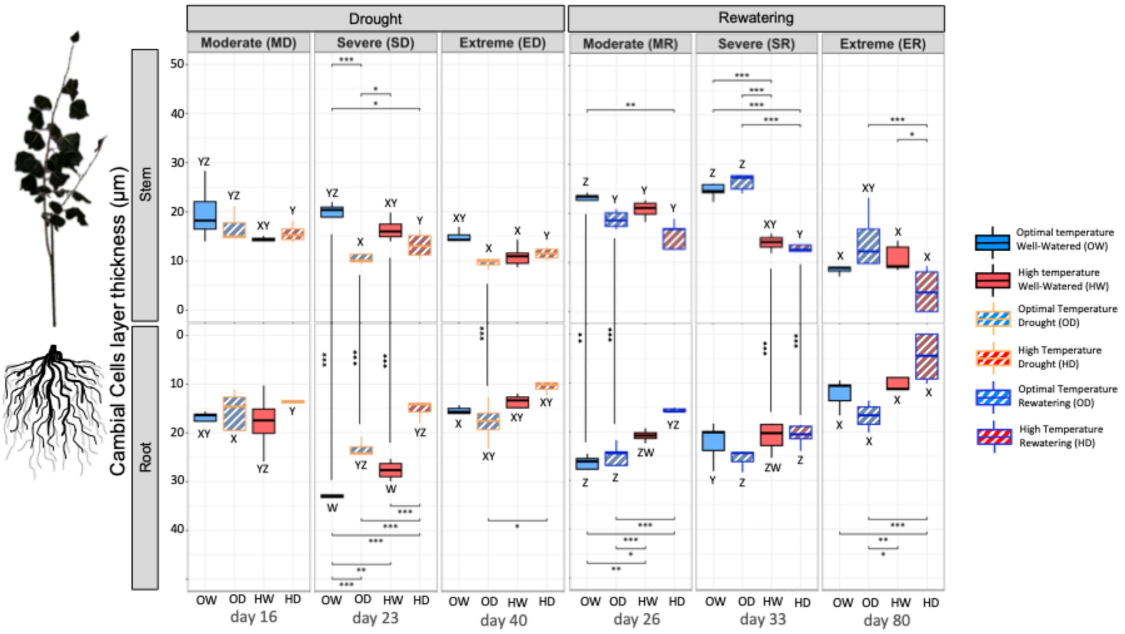

Figure 4: Variation in cambial cell thickness (µm) throughout the experiment. The bars ("caps") above the boxes indicate statistical differences between treatments within the same sampling point; capital letters denote statistical differences for the same treatment across different sampling points; black lines connecting the lower and upper panels indicate statistical differences between root and stem tissues, respectively.

During the early phase of drought (MD – day 16), cambial cell thickness was not significantly affected by the different treatments in either stem or root tissues, indicating a general tolerance of the cambial zone to moderate water deficit (Figure 4). However, during the severe drought phase (SD – day 23), clear treatment-specific differences emerged in the stem, where both drought treatments (HD and OD) resulted in a reduction in cambial thickness compared to the well-watered controls (HW and OW). In roots, OD-treated plants had significantly thicker cambial layers compared to HD, suggesting that high temperatures exacerbated the impact of drought. The difference became less pronounced during the extreme drought phase (ED – day 40). In roots, only HD plants consistently exhibited the lowest cambial thickness, supporting the idea that high temperatures strengthened drought-induced reductions in cambial activity. After rewatering, treatment-specific recovery patterns emerged. During moderate rewatering (MR – day 26), roots exposed to high temperatures (HW and HD) had significantly lower cambial thickness compared to OW and OD, highlighting the negative impact of elevated temperatures during rehydration in this tissue. In the stem, only the HD treatment was significantly different compared to the control (OW). Moving forward, in the severe rewatering phase, the situation changed: roots showed no statistical difference between treatments, whereas in the stem both high-temperature treatments (HD and HW) differed from the optimal temperature treatments (OW and OD). In the extreme rewatering phase (ER – day 80), HD-treated plants differed from HW and OD treatments in both root and stem tissues, with HW also differing from OD in roots.
At several time points (black bars), significant differences were observed between stem and root VC thickness. Across all treatments and sampling points, roots generally exhibited higher cambial thickness than stems. After 23 days, OW, OD, and HW treatments showed differences between roots and stems. Additionally, after 40 days, the OD treatment still showed differences. Following the rewatering phase, the first recovery highlighted differences between OW and OD. In contrast, during

severe rewatering, differences were found in HW and HD treatments. Finally, at the end of the experiment, no differences were observed between treatments.

Across all phases, OW and HW plants followed a seasonal growth curve, reaching peak values at different times. OW had its highest value after 33 days, while HW peaked earlier—at day 26 in the stem and day 23 in the root. Additionally, the droughted treatment OD in the stem tissue displayed good recovery after rewatering, especially during severe drought, with a statistically significant increase in cambial cell thickness. In contrast, the HD treatment in the stem failed to restore the situation to its pre-rewatering state. The OD treatment in the root tissue appeared to be mitigated. In the stem, cell thickness started to decrease after 16 days, while in the root, it increased significantly under severe drought conditions. However, in rewatered plants, cambial cell thickness significantly increased in the stem during severe rewatering, whereas in the root, the increase was already evident under moderate rewatering.

At several time points (black bars), significant differences were observed between stem and root VC thickness. Across all treatments and sampling points, roots generally exhibited higher cambial thickness than stems. After 23 days, OW, OD, and HW treatments showed differences between roots and stems. Additionally, after 40 days, the OD treatment still showed differences. Following the rewatering phase, the first recovery highlighted differences between root and stem in OW and OD treatments. In contrast, during severe rewatering, differences between the two organs were found in HW and HD treatments. Finally, at the end of the experiment, no differences were observed.

2.3.2 Post-Cambial Cells

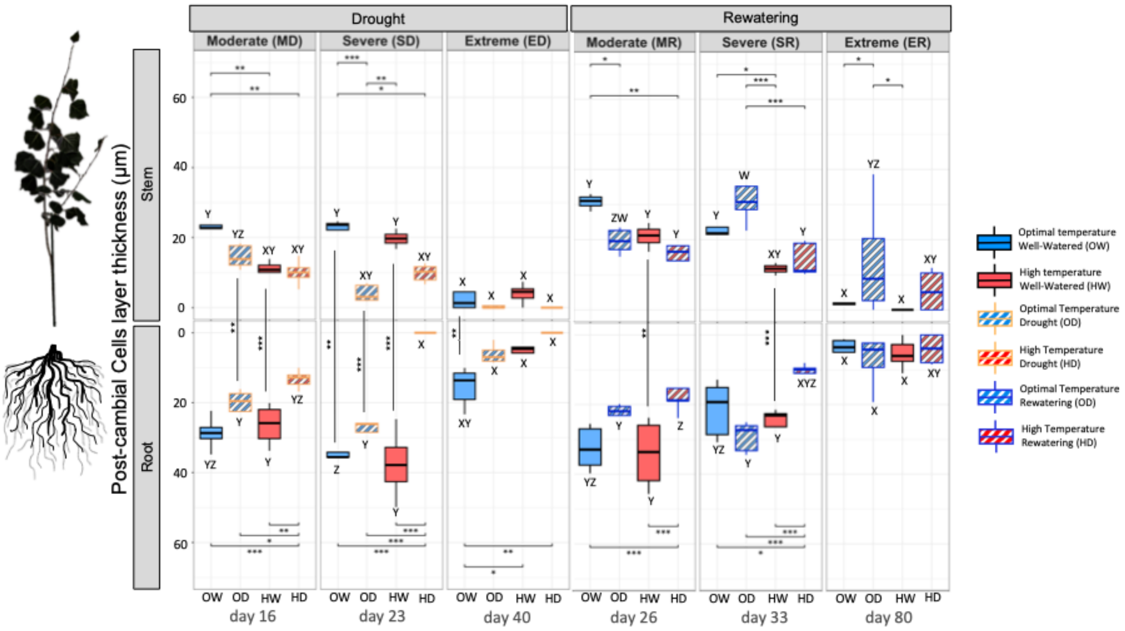

Figure 5: Variation in post-cambial cell thickness (µm) throughout the experiment. The bars ("caps") above the boxes indicate statistical differences between treatments within the same sampling point; capital letters denote statistical differences for the same treatment across different sampling points; black lines connecting the lower and upper panels indicate statistical differences between root and stem tissues, respectively.

Unlike the cambial cells, the Post-cambial (PC) cell layer thickness showed treatment-specific responses that appeared as early as the moderate drought phase (MD – day 16) (Figure 5). Both stem and root tissues experienced a significant reduction in PC thickness under HD treatment compared to

controls (OW), indicating that combined heat and drought had a rapid impact on cell development. In the stem, even HW treatment resulted in significantly less growth than the control.
At the severe level of drought (SD – day 23) in roots, PC thickness remained relatively high in OW, HW, and OD compared to the moderate drought. In stems, well-watered treatments maintained higher PC thickness, while both drought treatments (OD and HD) showed reduced values without significant differences between each other. Notably, in the root tissue, the HD treatment reduced post-cambial cell growth to zero, indicating an interruption of cambial activity. After 40 days, the thickness of post-cambial cells decreased sharply in all treatments. In the stem, all HD and the majority of OD individuals stop their activity, whereas in the root, this occurred only in the HD treatment, possibly reflecting resource depletion under prolonged stress. Additionally, OW and HW roots maintained slightly higher values, although these also showed signs of decline, consistent with the expected seasonal pattern of cambial activity.
Following rewatering, recovery was observed in HD e OD treatments, especially during the moderate and severe rewatering phases (MR – day 26 and SR - day 33). Notably, during the severe rewatering, OD treatment showed higher values compared to the corresponding drought phase on the stem side. Particular behavior was observed during the extreme rewatering phase at the stem level. Plants in the OW and HW treatments had their activity drastically reduced and stopped, while those in the OD and HD treatments remained active. At this stage, OD experienced significantly more growth than OW and HW. In contrast, root procambial cells showed a decrease in growth across all treatments as well, but they remained active.

Throughout all phases, OW and HW plants showed a seasonal growth pattern, with peak values at different sample points: OW peaked on day 26 both stem and root, while HW reached its maximum earlier, at day 26 in the stem and day 23 in the root. In the stem tissue, OD treatment significantly declined after 16 days, whereas in the root, the reduction began on day 23, indicating a slight resistance of this tissue to drought stress. Conversely, the HD treatment, under double stress, stopped growth after 23 days in the root, while growth in the stem remained active at that same point. After rewatering, both the OD and HD treatments in the stem recovered from all drought stress levels, even when activity had stopped in both tissues during the extreme level. Notably, around half of the OD and HD plants resumed activity during the extreme rewatering phase. It is particularly noteworthy that the OD treatment in the stem tissue showed a more pronounced recovery.

Unlike the cambial cells, at moderate drought levels, the single-stress treatments (OD and HW) revealed differences between stem and root, with roots exhibiting slightly more growth. After 23 days, OW, OD, and HD showed a statistical difference between stem and root, while at the extreme drought level, almost all treatments except OW showed no differences between organs. Conversely, during the moderate and severe rewatering phase, only the HW treatment exhibited a difference between stem and root.

2.3.3 Secondary Cell Wall-Forming Cells

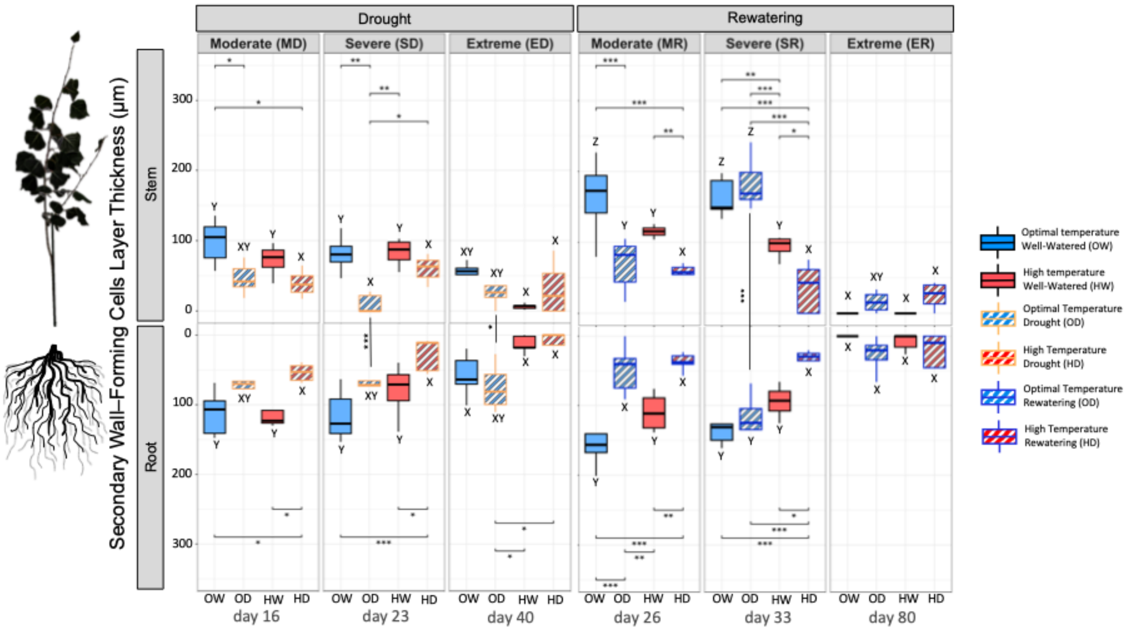

Figure 6: Variation in secondary cell wall-forming cell thickness (µm) throughout the experiment. The bars ("caps") above the boxes indicate statistical differences between treatments within the same sampling point; capital letters denote statistical differences for the same treatment across different sampling points; black lines connecting the lower and upper panels indicate statistical differences between root and stem tissues, respectively.

Speaking about the treatments, the stem under Moderate Drought showed statistically significant differences in OD and HD compared to the control (OW). Conversely, only HD in the root tissue exhibited differences with OW and HW. During severe drought in the stem, the secondary cell wall formation layer thickness in OD decreased significantly compared to other treatments, with only one plant remaining active. The root tissue showed a similar pattern to the previous drought level. After 40 days, there were no statistical differences among treatments in the stem, but in the root, both high-temperature treatments (HW and HD) differed significantly from OD. During the rewatering phase in moderate conditions, OD and HD in the stem differed statistically from OW, with HD also differing from HW. In the root, OD and HD differed from both well-watered treatments. By day 33, stem growth in OD had increased to match the control, with HW and OD differing from other treatments. In the root, only HD differed. After 80 days, growth in OW ceased in both tissues, although two individuals in HW remained active.

Throughout the drought phase, both OW and HW treatments followed a seasonal growth pattern, with the secondary wall-forming cell layer thickness increasing around 26 days. During the moderate rewatering phase, both droughted treatments (OD and HD) maintained the same growth as before the rewatering. Drought and heat-stressed plants (HD) showed no significant differences across sampling points in both stem and root, while the drought-stressed treatment, at the severe level in the stem tissue, exhibited a significant recovery after rewatering.

Across the experiment, the secondary wall-forming cell Layer thickness was consistent between root and stem, except for OD treatment, which showed a statistically significant difference between the two tissues during severe and extreme drought conditions, as well as after the severe rewatering stage. Under drought conditions, the root exhibited greater thickness than the stem, whereas during severe rewatering (SR), the stem's growth increased more than the root.

2.3.4 Mature Cells

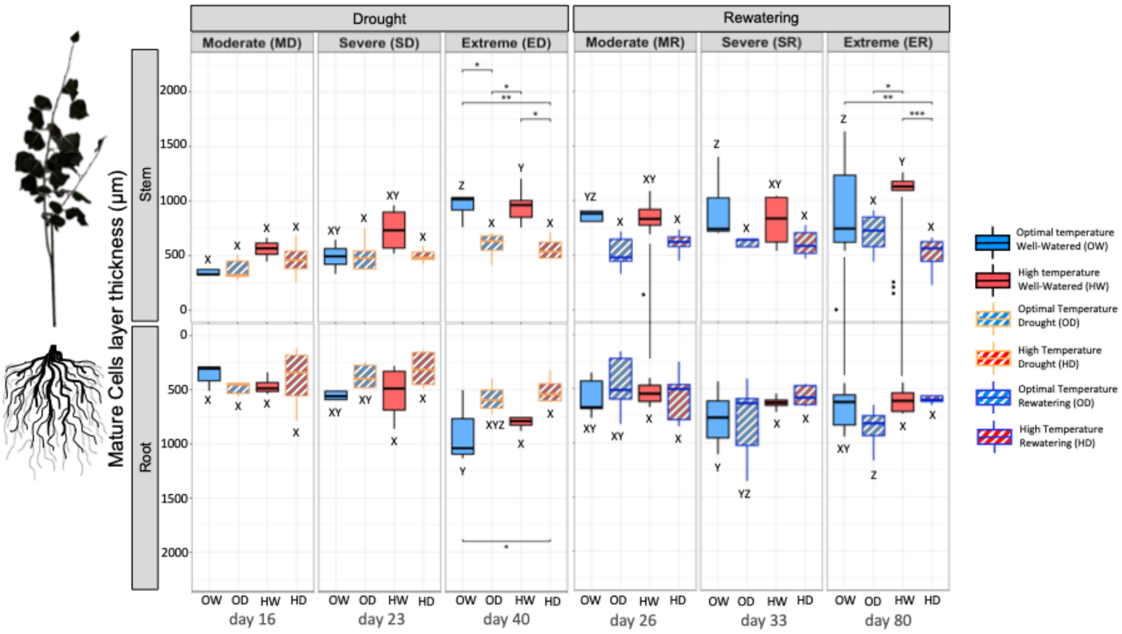

Figure 7: Variation in mature cell thickness (µm) throughout the experiment. The bars ("caps") above the boxes indicate statistical differences between treatments within the same sampling point; capital letters denote statistical differences for the same treatment across different sampling points; black lines connecting the lower and upper panels indicate statistical differences between root and stem tissues, respectively.

During the drought phase, moderate and severe stress levels did not lead to notable differences among treatments in either stem or root (Figure 7). However, under extreme drought (ED), differences became evident. The stem showed significantly thinner mature cell layer in droughted treatments (OD and HD) compared to well-watered conditions (OW and HW). In the root, only HD differed significantly from OW, indicating that the combination of high temperature and prolonged drought had a more severe impact on mature cells, whereas single stressors (OD or HW) did not cause similar reductions. During rewatering, most treatments recovered with no consistent differences observed. However, in the stem during extreme rewatering (ER), HD remained significantly lower than OW and HW, suggesting an incomplete recovery under combined stress.

Across sampling dates, well-watered treatments (OW and HW) maintained relatively stable mature cell thickness in both the stem and the root, with only minor fluctuations. No statistical differences were found when comparing each drought phase with the corresponding rewatering period.

Both tissues seemed to have the same mature cell development at every sampling point. The only exceptions were found in HW during moderate rewatering and in HW and OW in the extreme rewatering. In both situations, the stem showed higher values than the root side.

2.3.5 Non-Collapsed Phloem

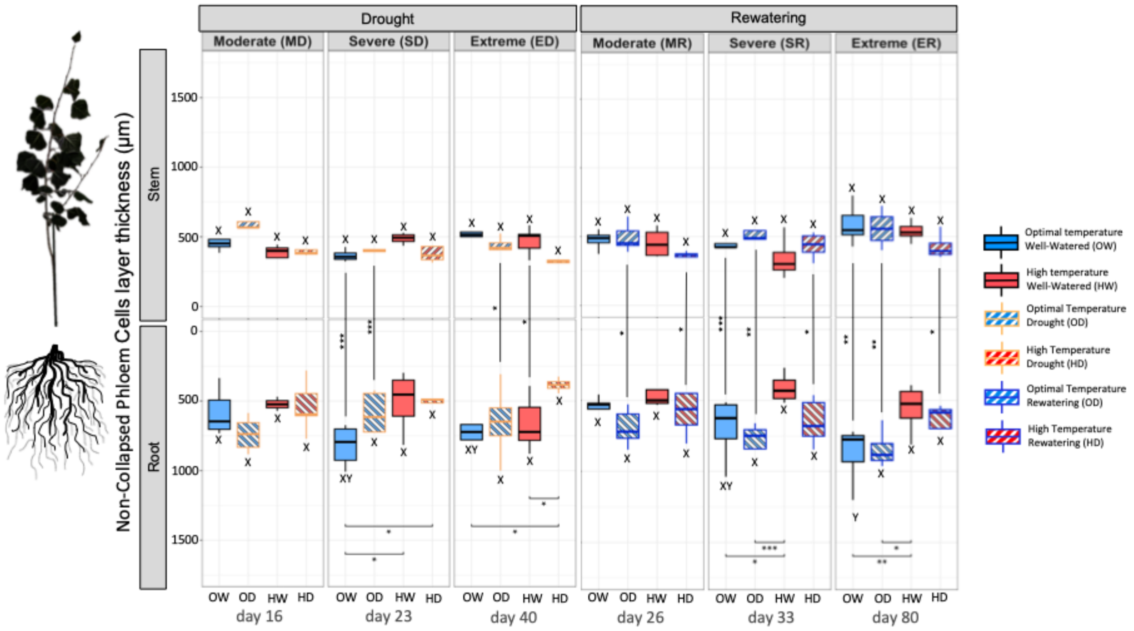

Figure 8: Variation in non-collapsed phloem cell thickness (µm) throughout the experiment. The bars ("caps") above the boxes indicate statistical differences between treatments within the same sampling point; capital letters denote statistical differences for the same treatment across different sampling points; black lines connecting the lower and upper panels indicate statistical differences between root and stem tissues, respectively.

As observed in other tissues such as the cambial cell layer, moderate drought (MD) did not significantly affect phloem thickness in either organ (Figure 8). In the stem, non-collapsed phloem thickness remained consistent across all treatments, with no significant differences at the same sample point. Conversely, in the root, significant differences emerged especially under severe (SD) and extreme (ED) drought. In the SD, high-temperature treatments (HW and HD) have reduced thickness compared to the control (OW), while in the ED, only HD displayed a significant difference, highlighting the root phloem's sensitivity to combined and prolonged stress. Even during rewatering, stem phloem thickness remained uniform among treatments. However, in the root, after 33 and 80 days , HW plants exhibited a significantly greater reduction in non-collapsed phloem thickness compared to OD and OW.

During sampling points, the phloem structure of all treatments remained relatively stable with minimal variation. In the stem, OD and HD plants showed a seasonal growth pattern with minor fluctuations, and there were no major declines during the drought and rewatering phases, indicating the absence of water deficit stress. Indeed, in the stem, none of the treatments showed a statistically significant difference. The situation in the root was similar, with no significant difference between before and after rewatering. The only notable finding was that in the root, the OW treatment experienced a significant increase in non-collapsed phloem growth from day 16 to day 80.

In general, roots showed thicker non-collapsed phloem layers than stems, likely reflecting a prioritization of belowground transport capacity. In particular, in the OD treatment, root tissues seemed to grow more than the stem at all sampling points except during the moderate drought. Conversely, the HW treatment showed a significant difference between tissues only in the extreme drought sampling point, while the HD treatment differed significantly only during the rewatering phases.

2.3.6 Relationship between water content and cell layer thickness (µm)

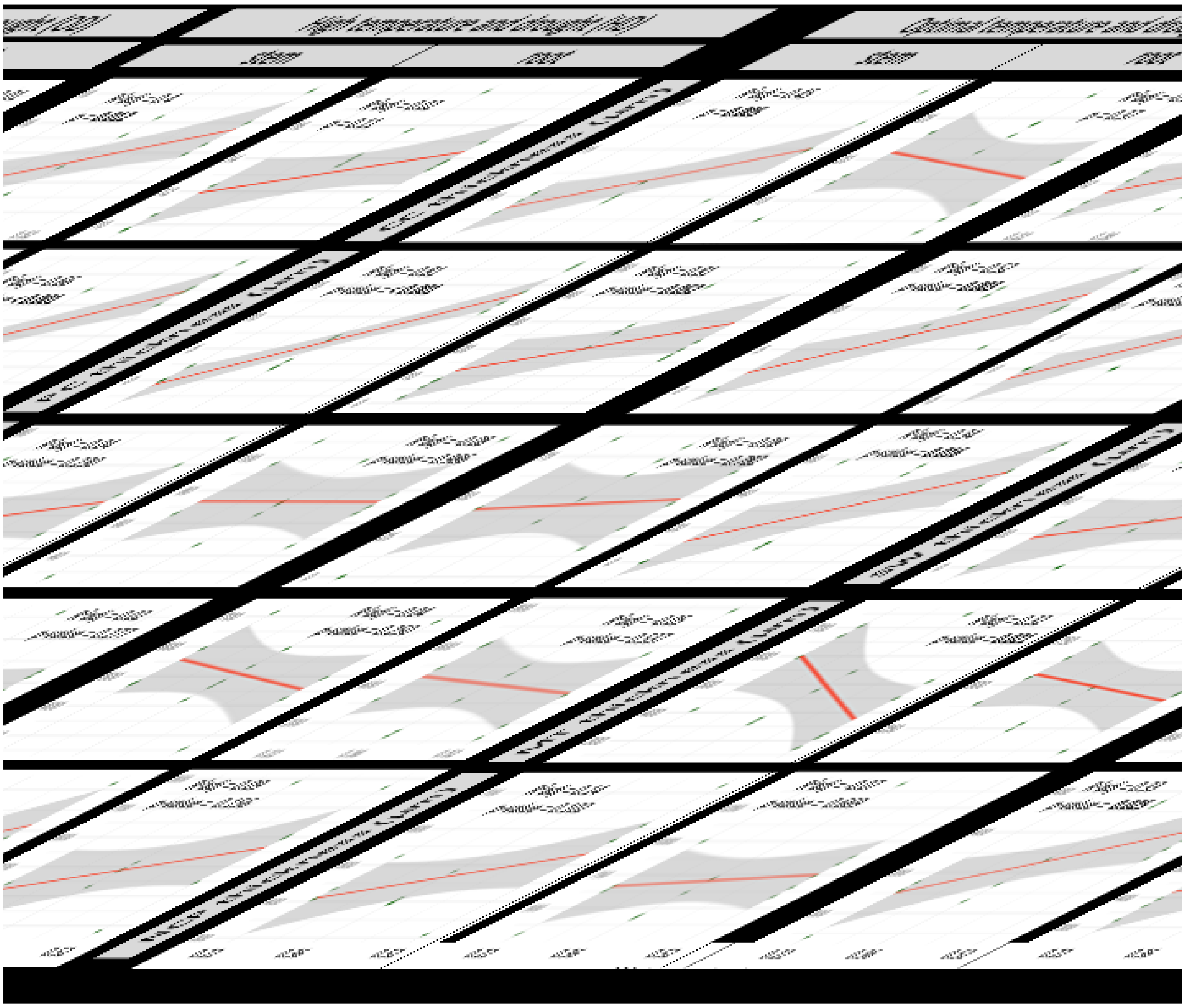

Figure 9: Linear relationships between water content (x-axis) and cell layer thickness (y-axis) for cambial and derivative cell types (five panels) in both root and stem tissues under OD and HD treatments. Significant relationships are shown in bold. Sampling points correspond to moderate drought (MD – day 16, 0.56 water content), severe drought (SD – day 22, 0.36 water content), and extreme drought (ED – day 40, 0.22 water content). In the post-cambial cell panels, some points in the post-cambial cell charts overlap at zero.

2.3.6.1 Cambial Cells

In the stems of OD and HD treatment, cambial cell thickness exhibited a significant positive relationship with water content, pointing out that water availability plays a key role in promoting cambial activity (Figure 9). In contrast, the linear regression analysis for root tissues across both treatments revealed no significant relationship, with adjusted R^2 values showing no linear trend as water content increased. Peak values in roots occurred at a water content of 0.36, suggesting non-linear patterns.

2.3.6.2 Post-Cambial Cells

In post-cambial cells, a positive linear relationship was observed across all treatments and both tissues. In the stem of OD treatment, at 0.22 water content (extreme drought), most plants lacked in post-cambial cells, while in the HD group, growth was completely halted. Conversely, the OD root exhibited a post-cambial development even at 0.22 water content. Meanwhile, in the root, most HD treated plants had already stopped growing at 0.36 water content, whereas all OD treated plants were still active.

2.3.6.3 Secondary Cells Wall formation Cells, Mature Cells, and Non-Collapsed Phloem Cells

In the secondary wall-forming cells, only the HD root showed a significant linear regression compared to the HD stem and OD stem and root. Differently, in the mature cells, only the OD stem exhibited a negative linear relationship between water content and the thickening of mature cell layers. As water content increased, the growth of mature cells decreased. Finally, only the HD stem in non-collapsed phloem cells highlighted a positive effect of water content increase on cell layer thickening.

2.4 DISCUSSION

Our results reveal complex, tissue- and treatment-specific responses to drought and heat stress across cambial and derivative cell types in stems and roots. Cambial cells showed resilience to moderate drought, maintaining consistent thickness early in stress, supporting previous findings on the drought tolerance of cambial meristems (Li & Jansen, 2017). They noted that *Corylus avellana* seedlings had undamaged cambial cells under moderate water stress.

Unlike well-watered treatments, which showed a bell-shaped growth pattern, drought treatments OD and HD in the stem exhibited a progressive decrease in cell thickness over the three drought periods. A reduction in cambial cell layers during drought has been reported in poplar (Arend & Fromm, 2007). In roots, HD followed the same declining trend as stems, but OD maintained growth until severe drought. However, at severe drought levels, both treatments showed less growth than the control. In the stem, severe drought similarly impacts OD and HD. It is known that drought-stressed stems produce smaller vessels to prevent embolism and control water flow (Brunner et al., 2015). In roots, although both treatments had a significant reduction in cambial activity compared to the control, HD showed the lowest value, indicating that temperature coupling with drought had a greater effect on root cambial activity, similar to the stem.

In woody plants, vessel size and number generally decrease from roots upward through stems, branches, and petioles, reflecting an adaptive balance between maximizing hydraulic efficiency and reducing embolism risk (Tyree & Zimmermann, 2002; Lintunen & Kalliokoski, 2010; Schuldt et al., 2013). This reduction allows plants to regulate water flow independently of pathway length, with the smallest vessels at the terminal points. In our control plants, this pattern was evident during early growth stages, where root cambial layers were thicker than those in stems, likely to optimize water uptake and transport during high-growth periods, even though we didn’t measure the cell size and the number. By the final drought sampling (day 40), plants exposed to moderate drought (OD) still exhibited this distinct root–stem difference, which may represent a strategy to improve the movement of limited water resources toward the canopy. Similar patterns have been observed in other tree treatments, where cambial and derived layer thickness in the root is maintained or even enlarged under water deficit to preserve conductivity. In contrast, combined heat and drought stress (HD) led to a complete cessation of cambial activity in both organs. Following rewatering after moderate drought (MR), root cambial activity in OD plants recovered faster than in stems, returning to control-like levels, while in HD plants, both roots and stems remained at similarly low levels. This highlights the greater resilience of root cambial tissues to single drought compared with combined heat–drought stress. In severe rewatering (SR), OD plants displayed a strong recovery in both roots and stems, matching control values, whereas in high-temperature treatments (HD and HW), the recovery was uneven: roots, in direct contact with re-supplied water, quickly resumed activity to control levels, but stems remained rather below the control values. At the end of the experiment (day 80), OW, OD, and HW had reduced their growth but remained alive. Although we were not focusing on plant death, half of the plants under double stress (HD) died, suggesting an irreversible effect after prolonged stress when heat and drought conditions occur together.

Post-cambial cells, responsible for cell expansion and differentiation, were more sensitive to early stress, showing significant reductions in thickness under HD treatments even at moderate drought levels in both roots and stems. The complete interruption of post-cambial activity in stems under extreme drought and heat indicates a critical vulnerability of cell enlargement processes (Pantin et al.

2012; Steppe et al. 2015; De Micco et al. 2019). In fact, by day 40 of the experiment, both drought treatments (OD and HD) the stem experienced a no production of post-cambial cells. A similar behavior was observed in Scots pine in different studies, where cambial activity and derivatives stopped when conditions became too severe (Gruber et al., 2010; Eilmann et al., 2011; Ren et al., 2015). Although not statistically significant, the OD root at extreme drought remained active compared to the stem. Notably, high temperature combined with well-watered conditions exhibited statistically higher thickening of post-cambial cells in roots than in stems from day 16 to day 33, likely to facilitate efficient water flow to counteract transpiration in the upper parts of the plant. It is well known that during warm temperatures, plants lose water through stomata in order to reduce leaf temperature (Trewavas 2009; Urban et al. 2017). Additionally, the same trend observed in cambial cells at day 33 was seen, where the OD treatment showed a recovery phase, indicating that cambial and post-cambial cells were able to resume well after severe drought stress. Ultimately, by day 80, nearly all well-watered plants in the stem reduce their growth entirely, while about half of the HD and OD plants remained active.

Cells involved in secondary cell wall formation, a process requiring significant energy during lignification and cellulose deposition, were affected across all drought conditions in all treatments. Even during the lignin deposition phase, the OD and HD treatments exhibited differences compared to the control. Specifically, in the stem, both OD and HD showed variations from the control, whereas in the root, only HD differed significantly, while OD displayed lower—but not statistically significant—values. For the HW treatment, the thickness of cells undergoing secondary wall formation was comparable to or slightly lower than the control, although this difference was not statistically significant.

An intriguing pattern was observed during the severe drought (SD) phase in the stem, where both high-temperature treatments recorded elevated values comparable to the control. As demonstrated in prior research, Zhao and colleagues (Zhao et al., 2022) established that short-term high-temperature exposure (three days) can enhance lignin content and its metabolic intermediates. Consistent with this, in our experiment, after 23 days, both high-temperature treatments exhibited this trend in the stem, whereas no such pattern was detected in the root.

Ultimately, under the most extreme drought conditions, nearly all stressed plants showed a drastic reduction in growth, with some individuals reaching zero growth after 40 days of drought. However, in the roots, plants subjected to the OD treatment demonstrated an increase in growth compared with the control, although this difference was not statistically significant.

Regarding the rewatering phase, the increase in the thickness of secondary cell-wall forming cells following recovery from drought mirrored the patterns observed for cambial and post-cambial cells: complete recovery in the OD treatment after the severe drought phase, and a total cessation of growth in the well-watered treatments by Day 80. Nevertheless, at this final sampling point, treatments subjected to drought, specifically OD and HD, still exhibited residual activity, indicating that cell-wall thickening had not been entirely ceased.

Mature cells remained relatively stable under moderate and severe drought conditions, but a similar pattern was observed in both stem and root during extreme drought, where both OD and HD treatments reduced growth. As reported in numerous studies (Thomas et al. 2007; Shtein et al. 2011, Shtein et al. 2021), drought tends to reduce vessel size and thickness, and our results followed a similar trend after a prolonged drought stress period, reducing the thickness. No clear patterns emerged during the recovery phase. Notably, in the stem, both OD and HD treatments showed reduced recovery during moderate and severe rewatering, remaining below the growth levels of the control.

Previous studies have shown that phloem formation remains relatively stable throughout the growing season and is less sensitive to environmental fluctuations than xylem (Alfieri & Evert, 1968; Panshin & DeZeeuw, 1980; Gričar et al., 2007; Gričar & Čufar, 2008). Our results align with this pattern, as stem non-collapsed phloem layers in our experiment exhibited only minor thickness changes during drought and heat stress, unlike the significant variations observed in xylem tissues. After 23 days, the HW stem reached its highest value at that sampling point, even though it was not statistically significant. At this stage, our findings confirm a previous study that reported an increase in phloem cross-sectional area at 34 °C in grapevine (Wolberg et al., 2024) for sucrose transport. In contrast, roots showed that phloem formation is variable throughout the growing season. The HD treatment under severe and extreme drought conditions resulted in a decrease in cell layer thickness compared to the control, with significant reductions observed after 33 and 80 days of HW treatment. Another noteworthy finding is that, except at the first sampling point, the thickness of the non-collapsed phloem differed between stem and root. This pattern was consistent throughout most of the experiment in the OD treatment, whereas in HD it only appeared during rewatering phases, highlighting a trend that deserves further investigation.

In our experiment, cambial cell layer thickness in the stem demonstrated a significant positive relationship with water content under both optimal temperature and drought (OD) and high temperature and drought (HD) conditions. This finding indicates that as water availability increased, cambial activity in the stem was consequently enhanced. These results are consistent with previous research reporting that cambial cell production in stems is highly sensitive to water content, with reduced water availability leading to declines in division rates and cell enlargement (Rossi et al., 2009; Arend & Fromm, 2007; Ziaco et al., 2018; Vieira et al., 2014; Balducci et al., 2016). Conversely, root cambial cells did not exhibit any significant relationship between water content and cell layer thickness, suggesting that factors other than water may also influence root cambial activity.
Regarding post-cambial cells, our study demonstrated a strong positive effect of soil water content, particularly when compared to cambial cells across both tissues. This pattern aligns with existing literature, which indicates that the cell enlargement phase is primarily driven by turgor pressure (Peters et al., 2021; Pantin et al., 2012; Steppe et al., 2015). Additionally, our findings reveal that all drought treatments ceased their activity at the extreme drought threshold of 0.22 water content. In contrast, high temperature conditions prompted an earlier cessation of root cambial activity at the severe drought level, thereby highlighting this tissue's sensitivity under combined drought and high temperature stress. This observation is supported by research demonstrating that the combination of drought and high temperature modifies plant metabolism, phytohormonal signaling, ABA biosynthesis, sugar transport, and gene expression (Rizhsky et al., 2004; Rasmussen et al., 2013; Jia et al., 2017).

The other cambial derivatives generally did not show a statistical link between cell layer thickness and water content, except for the stem under high temperature and drought (HD), the stem under optimal temperature and drought (OD), and the root under high temperature and drought (HD) in secondary cell wall-forming cells, mature cells, and non-collapsed phloem cells, respectively. In addition, a clear pattern emerged: secondary cell-wall forming cells (SW) and non-collapsed phloem cells (NCP) tend to increase as water content rises, while mature cells (MT) tend to decrease. When cambial activity declines due to reduced water availability, the growth of cambial derivatives also diminishes, whereas mature cells exhibit opposite trends. Balducci and colleagues (Balducci et al. 2015) observed that non-irrigated saplings had an increase in mature cell numbers similar to those irrigated until early June. Since mature cell formation and lignification can continue after cambial activity ceases (Rossi et al., 2006), the variation in mature cell layer thickness is likely more influenced by the number and size of cells formed during cambial activity rather than by direct water content reduction.

2.5 CONCLUSION

Our study showed that cambial cells in stems and roots had similar sensitivities, except during moderate drought, with stems being more vulnerable under severe and extreme drought. Roots, however, maintained greater resilience. The combination of heat and drought stress (HD) was the most damaging, causing early reduction in cambial activity and derivative cell development. In contrast, optimal temperature and drought (OD) allowed for partial recovery, especially after severe rewatering. Derivative cells, particularly post-cambial and secondary cell-wall-forming cells, were more sensitive than cambial cells themselves, with cell enlargement and lignification heavily restricted under drought, heat, and their combination.

We acknowledge that cambial activity and cell differentiation are dynamic processes, and that the tissues collected at each time point represent different developmental stages across treatments. Consequently, the observed differences among treatments may reflect the direct effect of water availability, temperature, and the intrinsic progression of tissue differentiation. However, our aim was not to isolate instantaneous treatment effects, but to characterize how different water regimes modulate the temporal dynamics of cambial activity and xylem differentiation. In this sense, the combined influence of growth stage and environmental condition provides an integrated view of the xylogenetic response to drought and rewatering under different temperature regimes.

Overall, these findings demonstrate that heat exacerbates the effects of drought and that xylogenesis outcomes depend on the interplay of stress type, intensity, and organ identity. The clear differences between stem and root responses highlight the importance of including root tissues—still less studied than stems—in understanding how whole plants respond to climate extremes. Moreover, although both xylem and phloem originate from the VC activity, their formation and dynamics are not strictly linked, and phloem anatomy and function still require more research in plant science. In summary, our findings suggest that future studies should consider root and phloem responses to better understand plant adaptation under combined stresses. The duration and severity of drought, along with their interaction with high temperatures, are key factors in designing experiments and predicting the resilience of woody plants in future climate scenarios.

2.6 REFERENCES

- Alfieri, F. J., & Evert, R. F. (1968). Seasonal development of the secondary phloem in Pinus. American Journal of Botany, 55(4), 518-528.
- Allen, C. D., Breshears, D. D., & McDowell, N. G. (2015). On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere, 6(8), 1-55.
- Aloni, R. (1980). Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. Planta, 150(3), 255-263.
- Arend, M., & Fromm, J. (2007). Seasonal change in the drought response of wood cell development in poplar. Tree physiology, 27(7), 985-992.
- Augustynczik, A. L. D., Gutsch, M., Basile, M., Suckow, F., Lasch, P., Yousefpour, R., & Hanewinkel, M. (2020). Socially optimal forest management and biodiversity conservation in temperate forests under climate change. Ecological Economics, 169, 106504.
- Baesso, B., Chiatante, D., Terzaghi, M., Zenga, D., Nieminen, K., Mahonen, A. P., ... & Montagnoli, A. (2018). Transcription factors PRE 3 and WOX 11 are involved in the formation of new lateral roots from secondary growth taproot in A. thaliana. Plant Biology, 20(3), 426-432.
- Baesso, B., Terzaghi, M., Chiatante, D., Scippa, G. S., & Montagnoli, A. (2020). WOX genes expression during the formation of new lateral roots from secondary structures in Populus nigra (L.) taproot. Scientific Reports, 10(1), 18890.
- Balducci, L., Deslauriers, A., Giovannelli, A., Rossi, S., & Rathgeber, C. B. (2013). Effects of temperature and water deficit on cambial activity and woody ring features in Picea mariana saplings. Tree Physiology, 33(10), 1006-1017.
- Balducci, L., Deslauriers, A., Giovannelli, A., Beaulieu, M., Delzon, S., Rossi, S., & Rathgeber, C. B. (2015). How do drought and warming influence survival and wood traits of Picea mariana saplings?. Journal of Experimental Botany, 66(1), 377-389.
- Balducci, L., Cuny, H. E., Rathgeber, C. B., Deslauriers, A., Giovannelli, A., & Rossi, S. (2016). Compensatory mechanisms mitigate the effect of warming and drought on wood formation. Plant, Cell & Environment, 39(6), 1338-1352.

- Balzano, A., Čufar, K., Battipaglia, G., Merela, M., Prislan, P., Aronne, G., & De Micco, V. (2018). Xylogenesis reveals the genesis and ecological signal of IADFs in Pinus pinea L. and Arbutus unedo L. Annals of botany, 121(6), 1231-1242.
- Barham, L., Duller, G. A., Candy, I., Scott, C., Cartwright, C. R., Peterson, J. R., ... & Nkombwe, P. (2023). Evidence for the earliest structural use of wood at least 476,000 years ago. Nature, 622(7981), 107-111.
-Battipaglia, G., De Micco, V., Brand, W. A., Linke, P., Aronne, G., Saurer, M., & Cherubini, P. (2010). Variations of vessel diameter and δ13C in false rings of Arbutus unedo L. reflect different environmental conditions. New Phytologist, 188(4), 1099-1112.
- Begum, S., Nakaba, S., Oribe, Y., Kubo, T., & Funada, R. (2007). Induction of cambial reactivation by localized heating in a deciduous hardwood hybrid poplar (Populus sieboldii× P. grandidentata). Annals of botany, 100(3), 439-447.
- Brunner, I., Herzog, C., Dawes, M. A., Arend, M., & Sperisen, C. (2015). How tree roots respond to drought. Frontiers in plant science, 6, 547.
- Campelo F, Gutiérrez E, Ribas M, Nabais C, Freitas H. (2007). Relationships between climate and double rings in Quercus ilex from Northeast Spain. Can J For Res 37:1915–1923.
- Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. Nature, 558(7711), 531-539.
- Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., ... & Valentini, R. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature, 437(7058), 529-533.
- Čufar, K., Prislan, P., De Luis, M., & Gričar, J. (2008). Tree-ring variation, wood formation and phenology of beech (Fagus sylvatica) from a representative site in Slovenia, SE Central Europe. Trees, 22(6), 749-758.
- Cuny, H. E., Rathgeber, C. B., Frank, D., Fonti, P., Mäkinen, H., Prislan, P., ... & Fournier, M. (2015). Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature plants, 1(11), 1-6.
- Daniels, L. D., Maertens, T. B., Stan, A. B., McCloskey, S. P., Cochrane, J. D., & Gray, R. W. (2011). Direct and indirect impacts of climate change on forests: three case studies from British Columbia. Canadian Journal of Plant Pathology, 33(2), 108-116.
- De Luis, M., Gričar, J., Čufar, K., Raventós, J. (2007). Seasonal dynamics of wood formation in Pinus halepensis from dry and semi-arid ecosystems in Spain. IAWA J 28:389–404.
- De Micco, V., Saurer, M., Aronne, G. Tognetti, R., Cherubini, P. 2007. Variations of wood anatomy and δ13C within-tree rings of coastal Pinus pinaster showing intra-annual density fluctuations. IAWA Journal 28: 61-74.
-De Micco, V., Balzano, A., Čufar, K., Aronne, G., Gričar, J., Merela, M., & Battipaglia, G. (2016). Timing of false ring formation in Pinus halepensis and Arbutus unedo in Southern Italy: outlook from an analysis of xylogenesis and tree-ring chronologies. Frontiers in plant science, 7, 705.
- De Micco, V., Carrer, M., Rathgeber, C. B., Camarero, J. J., Voltas, J., Cherubini, P., & Battipaglia, G. (2019). From xylogenesis to tree rings: wood traits to investigate tree response to environmental changes. IAWA journal, 40(2), 155-182.
-de Luis, M., Novak, K., Raventós, J., Gričar, J., Prislan, P., & Čufar, K. (2011). Climate factors promoting intra-annual density fluctuations in Aleppo pine (Pinus halepensis) from semiarid sites. Dendrochronologia, 29(3), 163-169.
- Denne, M. P. (1971). Temperature and tracheid development in Pinus sylvestris seedlings. Journal of Experimental Botany, 22(2), 362-370.
- D'Orsi, R., De Stefano, S., Durante, O., Ambrico, M., Aceto, D., Ambrico, P. F., ... & Di Bartolomeo, A. (2025). Tunable electrical properties of Lignin: Morphology-dependent ionic conduction in Kraft and organosolv-derived materials. Journal of Physics: Materials.
- Donat, M. G., Alexander, L. V., Yang, H., Durre, I., Vose, R., Dunn, R. J., ... & Kitching, S. (2013). Updated analyses of temperature and precipitation extreme indices since the beginning of the

twentieth century: The HadEX2 dataset. Journal of Geophysical Research: Atmospheres, 118(5), 2098-2118.

- Eilmann, B., Zweifel, R., Buchmann, N., Graf Pannatier, E., & Rigling, A. (2011). Drought alters timing, quantity, and quality of wood formation in Scots pine. Journal of Experimental Botany, 62(8), 2763-2771.

-FAO; UNEP. The State of the World's Forests 2020. In Forests, Biodiversity and People; FAO: Roma, Italy; UNEP: Nairobi, Kenya, 2020.

- Fonti, P., & Jansen, S. (2012). Xylem plasticity in response to climate. New Phytologist, 195(4), 734-736.

- Frei, E. R., Gossner, M. M., Vitasse, Y., Queloz, V., Dubach, V., Gessler, A., ... & Wohlgemuth, T. (2022). European beech dieback after premature leaf senescence during the 2018 drought in northern Switzerland. Plant Biology, 24(7), 1132-1145.

- Girona, M. M., Aakala, T., Aquilué, N., Bélisle, A. C., Chaste, E., Danneyrolles, V., ... & Gauthier, S. (2023). Challenges for the sustainable management of the boreal forest under climate change. In Boreal forests in the face of climate change: Sustainable management (pp. 773-837). Cham: Springer International Publishing.

- Gričar, J., Zupančič, M., Čufar, K., & Oven, P. (2007). Regular cambial activity and xylem and phloem formation in locally heated and cooled stem portions of Norway spruce. Wood Science and Technology, 41(6), 463-475.

- Gričar, J., & Čufar, K. (2008). Seasonal dynamics of phloem and xylem formation in silver fir and Norway spruce as affected by drought. Russian Journal of Plant Physiology, 55(4), 538-543.

- Gruber, A., Strobl, S., Veit, B., & Oberhuber, W. (2010). Impact of drought on the temporal dynamics of wood formation in Pinus sylvestris. Tree Physiology, 30(4), 490-501.

- Haynes, K. J., Allstadt, A. J., & Klimetzek, D. (2014). Forest defoliator outbreaks under climate change: effects on the frequency and severity of outbreaks of five pine insect pests. Global change biology, 20(6), 2004-2018.

- Hsiao, T. C. (1973). Plant responses to water stress. Annual review of plant physiology, 24(1), 519-570.

- Hu, X., Wu, L., Zhao, F., Zhang, D., Li, N., Zhu, G., ... & Wang, W. (2015). Phosphoproteomic analysis of the response of maize leaves to drought, heat and their combination stress. Frontiers in plant science, 6, 298.

- IPCC (2014): Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland.

- IPCC (2023): Summary for Policymakers. In: Climate Change 2023: Synthesis Report.Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.

- IPCC (2022): Summary for Policymakers [H.-O. Pörtner, D.C. Roberts, E.S. Poloczanska, K. Mintenbeck, M. Tignor, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem (eds.)]. In: Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA

- IUCN. 2025. The IUCN Red List of Threatened Species. Version 2025-1. https://www.iucnredlist.org. Accessed on [25 July 2025].

- Jentsch, A., & Beierkuhnlein, C. (2008). Research frontiers in climate change: effects of extreme meteorological events on ecosystems. Comptes Rendus Geoscience, 340(9-10), 621-628.

- Jia, J., Zhou, J., Shi, W., Cao, X., Luo, J., Polle, A., & Luo, Z. B. (2017). Comparative transcriptomic analysis reveals the roles of overlapping heat-/drought-responsive genes in poplars exposed to high temperature and drought. Scientific reports, 7(1), 43215.

- Kaennel, M., & Schweingruber, F. H. (1995). Multilingual glossary of dendrochronology. WSL FNP, Haupt, 133, 162-184.
- Körner, C. (2015). Paradigm shift in plant growth control. Current opinion in plant biology, 25, 107-114.
- Klesse, S., Peters, R. L., Alfaro-Sánchez, R., Badeau, V., Baittinger, C., Battipaglia, G., ... & Buras, A. (2024). No future growth enhancement expected at the northern edge for European beech due to continued water limitation. Global change biology, 30(10), e17546.
- Li, S., & Jansen, S. (2017). The root cambium ultrastructure during drought stress in Corylus avellana. IAWA journal, 38(1), 67-80.
- Lintunen, A., & Kalliokoski, T. (2010). The effect of tree architecture on conduit diameter and frequency from small distal roots to branch tips in Betula pendula, Picea abies and Pinus sylvestris. Tree physiology, 30(11), 1433-1447.
- López, R., Ramírez-Valiente, J. A., & Pita, P. (2022). How plants cope with heatwaves in a drier environment. Flora, 295, 152148.
- Martin-Benito, D., Anchukaitis, K. J., Evans, M. N., Del Río, M., Beeckman, H., & Cañellas, I. (2017). Effects of drought on xylem anatomy and water-use efficiency of two co-occurring pine species. Forests, 8(9), 332.
- McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., ... & Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought?. New phytologist, 178(4), 719-739.
- Melillo, J. M., Richmond, T. T., & Yohe, G. (2014). Climate change impacts in the United States. Third national climate assessment, 52, 150-174.
- Montagnoli, A., Terzaghi, M., Di Iorio, A., Scippa, G. S., & Chiatante, D. (2012). Fine-root morphological and growth traits in a Turkey-oak stand in relation to seasonal changes in soil moisture in the Southern Apennines, Italy. Ecological Research, 27(6), 1015-1025.
- Montagnoli, A., Di Iorio, A., Terzaghi, M., Trupiano, D., Scippa, G. S., & Chiatante, D. (2014). Influence of soil temperature and water content on fine-root seasonal growth of European beech natural forest in Southern Alps, Italy. European Journal of Forest Research, 133(5), 957-968.
- Montagnoli, A., Terzaghi, M., Baesso, B., Santamaria, R., Scippa, G. S., & Chiatante, D. (2016). Drought and fire stress influence seedling competition in oak forests: fine-root dynamics as indicator of adaptation strategies to climate change. Reforesta, (1), 86-105.
- Montagnoli, A., Dumroese, R. K., Terzaghi, M., Onelli, E., Scippa, G. S., & Chiatante, D. (2019). Seasonality of fine root dynamics and activity of root and shoot vascular cambium in a Quercus ilex L. forest (Italy). Forest Ecology and Management, 431, 26-34.
- Olano, J. M., Linares, J. C., García-Cervigón, A. I., Arzac, A., Delgado, A., & Rozas, V. (2014). Drought-induced increase in water-use efficiency reduces secondary tree growth and tracheid wall thickness in a Mediterranean conifer. Oecologia, 176(1), 273-283.
- Oleyar, M. D., Greve, A. I., Withey, J. C., & Bjorn, A. M. (2008). An integrated approach to evaluating urban forest functionality. Urban Ecosystems, 11(3), 289-308.
- Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., ... & Hayes, D. (2011). A large and persistent carbon sink in the world's forests. science, 333(6045), 988-993.
- Panshin, A. J., & Zeeuw, C. D. (1981). Textbook of wood technology (pp. 772-pp).
- Rasmussen, S., Barah, P., Suarez-Rodriguez, M. C., Bressendorff, S., Friis, P., Costantino, P., ... & Mundy, J. (2013). Transcriptome responses to combinations of stresses in Arabidopsis. Plant physiology, 161(4), 1783-1794.
- Pantin, F., Simonneau, T., & Muller, B. (2012). Coming of leaf age: control of growth by hydraulics and metabolics during leaf ontogeny. New Phytologist, 196(2), 349-366.
-Peñuelas, J., Castells, E., Joffre, R., & Tognetti, R. (2002). Carbon-based secondary and structural compounds in Mediterranean shrubs growing near a natural CO2 spring. Global Change Biology, 8(3), 281-288.

-Peters, R. L., Steppe, K., Cuny, H. E., De Pauw, D. J., Frank, D. C., Schaub, M., ... & Fonti, P. (2021). Turgor–a limiting factor for radial growth in mature conifers along an elevational gradient. New Phytologist, 229(1), 213-229.

- Pintilii, R. D. (2022). Forest recreation and landscape protection. Forests, 13(9), 1440.

Postharvest Biology and Technology, 61(2-3), 184-192.

- Plomion, C., Leprovost, G., & Stokes, A. (2001). Wood formation in trees. Plant physiology, 127(4), 1513-1523.
- Ren, P., Rossi, S., Gricar, J., Liang, E., & Cufar, K. (2015). Is precipitation a trigger for the onset of xylogenesis in Juniperus przewalskii on the north-eastern Tibetan Plateau?. Annals of Botany, 115(4), 629-639.
- Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., & Mittler, R. (2004). When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant physiology, 134(4), 1683-1696.
- Rossi, S., Deslauriers, A., Anfodillo, T., Morin, H., Saracino, A., Motta, R., & Borghetti, M. (2006). Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New phytologist, 170(2), 301-310.
- Rossi, S., Simard, S., Rathgeber, C. B., Deslauriers, A., & De Zan, C. (2009). Effects of a 20-day-long dry period on cambial and apical meristem growth in Abies balsamea seedlings. Trees, 23(1), 85-93.
- Sankar, M., Nieminen, K., Ragni, L., Xenarios, I., & Hardtke, C. S. (2014). Automated quantitative histology reveals vascular morphodynamics during Arabidopsis hypocotyl secondary growth. Elife, 3, e01567.
- Schiestl-Aalto, P., Kulmala, L., Mäkinen, H., Nikinmaa, E., & Mäkelä, A. (2015). CASSIA–a dynamic model for predicting intra-annual sink demand and interannual growth variation in S cots pine. New Phytologist, 206(2), 647-659.
- Schuldt, B., Leuschner, C., Brock, N., & Horna, V. (2013). Changes in wood density, wood anatomy and hydraulic properties of the xylem along the root-to-shoot flow path in tropical rainforest trees. Tree physiology, 33(2), 161-174.
- Senf, C., Buras, A., Zang, C. S., Rammig, A., & Seidl, R. (2020). Excess forest mortality is consistently linked to drought across Europe. Nature communications, 11(1), 6200.
- Serra, O., Mähönen, A. P., Hetherington, A. J., & Ragni, L. (2022). The making of plant armor: the periderm. Annual review of plant biology, 73(1), 405-432.
- Shah, N. H., & Paulsen, G. M. (2003). Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant and soil, 257(1), 219-226.
- Shtein, I., Meir, S., Riov, J., & Philosoph-Hadas, S. (2011). Interconnection of seasonal temperature, vascular traits, leaf anatomy and hydraulic performance in cut Dodonaea ‘Dana’branches.
- Shtein, I., Wolberg, S., Munitz, S., Zait, Y., Rosenzweig, T., Grünzweig, J. M., ... & Netzer, Y. (2021). Multi-seasonal water-stress memory versus temperature-driven dynamic structural changes in grapevine. Tree Physiology, 41(7), 1199-1211.
- Steppe, K., Sterck, F., & Deslauriers, A. (2015). Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends in plant science, 20(6), 335-343.
- Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M. A., & Steppe, K. (2015). Responses of tree species to heat waves and extreme heat events. Plant, cell & environment, 38(9), 1699-1712.
- Thomas, D. S., Montagu, K. D., & Conroy, J. P. (2007). Temperature effects on wood anatomy, wood density, photosynthesis and biomass partitioning of Eucalyptus grandis seedlings. Tree Physiology, 27(2), 251-260.
- Trewavas, A. (2009). What is plant behaviour?. Plant, cell & environment, 32(6), 606-616.
- Tyree, M. T., & Zimmermann, M. H. (2002). Hydraulic architecture of whole plants and plant performance. In Xylem structure and the ascent of sap (pp. 175-214). Berlin, Heidelberg: Springer Berlin Heidelberg.

- Ummenhofer, C. C., & Meehl, G. A. (2017). Extreme weather and climate events with ecological relevance: a review. Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1723), 20160135.
- Urban, J., Ingwers, M. W., McGuire, M. A., & Teskey, R. O. (2017). Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in Pinus taeda and Populus deltoides x nigra. Journal of experimental botany, 68(7), 1757-1767.
- Van der Werf, GW.; Sass-Klaassen, UG.; Mohren GM, J. The impact of the 2003 summer drought on the intra-annual growth pattern of beech (Fagus sylvatica L.) and oak (Quercus robur L.) on a dry site in the Netherlands. Dendrochronologia 2007, 25, 103–112.
-Vieira, J., Rossi, S., Campelo, F., Freitas, H., & Nabais, C. (2014). Xylogenesis of Pinus pinaster under a Mediterranean climate. Annals of Forest Science, 71(1), 71-80.
- Vile, D., Pervent, M., Belluau, M., Vasseur, F., Bresson, J., Muller, B., ... & Simonneau, T. (2012). Arabidopsis growth under prolonged high temperature and water deficit: independent or interactive effects?. Plant, cell & environment, 35(4), 702-718.
- Von Buttlar, J., Zscheischler, J., Rammig, A., Sippel, S., Reichstein, M., Knohl, A., ... & Mahecha, M. D. (2018). Impacts of droughts and extreme-temperature events on gross primary production and ecosystem respiration: a systematic assessment across ecosystems and climate zones. Biogeosciences, 15(5), 1293-1318.
- Wolberg, S., Dumbur, R., Netzer, Y., Grünzweig, J. M., & Shtein, I. (2024). High temperature induces investment in phloem and increases sucrose accumulation in grapevine. Trees, 38(5), 1151-1159.
- Zhao, X., Li, P., Liu, X., Xu, T., Zhang, Y., Meng, H., & Xia, T. (2022). High temperature increased lignin contents of poplar (Populu s spp) stem via inducing the synthesis caffeate and coniferaldehyde. Frontiers in genetics, 13, 1007513.
- Ziaco E, Truettner C, Biondi F, Bullock S. Moisture-driven xylogenesis in Pinus ponderosa from a Mojave Desert mountain reveals high phenological plasticity. Plant, Cell Environ. 2018;41(4):823–36. https:// doi. org/ 10. 1111/ pce. 13152.
- Zscheischler, J., Mahecha, M. D., Von Buttlar, J., Harmeling, S., Jung, M., Rammig, A., ... & Reichstein, M. (2014). A few extreme events dominate global interannual variability in gross primary production. Environmental Research Letters, 9(3), 035001.

Chapter III: Above- and below-ground morpho-physiological traits indicate that biochar is a potential peat substitute for grapevine cuttings nursery production.

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Abstract: The growing demand for grapevine planting materials, due to growing global viticulture, is promoting research studies to improve vineyard sustainability. In greenhouse nurseries, peat is the most common growing medium component used although is an expensive and non-renewable material. Indeed, the reduction of peat exploitation is receiving great attention, and currently, several materials arebeing investigated as peat substitutes for composing the cultivation substrates. Biochar, a carbon- rich, recalcitrant charred organic co-product of the pyrolysis or gasification process, has emerged as a potentially promising replacement for soilless substrates in nursery plant material propagation. Although several studies carried out at greenhouse nurseries have shown that biochar, can improve plant growth, only a few studies have focused on the production of grapevine plant material. To fulfil this knowledge gap and push forward the sustainability of the nursery sector, we evaluated above. and below-ground morpho-physiological traits of one-year-old potted grapevine cuttings growing with 30% volume of four different biochar types (i.e., from pyrolysis and gasification) mixed with commercial peat. The present study shows that biochar can be used in growing media mixes without adverse effects on roots, improves soil water retention and leaf water potential, and improves the effects on soil microbiology.

3.1 INTRODUCTION

The increase in viticulture worldwide reflects a higher demand for grapevine (*Vitis vinifera* L.) planting materials raising the urgent need to improve the sustainability of nursery - activities[1]. Indeed, in greenhouse nurseries, peat is the most common material composing growing media although is expensive and non-renewable[2]. In addition, peatlands exploited for peat extraction are important ecosystems for carbon (C) reserves and for regulating local water quality and - regime[3]. Therefore, to reduce this exploitation impact several alternative-to-peat materials are currently receiving great attention from researchers being investigated as growing media - components[4]. Biochar, a carbon-rich coproduct of biomass pyrolysis or gasification, can be produced from renewable organic waste material and is a potential candidate material for composing alternative and sustainable growing - media[5,6–8]. Indeed, the majority of the studies testing the application of biochar to soils, soil-less growing media, and plant growth showed a series of positive - properties[9,10], such as the increase in plant - growth[11,12] mainly due to: (i) the higher soil water content occurring during the dry growing - period[13,14], (ii) the increase in beneficial microbial - microfauna[15,16] and (iii) the increase in cation exchange capacity and micro–macro nutrients - availability[17]; Therefore, the valorization through the biochar production of agro-industrial by-products may represent a potential material alternative-to-peat in composing the growing media. Besides environmental - concerns[18] the nursery production of forced or dormant grafts grown in containers may represent an effective alternative to open-field propagation, and the selection of alternative-to-peat materials should take into consideration their ability to produce high-quality plant material to be transplanted at any time of the year. To this aim, replacing peat with biochar could represent a powerful tool for improving plant growth and substrate characteristics while reducing water consumption. In particular, problems related to biotic and abiotic stresses, such as root dehydration, contamination by soil pathogens, and frost damage, can negatively affect vineyard establishment of dormant root vines and ensuing field - production[19]. Furthermore, a further critical issue in the nursery sector concerns the quantity and quality of the water used for irrigation since the annual water consumption for irrigation is currently around 1000–1500 mm for each plant in a - container[20]. Therefore, the peat replacement with biochar is linked to its inherent characteristics of an increase in water availability of the growing - media[21,22] and, thus, a reduction in the quantities of irrigation water needed. Also, container production reduces production cycle timespan, and improves plant quality and seedling performance in the field, especially under harsh site - conditions[23]. Specifically, for the soil chemical characteristics of the growing media, an ideal substrate should have balanced air porosity, optimal bulk density, and adequate water-holding capacity. These characteristics guarantee an efficient exchange of oxygen and carbon dioxide, promoting the correct development of fine - roots[2,12]. Achieving the right

balance between these physical characteristics is important to stimulate root and plant growth throughout the container usage. Finally, most studies on biochar and growing media have focused on increasing crop growth or reducing non-peat environmental concerns, such as carbon sequestration, remediation of contaminants, and reduction of greenhouse gas - emissions[24,25]. There is also a fair number of studies focused on the production, characterization, and engineering of - biochar[26,27] in soilless - substrates[28,12, 29]. All of these studies indicated that plant responses to the addition of biochar to substrates are similar to those found with standard peat-containing substrates, with the added benefits of reducing nutrient and water loss and bulk density and creating a benefit for microbes. Although this plethora of studies done in a greenhouse nursery shows that biochar, used at low doses, can improve plant - growth[29], few studies have been done on the production of grapevine planting - material[8,30,31]

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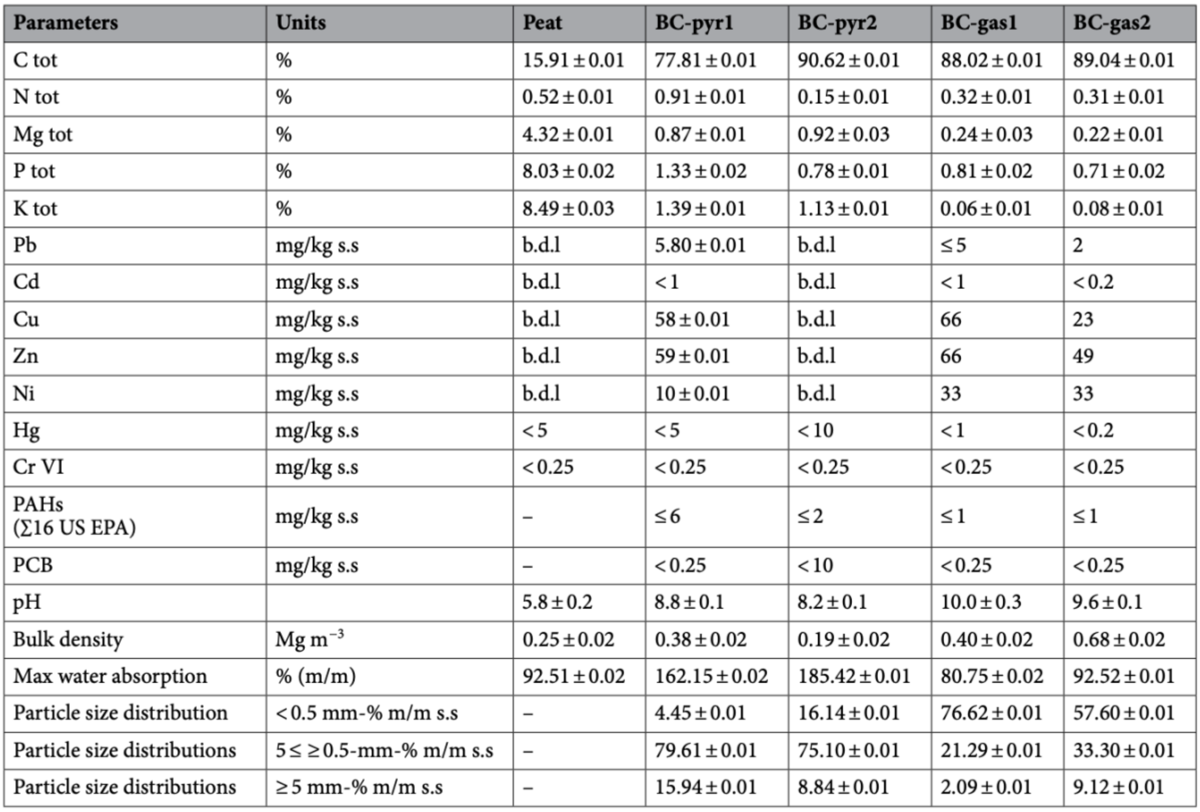
Therefore, the present research aims to evaluate biochar as a possible substitute for peat in the pot growth of grapevine cuttings. The biochar types used come from pyrolysis and gasification of waste biomass and different temperatures allowing to evaluation of the performance of the different biochar types on the characteristics of the substrate and plant growth development in terms of water retention, microbial biomass, root functional traits, ecophysiological performance, and production. We hypothesized that biochar is a soil component that can be used in growing media mixes in quantities of 30% v/v conferring ideal physicochemical properties to the substrate and, in turn, (i) increasing the substrate water retention and plant water status and reducing the irrigation requirement (ii), improving the fine root development by increasing both length and biomass in all diameter classes, and (iii) increase the community-level physiological profiling (CLPP) of rhizosphere. To test our multiple hypotheses, we used four different biochars in terms of the bioenergy production process (i.e., pyrolysis and gasification) to analyze the morpho-physiological above- and below-ground traits of potted grapevine plants together with the chemical-physical substrate characteristics and the physiological profile of rhizosphere microbial community function.

3.2 Results

3.2.1 Growing media characteristics

All types of biochar used, shown in Table 1, have a high total carbon content - (Ctot) that was higher than 70 compared to peat which contains 15.91 ± 0.01%. The total macronutrients analyzed (Mg_{tot}, - P_{tot}, and - K_{tot}) have very



Parameters	Units	Peat	BC-pyr1	BC-pyr2	BC-gas1	BC-gas2
C tot	%	15.91 ± 0.01	77.81 ± 0.01	90.62 ± 0.01	88.02 ± 0.01	89.04 ± 0.01
N tot	%	0.52 ± 0.01	0.91 ± 0.01	0.15 ± 0.01	0.32 ± 0.01	0.31 ± 0.01
Mg tot	%	4.32 ± 0.01	0.87 ± 0.01	0.92 ± 0.03	0.24 ± 0.03	0.22 ± 0.01
P tot	%	8.03 ± 0.02	1.33 ± 0.02	0.78 ± 0.01	0.81 ± 0.02	0.71 ± 0.02
K tot	%	8.49 ± 0.03	1.39 ± 0.01	1.13 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
Pb	mg/kg s.s	b.d.l	5.80 ± 0.01	b.d.l	≤5	2
Cd	mg/kg s.s	b.d.l	< 1	b.d.l	< 1	< 0.2
Cu	mg/kg s.s	b.d.l	58 ± 0.01	b.d.l	66	23
Zn	mg/kg s.s	b.d.l	59 ± 0.01	b.d.l	66	49
Ni	mg/kg s.s	b.d.l	10 ± 0.01	b.d.l	33	33
Hg	mg/kg s.s	< 5	< 5	< 10	< 1	< 0.2
Cr VI	mg/kg s.s	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
PAHs (Σ16 US EPA)	mg/kg s.s	–	≤6	≤2	≤1	≤1
PCB	mg/kg s.s	–	< 0.25	< 10	< 0.25	< 0.25
pH		5.8 ± 0.2	8.8 ± 0.1	8.2 ± 0.1	10.0 ± 0.3	9.6 ± 0.1
Bulk density	Mg m^{-3}	0.25 ± 0.02	0.38 ± 0.02	0.19 ± 0.02	0.40 ± 0.02	0.68 ± 0.02
Max water absorption	% (m/m)	92.51 ± 0.02	162.15 ± 0.02	185.42 ± 0.01	80.75 ± 0.02	92.52 ± 0.01
Particle size distribution	< 0.5 mm-% m/m s.s	–	4.45 ± 0.01	16.14 ± 0.01	76.62 ± 0.01	57.60 ± 0.01
Particle size distributions	5 ≤ ≥ 0.5-mm-% m/m s.s	–	79.61 ± 0.01	75.10 ± 0.01	21.29 ± 0.01	33.30 ± 0.01
Particle size distributions	≥ 5 mm-% m/m s.s	–	15.94 ± 0.01	8.84 ± 0.01	2.09 ± 0.01	9.12 ± 0.01

Table 1. Main chemical and physical characteristics of peat and biochar. b.d.l., below detection limit.

variable values. In particular, - $\mathrm{Mg_{tot}}$ and - $\mathrm{K_{tot}}$ values are much higher in pyrolysis than in gasification biochar. The pyrolysis biochars (BC-pyr1 and BC-pyr2) have a pH value of 8.8 ± 0.1 and 8.2 ± 0.1 respectively, while gasification biochars have a relatively more basic pH with values of 10.0 ± 0.3 in BC-gas1 and values of 9.6 ± 0.1 in BC-gas2 (Table 1). Peat has an acidic pH of 5.8 ± 0.2. All types of biochar used in the present study fall within the parameters required by Italian Legislative Decree 75/10 which defines biochar as an agronomic amendment in Italy for PAH, PCB, and heavy metals content (Table 1). Furthermore, all types of biochar analyzed could also fall under the new regulation on fertilizer products (EU/2019/1009), recently adopted by the European Union. All types of biochar used were characterized by a very low bulk density (BD) value with lower values in pyrolysis biochar (0.38 ± 0.02 and 0.19 ± 0.02 Mg m^{-3} respectively in BC-pyr1 and BC-pyr2), compared to gasification biochar (0.40 ± 0.02 and 0.68 ± 0.02 Mg m^{-3} respectively in BC-gas1 and BC-gas2; Table 1). Maximum water absorption was on average 174 ± 1% (w w^{-1}) in pyrolysis biochar and 87 ± 2% (w w^{-1}) in gasification biochar (Table 1). The largest percentage of particle size of pyrolysis biochar is concentrated between 5 and 0.5 mm, while in biochar obtained from gasification, the particle size is concentrated in values lower than 5 mm in dry matter (Table 1).

3.2.2 Growing media measurements

Substrates with biochar from pyrolysis and gasification have a more basic pH than the control, with an average value of 7.3 ± 0.2 for both the substrates (pyrolysis and gasification biochar) compared to the control with a pH of 5.9 ± 0.4 (Table 2). Bulk density values are lower in growing media with pyrolysis biochar (BC-pyr) compared to control. The bulk density values of the substrates with biochar from gasification (BC-gas) showed higher apparent density values than the control (Table 2).

3.2.3 Plant measurements

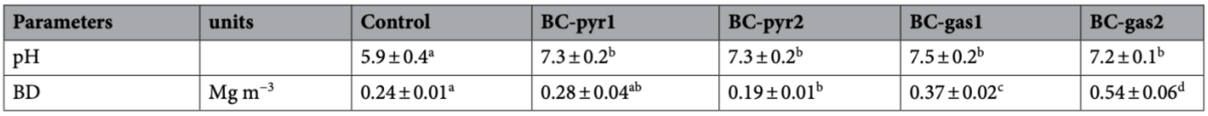
Plant height did not differ between biochar-amended substrates and control independently of the biochar type considered and during the entire growing period with the only exception of the last sampling point (Fig. 1). In particular, on the last sampling date, the height of the plants grown with BC-pyr1, BC-pyr2, and BC-gas1 was significantly higher than both control and BC-gas2-treated plants, which had similar values (Table 3). The total plant biomass did not differ among control and treatments, independently of the type of biochar analyzed (Table 3). The highest and lowest values of the third internode length were measured respectively in BC-pyr1 and BC-pyr2, and in BC-gas2 treated plants, while control and BC-gas1 treated plants had intermediate values (Table 3). Figure 2 shows the leaf water potential values measured at midday. The data showed that the leaf water potential values for biochar-treated plants, both pyrolysis and gasification were significantly lower to control plants on 16th June and 12th July. On 11th August there were still differences in BC-pyr1 and BC-pyr2, but not statistically different in BC-gas1 and BC-gas2 from the control. However, there are no differences between the two biochar treatments (BC-pyr and BCgas) (Fig. 2). Although leaf temperatures (Fig. 3) show lower values in plants treated with biochar, there are no statistically significant differences. Statistically significant differences were noted on the date of - 11th August between BC-pyr and C.

3.2.4 Community-level physiological profile of rhizosphere

The community-level physiological profile (CLPP) was analyzed on the rhizosphere. The microbial response in each microplate that expressed average well-colour development (AWCD) index increased proportionally and the most intensive metabolism of carbon substrates was observed at 120 h. The biochar treatments showed an increase in the AWCD compared to the control (Fig. 4a). The highest value of AWCD was found in the rhizosphere of BC-gas2-treated plants, without significant differences with the other biochar treatments, which were similar to the control (Fig. 4a). Concerning the microbial level of substrate uses, no significant differences between different biochar types and controls were found for carboxylic acids, carbohydrates, amino acids, polymers, and amine except phenolic compounds (Fig. 4b-g) that showed the highest value in samples characterized by the BC-pyr1 application (Fig. 4e).

3.2.5 Root growth length and biomass

Both the length and biomass of the totality of fine roots (d < 2 mm) did not differ among the control and the four biochar treatments (Fig. 5a,e), with the only exception of BC-gas2-treated plants that had the highest biomass than control (Fig. 5e). The fine-roots analysis performed according to three different diameter classes revealed that this pattern remained the same (Fig. 5b,d,f,h). Moreover, the BC-gas2 treated plants showed significantly higher biomass than control plants only in the 0.5–1 mm root class (Fig. 5g). Independently of the treatment,



Parameters	units	Control	BC-pyr1	BC-pyr2	BC-gas1	BC-gas2
pH		5.9 ± 0.4^{a}	7.3 ± 0.2^{b}	7.3 ± 0.2^{b}	7.5 ± 0.2^{b}	7.2 ± 0.1^{b}
BD	Mg m^{-3}	0.24 ± 0.01^{a}	0.28 ± 0.04^{ab}	0.19 ± 0.01^{b}	0.37 ± 0.02^{c}	0.54 ± 0.06^{d}

Table 2. Growing media physical characteristics at the end of the experiment (23rd September 2022). Data (n = 5) are reported as mean ± standard deviation. Statistically significant differences ($p < 0.05$) were identified by the post hoc Dunnett's test for multiple comparisons (SPSS Statistics 25 IBM) and marked with the letters a, b, c.

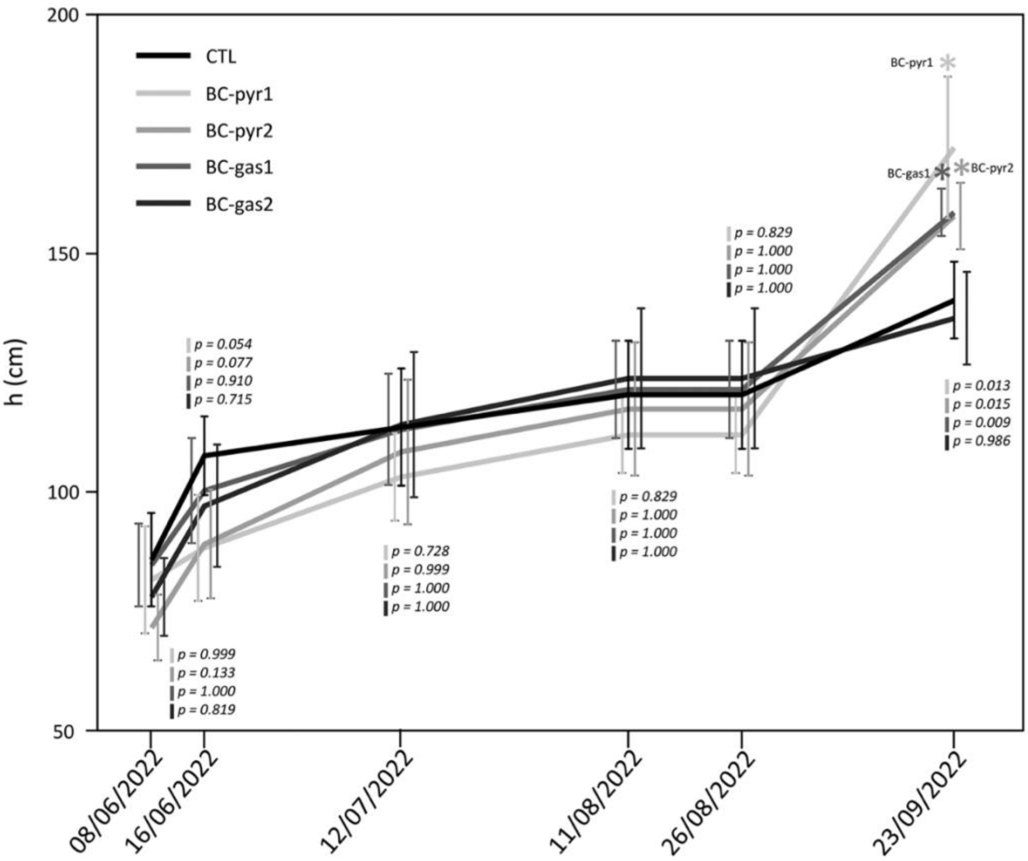


Figure 1. Plant growth rate (cm) during the period of measurement. The curves represent the mean values of 10 plants for treatment (n = 10). Error bars represent the 95% confidence interval while asterisks ($p < 0.05$) and *p* values indicate statistical differences between the control and the respective treatment.

Parameters	Units	Control	BC-pyr1	BC-pyr2	BC-gas1	BC-gas2
Total biomass	(g)	71 ± 0^{a}	80 ± 1^{a}	80 ± 1^{a}	76 ± 0^{a}	75 ± 1^{a}
3rd internode length	cm	1.7 ± 0.5^{ab}	2.2 ± 0.2^{b}	2.5 ± 0.0^{b}	2.2 ± 0.6^{ab}	1.5 ± 0.3^{a}
Total height	cm	140 ± 6^{a}	172 ± 12^{b}	158 ± 6^{b}	159 ± 4^{b}	136 ± 8^{a}

Table 3. Morphological and physiological parameters of the plants at the end of experiment (23rd September 2022). Data (n = 5) are reported as mean ± standard deviation. Statistically significant differences ($p < 0.05$) were identified by the post hoc Dunnett's test for multiple comparisons (SPSS Statistics 25 IBM) and marked with the letters a, b, c.

the fine-root length decreased with the increase of the root diameter (Fig. 5b,d), and, vice versa, the fine-root biomass proportionally increased with the different root sizes (Fig. 5f,h).

3.3 DISCUSSION

Grapevine propagation in the nursery greenhouse is relatively easy. However, high skills and organization are required to produce planting materials with the high-quality standards required every year by growers for new plantings, replanting of uneconomical vineyards, or replacing plants affected by stem disease- pathogens[19].

The chemical and physical characteristics of the growing media can influence the cutting - system[32]. Growing media should be friable, free of weeds and pathogens, with good water capacity and - drainage[33]. Peat is the most commonly used growing media due to its positive hydrological, physicochemical, and agronomic- characteristics[34,19]. However, to improve agricultural sustainability, researchers are called to study alternatives to the use of peat as innovative growing substrates. Among the different components suitable for growing media, biochar materials belong to a relatively new and not yet established group in the growing media market.

The main focus of the growing media industry is on growth-supporting properties such as slightly acidic pH, high cation exchange capacity, good aeration, and high-water holding capacity to ensure germination and good plant growth in the youth. In areas where peat is not available, carbonized materials are often used successfully as reported by Steiner et al.[35] using rotten tree trunks and biochar in the Brazilian Amazon. Careful consideration of the use of peat has led to various alternatives being considered, some of which have been evaluated as 1:1 substitute. When considering new growing media ingredients, whether mixed with peat or alone, on a technical level there are important physical, chemical, and biological factors to consider. From a physical point of view, the bulk density of the growing medium components should be low, but the

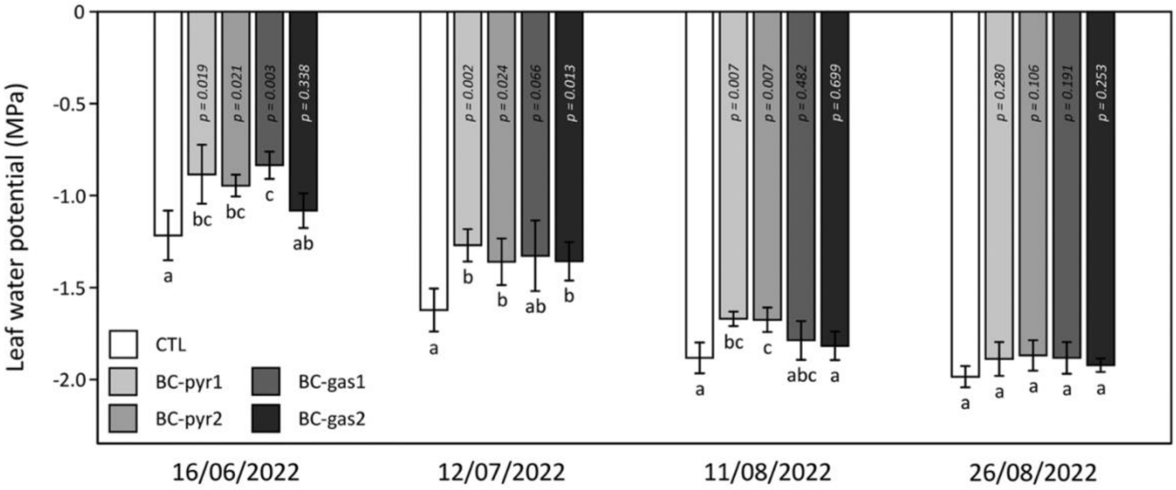


Figure 2. Leaf water potential (MPa) on midday measurement. Each column represents the mean value of 5 measurements (n = 5). Error bars represent the 95% confidence interval while *p* values indicate statistical differences between the control and the respective treatment.

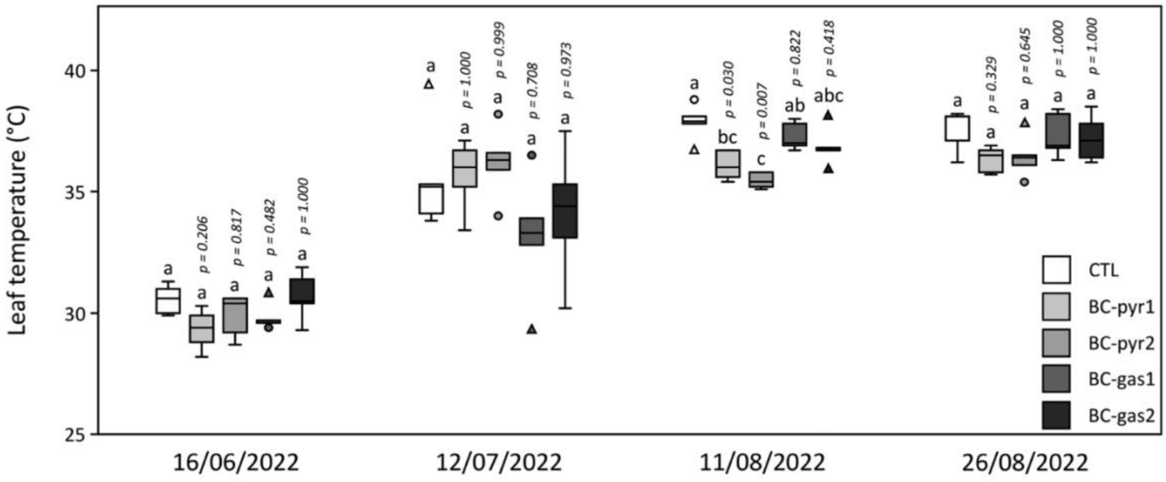


Figure 3. Leaf temperature measurement (°C) made on 5 independent plants (n = 5). Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% values of the distribution. Within each box, the solid horizontal line is the median value, while circles and triangles represent outliers and extreme outliers. *p* values indicate statistical differences between the control and the respective treatment.

mechanical stability and total pore space should be - high[36]. Particle size distribution affects aeration and water-holding - capacity[37] The presented results support our general hypothesis demonstrating that biochar can be used as a partial replacement for peat in growing media, in agreement with Dumroese et al.[38] and Steiner and - Harttung[3], since it confers ideal physicochemical properties desired in soilless cultivation substrates. According to Yao et al.[39], carbon content is the most significant parameter for biochar quality, and values above the 70% characterize a high-quality biochar. Although the starting material primarily influences the characteristics of the obtained biochar, those used in the present experiment all exceed 70% of carbon content and have little differences in the elemental content and no permanent differences in the chemical characteristics. In agreement with other studies and in support of the first part of the hypothesis, in our study, the biochar application improves the water availability and decreases the leaf water potential, which can be related to the intrinsic characteristics of the biochar used, especially biochar from pyrolysis which showed very high values of maximum water absorption. The biochar used in this experiment has the highest particle size distribution concentration between 0.5 and 5 mm and, as noted by Kern et al.[40], in particular, the < 1 mm fraction plays a key role in water availability and air capacity. Furthermore, the biochar used in our experiment shows lower bulk density values than peat only in the biochar from pyrolysis (BC-pyr2). These properties probably explain how, in our experiment, the addition of biochar improved the leaf water potential during the growing season, confirming and extending the results of other - authors[41]. Other - studies[42,43, 44] highlighted how the biochar production method influences the intrinsic characteristics of the biochar itself

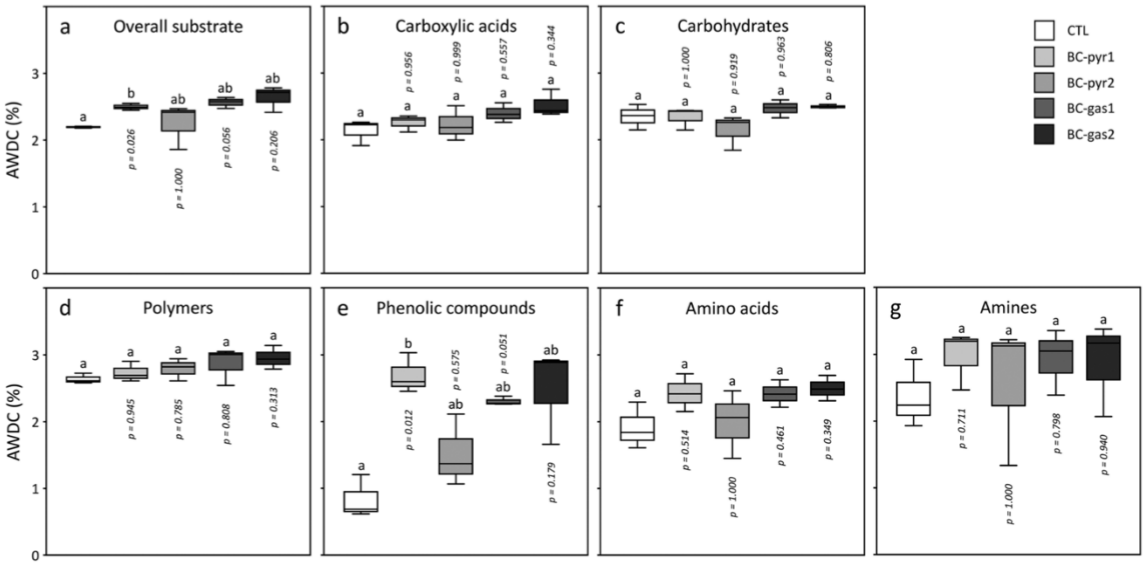


Figure 4. Community-level physiological profiling of the rhizosphere using Biolog EcoPlates. (**a**) Average (n = 3) of well color development (AWCD) at the maximum development drawn from the overall community substrate utilization profile during an incubation period of 12–120 h. (**b–g**) AWCD analyzed considering the microbial activity degrading of each carbon source: (**b**) AWCD at the maximum development of carboxylic acids; (**c**) AWCD at the maximum development of carbohydrates; (**d**) AWCD at the maximum development of polymers; (**e**) AWCD at the maximum development of phenolic compounds; (**f**) AWCD at the maximum development of aminoacidic; (**g**) AWCD at the maximum development of amines. Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% values of the distribution. Within each box, the solid horizontal line is the median value. *p* values indicate statistical differences between the control and the respective treatment.

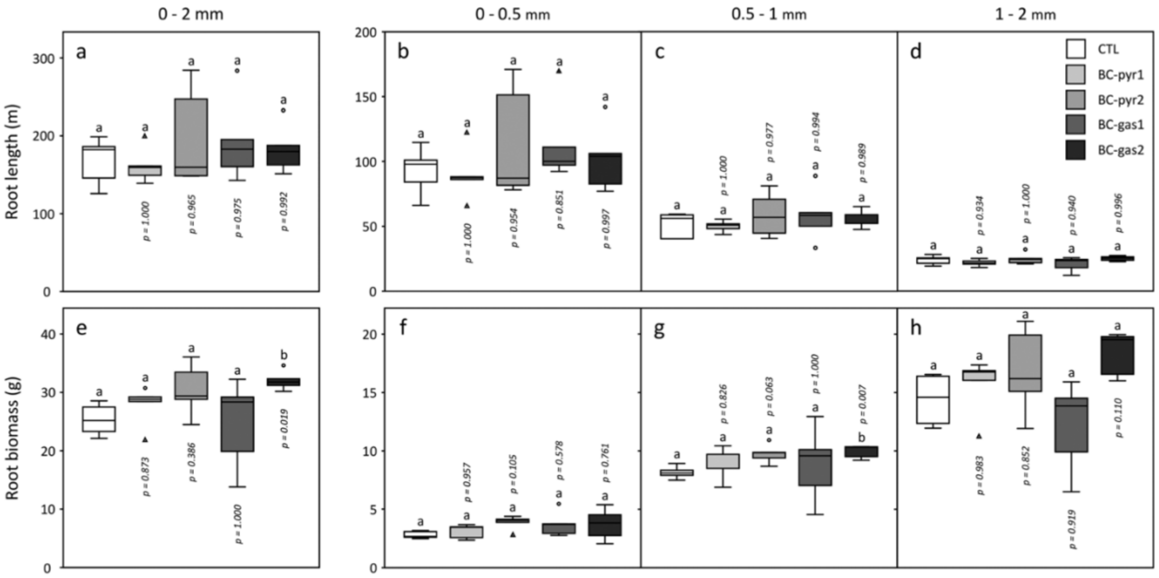


Figure 5. Mean fine-root standing length (m) (**a–d**) and biomass (g) (**e–h**) according to different diameter classes: (**a, e**) 0–2 mm, (**b, f**) 0–0.5 mm, (**c–g**) 0.5–1 mm, and (**d–h**) 1–2 mm. Vertical boxes represent approximately 50% of the observations (n = 5) and lines extending from each box are the upper and lower 25% values of the distribution. Within each box, the solid horizontal line is the median value, while circles and triangles represent outliers and extreme outliers. *p* values indicate statistical differences between the control and the respective treatment.

and, consequently, the interactions with the soil. Furthermore, understanding water-biochar behavior could be further studied by considering molecular interactions at the micro-nano level. For example, Conte et al.[45] suggest that water molecules are bonded to solid carbonaceous material through unconventional hydrogen bonds. The modification of plant water availability induced by the application of biochar could increase the resilience of grapevine cuttings nursery production to water shortage (drought), as demonstrated by the lower leaf potential measured in treated plots compared to control plots. The effect of biochar and its influence on the water-soil relationship depended directly on the type of biochar and the data demonstrate that pyrolysis biochar responds better to the need due to their intrinsic characteristics (Table 1) in terms of maximum water absorbent. The gasification biochars, however, show higher bulk density values compared to the control. In our study we find that replacing peat with biochar leads to an increase in pH, being an alkaline material, as found by Vaughn et al.[4], and a slight increase in bulk density but without negatively affecting plant growth throughout the analyzed period. Indeed, the increase in pH is in line with numerous studies on biochar-based - material[46,47].

The increase in pH following the addition of biochar is beneficial for acidic soil or growing media (as is the case for peat in the present work), with the biochar acting as a liming agent and likely replacing the calcium oxide used to increase the pH[48,49]. The biochars ratio used in the present study, maintained pH values around 7.0, as the ideal substrate pH for peat substrate is between 5.0 and 5.550. Furthermore, C_{tot} values are higher in biochar-based growing media.

Moreover, results of plants treated with the two different biochar types showed a similar fine root development independently of the diameter class considered partially supporting the second part of our hypothesis. Also, the ameliorating of the community-level physiological profile observed in biochar-treated rhizosphere supported the third part of our hypothesis.

The results of this experiment were substantially in agreement with those of previous works, which reported that an increase in plant biomass is one of the macroscopic effects induced by the partial replacement of peat in the cultivation substrates with agro-industrial by-products, such as compost, digestate, and - biochar[8]. The ability of biochar to increase plant biomass is probably due to better availability and absorption of nutrients by the plant and/or the presence of some microorganisms and compounds capable of increasing plant growth as secondary-metabolites[51]. When these substances are added to growing media, they can improve the biochemical activity of plants, similar to plant hormone-like - promoters[52]. Interactions among microorganisms and plant roots are essential for the nutritional requirements of the plant. Our results showed how the microbial community was positively influenced by the biochar application for the use of phenolic compounds, which are of great significance in diverse processes of plant development, including rhizogenesis[53].

To widen the practical application of the present study, a few considerations about the economic analysis of the biochar are included. Considering the wholesale prices of the main constituents of the media in cultivation, such as peat, coconut fiber, or green compost, these are much lower than those of biochar and range from €25,00 m^{-3} for peat to €20,00 m^{-3} for coconut fiber, or €12,50 m^{-3} for green compost. If we use peat as a reference and consider an average biochar bulk density of 0.20 $t\ m^{-3}$, the reference price level per ton of biochar could stand around €125,00, making it difficult to imagine a future for this material in nursery activities and horticulture (oral communication COST ACTION TD1107 "Biochar as option for sustainable resource management").

However, biochar was recently recognized as a negative emissions technology by the Intergovernmental Panel on Climate Change (IPCC)[54], representing a powerful tool for moving these nursery activities toward higher levels of environmental sustainability. Finally, since environmental considerations have become as important as performance and economic costs, we suggest the need to integrate the environmental and economic assessment to better illustrate the additional costs that could be incurred to reach - CO2 reduction - target[55,36]. For example, very recently, Hashemi et al.[56] demonstrated that bio-based peat alternatives (wood fiber, compost, and hydrochar based on willow and degassed fiber from agricultural waste) and their mixtures as growing media (GM) for plant production in Denmark may significantly reduce greenhouse gas emissions and global warming potential compared to peat.

3.4 CONCLUSION

The results obtained in the present study further contribute to expanding the biochar potential applicability and suggesting that it could be a suitable ingredient for alternative-to-peat growing media and an effective strategy to reduce high-quality water requirements in the vine nursery sector. In particular, the different biochars used showed a well above- and below-ground plant and microbial morpho-physiological performance. However, the complexity of interaction between biochar, plant, rhizosphere microbial community, and nutrient-water deserves further investigation, especially considering the wide variability due to different starting materials and thermochemical processes. Finally, although the sale price of biochar is still high when compared with other growing media ingredients, the en vironmental advantages should be included in a complete analysis of the performance and economic costs.

3.5 MATERIALS AND METHODS

3.5.1 Experimental design

The experiment was made in 2022 at Vivai New Plants di Barbara Gini, Cenaia (Pisa, Italy). Trials involved 100 grafted cuttings of V. vinifera cv. Sangiovese (clone I-SS-F9-A5-48) grafted in 2021 on rootstock 1103 Paulsen (*Vitis berlandieri* × *Vitis rupestris*), grown in pots (15x15x20 cm, approx 4.5 L volume). A randomized block design experiment, with 20 replicates (each replicate composed of one pot), was set up considering five treatments (4 different growing media with biochar and peat) and control without biochar. The experiment started at the end of April (22nd April 2022) transplanting one-year-cuttings of V. vinifera for each pot and the plants were harvested at the end of September 2022 (23th September 2022). During the experiment, drip watering was performed once a day at 10 a.m. at a rate of approximately 0.7 l per pot. The evaluated growing media included replacing half of the peat for a 30% in volume of the whole media composition (i.e., Control: peat/pumice 2:1; Treatment: peat/biochar/pumice 1:1:1) with 4 different types of biochar, called BC-pyr1, BC-pyr2, BC-gas3, and BC-gas4.

3.5.2 Growing media (peat and biochar) characteristics

First of all, a series of parameters are recognized that will contribute to a large extent to the different properties of the biochar, among which the production temperature and the type of conversion process influence the characteristics of the - biochar[57]. For this reason, we decided to use 4 different types of biochar, 2 of which come from gasification plants and two from pyrolysis plants.

The total content of different elements (Mg, P, K, Pb, Cd, Cu, Zn, Ni, Hg, Cr) was determined after acid digestion with a microwave oven, according to EPA - 3052[58], with an ICP-OES (iCAP 6000 Series, Thermo Scientific, Waltham, MA, USA) based on the EPA 6010D 2014 standard. For total carbon - (C_{tot}) and total nitrogen - (N_{tot}) of biochars and peat, samples were oven-dried at 105 °C for 24 h, acid digested with a microwave oven (CEM, MARSXpress) according to the EPA method - 305258 and determined using a CHN Elemental Analyzer (Carlo Erba Instruments, mod 1500 series 2). The polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) analysis was carried out via the US-EPA - 3550C[59] method; respectively the maximum water retention and the particle size fraction were determined using the method indicated in the DM 1/08/97 SO n. 173 GU 204 2/09/1997 Met.4 and UNI EN - 15428[60].

Biochar and peat bulk density were determined using the UNI EN 13041 - method[61]. The peat was a commercial type. Following is a brief description of each biochar type and in Table 1 their chemical-physical characteristics along with those of the peat used for composing the growing media.

- Control (C): peats
- Biochar 1 (BC-pyr1) was obtained from the orchard (i.e., olive tree, vines, apricot, and apple tree) pruning biomass through a slow pyrolysis process at a temperature of 500 °C in a transportable ring kiln of 2.2 m in diameter and holding around 2 t of feedstock.
- Biochar 2 (BC-pyr2) was obtained from olive tree pruning biomass through a slow pyrolysis process at a temperature of 550 °C in a rotary kiln of 100 kg per hour process capacity.
- Biochar 3 (BC-gas1) was obtained from the gasification of coppicing residues of beech (Fagus sylvatica L.) and oak (Quercus spp.) forests at a temperature of 900 °C.
- Biochar 4 (BC-gas2) was obtained from the gasification of coppicing residues of beech (F. sylvatica L.), hazel (Corylus avellana L.), oak (Quercus spp.), and birch (Betula alba L.) at a temperature of 900 °C.

3.5.3 Growing media measurements

At the end of the experiment, growing media samples were collected from 10 plots per treatment including the control. Soil pH was measured in water solution (1:2.5 ratio) using the pH Meter Mettler Toledo S220. The soil bulk density (BD, $Mg\ m^{-3}$) was measured using a cylindrical core of 100 - cm^3 volume (V)[62]. Samples were weighed at field conditions (FW), dried in an oven at 105 °C for 48 h, and reweighed (DW) for calculating the gravimetric soil moisture content ($g\ g^{-1}$) as:

$[(FW - DW) \div DW]$ *(1)*

and BD ($Mg\ m^{-3}$) as:

$[DW \div V]$ *(2)*

3.5.4 Plant measurements

During the experiment, periodic measurements (8th June; 16th June; 12th July; 11th August; 26th August) of plants' height were done. Dry biomass of stems and leaves (at 80 °C in a ventilated oven for 48 h), 3rd internode length, and total height were measured at the final sampling point (23rd September). During summer, the leaf water potential (MPa) was measured on four cloudless and representative days (16th June; 12th July; 11th August; 26th August). Leaf water potential was measured according to the procedures of Padgett-Johnson et al.[63], using a pressure chamber (PMS, Instrumentation Co. Corvallis, OR, USA), on 5 randomly selected plants for each treatment, 25 in total. The measurements were made on the plants after the water had been removed for 48 h, only on mid-day leaf water potential (ψmd) because this method is considered the most suitable for the control of vine water - status[64–66]. Specifically, mid-day measurements were taken between 12.30 and 1.30 pm. The time between leaf excision and chamber pressurization was generally less than 15 s. For each sampled plant, the fourth fully expanded and sun-exposed leaf (1 leaf per plant, 5 leaves in total for mid-day measurement for each treatment). Leaf temperature measurements were achieved with a handheld thermal camera Flir i7 (FLIR Systems Inc., Wilsonville, OR, USA). Leaf thermal images have been acquired before sampling on the same leaves used for leaf water potential measurements; then the average surface temperature for each leaf has been calculated with the FLIR Tools software.

3.5.5 Community-level physiological profile of rhizosphere

to stabilize the microbial biomass before the extraction. The CLPP analysis was attained according to a method first developed by Garland and - Mills[67] and it's based on the community-level substrate utilization. The soil was inoculated in 96-well plates (Biolog Ecoplate) containing 31 different carbon sources (carbohydrates, carboxylic acids, polymers, phenolic compounds, amino acids, and amine) plus a control well, in three replications. Tetrazolium violet redox dye was used for each well as a color indicator if added microorganisms utilize the substrates[68]. The soil was shaken for 30 min at 250 rotation per minute in NaCl (0.9%) solution in a ratio 1:10 containing glass beads and then centrifugated for 3' at 3000 rotation per minute. Next 150 µL of each sample were inoculated into each well of Biolog EcoPlates and incubated at 25 °C. The rate of utilization was indicated by the reduction of tetrazolium, a redox indicator dye that changes from colorless to purple[69]. Data were recorded with a plate reader at 590 nm every 24 h until 216 h. Microbial response in each microplate that expressed average well-color development (AWCD) was determined as follows70:

$$\mathrm{AWCD} = \sum \mathrm{ODi}/31$$

where ODi is the optical density value from each well, corrected by subtracting the blank well (inoculated, but without a carbon source) values from each plate well[68]. AWCD was also analyzed considering the microbial activity degrading each carbon source.

3.5.6 Root analysis

Five plants for each treatment were used for root morphological traits analysis. The stem was cut and the soil clod was carefully pulled out of the pot taking care to leave it intact. Roots were freed from the soil by washing it away over a 2 mm sieve. Grapevine roots were sorted from other species roots, immersed in water/ethanol (5:1 vol.) solution in Petri dishes and stored at 5 °C until further processed. Roots were scanned with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Expression 10000 XL, Epson America Inc., Long Beach, CA, USA) and images were analyzed by WinRHIZO software to measure root length (m) according to different diameter classes (class 1 0–0.5 mm; class 2 0.5–1 mm; class 3 1–2 mm). Afterward, the plant tissues were separately oven-dried at 70 °C for 72 h (until constant weight) and weighed to obtain the dry mass (g) of different diameter roots.

3.5.7 Statistical analysis

SPSS Statistics 25 (IBM) was used to run the post hoc Dunnett's test for multiple comparisons. Statistically significant differences ($p < 0.05$) between the means were marked with the letters a, b, c. In box plots, vertical boxes represent approximately 50% of the observations, and lines extending from each box are the upper and lower 25% values of the distribution. Circles and triangles represent outliers and extreme outliers. In bar charts error bars represent the 95% confidence interval.

3.6 ETHICAL POLICIES

The use of plant materials in this study was carried out in compliance with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

3.7 DATA AVAILABILITY

The data presented in this study are available on request from the corresponding author.

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3.8 REFERENCES

1. Reynolds, A. G. Managing Wine Quality: Viticulture and Wine Quality (Woodhead Publishing, 2010).
2. Mariotti, B. et al. A global review on innovative, sustainable, and effective materials composing growing media for forest seedling production. Curr. For. Rep. 9, 1–16 (2023).
3. Steiner, C. & Harttung, T. Biochar as a growing media additive and peat substitute. Solid Earth 5, 995 (2014).
4. Vaughn, S. F., Deppe, N. A., Palmquist, D. E. & Berhow, M. A. Extracted sweet corn tassels as a renewable alternative to peat in greenhouse substrates. Indic. Crops Prod. 33, 514–517 (2011).
5. Ceriani, A., Dalle Fratte, M., Agosto, G., Montagnoli, A. & Cerabolini, B. E. L. Using plant functional traits to define the biomass energy potential of invasive alien plant species. Plants 12, 3198 (2023).
6. Ceriani, A. et al. Woody and herbaceous invasive alien plant species-derived biochars are potentially optimal for soil amendment, soil remediation, and carbon storage. GCB Bioenergy 16, e13117. https:// doi. org/ 10. 1111/ gcbb. 13117 (2024).
7. Glaser, B. & Asomah, A. Plant growth and chemical properties of commercial biochar-versus peat-based growing media. Horticulturae 8(4), 339 (2022).
8. Ronga, D. et al. Valorisation of vineyard by-products to obtain composted digestate and biochar suitable for nursery grapevine (Vitis vinifera L.) production. Agronomy 9(8), 420 (2019).
9. Atkinson, C., Fitzgerald, J. & Hipps, N. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant Soil 337, 1–18 (2010).
10. Lehmann, J. & Joseph, S. Biochar for Environmental Management: Science and Technology 2nd edn. (Earthscan, 2015).
11. Polzella, A. et al. Morpho-physiological responses of Pisum sativum L. to different light-emitting diode (LED) light spectra in combination with biochar amendment. Agronomy 10(3), 398 (2020).
12. Simiele, M. et al. Biochar and/or compost to enhance nursery-produced seedling performance: A potential tool for forest restoration programs. Forests 13(4), 550 (2022).
13. Montagnoli, A. et al. Pioneer and fibrous root seasonal dynamics of Vitis vinifera L. are affected by biochar application to a low fertility soil: A rhizobox approach. Sci. Total Environ. 751, 141455 (2021).
14. Singh, B., Singh, B. P. & Cowie, A. L. Characterisation and evaluation of biochars for their application as a soil amendment. Soil Res. 48, 516–525 (2010).
15. Akhter, A., Hage-Ahmed, K., Soja, G. & Steinkellner, S. Compost and biochar alter mycorrhization, tomato root exudation, and development of Fusarium oxysporum f. Sp. lycopersici. Front. Plant Sci. 6, 529 (2015).
16. Nielsen, S. et al. Comparative analysis of the microbial communities in agricultural soil amended with enhanced biochars or traditional fertilisers. Agric. Ecosyst. Environ. 191, 73–82 (2014).
17. Omara, P. et al. Short-term effect of field application of biochar on cation exchange capacity, pH, and electrical conductivity of sandy and clay loam temperate soils. Technol. Agron. 3(1), 16 (2023).
18. Verhagen, et al., Scientific Assessment and Policy Analysis, the Netherlands Programme on Scientific assessment and Policy Analysis Climate Change (WAB). Report nr. 500102 027, Peatlands and carbon flows, Outlook and importance for the Netherlands (2009).
19. Waite, H., Whitelaw-Weckert, M. & Torley, P. Grapevine propagation: Principles and methods for the production of high-quality grapevine planting material. N. Z. J. Crop Hortic. Sci. 43, 144–161 (2015).
20. Incrocci, L. et al. Sensor-based management of container nursery crops irrigated with fresh or saline water. Agric. Water Manag. 213, 49–61 (2019).
21. Gugliuzza, G., Verduci, A., & Iovino, M. Water retention characteristics of substrates containing biochar and compost as peat and perlite replacements for ornamental plant production. In III

International Symposium on Growing Media, Composting and Substrate Analysis,1305, 507–512 (2019).

22. Mendez, A., Cárdenas-Aguiar, E., Paz-Ferreiro, J., Plaza, C. & Gasco, G. The effect of sewage sludge biochar on peat-based growing media. Biol. Agric. Hortic. 33(1), 40–51 (2017).

23. Montagnoli, A. et al. Asymmetrical copper root pruning may improve root traits for reforesting steep and/or windy sites. New For. 53, 1093–1112 (2022).

24. Bolan, N. et al. Multifunctional applications of biochar beyond carbon storage. Int. Mater. Rev. 67(2), 150–200 (2022).

25. Das, S. K., Ghosh, G. K. & Avasthe, R. Biochar application for environmental management and toxic pollutant remediation. Biomass Convers. Biorefin. 13, 5585–6556 (2020).

26. Basak, B. B. et al. Revamping highly weathered soils in the tropics with biochar application: What we know and what is needed. Sci. Total Environ. 822, 153461 (2022).

27. Bolan, N. et al. The potential of biochar as a microbial carrier for agricultural and environmental applications. Sci. Total Environ. 886, 163968 (2023).

28. Di Lonardo, S. et al. Biochar-based nursery substrates: The effect of peat substitution on reduced salinity. Urban For. Urban Gree 23, 27–34 (2017).

29. Schulz, H. & Glaser, B. Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. J. Plant Nutr. Soil Sci. 175, 410–422 (2012).

30. Arianna, B., Diego, P., Alessandra, C., Francioso, O. & Serenella, N. Effects of moderate and high rates of biochar and compost on grapevine growth in a greenhouse experiment. AIMS Agric. Food 2(1), 113–128 (2017).

31. Ronga, D. et al. Using digestate and biochar as fertilizers to improve processing tomato production sustainability. Agronomy 10(1), 138 (2020).

32. Baker, K. F., Chandler, P. A. UC System for Producing Healthy Container-Grown Plants 23–331 (University of California Experimental Station Service, Riverside, CA, USA, 1957).

33. Daughtrey, M. L. & Benson, D. M. Principles of plant health management for ornamental plants. Annu. Rev. Phytopathol. 43, 141–169 (2005).

34. Schmilewski, G. The role of peat in assuring the quality of growing media. Mires Peat 3, 2 (2008).

35. Steiner, C., Teixeira, W. G., Woods, W. I. & Zech, W. Indigenous knowledge about Terra Preta formation. In Amazonian Dark Earths: Wim Som-broek's Vision (eds Woods, W. I. et al.) (Springer, 2009).

36. Gruda, N. S. Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. Agronomy 9(6), 298 (2019).

37. Verheijen, F. G. et al. The influence of biochar particle size and concentration on bulk density and maximum water holding capacity of sandy vs sandy loam soil in a column experiment. Geoderma 347, 194–202 (2019).

38. Dumroese, R. K., Heiskanen, J., Englund, K. & Tervahauta, A. Pelleted biochar: Chemical and physical properties show potential use as a substitute in container nurseries. Biomass Bioenergy 35, 2018–2027 (2011).

39. Yao, Z., You, S., Ge, T. & Wang, C. H. Biomass gasification for syngas and biochar co-production: Energy application and economic evaluation. Appl. Energy 209, 43–55 (2018).

40. Kern, J. et al. Synergistic use of peat and charred material in growing media—an option to reduce the pressure on peatlands?. J. Environ. Eng. Landsc. Manag. 25(2), 160–174 (2017).

41. Baronti, S. et al. Long term effect of biochar on soil plant water relation and fine roots: Results after 10 years of vineyard experiment. Sci. Total Environ. 851, 158225 (2022).

42. Fellet, G. et al. Changes in physicochemical properties of biochar after addition to soil. Agriculture 12, 320 (2022).

43. Conte, P. et al. Recent developments in understanding biochar's physical-chemistry. Agronomy 11, 615 (2021).

44. Joseph, S. et al. Microstructural and associated chemical changes during the composting of a high temperature biochar: Mechanisms for nitrate, phosphate and other nutrient retention and release. Sci. Total Environ. 618, 1210–1223 (2018).
45. Conte, P. et al. Nature of water biochar interface interactions. GCB Bioenergy 5, 116–121 (2012).
46. Cooper, J. et al. Effect of biochar and compost on soil properties and organic matter in aggregate size fractions under field condi- tions. Agric. Ecosyst. Environ. 295, 106882 (2020).
47. Zhang, L., Jing, Y., Xiang, Y., Zhang, R. & Lu, H. Responses of soil microbial community structure changes and activities to biochar addition: A meta-analysis. Sci. Total Environ 643, 926–935 (2018).
48. Bedussi, F., Zaccheo, P. & Crippa, L. Pattern of pore water nutrients in planted and non-planted soilless substrates as affected by the addition of biochars from wood gasification. Biol. Fertil. Soils 51, 625–635 (2015).
49. Sun, L., Li, L., Chen, Z., Wang, J. & Xiong, Z. Combined effects of nitrogen deposition and biochar application on emissions of N2O, CO2 and - NH3 from agricultural and forest soils. Soil Sci. Plant Nutr. 60, 254–265 (2014).
50. Bunt, A. Media and Mixes for Container-Grown Plants: A Manual on the Preparation and Use of Growing Media for Pot Plants 1st edn. (Unwin Hyman Ltd., 1988).
51. Bernal-Vicente, A., Ros, M., Tittarelli, F., Intrigliolo, F. & Pascual, J. A. Citrus compost and its water extract for cultivation of melon plants in greenhouse nurseries. Evaluation of nutriactive and biocontrol effects. Bioresour. Technol. 99, 8722 (2008).
52. Jindo, K. et al. Root growth promotion by humic acids from composted and non-composted urban organic wastes. Plant Soil 353, 209–220 (2012).
53. Fries, L. L., Pacovsky, R. S., Safir, G. R. & Siqueira, J. O. llora Plant growth and arbuscular mycorrhizal fungal colonization affected by exogenously applied phenolic compounds. J. Chem. Ecol. 23, 1755–1767 (1997).
54. Rogelj, J. et al. Scenarios towards Limiting Global Mean Temperature Increase below 1.5 °c. Nat. Clim. Chang. 8, 325–332. https://doi. org/ 10. 1038/ s41558- 018- 0091-3 (2018).
55. Barrett, G. E., Alexander, P. D., Robinson, J. S. & Bragg, N. C. Achieving environmentally sustainable growing media for soilless plant cultivation systems: A review. Sci. Hortic. (Amst.) 212, 220–234 (2016).
56. Hashemi, F., Mogensen, L., Smith, A. M., Larsen, S. U. & Knudsen, M. T. Greenhouse gas emissions from bio-based growing media: A life-cycle assessment. Sci. Total Environ. 907, 167977 (2024).
57. Weber, K. & Quicker, P. Properties of biochar. Fuel 217, 240–261. https:// doi. org/ 10. 1016/j. fuel. 2017. 12. 054 (2018).
58. US-EPA. Method 3052—Microwave assisted acid digestion of siliceous and organically based matrices. Tests Methods Eval. Solid Waste, Phys. Methods SW 846 - US Gov. Print. Off. 20 (1996).
59. US-EPA. Method 3550C. Ultrasonic extraction. In Test Methods for Evaluating Solid Waste, Physical/Chemical Methods 3, 1–17 (2007).
60. UNI EN 15428; Soil Improvers and Growing Media—Determination of Particle Size Distribution (UNI Ente Italiano di Normazione, Milano, Italy, 2008).
61. UNI EN 13041; Soil Improvers and Growing Media. Determination of Physical Properties. Dry Bulk Density, Air Volume, Water Volume, Shrinkage Value and Total Pore Space (UNI Ente Italiano di Normazione, Milano, Italy, 2012).
62. Grossman, R. B. & Reinsch, T. G. The Solid Phase 2.1 Bulk Density and Linear Extensibility (2002).
63. Padgett-Johnson, M., Williams, L. E. & Walker, M. A. The influence of vitis ripariarootstock on water relations and gas exchange of vitis vinifera cv. carignane scion under non-irrigated conditions. Am. J. Enol. Vitic. 51, 137–143. https:// doi. org/ 10. 5344/ ajev. 2000. 51.2. 137 (2000).
64. Deloire, A., Carbonneau, A., Wang, Z. & Ojeda, H. Vine and water: A short review. OENO One 38, 1. https:// doi. org/ 10. 20870/oeno- one. 2004. 38.1. 932 (2004).
65. Carbonneau, M. A., Léger, C. L., Descomps, B., Michel, F. & Monnier, L. Improvement in theantioxidant status of plasma and low-density lipoprotein in subjects receiving a red wine phenolics mixture. J. Am. Oil Chem. Soc. 75, 235–240. https:// doi. org/ 10. 1007/ s11746- 998- 0036-5 (1998).

66. Taylor, J. A., Acevedo-Opazo, C., Ojeda, H. & Tisseyre, B. Identification and significance ofsources of spatial variation in grapevine water status. Aust. J. Grape Wine Res. 16, 218–226. https:// doi. org/ 10. 1111/j. 1755- 0238. 2009. 00066.x (2010).
67. Garland, J. L. & Mills, A. L. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57, 2351–2359 (1991).
68. Frąc, M., Oszust, K. & Lipiec, J. Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. Sensors (Basel) 12(3), 3253–3268 (2012).
69. Islam, R., Chauhan, P. S., Kim, Y., Kim, M. & Sa, T. Community level functional diversity and enzyme activities in paddy soils under different long-term fertilizer management practices. Biol. Fertil. Soils. 47, 599–604 (2010).
70. Gomez, E., Garland, J. & Conti, M. Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient. Appl. Soil Ecol. 26, 21–30 (2004).

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3.10 AUTHOR CONTRINUTION

S.B. and S.F.D.G. conceived the research project. F.P.V. supervised the project. S.B. and A.Ma. collected and interpreted aboveground data. A.D. processed the roots and collected data. A.Mo. supervised the root analysis and interpreted root data. P.B. performed the data analysis and provided chart visualization. D.C. produced the biochar. S.B. wrote the manuscript draft. A.Mo., P.B., F.P.V., A.Ma., D.C. revised the manuscript draft. All authors contributed to the article and approved the submitted version.

3.11 COMPETING INTEREST

The authors declare no competing interests.

3.12 ADDITIONAL INFORMATION

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Chapter IV: Functional Wood and Root traits in Mediterranean successional sequence for understanding plant response to climate change and land use change

Abstact: The Mediterranean ecosystem is among the most vulnerable to climate change, especially in relation to the escalation of drought conditions events. Investigating plant functional traits, such as wood anatomical features and fine root morphological traits, provides valuable insights into how plants cope with environmental constraints. Tree-ring analysis allows for the reconstruction of cambial dynamics under long-term climatic and ecological pressures, whereas fine root traits reflect more immediate responses, highlighting how soil temperature and moisture influence growth. Together, these approaches shed light on plant strategies for resource allocation under changing environmental conditions. To gain a better understanding, stable isotope analyses of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$), and oxygen ($\delta^{18}O$) were performed to enhance the retrospective understanding of past climate and how trees responded to environmental variability. In this regard, two field campaigns were conducted on Pianosa Island in May 2023 and 2024 to collect fine root samples, as well as coarse root and stem cross-sections, respectively.

Dendrochronological analyses revealed that *Pistacia lentiscus* individuals growing in abandoned agricultural crops exhibited greater ring width compared to those in Mediterranean macchia, suggesting that less competitive and more favorable environments promote secondary growth. In addition, stem and root growth trends were more aligned in abandoned crops, while in the harsher shrubland environment, such as Mediterranean macchia, stem–root growth dynamics diverged significantly. In addition, isotopic analyses indicated that former agricultural practices influenced isotope content in tree rings, and that $\delta^{18}O$ in the most recent rings varied with vegetation type, suggesting a potential enrichment of leaf ^{18}O due to the higher evapotranspiration in Mediterranean shrublands compared to abandoned crops.

Finally, fine root traits indicated that root length (RL) and root dry weight (RDW) were higher in macchia than in abandoned crops or woodland, reflecting the need for soil exploration and efficient transport capacity under competitive and water-limited conditions.

4.1INTRODUCTION

Human-induced climate change considerably influences the conditions for plant and forest development. Recent decades have seen numerous studies focused on understanding how vegetation responds and adapts to this rapidly changing environment (Shah & Paulsen, 2003; Montagnoli et al., 2016, 2019). One of the most visible consequences of climate change is the increasing frequency and severity of Extreme Weather Events (EWEs), which have significantly intensified in recent years (IPCC, 2014; Melillo et al., 2014; Donat et al., 2013). EWEs are rare climate anomalies that occur at or beyond the 10th or 90th percentile of a given probability distribution, based on historical data. Unlike gradual climate variations, these events are sudden, localized, and directly observable (IPCC, 2022). They include heatwaves, heavy rainfall, droughts, flooding, windstorms, and wildfires, all representing critical stressors for plants and ecosystems (Jentsch & Beierkuhnlein, 2008; Ummenhofer & Meehl, 2017).

Among these phenomena, heatwaves and droughts have received particular attention due to their pronounced and often synergistic impacts on plant functioning (Ciais et al., 2005; Lopez et al., 2022). Heat stress has been demonstrated to suppress photosynthesis, exacerbate oxidative damage, accelerate leaf senescence and abscission, and diminish both leaf expansion and overall biomass accumulation (Teskey et al., 2015). These effects become increasingly severe when elevated temperatures are combined with water scarcity, a combination that frequently occurs during the summer months across numerous regions. One notable example is the extreme heatwave in Europe in 2003, where July temperatures were up to 6 °C above the long-term average, and rainfall deficits were around 300 mm—nearly half of the annual precipitation in some areas. Under these extreme conditions, a 30% reduction in gross primary productivity was observed, resulting in Europe transitioning from a carbon sink to a temporary carbon source, thereby releasing approximately 0.5 PgC into the atmosphere (Ciais et al., 2005).

Such episodes underscore how Extreme Climatic Events (ECEs), especially when prolonged over extensive regions, can disrupt ecosystems across multiple scales (Smith, 2011). They not only impair physiological processes at the individual plant level but also have the potential to alter species composition, biodiversity, and ecosystem functionality (Parmesan, 2000). This consideration is particularly pertinent given that the terrestrial biosphere currently sequestred approximately 30% of anthropogenic CO_2 emissions from fossil fuels (2008–2017), serving as a vital buffer against global warming (Le Quéré et al., 2018). Future projections consistently indicate that ongoing climate change will further intensify these dynamics, with increases in mean temperatures, shifts in precipitation patterns, altered hydrological cycles, and an overall escalation of extreme events (Bates et al., 2008; Blöschl et al., 2019; Hirabayashi et al., 2013; Milly et al., 2005; Trenberth, 2011). Consequently, understanding the ecological impact of such transformations, particularly regarding recurrent extreme weather events (EWEs), is essential for evaluating the resilience and adaptive capacity of terrestrial ecosystems in the forthcoming decades.

The Mediterranean Basin has been extensively identified as one of the world's climate change hotspots (Giorgi, 2006). Its climate is predominantly characterized by mild, rainy winters followed by hot, dry summers, although notable variability exists, particularly in the intensity and duration of the dry season, which increases from higher to lower latitudes (Castri et al., 1981). This climatic regime, together with a complex historical biogeography and significant environmental heterogeneity, has contributed to a remarkable diversity of landscapes and vegetation. Indeed, the Mediterranean is recognized as one of the thirty-five terrestrial biodiversity hotspots worldwide (Médail & Myers, 2004). Forests and shrublands are essential for maintaining this biodiversity. They contribute directly through various tree species (Infusino et al., 2016; Santoja et al., 2018) and indirectly by hosting microbes (Oria-de-Rueda et al., 2010; Liu et al., 2022), fungi (Buscardo et al., 2011), lichens (Brunialti et al., 2010), and animals (Herrera et al., 2016). Additionally, the region's woody vegetation mainly consists of sclerophyllous species—evergreen shrubs and small trees with tough, leathery leaves—adapted to prolonged water scarcity.

However, this ecological diversity faces increasing threats from ongoing climate change. In recent decades, the Mediterranean Basin has experienced a marked increase in aridity, driven by rising temperatures across southern Europe and northern Africa (Medrano et al. 2009). Observational data confirm this trend (Piñol et al. 1998; Sillmann et al. 2013), while climate models consistently project further intensification of drought conditions (Vicente-Serrano et al., 2010; Taibi et al., 2019). Projections indicate that, under a warming of 3–4 °C and a 20% reduction in rainfall, forest ecosystems will be severely affected (Barredo et al., 2016; FAO, 2018). Notably, while global temperatures have increased by 0.85 °C during the last century, the Mediterranean has warmed by about 1.3 °C compared to 1880–1920 levels (Solomou et al., 2017). In addition, Barredo et al. (2016) projected a 15–23% contraction of the Mediterranean climatic zone by the end of the century, particularly in southern Iberia, southern Italy, North Africa, and parts of Greece and Turkey. Besides limited water, Mediterranean soils commonly suffer from nutrient deficiencies, especially nitrogen (N) and phosphorus (P), which further restricts vegetation growth (Sardana & Peñuelas, 2013; Sardana et al., 2004; Hanley, 2007).

Climate is not the only driver of ecological change in this region. Over the last century, large-scale land-use changes—the abandonment of rural areas —have led to forest expansion across many parts of the basin (Cervera et al., 2019). Such land-use change is among the most significant human pressures, disrupting biophysical processes and ecosystem structures (Newbold et al. 2015). In this setting, secondary succession plays a vital ecological role. As formalized by Odum (1969), secondary succession occurs after disturbances and progresses as a sequential, directional process, leading plant communities toward increased stability, biomass, and functional integration (Odum, 1969; Bazzaz, 1979; Chang & Turner, 2019).

Yet, succession in the Mediterranean is particularly complex. In spite of being a global biodiversity hotspot—covering only 2% of Earth's surface but containing about 20% of the world's vascular plant species (Klausmeyer & Shaw, 2009)—the region faces severe threats from climate change and human activities (Milano et al., 2013). In fact, studying succession here is challenging because frequent wildfires and other disturbances often interrupt or reset the process, resulting in persistent grasslands or degraded stages instead of mature forests (La Mantia et al., 2008). Therefore, understanding how secondary succession develops under these difficult conditions is essential for evaluating the resilience and adaptive capacity of Mediterranean ecosystems under environmental changing (Pérez-Hernández & Gavilán, 2021).

The vascular cambium (VC) is a bifacial lateral meristem that plays a crucial role in radial growth, producing new cells both inward and outward. On one side, it generates secondary xylem, which is essential for water transport and structural support; on the other, it produces secondary phloem, which ensures the movement of photosynthates throughout the plant (Plomion et al., 2001). While phloem has a primarily conductive role, xylem is central to both the hydraulic and mechanical properties of trees, influencing transpiration, carbon fixation, and plant stability (Tyree & Zimmermann, 2002; Fonti & Jansen, 2012; Cuny et al., 2015).

Temperate forests have a clear seasonal cycle. It typically stops during winter due to low temperatures, which stop cell division, then resumes in spring with the production of new xylem forming on the inside of the stem and new phloem forming on the outside. Xylem formation, also known as xylogenesis, involves several steps. First, the cambial initials divide through mitosis to produce new cells. Next, these cells expand outward. Then, the cell walls begin to harden and thicken through lignification. Finally, the cells die in a process called programmed cell death, leaving behind mature conduits for water transport (Wilson, 1970; Rathgeber et al., 2016).

The regulation of these processes depends on both internal factors, such as hormones, genes, and signaling pathways (Aloni 1980; Funada et al., 2001; Baesso et al. 2018, 2020), and external drivers, including temperature, soil nutrients, and water availability (Denne 1971; Montagnoli et al. 2019; De Micco et al. 2019). For example, early in the growing season, xylem cells typically develop into large conduits with thin walls, known as earlywood, which maximize hydraulic efficiency. Conversely, towards the end of the season, cells become narrower and develop thick walls, forming latewood, which provides mechanical support at the expense of conductive efficiency (Frits 1976; Creber 1977; Chave et al. 2009). This cyclical alternation between early and latewood ultimately shapes the tree-ring structure, which closes with the winter dormancy period. Beyond its anatomical and physiological roles, this process is crucial for carbon sequestration. Globally, forests absorb about 26% of human-caused CO_2 emissions (Pan et al. 2011). Much of this carbon is stored in wood, as the cambium continuously converts photosynthates into long-lived biomass through xylogenesis (Wodzicki 1971; Rathgeber et al. 2016).

However, cambial activity may not cease exclusively due to winter dormancy. In Mediterranean environments, for instance, extended summer droughts can also induce a temporary halt in xylem formation, leading to anomalies in the annual growth pattern. These interruptions may give rise to intra-annual density fluctuations (IADFs), indicative of the so-called "double stress" characteristic of Mediterranean climates, driven by both cold winters and hot, arid summers (Campelo et al. 2007; De Luis et al. 2007; De Micco et al. 2007).

Generally, when cambial activity is prematurely interrupted—whether due to drought, heat, or other stress factors—the capacity for carbon sequestration can be diminished, thereby impacting the forest's ability to mitigate atmospheric CO_2 accumulation. Consequently, alterations in tree-ring formation may influence the global carbon cycle (Babst et al., 2014).

Despite these challenges, tree rings constitute powerful ecological proxies. As long-lived organisms, trees integrate environmental signals into their wood structure, offering valuable archives for reconstructing historical climates and ecosystem dynamics (Esper et al., 2002; Treydte et al., 2006; Trouet et al., 2009). Parameters such as tree-ring width or latewood density are highly sensitive to climatic factors like temperature and precipitation, making them widely applicable in climate reconstructions (IPCC, 2007; Jones et al., 2009). More recently, quantitative wood anatomy has been utilized to analyze anatomical traits, including conduit diameter, cell wall thickness, and tissue composition, thereby enhancing our understanding of the relationship between growth and environmental variables (Fonti et al., 2010; Bräuning et al., 2016). As previously noted, dendroecological studies in Mediterranean regions remain relatively limited, owing to the less regular seasonal dynamics of cambial activity and wood formation, which pose challenges for tracking these processes (Cherubini et al., 2003), making Mediterranean tree rings more difficult to interpret in comparison to those from temperate zones.

Stable isotope ratios in tree rings serve as a valuable record for reconstructing historical environmental conditions and ecosystem dynamics. Key isotopes like carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$), and nitrogen ($\delta^{15}N$) are especially useful because they offer different insights into tree physiology and external influences such as climate change, water supply, and nutrient cycling (McCarroll & Loader 2004; Cherubini & Battipaglia 2021). Using these isotopes together helps develop a fuller understanding of how vegetation reacts to natural and human-made disturbances, including land-use changes and the end of agricultural practices.

In dendroecology, carbon isotopes in tree rings are extensively used because they closely relate to plant carbon uptake and stomatal behavior (Belmecheri & Lavergne 2020). During photosynthesis, when plants absorb CO_2, they discriminate against heavier carbon isotopes, resulting in organic matter that is depleted in ^{13}C compared to atmospheric CO_2 (Craig 1957). This discrimination occurs through two fractionation steps: the diffusion of CO_2 through stomata, which reduces $\delta^{13}C$ by about 4.4‰, and enzymatic fixation by Rubisco, causing an additional reduction of approximately 27‰ (Farquhar et al. 1989). The ratio of intercellular CO_2 (Ci) to ambient CO_2 (Ca) is crucial, as it reflects the balance between stomatal conductance (gs) and photosynthetic capacity, and it is strongly affected by environmental stresses. For example, during droughts, stomata tend to close, influencing the $^{13}C/^{12}C$ ratio (Farquhar et al., 1989b; Scheidegger et al., 2000).

Instead, oxygen isotopes offer complementary insights into tree–water relations and atmospheric conditions (Roden & Ehleringer 2000; Barbour et al. 2004). Inside the leaf, transpiration causes water to become enriched in ^{18}O because $H_2{}^{18}O$ molecules evaporate more slowly than the lighter $H_2{}^{16}O$ (Farquhar et al., 1989). Generally, Tree $\delta^{18}O$ is influenced by three main factors beyond the source water variability: (1) leaf water enrichment—affected by the Péclet effect and moderated by heterogeneity within leaf water at evaporation sites; (2) biochemical fractionation between carbonyl oxygen and water during cellulose synthesis; and (3) $\delta^{18}O$ of ambient vapor, mainly governed by relative humidity (Scheidegger et al., 2000). Sugars formed during photosynthesis carry this enrichment, which is partly altered during transport and cellulose formation (Sternberg et al. 1986; Anderson et al. 2002). The resulting $\delta^{18}O$ in tree-ring cellulose reflects both the isotopic composition and the extent of evaporative enrichment at the leaf level. Notably, the latter strongly depends on relative humidity and vapor pressure deficit, making $\delta^{18}O$ a useful indicator of hydroclimatic conditions (Barbour 2007). Variations in $\delta^{18}O$ over the years have been employed to reconstruct changes in rainfall patterns, humidity, and even atmospheric circulation (Roden et al. 2000).

Compared to carbon and oxygen isotopes, nitrogen isotopes are less often used in dendroecology mainly because of analytical difficulties and the high mobility of nitrogen within wood tissues, which can hide annual signals (Cowling & Merrill, 1966; Hart & Classen, 2002). Naturally nitrogen exists as two stable isotopes: the common ^{14}N and the rarer ^{15}N, which has seven protons and eight neutrons, giving it an atomic mass of 15. The isotopic composition of soil nitrogen usually falls within a narrow range of –4 to +14‰ $\delta^{15}N$ (Peterson & Fry, 1987). However, the $\delta^{15}N$ value of bioavailable nitrogen in soils is heavily influenced by microbial and chemical transformations, including fixation, mineralization, volatilization, nitrification, and denitrification (Hopkins et al., 2010). Fixation and mineralization generally cause only small isotopic fractionation, while volatilization, nitrification, and denitrification tend to favor removing ^{14}N, leaving the remaining nitrogen enriched in ^{15}N (Högberg, 1997; Pardo & Nadelhoffer, 2010; Hobbie & Högberg, 2012). In contrast, isotopic discrimination during plant uptake is relatively minimal (Handley & Raven, 1992). Agricultural practices can significantly change soil $\delta^{15}N$. Organic manures and urea-based fertilizers are typically enriched in ^{15}N because of the preferential loss of ^{14}N through volatilization, as previously mentioned (Kreitler, 1979; Högberg, 1991; Wassenaar, 1995), while synthetic mineral fertilizers are often depleted in ^{15}N (Yoneyama, 1996). Therefore, fertilization, grazing, leaching, fire, or other disturbances may alter the nitrogen isotopic composition in soils and, thus, the nitrogen taken up by plants (Amundson et al., 2003; Zech et al., 2011). Despite these insights, applications of $\delta^{15}N$ in tree rings are still rare. A major challenge is the potential for post-depositional nitrogen mobility within xylem tissues, which can obscure the direct link between nitrogen uptake in a specific year and location, and the isotopic signature found in the corresponding growth ring (Levy et al., 1996). This uncertainty has limited the use of nitrogen isotopes in dendroecology.

The root system constitutes a fundamental component of plants, ensuring their growth, survival, and ecological functions. Roots not only provide anchorage (Nicoll & Ray, 1996) and store non-structural carbohydrates (Fitter, 2002), but also regulate water and nutrient absorption (Montagnoli et al., 2019), alter soil physical and chemical properties (Gill & Jackson, 2000; Jin et al., 2017), establish symbiotic relationships with mycorrhizae (Cudlin et al., 2007), and contribute to slope stabilization by mitigating erosion and landslides (Reubens et al., 2007). Given that the vascular cambium governs secondary growth in both stem and root tissues, the belowground compartment is likewise influenced by the interaction between endogenous factors (such as species genotype or ontogenetic stage) and exogenous variables (including temperature, precipitation, soil fertility, and competition) (Majdi et al., 2005). This interaction delineates the so-called Root System Architecture (RSA), whose structural configuration profoundly impacts aboveground development and ecosystem performance (Szymanowska-Pułka, 2013).

Within this framework, fine roots (<2 mm in diameter) play pivotal role. Despite representing only 2–3% of total biomass, they account for up to two-thirds of annual net primary production in forests (Vogt et al., 1995; Finér et al., 2011; McCormack et al., 2015). These short-lived, non-woody structures are typically associated with mycorrhizal symbioses and are essential for nutrient, oxygen, and water uptake (Eissenstat et al., 2000, 2015; Montagnoli et al., 2016, 2018; Brunner et al., 2015). Besides their absorptive function, fine roots influence soil processes through carbohydrate exudation that fuels microbial activity, accelerates organic matter decomposition, and enhances nutrient availability in the rhizosphere (Kaiser et al., 2015; Sun et al., 2021).

Environmental variability strongly shapes fine root traits. Under dry conditions, plants tend to develop thinner and longer roots, a similar adaptation to producing smaller and thinner leaves, which increases the specific root length (SRL, root length per unit mass) (Withington et al., 2006; Ostonen et al., 2007; Metcalfe et al., 2008). SRL has been proposed as a functional indicator of the trade-off between benefits, proportional to root length and resource capture, and costs, proportional to root mass and maintenance (Eissenstat & Yanai, 1997; Ostonen et al., 2007). Accordingly, species may adopt an "extensive" strategy, allocating more carbon belowground, or an "intensive" strategy, optimizing root morphology to maximize exploration and water uptake (Montagnoli et al., 2012).

These adjustments reflect expressions of phenotypic plasticity, enabling plants to cope with rapid shifts in soil moisture, nutrient availability, or competition (Brunner et al., 2019; Iversen & McCormack, 2021). Yet, when such responses fail or become insufficient, tree mortality may occur (McCarthy et al., 2010; Laginha Pinto Correia et al., 2019).

Fine roots are fundamental to the terrestrial carbon cycle because of their ongoing production and turnover. Their short lifespan makes them a highly dynamic interface between plants and soil, contributing organic matter when they die and influencing microbial decomposition processes (Finér et al., 2011; Montagnoli et al., 2014; Baesso et al., 2018). Turnover rates directly impact soil carbon sequestration and nutrient cycling, emphasizing their ecological importance (Freschet et al., 2021; Montagnoli et al., 2023). By measuring length, diameter, branching, and lifespan, roots serve as functional indicators of plant strategies for resilience and adaptation under climate change (Zadworny et al., 2016, 2017; Cudlin et al., 2007).

Despite their ecological significance, belowground parts are less studied than aboveground structures like stems or leaves (Canadell & Rodà, 1991). This knowledge gap is especially noticeable in Mediterranean ecosystems, where sclerophyllous species like *Pistacia lentiscus L.* endure seasonal droughts in summer and winter (Bussotti & Pollastrini, 2020). The limited data on root dynamics in these environments hinder our understanding of how these species cope with the increasingly intense and unpredictable droughts caused by global change (Giorgi & Lionello, 2008). Exploring the functional traits of Mediterranean fine roots can offer valuable insights into plant adaptation, ecosystem resilience, and carbon cycling in the face of current and future environmental challenges.

The root traits were also compared to leaf traits such as specific leaf area (SLA) and leaf dry matter content (LDMC). Overall, species that adopt a rapid resource-use strategy tend to exhibit higher specific leaf area values. Conversely, traits like higher LDMC are typically associated with a more conservative resource utilization (Pierce et al. 2007; Freschet et al. 2010; de la Riva et al. 2018).

This chapter aims to explore how climate variability and land abandonment have influenced the growth dynamics *of P. lentiscus* and nutrient cycling in a Mediterranean island ecosystem. Specifically, we hypothesize that: (i) The end of agricultural activities following prison closure has changed soil conditions and water availability, affecting cambial activity and carbon allocation to wood formation. These changes in vascular development are expected to be observable in the width of annual rings in stems and coarse roots. (ii) The isotopic signatures of carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$), and nitrogen ($\delta^{15}N$) in tree rings will serve as integrated indicators of past environmental conditions. We predict that isotopic values from the first five years of growth compared to the last five will reveal signals of climate variability and land-use change, indicating long-term shifts in nutrient cycling and water-use efficiency. (iii) Under secondary succession and changing Mediterranean climate conditions, fine roots will show distinctive growth patterns and significantly contribute to belowground biomass. Due to their critical role in carbon assimilation and nutrient uptake, fine roots are investigated as key drivers of net primary production through photosynthetic carbon allocation. (iv) The behavior of fine roots and leaves mirrors environmental conditions and the plant's growth environment.

4.2 MATERIALS AND METHODS

4.2.1 Study site: Pianosa Island

Pianosa Island (42°35′07″ N, 10°04′44″ E) is located in the northern Tyrrhenian Sea within the western Mediterranean basin (see Fig. 1A). It is about 13 km southeast of Elba Island, 45 km east of Corsica, and 55 km west of the Tuscan coast. Covering roughly 10.2–10.3 km² with a coastline of 18–20 km, Pianosa is the fifth-largest island in the Tuscan Archipelago, which also includes Elba, Giglio, Capraia, Montecristo, Gorgona, and Giannutri (De Giuli, 1970). Unlike the mostly hilly and mountainous terrain of the other islands, Pianosa is nearly flat, with an average elevation of 18 meters above sea level (a.s.l.) and a highest point of 29 meters in the northern part. The island has a roughly triangular shape, featuring a broad southern plateau and a narrow promontory extending northward (Nicotra, 2008).

The climate of Pianosa is heavily influenced by its flat topography and insular position in the Tyrrhenian Sea, which restricts the condensation of moist air masses and results in less rainfall compared to other islands in the archipelago (Vaccari et al., 2012; Natali, 2023). According to the Köppen classification, the climate is Mediterranean (Csa; Köppen, 1931). Meteorological data collected from 1951 to 2007 by the LaMMA Consortium (http://www.lamma.rete.toscana.it/) show an average annual rainfall of 481–497 mm, with yearly fluctuations ranging from 176 mm (1999) to 716 mm (1984) (Nicotra, 2008; Natali et al., 2023).

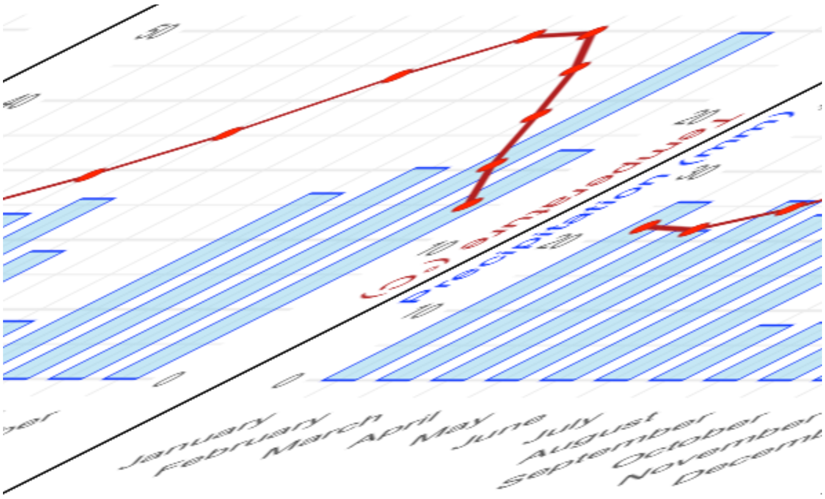

Figure 1: Climatic diagram showing monthly mean temperatures (red line) and total monthly precipitation (blue bars) for the period 2006–2024, based on data from http://www.lamma.rete.toscana.it/

Precipitation mainly occurs in autumn, peaking between October and December, while summer remains notably dry, with July being the driest month. The average annual temperature varies from 14.4 to 15.8 °C, with the lowest temperatures in January–February and the highest in July–August. Autumn tends to be warmer than spring, reflecting the influence of sea surface heat release on the local climate (Nicotra, 2008). Geologically, Pianosa is composed entirely of sedimentary rocks. Its primary materials are Plio-Pleistocene marine calcarenites and cemented sands, situated above Miocene marls and clays mixed with conglomerates. The sequence ends with Quaternary marine and continental deposits (Graciotti et al., 2008). These lithologies are highly permeable, and the absence of hills and surface water networks promotes rainfall infiltration and sustains aquifers with substantial groundwater resources, despite the island's small size and low precipitation (Doveri et al., 2012). Karst formations, driven by carbonate-rich rocks, are well-developed, shaping surface features like dolines and underground systems such as caves and tunnels. Notably, the Cervo and Lancia caves on the northeastern coast contain speleothems like stalactites, stalagmites, and flowstones (Natali et al., 2023). The soils on Pianosa originate from marine and continental sediment deposits (Bossio et al., 2000), characterized by sandy-loam to sandy textures, alkalinity, and high carbonate content, along with moderate rock fragment presence. According to the World Reference Base for Soil Resources, they are mainly classified as Leptosols (ISSS-ISRIC-FAO, 1998). A comprehensive pedological study is available in D'Acqui et al. (2007).

The natural vegetation of Pianosa consists of Mediterranean macchia, with communities dominated by *Rosmarinus officinalis*, *Cistus* spp., and *Juniperus phoenicea* (Baldini, 2000). This plant community exists at various successional stages due to ongoing re-naturalization after the abandonment of agricultural activities. Additionally, remnants of natural coastal vegetation remain, especially along the coastline, where the Crithmo–Limonietea association can be identified (Braun-Blanquet et al., 1952), hosting the endemic Limonium planesiae (Baldini, 2000).

Vegetation across the island can be classified into three main formations: cliffs, grasslands, and scrublands (Colom et al., 2004; Natali et al., 2023). Cliff habitats support cormophytic and aerohaline communities, including the island's endemic *Limonium planesiae*. Rocky shelves near sea level support halophilous plants such as *Spergularia salina* and *Sagina maritima*. Garrigue communities, dominated by *Helichrysum litoreum*, are common, while abandoned pastures and more developed soils host *Thymelaea hirsuta*. Dry grasslands are rich in therophytes, including *Lagurus ovatus*, *Trachynia distachya*, *Festuca ciliata*, *Medicago minima*, *Trifolium scabrum*, and *T. stellatum*. Recently abandoned croplands are typically colonized by *Asphodelus ramosus* and *P. lentiscus*. Low scrub dominated by evergreen shrubs and *Cistus* spp., along with *P. lentiscus*, *Salvia rosmarinus*, and *Coronilla valentina*, occupy intermediate elevations. At higher points, *J. phoenicea* forms dense stands, often accompanied by *Teucrium fruticans* and *Stachys majus*. Woodlands are scarce, with *Pinus halepensis* particularly abundant along the eastern coast and scattered patches of Quercus ilex (Natali et al., 2023).

4.2.2 Land-use history

Human activity has significantly influenced Pianosa's ecosystems since Roman times. Archaeological remains indicate settlements dating back to prehistoric periods and Roman colonization (Rapetti, 1835). More recently, in 1858, Pianosa was designated as an agricultural penal colony, and for over a century, prisoners carried out extensive farming and livestock management (La Provincia di Livorno, 1962). Historical records from the Penitentiary of Portoazzurro show the development of land-use practices. Early in the 20th century, simple crop rotations were common, but later agriculture intensified with the use of mineral fertilizers, irrigation, and forage imports (Colom et al., 2004). From 1910 to 1990, the share of fodder crops increased from 13% to 40% of the Utilized Agricultural Area. These practices greatly altered the natural vegetation, which now mainly survives in marginal coastal zones (Baldini, 2000). All agricultural and pastoral activities were discontinued by the end of the 1990s, setting off a gradual process of ecological re-naturalization. By 2001, surveys identified three main ecosystems: abandoned croplands and pastures (covering 52% of the island), Mediterranean macchia, and woodlands (Colom et al., 2004). Despite their extensive coverage, abandoned fields comprise a relatively small amount of the total island biomass (26%).

In this context, two field campaigns were carried out from May 9 to 16, 2023, and from May 7 to 14, 2024. These surveys focused on collecting fine roots and woody tissues, including both stem and coarse root samples, to study below- and above-ground growth dynamics and isotopic signals. For fine root sampling, three plots were established within each ecosystem type—abandoned croplands and pastures, Mediterranean macchia, and woodlands. Within each plot, five individual plants were selected, resulting in replicated samples across the three ecosystems. In contrast, for wood sampling and isotopic analyses, the same three plots per ecosystem were maintained; however, due to time constraints, the number of individuals sampled per plot was reduced to three (Fig. 2).

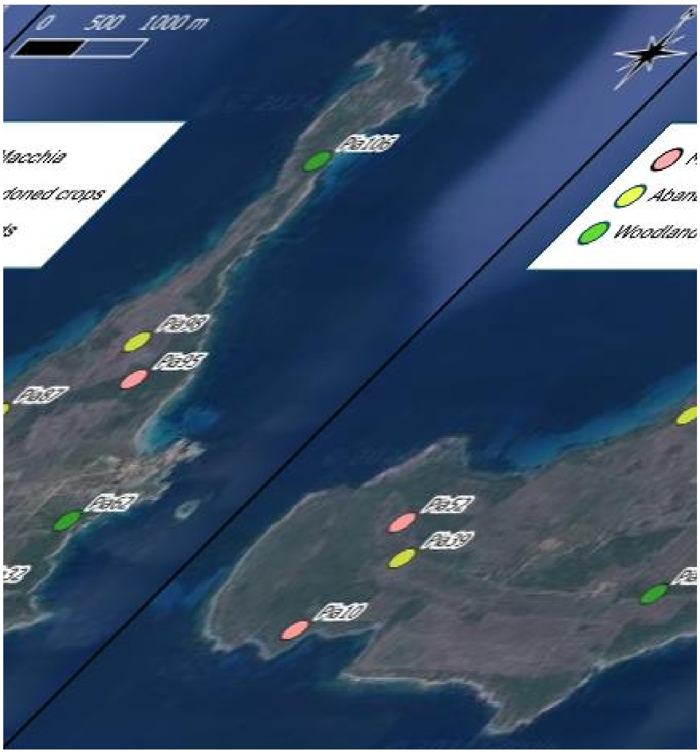

Figure 2: Pianosa island and sample sites. Image source: Google Satellite

4.2.3 Tree rings analysis

During the second campaign, conducted in May 2024, three individuals per treatment (Mediterranean macchia and abandoned croplands) were selected, choosing samples with the least disturbed growth form. Each stem was destructively sampled using a chainsaw, obtaining cross-sections just above the soil surface between 20 and 50 cm. For the root component, a shovel was used to excavate a pit near the stem in order to locate a sufficiently large root for dendrochronological and dendrochemical analyses (Fig. 3). Once the root was identified, it was sampled using a hand saw. In total, 36 samples were collected: 2 treatments, 3 plots per treatment, 3 individuals per plot, and 2 tissues (stem and root). Stem and root disks were then cut into smaller, more manageable samples and progressively sanded using sandpaper with increasing grit sizes, from 80 up to 5000, to enhance ring visibility.

Growth rings were then visually identified and counted from the bark towards the pith using a stereomicroscope (magnification 6±4–40×, Wild M3Z Leica, Germany). Because ring boundaries were often challenging to discern, each ring was marked on both stem and root disks, and the counts were repeated on the opposite side of the cross-section until the two consecutive measurements matched. Each prepared cross-section was positioned on a customized Atrics system (Levanič, 2007), which consists of a motorized stage (minimum precision 0.01 mm) controlled to move the camera systematically from the bark toward the pith (Gärtner et al., 2024). High-resolution images were captured using a Canon 5DsR camera equipped with a 100 mm macro lens (Canon Inc., Tokyo, Japan). Multiple images were stitched together using PTGui software (New House Internet Services B.V., Rotterdam, NL; von Arx et al., 2016) to produce complete, high-quality images of each sample. Calibration was performed by measuring a standard mm-paper within each image to ensure accurate scaling.

The cross-dating was performed using with the image analysis software CooRecorder 8.1 (Cybis Elektronik & Data AB), and the ring-width measurements were carried out along two radii of each cross-section to the nearest 0.01 mm using TsapWin (Time Series Analysis and Presentation, Frank Rinn, Heidelberg, Germany).

Figure 3: Cross-section of P. lentiscus collected in abandoned crop fields (top) and Mediterranean macchia (bottom). The images illustrate two stems and their annual rings, with a millimeter grid included as a scale reference.

4.2.4 Tree rings isotopes measurements

For isotope analyses, 36 cross-sections from abandoned crops and Mediterranean macchia were initially Soxhlet-extracted with hot ethanol for 72 hours to remove resins and other soluble compounds. After air-drying to eliminate residual ethanol, two sets of five rings—taken from the innermost and outermost parts of each sample—were selected and finely milled with an ultracentrifugal mill (Model ZM 300, Retsch GmbH, Haan, Germany). The resulting powders were then weighed on a high-precision balance (Mettler MT5 model, Mettler Toledo Ltd., Greifensee, Switzerland) and placed in silver cups (3.3 × 5.0 mm) for carbon and oxygen isotope analyses (~1.0 mg) and in larger silver capsules (5 × 9 mm) for nitrogen (~10.0 mg). Additionally, ten soil samples collected at the tree sampling sites were prepared for isotope analysis by weighing approximately 3.0 mg of material into tin capsules (3.2 × 6.0 mm). The isotope analyses were performed using two complementary approaches: one focused on nitrogen combustion, and the other on carbon and oxygen pyrolysis. For nitrogen isotopes, about 10 ± 0.5 mg of homogenized wood powder was combusted in an elemental analyzer (iso-Earth, Sercon, UK) connected to an isotope-ratio mass spectrometer (HS2022, Sercon, UK). During this process, CO_2 was removed to prevent interference with $\delta^{15}N$ measurements. Calibration used both laboratory and international reference materials with known $\delta^{15}N$ values, achieving an analytical precision of approximately 0.3‰. Isotopic values were expressed in delta notation (‰) relative to atmospheric N_2, and total nitrogen concentrations were calculated as a percentage of dry mass.

Carbon and oxygen isotopes ($\delta^{13}C$ and $\delta^{18}O$) were measured in a separate sequence using a continuous-flow isotope ratio mass spectrometer (CF-IRMS) coupled to a pyrolysis unit operating at 1420 °C (PYRO-cube, Elementar, Hanau, Germany). The system was connected to a Delta Plus XP mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) via a Conflo III open-split interface (Thermo Fisher Scientific, Bremen, Germany), following the setup described by Woodley et al. (2012). Carbon isotope ratios were expressed relative to the Vienna Pee Dee Belemnite (VPDB) standard, and oxygen isotope ratios relative to the Vienna Standard Mean Ocean Water (VSMOW). Measurement reproducibility was better than 0.3‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{18}O$.

4.2.5 Fine-root and leaf traits

Fine roots were sampled using a portable, motor-driven corer adapted from Ponder and Alley (1997) in three different vegetation types: abandoned crops, Mediterranean macchia, and woodland. In each plot, a single soil core (4 cm in diameter, 30 cm deep) was randomly extracted. Overall, 135 cores were obtained, with one core per tree, across three soil layers (0–10, 10–20, and 20–30 cm), from five individuals per plot, across three plots and three treatments. After collection, cores were placed in plastic bags and stored at 4 °C until further processing. Root extraction was performed by washing the soil cores in nylon mesh bags (300 µm) using an automated washing system, following Benjamin and Nielsen (2004). Cleaned roots were then sorted and identified according to color, texture, and morphology under a stereomicroscope at 10× magnification (Vogt and Persson, 1991). Living fine roots were scanned at 600 dpi with a flatbed scanner (EPSON 12000 XL), and measurements were performed using WinRhizo Pro v. 2007d software (Regent Instruments Inc., Quebec). For each category, the following root traits were measured: mean Root Length (RL - mm^{-2}), mean Root Biomass (RB – g m^{-2}), and Specific Root Length (SRL - m g^{-1}). To gain a broader overview about the *P.lentiscus* behavior, we also investigated leaf functional traits. Specifically, laboratory analyses followed the standardized protocol described by Pérez-Harguindeguy et al. (2013). Leaf samples were kept at 4 °C overnight to ensure full hydration before measuring leaf fresh weight (LFW) and leaf area (LA; defined as the surface area of fully expanded leaves). LA was quantified using a digital scanner and the Leaf Area Measurement software (LAM v.1.3; University of Sheffield, UK). Leaves were then dried at 105 °C for 24 hours to determine leaf dry weight (LDW), from which leaf dry matter content was calculated. Finally, specific leaf area was derived as the ratio of LA to LDW.

4.2.6 Statistical analysis

Since Raw ring-width (RW) values are heteroscedastic (variance proportional to mean growth), values were log-transformed before the analysis. By taking the logarithm, growth values become more comparable across trees and years, reducing the influence of year- and size-related bias.

Tree growth dynamics were then analyzed using Generalized Additive Models (GAMs) in the mgcv package of R (version 2024.12.1). The full model included a smooth term for tree age, allowing the estimation of a separate non-linear growth curve for each treatment. This approach was used because tree growth is not linear over time, with rapid growth at early ages followed by a deceleration in later years. In the model, we included treatment (macchia vs. abandoned crops), organ type (stem vs. root), year, and their interactions, to test how these factors influenced growth patterns.

Since several growth measurements were taken from the same tree over time, and trees were located within the same plots, the model accounted for this structure by including random effects. This means that repeated measures from the same tree were not treated as independent observations, and that trees growing close to each other within the same plot could share similar environmental conditions. To further capture the fact that growth in one year is influenced by growth in the previous year, we also included a temporal autocorrelation term (first-order autoregressive structure).

Pairwise comparisons between treatments for each year were performed using estimated marginal means (emmeans), with Tukey adjustment for multiple comparisons, and significant differences (p-value < 0.05) were indicated with asterisks on the growth curves.

To ensure comparability among individuals, the last five growth rings were selected from both stems and roots. This method allowed each sample to represent the same time period- the last five years of growth- making comparisons between organs and treatments statistically consistent. For each isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$), values were modeled using linear models that included treatment (macchia vs abandoned crops), organ type (root vs stem), their interaction, and plots as a factor to account for potential spatial heterogeneity, since environmental conditions and micro- site characteristics can vary locally and influence isotopic signatures. The significance of main effects and interactions was tested with analysis of variance (ANOVA). When significant effects or interactions were found, pairwise comparisons were conducted using Tukey' s Honest Significant Difference (HSD) test to identify specific differences.

The isotopic composition of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{18}O$ was examined from the five innermost tree rings, representing early growth stages, through to the five outermost rings. Although the sampled individuals varied in age- which could introduce some bias- this approach allowed us to reconstruct temporal patterns of isotopic variation from each individual' s establishment to the time of sampling across treatments.

Stable isotope analyses ($\delta^{13}C$, $\delta^{18}O$, $\delta^{15}N$) were based on the difference between innermost and outermost rings for each individual (Δ[in–out]). This index was calculated as the difference between the average isotopic value of the first five rings and that of the last five rings, providing a standardized measure of within- tree isotopic change over time while minimizing the impact of nitrogen mobility across rings. To analyze isotope patterns, linear models including treatment, organ type, and their interaction were used. We also included “plot” as a factor to account for variations in soil or microclimatic conditions across different plots. This approach helps separate location- specific differences from the treatment effects, ensuring that the results mainly reflect the influences of treatments rather than plot conditions. The significance of effects was tested with ANOVA, and pairwise comparisons were made using Tukey' s HSD test.

To evaluate differences among treatments, leaf functional traits and fine root traits (considering the entire 0–30 cm soil layer) were analyzed using Dunnett' s test for multiple comparisons, with a significance level of p < 0.05. 05. Statistically significant differences among treatments are indicated by different lowercase letters (a, b, c).

Regarding the analysis of fine root traits at various soil depths (0–10 cm, 10–20 cm, 20–30 cm), the same post hoc procedure was employed. In this context, lowercase letters (a, b, c) denote statistically significant differences among treatments within the same depth, whereas uppercase letters (X, Y, Z) signify statistically significant differences among soil depths within the same treatment. Pairwise correlations among variables were evaluated by constructing a correlation matrix utilizing Pearson’s product–moment correlation coefficient. This approach was selected because it presumes linear relationships between continuous variables and exhibits robustness under the assumption of normality. Correlation coefficients (r) span from –1 (indicating a perfect negative correlation) to +1 (indicating a perfect positive correlation), with values near 0 suggesting the absence of a linear

association. The statistical significance of these correlations was tested, and p-values below 0.05 were deemed to indicate statistically significant relationships.
All statistical analyses were performed using R software (version 2024.12.1).

4.3 RESULTS

4.3.1 Sample description and sample depth of tree-ring chronologies

treatment	organ	mean age (sd)	max age	series start year	n° of samples
macchia	stem	21.0 ± 14.1	62	1962	9
macchia	root	20.0 ± 13.9	61	1963	9
abandoned crops	stem	9.6 ± 5.4	21	2003	9
abandoned crops	root	8.6 ± 4.9	19	2005	9

Table 1: Summary of the sampled trees from macchia and abandoned crop sites. Values represent the mean age (± standard deviation), maximum age, series start year (earliest year of the tree-ring record), and the number of samples analyzed for each organ (stem and root).

Individuals from the macchia exhibited a broader age range, with mean ages of 21.0 ± 14.1 years in stems and 20.0 ± 13.9 years in roots. The oldest sample reached 62 years (stem) and 61 years (root), with the earliest rings dating back to 1962 and 1963, respectively
In contrast, individuals from abandoned crops showed a mean age of 9.6 ± 5.4 years in stems and 8.6 ± 4.9 years in roots. The oldest tree reached 21 years (stem) and 19 years (root), with ring series extending back to 2003 and 2005, respectively (Tab 1).

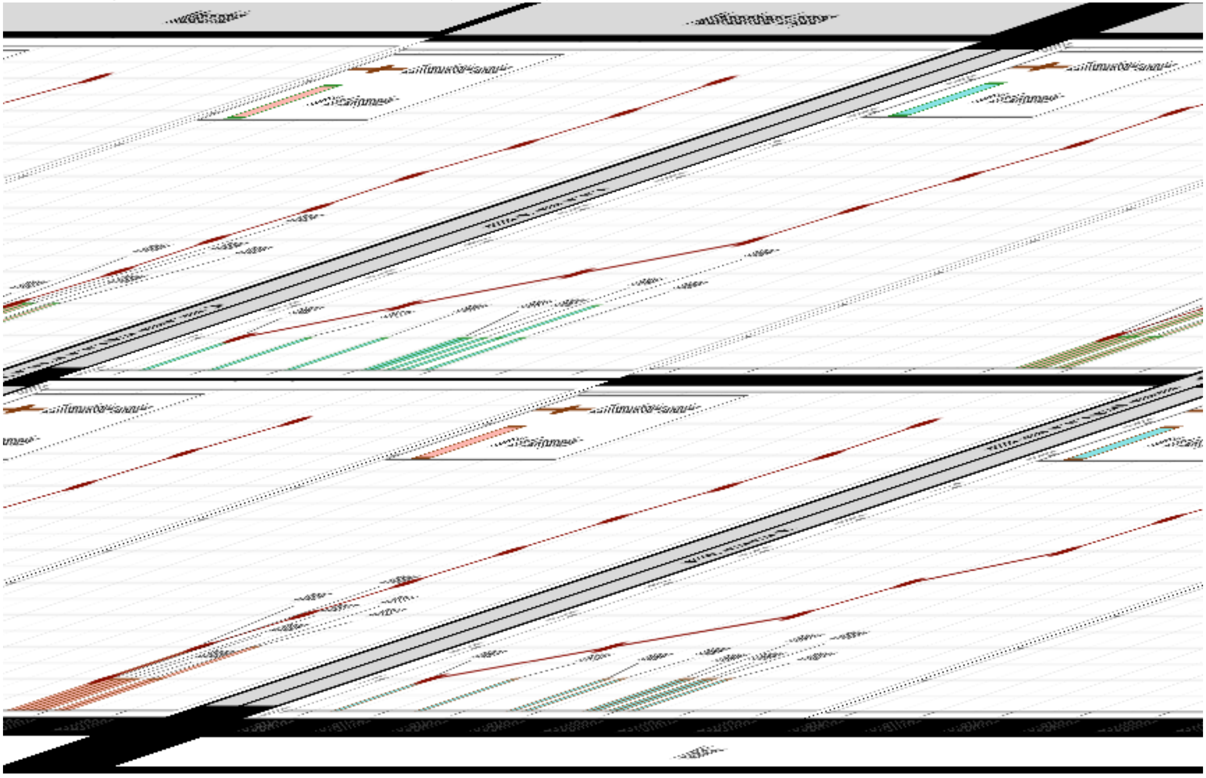

Figure 4: Sample depth of root and stem chronologies from abandoned crops and Mediterranean macchia

The sample depth indicates the number of individuals contributing to the ring-width chronology in a given year. It reflects both the year each individual was established and the cumulative number of samples over time. In the graphs (Fig. 4), black bars represent the recruitment of new individuals (i.e., year of germination), while the orange line shows the total number of samples included in the analysis. Therefore, in the earliest years, the chronology depends on a limited number of individuals, but in

later years, the sample depth increases as more trees establish and are added. This information is vital for assessing the robustness of the dendrochronological signal, as greater replication generally improves the reliability of growth trend interpretations.
As the charts display, in the abandoned crops, the first year of root establishment was 2005, while for the stem it was 2003. Regarding the Mediterranean macchia, the root time series starts in 1964, while the stem series begins a year earlier, in 1964.

4.3.2 Ring-width chronologies

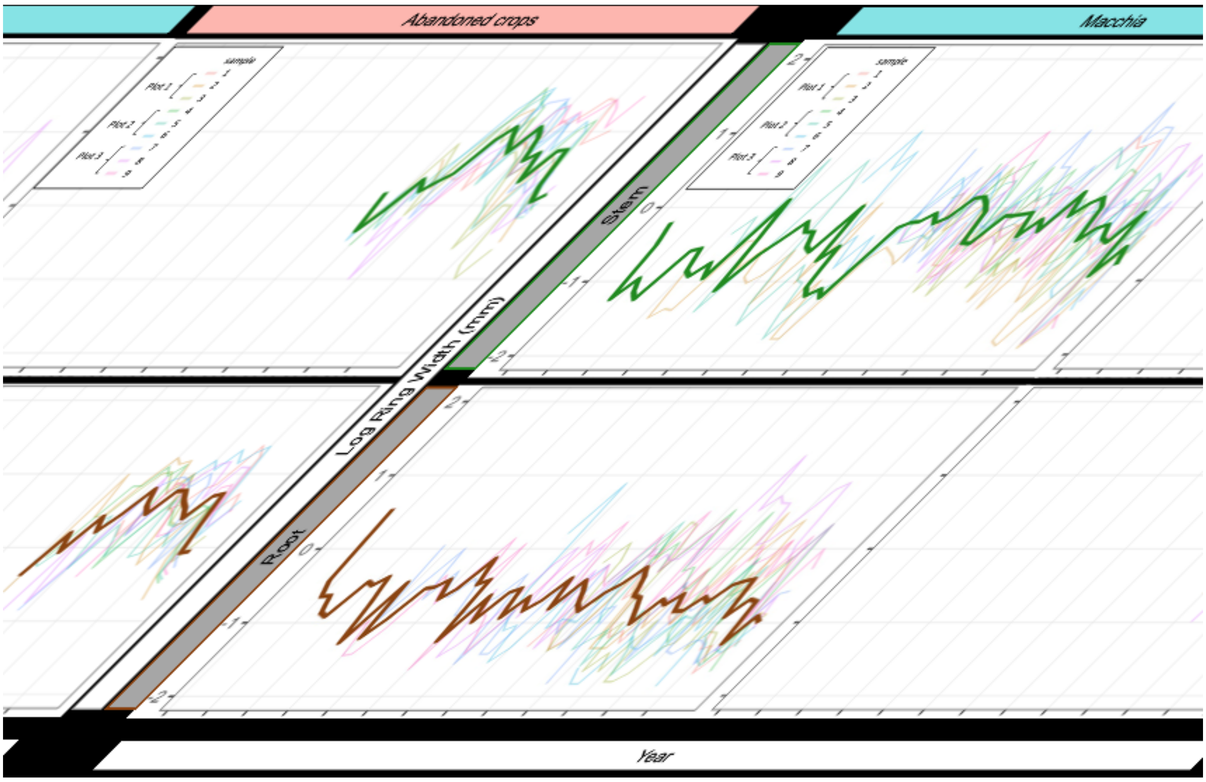

Figure 5: logarithmic tree ring-width (mm) chronology of stem and root from abandoned crops and Mediterranean macchia. Colored lines represent individual growth curves, while the darkest line shows the mean chronology.

The graphs depict annual ring-width chronologies derived from root and stem samples subjected to abandoned crop and Mediterranean macchia treatments. The x-axis delineates the time series, whereas the y-axis indicates ring width in centimeters. Each colored line corresponds to the growth trend of a single tree, thereby emphasizing variability among sampled specimens. The thick black line represents the arithmetic mean across all samples for each year, thereby illustrating an overarching growth trajectory. The number of sampled trees varies throughout the years, with fewer samples at the initial stages and an increased number during the central and subsequent periods, as depicted in the sample depth charts positioned above. This variation influences the robustness of the mean chronology. Overall, the graphs visually represent individual growth patterns and the mean trend over the entire duration, without inferring causality.

4.3.3 Mean average rings growth

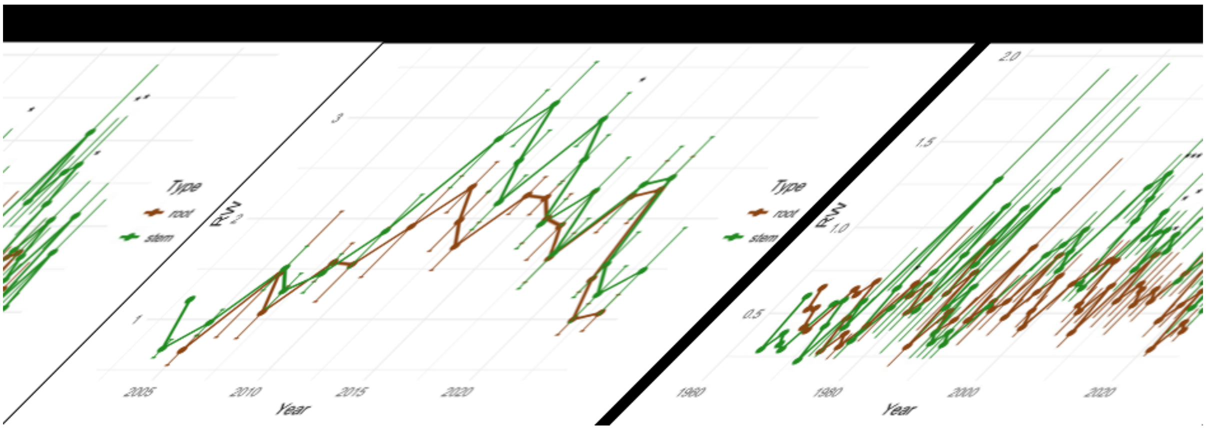

Figure 6:Temporal dynamics of tree-ring growth (Ring Width, RW) in roots (brown line) and stems (green line) under two treatments: abandoned crops (left) and macchia vegetation (right). Error bars indicate standard errors, and asterisks mark significant differences between roots and stems (p < 0.05).

The chart above illustrates the tree-ring growth (Ring Width, RW) of roots (brown line) and stems (green line) over time for two treatments: abandoned crops (left panel) and Mediterranean macchia (right panel). The y-axis shows RW, while the x-axis marks the year of ring formation. In abandoned crops, root and stem growth followed a generally similar trend, with notable differences only appearing in 2014, but with the same trend. In contrast, the macchia displayed more divergent growth patterns between roots and stems, with several years showing statistically significant differences (asterisks, $p < 0.05$). Overall, this comparison reveals a stronger difference between below- and aboveground growth in the macchia than in abandoned crops.

4.3.4 Comparison between treatments

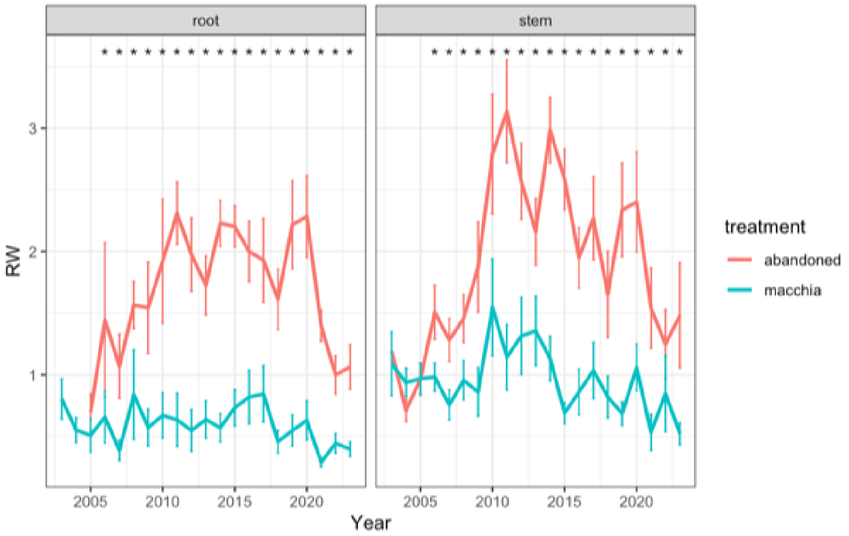

Figure 7: Mean annual ring width (RW, mm) of P. lentiscus from 2003 onwards in two treatments: abandoned crops (red) and Mediterranean macchia (blue). Growth curves are shown separately for root (left) and stem (right). Lines represent yearly means, error bars indicate ± standard error. Asterisks denote years with significant differences between treatments (p < 0.05).

The comparison of *P.lentiscus* tree ring width (RW) revealed clear differences in growth patterns between plants established in abandoned crops and those growing in Mediterranean macchia (Fig. 7). Although the main effect of treatment was not statistically significant ($p = 0.063$), the significant treatment × type interaction (p = 0.028) suggests that treatment effects differed between roots and stems.

For the root rings, individuals from abandoned crops consistently exhibited larger annual RW compared to those from the macchia. The mean root RW in the abandoned treatment was (1.77 ± 0.071), whereas in the macchia it was only (0.6 ± 0.026). These differences were statistically significant across most of the studied years, as shown by the statistical comparisons (except for 2005).

Similarly, for the stem rings, plants from abandoned plants exhibited markedly higher growth rates than those from the macchia. The mean stem RW was 2.05 ± 0.09 in the abandoned crops, while plants from the macchia were 0.89 ± 0.03. The difference between treatments persisted throughout the time series, with abandoned plants displaying wider rings, especially from 2006 to May 2023.

Overall, both roots and stems demonstrated that *P. lentiscus* growing in abandoned crops developed significantly wider annual rings compared to those in the Mediterranean macchia, indicating a continued treatment effect over time. The only exception was before 2005, when both tissues showed similar growth. Unfortunately, climatic data for those periods are not available.

4.3.5 Annual mean Ring Width growth

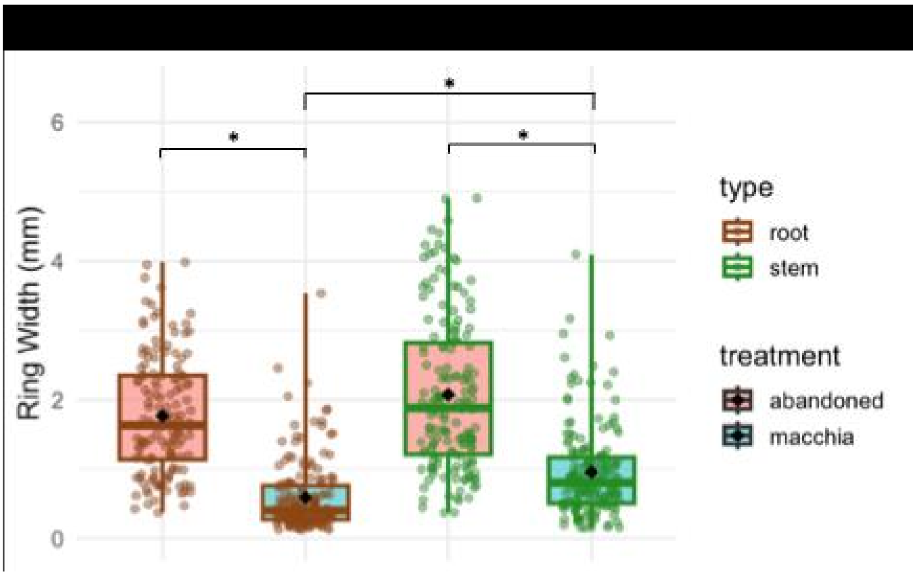

Figure 8: Mean ring width of P. lentiscus from 2005 onwards in abandoned crops (pink) and Mediterranean macchia (light blue). Box outlines indicate root (brown) and stem (green). Asterisks denote significant differences between organs and treatments (p < 0.05).

Annual ring width showed clear differences between treatments and organs. In the abandoned plots, both roots and stems exhibited relatively large values, with stems averaging 2.05 ± 0.087 mm and roots 1.77 ± 0.071 mm. Variability was higher in stems compared to roots, but the overall distribution of values was similar, indicating comparable growth between below- and above-ground organs under these conditions. In contrast, individuals growing in the Mediterranean macchia displayed markedly reduced ring width. Roots had the lowest values (0.599 ± 0.026 mm), while stems were slightly wider (0.895 ± 0.033 mm), highlighting a more pronounced difference between organs in this harsher environment.

4.3.6 Isotope analysis

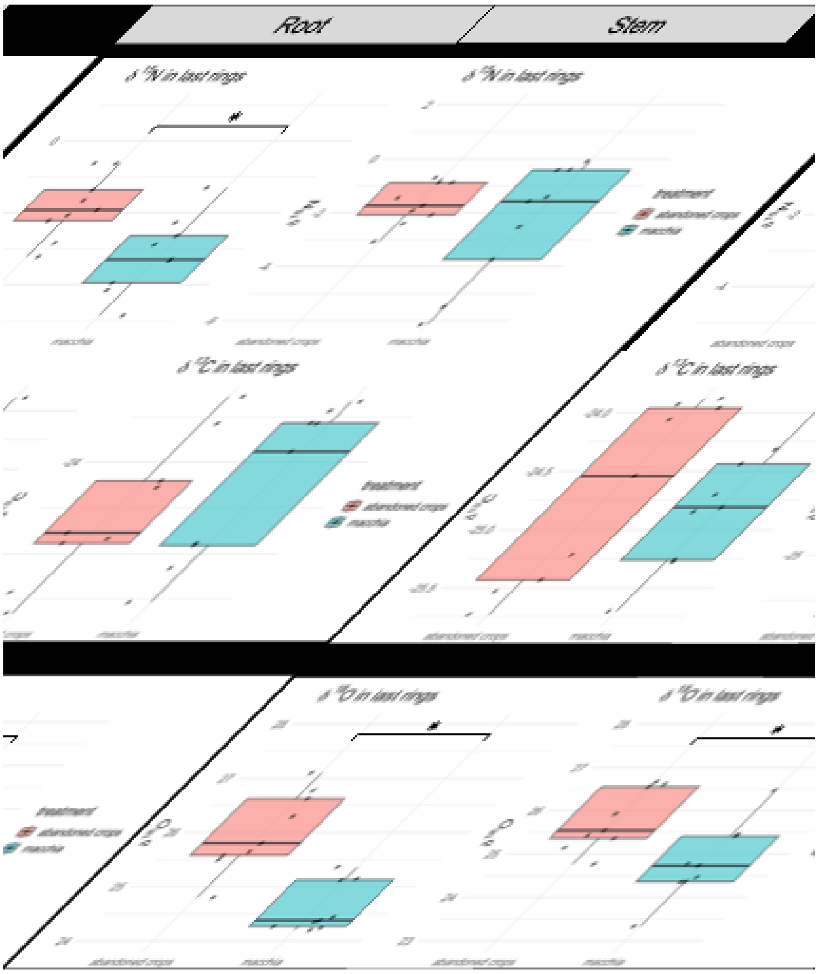

Figure 9: Stable isotope composition (δ¹⁵N, δ¹³C, and δ¹⁸O) in the outermost five tree rings of P.lentiscus roots (left panels) and stems (right panels) from abandoned crops (pink) and Mediterranean macchia (blue). Significant differences between treatments are indicated by asterisks (p < 0.05).

The isotopic analysis of the outermost five tree rings provided insights into how land-use history affects plant tissues. Results are presented separately for nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$), and oxygen ($\delta^{18}O$) isotopes (Fig. 9). For $\delta^{15}N$, a significant land-use effect ($p < 0.05$) was observed in the root system. Roots from abandoned crops showed higher values (–1.84 ± 0.30) compared to those from Mediterranean macchia (–3.19 ± 0.36). In stems, however, no significant differences appeared between the two treatments, with mean values of –1.68 ± 0.26 in abandoned crops and –2.43 ± 0.75 in macchia. Conversely, the $\delta^{13}C$ analysis revealed no significant treatment differences in either organ. Root $\delta^{13}C$ averaged –24.7 ± 0.26 in abandoned crops and –24.8 ± 0.19 in macchia, while stem values were –24.5 ± 0.26 and –24.3 ± 0.29, respectively. Lastly, $\delta^{18}O$ showed clear and consistent differences across treatments in both organs. Root $\delta^{18}O$ values were significantly higher in abandoned crops (26.0 ± 0.25) than in macchia (24.6 ± 0.15; $p < 0.05$). A similar enrichment was found in stems, with values of 25.8 ± 0.23 in abandoned crops compared to 24.8 ± 0.30 in macchia ($p < 0.05$).

Taken together, the results demonstrate that the isotopic composition of the outermost tree rings varies between land-use types mainly for $\delta^{15}N$ and $\delta^{18}O$, with $\delta^{15}N$ differences occurring only in roots, while $\delta^{18}O$ consistently distinguishes abandoned crops from macchia in both roots and stems. In contrast, $\delta^{13}C$ values remained stable across treatments and organs.

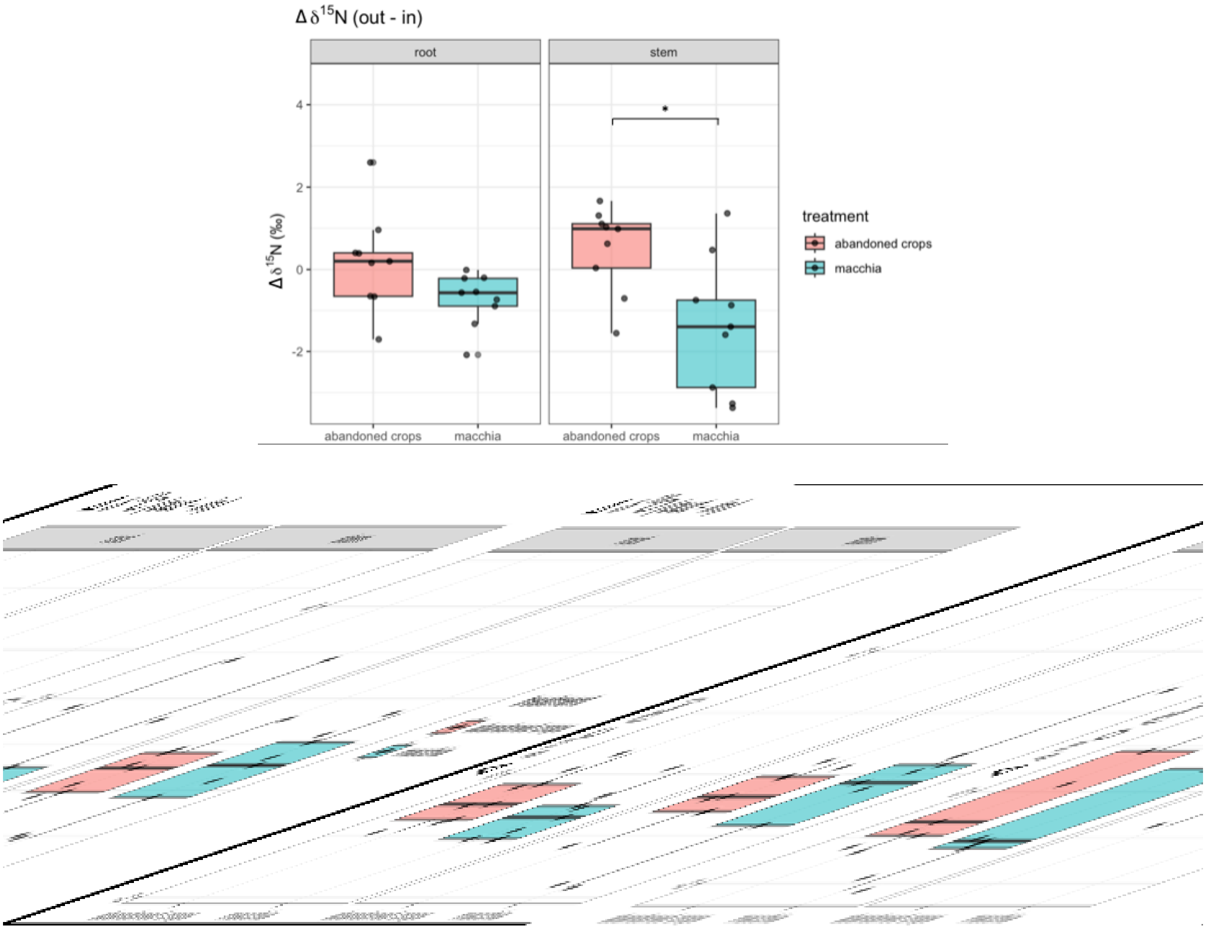

*Figure 10: differences between the innermost and outermost values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ stable isotope of P.lentiscus roots and stems from abandoned crops (pink) and Mediterranean macchia (blue). Significant differences between treatments are indicated by * ($p < 0.05$).*

Although individuals differed in age, the isotopic composition of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{18}O$ was measured from the innermost five tree rings, representing the early years of plant growth, to the outermost five rings. Although this comparison could be affected by age-related biases, it allowed for the reconstruction of temporal trends in isotopic variation from the establishment of each plant up to the sampling date for each treatment. The comparison of Δ $\delta^{15}N$ (in–out) values showed clear differences between organs and treatments (Fig. 10). In roots, $\Delta\ \delta^{15}N$ values were close to zero in both treatments, with averages of 0.19 ± 0.40 in abandoned crops and -0.73 ± 0.22 in macchia, with no significant differences. However, in stems, macchia had higher $\Delta\ \delta^{15}N$ values (-1.36 ± 0.55) compared to abandoned crops (0.5 ± 0.35), and this difference was statistically significant ($p < 0.05$). Overall, fractionation of $\Delta\ \delta^{15}N$ between inner and outer rings was minimal in roots but more noticeable in stems, especially in macchia. In contrast to $\Delta\ \delta^{15}N$ (in–out), no significant differences were found in $\Delta\ \delta^{13}C$ (in–out) and $\Delta\ \delta^{18}O$ (in–out) values between plants from the macchia and those from abandoned crops, whether in roots or stems. Mean $\Delta\ \delta^{13}C$ values were slightly higher in roots than in stems, ranging from -0.38 ± 0.14 in roots of abandoned crops to -0.68 ± 0.13 in roots of macchia. Stem values were similar across treatments (-0.33 ± 0.15 in abandoned crops and -0.33 ± 0.34 in macchia). Likewise, $\Delta\ \delta^{18}O$ values showed no differences between treatments and organs, with root means of 0.07 ± 0.49 (abandoned crops) and -0.41 ± 0.56 (macchia), and stem means of 0.39 ± 0.22 (abandoned crops) and 0.35 ± 0.32 (macchia). Overall, these results indicate that, unlike $\Delta\ \delta^{15}N$, neither $\Delta\ \delta^{13}C$ nor $\Delta\ \delta^{18}O$ signals differed significantly between vegetation types and treatments.

4.3.7 Fine root and leaf traits

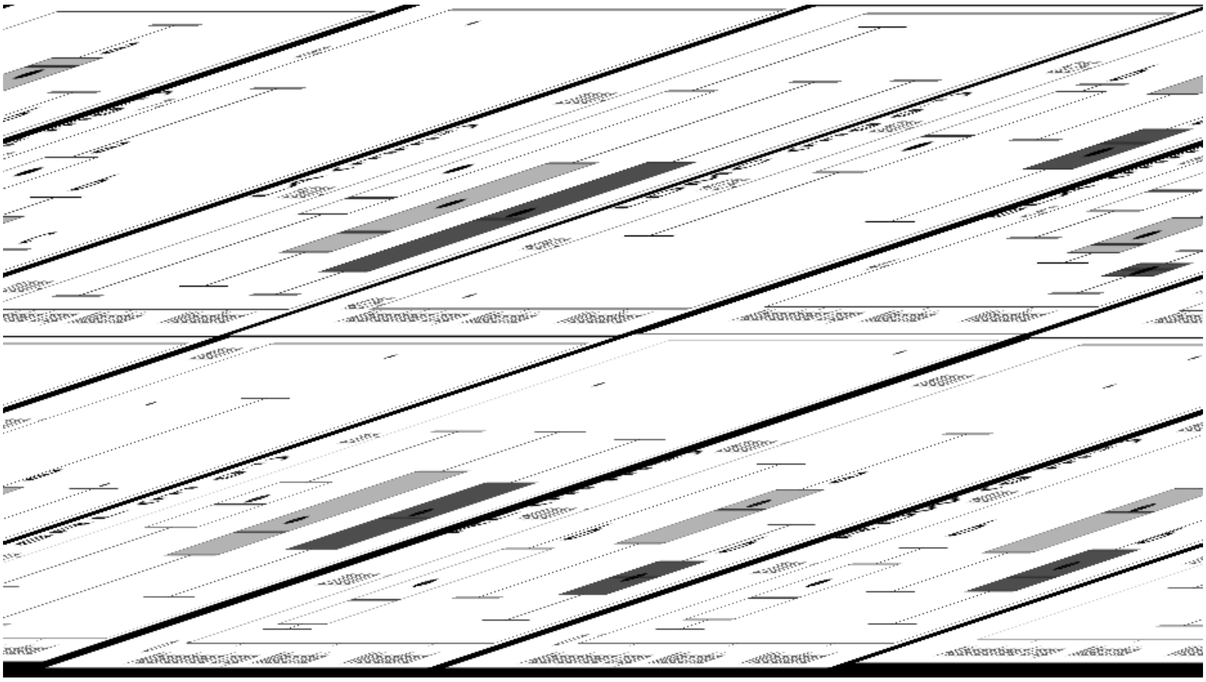

Figure11: Comparison between leaf traits (top panels) and fine root traits (bottom panels) across vegetation types (Abandoned crop, Macchia, Woodland). Boxplots show the median, interquartile range, and variability; black dots represent mean values. Different letters indicate statistically significant differences among treatments at p < 0.05

The multipanel figure presents a comparison between leaf traits (top panels) and fine root traits (bottom panels) across different vegetation types (Figure 11). Leaf area (LA) showed no statistically significant differences among treatments. Leaf dry matter content (LDMC) was significantly higher in macchia than in abandoned crop and woodland, which did not differ from each other. Specific leaf area was significantly different among all treatments, with the highest values in abandoned crop, intermediate in macchia, and the lowest in woodland.

For fine root traits, root length (RL) was significantly greater in Mediterranean macchia than in both abandoned crop and woodland, which did not differ from each other. Root dry weight (RDW) followed the same pattern, with significantly higher values in macchia compared to the other two treatments. Specific root length (SRL) did not differ significantly among treatments.

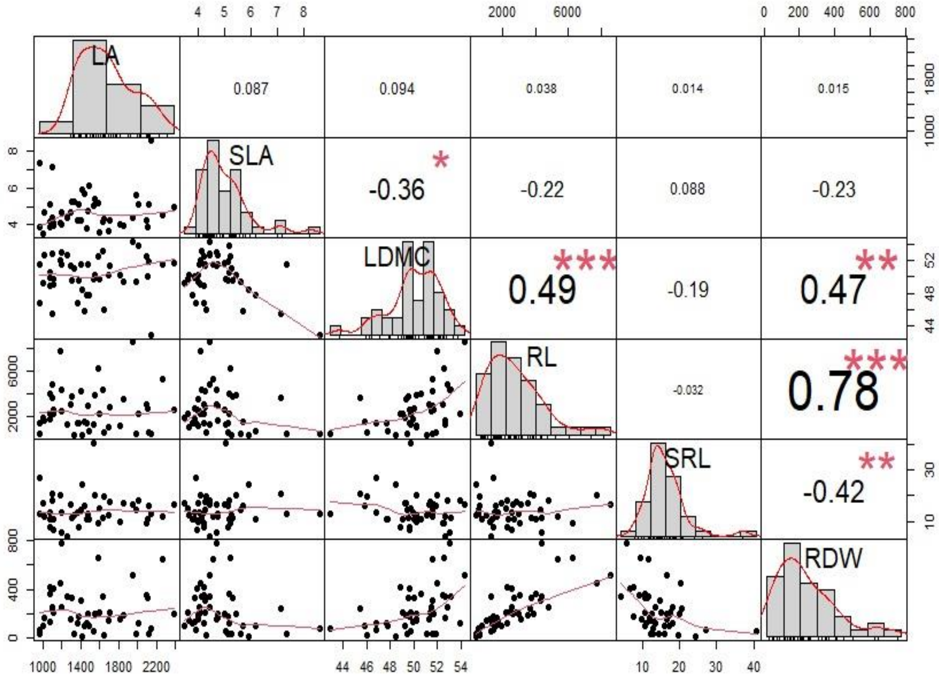

*Figure 12: Pearson's correlation matrix among leaf and root traits of P. lentiscus. Traits include leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), root length (RL), specific root length (SRL), and root dry weight (RDW). Values on the right represent correlation coefficients (r), with significance levels indicated as *p < 0.05, **p < 0.01, ***p < 0.001. Histograms on the diagonal show trait distributions, while scatterplots in the lower panels depict pairwise relationships with fitted trend lines.*

Pearson's correlation matrix for leaf and root traits is shown in Figure 12. Significant positive correlations were found between LDMC and RL (r = 0.49, ***p < 0.001) and between LDMC and RDW (r = 0.47, **p < 0.01). A notably strong positive relationship also existed between RL and RDW (r = 0.78, ***p < 0.001), indicating that increased root length is linked to higher root biomass. In contrast, SRL was negatively correlated with RDW (r = –0.42, **p < 0.01), suggesting that thicker roots tend to have lower specific root length. SLA showed a weak negative correlation with LDMC (r = –0.36, *p < 0.05).

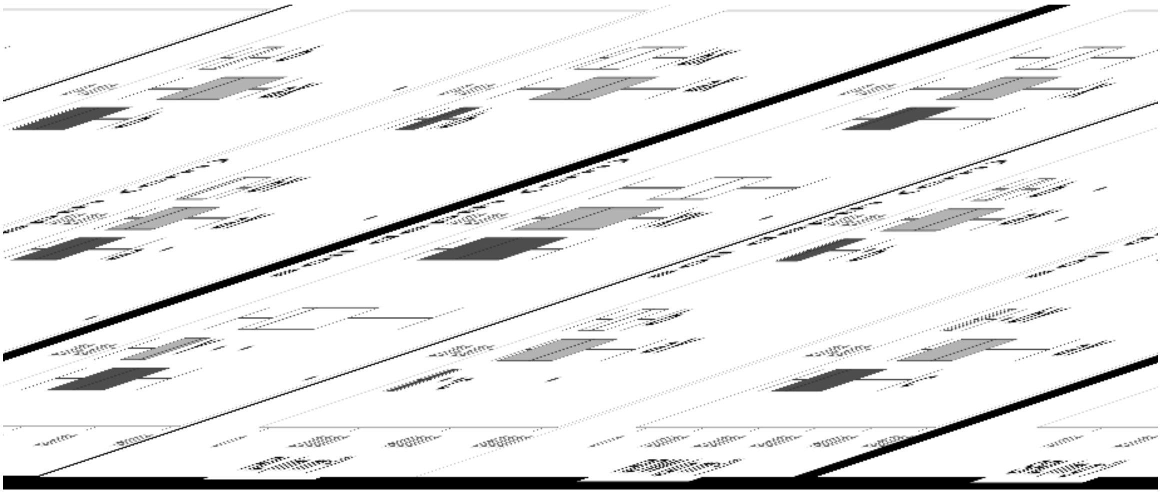

Figure 13: Fine root traits across soil depths (0–10 cm, 10–20 cm, 20–30 cm) for different vegetation types (Abandoned crop, Macchia, Woodland). Panels show root length (RL, left), root dry weight (RDW, center), and specific root length (SRL, right). Lowercase letters (a, b, c) indicate significant differences among treatments within the same depth, while uppercase letters (X, Y) denote significant differences among soil depths within the same treatment (p < 0.05)

Fine root traits across soil depths are shown in Figure 13. For root length (RL, left panel), Macchia had significantly higher values compared to both Abandoned crop and Woodland across all depths (0–10, 10–20, and 20–30 cm), as indicated by lowercase letters. Additionally, depth-related differences within treatments were observed, with higher values at 0–10 cm compared to deeper layers, as denoted by uppercase letters. For root dry weight (RDW, central panel), Macchia exhibited significantly higher values than the other treatments at both 0–10 cm and 10–20 cm, while at 20–30 cm Woodland showed the lowest values, as indicated by lowercase letters. Depth-related differences were also present within treatments, with RDW decreasing from the upper to deeper soil layers, as shown by uppercase letters. For specific root length (SRL, right panel), no significant differences were found among treatments within each depth, as indicated by the absence of distinct lowercase letters. However, significant differences across depths within the same treatment were detected, with higher SRL values generally observed in the upper layers compared to deeper layers, as indicated by uppercase letters.

4.4 DISCUSSION:

The analysis of wood anatomical traits of *P. lentiscus* revealed distinct growth patterns between roots and stems across treatments over the time series (Fig. 6). In abandoned crops, root and stem growth were mainly similar, with only a significant difference in magnitude observed in 2014. In contrast, in the Mediterranean macchia, the two tissues displayed slightly different behaviors: in some years, their growth trends increased or decreased together, while in others, they did not. These results suggest that in harsh environments, such as the Mediterranean macchia, roots and stems respond differently to environmental and competitive constraints, whereas in abandoned crops, where competition is minimal, this pattern is not observed. Notably, in both treatments, roots tended to grow less than stems, especially in the Mediterranean macchia.

Comparing stem and root ring widths over the past 20 years, based on the establishment of *P. lentiscus* in abandoned crops, plants in abandoned crops showed higher growth trends than those in the Mediterranean macchia (Fig. 7). This indicates that plants in abandoned crops may benefit from the heritage of past agricultural activity. In fact, the growth trends of both treatments were generally similar for most years, although they differed in magnitude. This pattern may reflect the similar influence of climate conditions on growth, while, as mentioned above, reduced competition in abandoned crops could have promoted better development in these plants.

Regarding isotopes, the analysis of the outermost rings revealed an enrichment of $\delta^{15}N$ in the roots of plants from abandoned fields compared to those from the Mediterranean macchia, supporting our hypothesis of a ^{15}N-enriched soil in these environments. Such enrichment was not observed in the stem. In parallel, the comparison of $\delta^{18}O$ values highlights how this isotope reflects physiological adjustments under different treatments. A previous study on Pianosa Island (Scartazza et al., 2014) reported that, while most climatic variables such as temperature were comparable, vapor pressure deficit (VPD) was higher in the abandoned fields. It is well established that under conditions of low to moderate humidity and limited soil water availability, stomata tend to close, thereby greatly reducing transpiration without completely suppressing it. As a result, leaf water becomes enriched in $H_2{}^{18}O$, and this enrichment is subsequently recorded in the plant biomass (Andreu-Hayles et al., 2022). In contrast, $\delta^{13}C$ did not exhibit significant differences between treatments and organs.

Furthermore, our analysis revealed distinct patterns in the differences between $\delta^{15}N$ values of innermost and outermost rings. Trends were similar across treatments, with lower $\delta^{15}N$ values in Mediterranean macchia compared to abandoned crops; however, statistical significance was observed only in stem tissue. Roots and stems from abandoned crops exhibited a positive trend (values greater than zero), indicating an increase in $\delta^{15}N$ from the innermost to outermost rings. In contrast, both tissues in Mediterranean macchia showed a negative trend (values less than zero), suggesting a reduction in $\delta^{15}N$ over time. This pattern may reflect the enrichment of $\delta^{15}N$ in fertilized soils through organic matter mineralization and microbial-mediated chemical transformations, which preferentially use the lighter ^{14}N isotope (Högberg, 1997; Pardo et al., 2006; Pardo & Nadelhoffer, 2009; Hobbie & Högberg, 2012).

The differences observed in both leaf and root traits generally reflect the resource allocation strategies adopted by plants (Grime, 1965). According to Grime and Mackey (Grime and Mackey 2002), these strategies can be positioned along a continuum of functional types, ranging from “acquisitive” species that rapidly exploit resources to “retentive” ones that are adapted to persist in chronically unproductive environments. In our case, the analysis of fine-root morpho-functional traits showed that both Root Length (RL) and Root Dry Weight (RDW), which are correlated according to the Pearson’s correlation matrix, were higher in the Mediterranean macchia compared with abandoned crops and woodland. Such increases can be interpreted as an adaptive response to water and nutrient scarcity, whereby plants allocate more resources to elongating fine roots and enhancing root biomass to maximize soil exploration and resource uptake.

Similar patterns have been documented previously. For example, Montagnoli et al. (2012) investigated fine-root dynamics of Quercus cerris L. across the growing season and reported that during periods of declining soil water content, fine-root length and biomass increased, thereby improving water uptake capacity. In that study, root traits were found to be negatively correlated with soil moisture. Consistent with this interpretation, earlier observations from Pianosa Island (2006–2009) showed that in May—the period corresponding to our sampling campaign—soil water content was lower in the Mediterranean macchia compared to abandoned crops (Scartazza et al., 2014). The behavior of Root Length (RL) and Root Dry Weight (RDW) in our analysis reflects these previous climatic data.

Regarding leaf traits, only Leaf Dry Matter Content (LDMC) showed a positive correlation with Root Dry Weight (RDW), with higher values observed in the Mediterranean macchia. In contrast, specific leaf area (SLA)—defined as the ratio of leaf area to leaf dry mass—reflects the ecological strategies plants adopt to optimize resource acquisition (Liu et al., 2021; Wright et al., 2004) and indicates the amount of new leaf area produced per unit of biomass (Niklas et al., 2007). Under environmental stress, such as that typical of the Mediterranean macchia, plants generally follow a conservative strategy characterized by lower SLA and higher LDMC (Blanco-Sanchez et al., 2022). This pattern was also observed in our study, whereas in abandoned fields, the opposite pattern was found, likely due to higher nutrient availability and soil water content. In the woodland, however, no clear trend emerged: SLA values were low, suggesting that other limiting factors may influence leaf traits in this environment.

Finally, Root Length (RL) and Root Dry Weight (RDW) measured at various soil depths followed the same overall pattern, providing no additional information. Roots in the Mediterranean macchia consistently showed greater elongation compared to those in abandoned fields and woodland.

4.5 CONCLUSION

This study demonstrates that *P. lentiscus* exhibits organ-specific growth strategies that vary across different environmental contexts. In abandoned crops, roots and stems followed a comparable growth pattern, whereas in the Mediterranean macchia, the two organs diverged, indicating that aboveground and belowground tissues may respond differently to environmental and competitive pressures. Stable isotope analyses also revealed contrasting patterns between treatments, highlighting differences in nutrient dynamics after the abandonment of the island and water-use strategies. Overall, these findings emphasize that roots and stems should not be considered separately when assessing plant responses to environmental constraints.

A key outcome of this research is the understanding that Mediterranean species like *P. lentiscus* rely on integrated strategies depending on environmental limitations, involving both aboveground and belowground organs to survive in challenging environments. Therefore, focusing only on stem traits may miss important aspects of plant adaptation and resilience. Future studies should include root traits along with stem and leaf characteristics to better understand plant functional responses to environmental stressors. This integrated view is vital not only for interpreting growth patterns in variable landscapes but also for predicting how Mediterranean ecosystems will adapt to ongoing climate change.

4.6 REFERENCE:

- Amundson, R., Guo, Y., & Gong, P. (2003). Soil diversity and land use in the United States. Ecosystems, 6(5), 470-482.
- Andreu-Hayles, L., Lévesque, M., Guerrieri, R., Siegwolf, R. T., & Körner, C. (2022). Limits and strengths of tree-ring stable isotopes. In Stable isotopes in tree rings: Inferring physiological, climatic and environmental responses (pp. 399-428). Cham: Springer International Publishing.
- Aloni, R. (1980). Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. Planta, 150(3), 255-263.

Babst, F., Bouriaud, O., Alexander, R., Trouet, V., & Frank, D. (2014). Toward consistent measurements of carbon accumulation: A multi-site assessment of biomass and basal area increment across Europe. Dendrochronologia, 32(2), 153-161.

- Baesso, B., Chiatante, D., Terzaghi, M., Zenga, D., Nieminen, K., Mahonen, A. P., ... & Montagnoli, A. (2018). Transcription factors PRE 3 and WOX 11 are involved in the formation of new lateral roots from secondary growth taproot in A. thaliana. Plant Biology, 20(3), 426-432.
- Baesso, B., Terzaghi, M., Chiatante, D., Scippa, G. S., & Montagnoli, A. (2020). WOX genes expression during the formation of new lateral roots from secondary structures in Populus nigra (L.) taproot. Scientific Reports, 10(1), 18890.
- Baldini, R. M. (2000). Flora vascolare dell'Isola di Pianosa (Arcipelago Toscano): revisione tassonomica ed aggiornamento. Webbia, 55(1), 107-189.
- Barbour, M. M., Roden, J. S., Farquhar, G. D., & Ehleringer, J. R. (2004). Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect. Oecologia, 138(3), 426-435.
- Barbour, M. M. (2007). Stable oxygen isotope composition of plant tissue: a review. Functional Plant Biology, 34(2), 83-94.
- Barredo, D., De Léséleuc, S., Lienhard, V., Lahaye, T., & Browaeys, A. (2016). An atom-by-atom assembler of defect-free arbitrary two-dimensional atomic arrays. Science, 354(6315), 1021-1023.
- Barredo, J. I., Mauri, A., Caudullo, G., & Dosio, A. (2019). Assessing shifts of Mediterranean and arid climates under RCP4. 5 and RCP8. 5 climate projections in Europe. In Meteorology and climatology of the Mediterranean and black seas (pp. 235-251). Cham: Springer International Publishing.
- Bates, B., Kundzewicz, Z., & Wu, S. (2008). Climate change and water. Intergovernmental Panel on Climate Change Secretariat.
- Battipaglia, G., De Micco, V., Brand, W. A., Linke, P., Aronne, G., Saurer, M., & Cherubini, P. (2010). Variations of vessel diameter and δ13C in false rings of Arbutus unedo L. reflect different environmental conditions. New Phytologist, 188(4), 1099-1112.
- Bazzaz, F. A. (1979). The physiological ecology of plant succession. Annual review of Ecology and Systematics, 10, 351-371.
- Belmecheri, S., & Lavergne, A. (2020). Compiled records of atmospheric CO2 concentrations and stable carbon isotopes to reconstruct climate and derive plant ecophysiological indices from tree rings. Dendrochronologia, 63, 125748.
- Benjamin, J. G., & Nielsen, D. C. (2004). A method to separate plant roots from soil and analyze root surface area. Plant and Soil, 267(1), 225-234.
- Blanco-Sánchez, M., Ramos-Muñoz, M., Pías, B., Ramírez-Valiente, J. A., Díaz-Guerra, L., Escudero, A., & Matesanz, S. (2022). Natural selection favours drought escape and an acquisitive resource-use strategy in semi-arid Mediterranean shrubs. Functional Ecology, 36(9), 2289-2302.
- Blöschl, G., Hall, J., Viglione, A., Perdigão, R. A., Parajka, J., Merz, B., ... & Živković, N. (2019). Changing climate both increases and decreases European river floods. Nature, 573(7772), 108-111.

- Bossio, A., Cornamusini, G., Ferrandini, J., Ferrandini, M., Foresi, L. M., Mazzanti, R., ... & Sandrelli, F. (2000). Dinamica dal Neogene al Quaternario della Corsica orientale e della Toscana. In PROGETTO INTERREG II Toscana-Corsica. L’attività scientifica delle Università di Pisa e Corte (pp. 87-95). ETS.
- Braun-Blanquet J. 1952. Les grupements végétaux de la France Méditerranéénne. CNRS, Montpellier.
- Bräuning, A., De Ridder, M., Zafirov, N., García-González, I., Dimitrov, D. P., & Gärtner, H. (2016). Tree-ring features: indicators of extreme event impacts. Iawa Journal, 37(2), 206-231.
- Brunialti, G., Frati, L., Aleffi, M., Marignani, M., Rosati, L., Burrascano, S., & Ravera, S. (2010). Lichens and bryophytes as indicators of old-growth features in Mediterranean forests. Plant Biosystems, 144(1), 221-233.
- Brunner, I., Herzog, C., Dawes, M. A., Arend, M., & Sperisen, C. (2015). How tree roots respond to drought. Frontiers in plant science, 6, 547.
- Brunner, I., Herzog, C., Galiano, L., & Gessler, A. (2019). Plasticity of fine-root traits under long-term irrigation of a water-limited scots pine forest. Frontiers in Plant Science, 10, 701.
-Buscardo, E., Rodriguez-Echeverria, S., Freitas, H., De Angelis, P., Pereira, J. S., & Muller, L. A. (2015). Contrasting soil fungal communities in Mediterranean pine forests subjected to different wildfire frequencies. Fungal Diversity, 70(1), 85-99.
- Bussotti, F., & Pollastrini, M. (2020). Opportunities and threats of Mediterranean evergreen sclerophyllous woody species subjected to extreme drought events. Applied Sciences, 10(23), 8458.
- Campelo F, Gutiérrez E, Ribas M, Nabais C, Freitas H. 2007. Relationships between climate and double rings in *Quercus ilex* from Northeast Spain. Can J For Res 37:1915–1923.
- Canadell, J., & Roda, F. (1991). Root biomass of Quercus ilex in a montane Mediterranean forest. Canadian Journal of Forest Research, 21(12), 1771-1778.
- Castri, F. D., Goodall, D. W., & Specht, R. L. (Eds.). (1981). Ecosystems of the world. 11. Mediterranean-type shrublands.
- Cherubini, P., Gartner, B. L., Tognetti, R., BRAeKER, O. U., Schoch, W., & Innes, J. L. (2003). Identification, measurement and interpretation of tree rings in woody species from Mediterranean climates. Biological Reviews, 78(1), 119-148.
- Cherubini, P., Battipaglia, G., & Innes, J. L. (2021). Tree vitality and forest health: can tree-ring stable isotopes be used as indicators?. Current Forestry Reports, 7(2), 69-80.
- Craig, H. (1957). The natural distribution of radiocarbon and the exchange time of carbon dioxide between atmosphere and sea. Tellus, 9(1), 1-17.
- Creber, G. T. (1977). Tree rings: a natural data-storage system. Biological Reviews, 52(3), 349-381.
- Cervera, T., Pino, J., Marull, J., Padró, R., & Tello, E. (2019). Understanding the long-term dynamics of forest transition: From deforestation to afforestation in a Mediterranean landscape (Catalonia, 1868–2005). Land use policy, 80, 318-331.
- Chang, C. C., & Turner, B. L. (2019). Ecological succession in a changing world. Journal of Ecology, 107(2), 503-509.
- Chave, J., Coomes, D., Jansen, S., Lewis, S. L., Swenson, N. G., & Zanne, A. E. (2009). Towards a worldwide wood economics spectrum. Ecology letters, 12(4), 351-366.
- Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., ... & Valentini, R. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature, 437(7058), 529-533.
- Colom, M. R., Vaccari, F. P., Scartazza, A., Brugnoli, E., Zerbi, G., Sforzi, S., ... & Spaccino, L. (2004). Pianosa island: structure, functioning and biodiversity of main ecosystems. Journal of Mediterranean Ecology, 5(1), 31-40.
- Cowling, E. B., & Merrill, W. (1966). Nitrogen in wood and its role in wood deterioration. Canadian Journal of Botany, 44(11), 1539-1554.
- Cudlin, P., Kieliszewska-Rokicka, B., Rudawska, M., Grebenc, T., Alberton, O., Lehto, T., ... & Kuyper, T. W. (2007). Fine roots and ectomycorrhizas as indicators of environmental change. Plant Biosystems, 141(3), 406-425.

- Cuny, H. E., Rathgeber, C. B., Frank, D., Fonti, P., Mäkinen, H., Prislan, P., ... & Fournier, M. (2015). Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature plants, 1(11), 1-6.
- D'Acqui, L. P., Santi, C. A., & Maselli, F. (2007). Use of ecosystem information to improve soil organic carbon mapping of a Mediterranean island. Journal of environmental quality, 36(1), 262-271.
- De Giuli, C. (1970). Lineamenti geologici, morfologici e paleontologici dell'Isola di Pianosa. Parco Nazionale Insulare di Pianosa nel Mar Tirreno. Gruppo Ricerche Scient. Tecn. Subacquee, Firenze, 1.
- de la Riva, E. G., Violle, C., Pérez-Ramos, I. M., Marañón, T., Navarro-Fernández, C. M., Olmo, M., & Villar, R. (2018). A multidimensional functional trait approach reveals the imprint of environmental stress in Mediterranean woody communities. Ecosystems, 21(2), 248-262.
- De Luis, M., Gričar, J., Čufar, K., Raventós, J. (2007). Seasonal dynamics of wood formation in Pinus halepensis from dry and semi-arid ecosystems in Spain. IAWA J 28:389–404.
- De Micco, V., Saurer, M., Aronne, G. Tognetti, R., Cherubini, P. 2007. Variations of wood anatomy and δ13C within-tree rings of coastal Pinus pinaster showing intra-annual density fluctuations. IAWA Journal 28: 61-74.
- De Micco, V., Carrer, M., Rathgeber, C. B., Camarero, J. J., Voltas, J., Cherubini, P., & Battipaglia, G. (2019). From xylogenesis to tree rings: wood traits to investigate tree response to environmental changes. IAWA journal, 40(2), 155-182.
- Denne, M. P. (1971). Temperature and tracheid development in Pinus sylvestris seedlings. Journal of Experimental Botany, 22(2), 362-370.
- Donat, M. G., Alexander, L. V., Yang, H., Durre, I., Vose, R., Dunn, R. J., ... & Kitching, S. (2013). Updated analyses of temperature and precipitation extreme indices since the beginning of the twentieth century: The HadEX2 dataset. Journal of Geophysical Research: Atmospheres, 118(5), 2098-2118.
- Doveri, M., Giannecchini, R., Mussi, M., Nicotra, I., & Puccinelli, A. (2012). Aspetti geologici e idrogeologici del peculiare acquifero dell’Isola di Pianosa (Arcipelago Toscano). Ital. J. Eng. Geol. Environ, 15, 17-30.
- Eissenstat, D. M., & Yanai, R. D. (1997). The ecology of root lifespan. In Advances in ecological research (Vol. 27, pp. 1-60). Academic Press.
- Eissenstat, D. M., Wells, C. E., Yanai, R. D., & Whitbeck, J. L. (2000). Building roots in a changing environment: implications for root longevity. The New Phytologist, 147(1), 33-42.
- Eissenstat, D. M., Kucharski, J. M., Zadworny, M., Adams, T. S., & Koide, R. T. (2015). Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytologist, 208(1), 114-124.
- Esper, J., Cook, E. R., & Schweingruber, F. H. (2002). Low-frequency signals in long tree-ring chronologies for reconstructing past temperature variability. science, 295(5563), 2250-2253.
- Farquhar, G. D., Hubick, K. T., Condon, A. G., & Richards, R. A. (1989a). Carbon isotope fractionation and plant water-use efficiency. In Stable isotopes in ecological research (pp. 21-40). New York, NY: Springer New York.
- Farquhar, G. D., Wong, S. C., Evans, J. R., & Hubick, K. T. (1989b). Photosynthesis and gas exchange. Plants under stress. Cambridge University Press, Cambridge, 39, 47-69.
- Finér, L., Ohashi, M., Noguchi, K., & Hirano, Y. (2011). Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics. Forest Ecology and Management, 262(11), 2008-2023.
- Fitter, A. (2002). Characteristics and functions of root systems. In Plant roots (pp. 49-78). CRC Press.
- Fonti, P., von Arx, G., García-González, I., Eilmann, B., Sass-Klaassen, U., Gärtner, H., & Eckstein, D. (2010). Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. New Phytologist, 185(1), 42-53.
- Fonti, P., & Jansen, S. (2012). Xylem plasticity in response to climate. New Phytologist, 195(4), 734-736.

- Freschet, G. T., Aerts, R., & Cornelissen, J. H. (2012). A plant economics spectrum of litter decomposability. Functional Ecology, 26(1), 56-65.
- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., ... & Stokes, A. (2021). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. New Phytologist, 232(3), 1123-1158.
- Fritts HC. 1976. Tree rings and climate. The Blackburn Press, Caldwell.
- Funada, R., Kubo, T., Tabuchi, M., Sugiyama, T., & Fushitani, M. (2001). Seasonal variations in endogenous indole-3-acetic acid and abscisic acid in the cambial region of Pinus densiflora Sieb. et Zucc. stems in relation to earlywood-latewood transition and cessation of tracheid production.
- Gärtner, H., Schneider, L., & Cherubini, P. (2024). A new workflow for sampling and digitizing increment cores. J. Visualized Exp.(JoVE), 211, e67098.
- Giorgi, F. (2006). Climate change hot-spots. Geophysical research letters, 33(8).
- Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. Global and planetary change, 63(2-3), 90-104.
- Gill, R. A., & Jackson, R. B. (2000). Global patterns of root turnover for terrestrial ecosystems. The New Phytologist, 147(1), 13-31.
- Graciotti, R., Pantaloni, M., & Foresi, L. (2008). Geomorphological map of the Pianosa Island (Tuscan Archipelago, Italy). Journal of Maps, 4(1), 463-471.
- Grime, J. P., & Mackey, J. M. L. (2002). The role of plasticity in resource capture by plants. Evolutionary ecology, 16(3), 299-307.
- Handley, L. L., & Raven, J. A. (1992). The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant, Cell & Environment, 15(9), 965-985.
- Hanley, M. E., Cordier, P. K., May, O., & Kelly, C. K. (2007). Seed size and seedling growth: differential response of Australian and British Fabaceae to nutrient limitation. New Phytologist, 174(2), 381-388.
- Hart, S. C., & Classen, A. T. (2003). Potential for assessing long-term dynamics in soil nitrogen availability from variations in δ 15N of tree rings. Isotopes in Environmental and Health Studies, 39(1), 15-28.
- Herrera, C. M., & Bazaga, P. (2016). Genetic and epigenetic divergence between disturbed and undisturbed subpopulations of a Mediterranean shrub: A 20-year field experiment. Ecology and Evolution, 6(11), 3832-3847.
- Hirabayashi, Y., Mahendran, R., Koirala, S., Konoshima, L., Yamazaki, D., Watanabe, S., ... & Kanae, S. (2013). Global flood risk under climate change. Nature climate change, 3(9), 816-821.
- Hobbie, E. A., & Högberg, P. (2012). Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. New phytologist, 196(2), 367-382.
- Högberg, P. (1991). Development of 15N enrichment in a nitrogen-fertilized forest soil-plant system. Soil Biology and Biochemistry, 23(4), 335-338.
- Högberg, P. (1997). Tansley review no. 95 15N natural abundance in soil–plant systems. The New Phytologist, 137(2), 179-203.
- Hopkins, D. W., & Dungait, J. A. (2010). Soil microbiology and nutrient cycling. In Soil microbiology and sustainable crop production (pp. 59-80). Dordrecht: Springer Netherlands.
- Infusino, M., Greco, S., Turco, R., Bernardini, V., & Scalercio, S. (2016). Managed mountain forests as diversity reservoirs in Mediterranean landscapes: new data on endemic species and faunistic novelties of moths.
- IPCC (2007) Climate Change 2007. The Fourth Assessment Report. Impacts, Adaptation and Vulnerability. Cambridge University Press, Cambridge.
- IPCC (2014): Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland.
-IPCC (2022): Summary for Policymakers [H.-O. Pörtner, D.C. Roberts, E.S. Poloczanska, K. Mintenbeck, M. Tignor, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem (eds.)]. In: Climate Change

2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C.

- IPCC (2023): Summary for Policymakers. In: Climate Change 2023: Synthesis Report.Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.
- Iversen, C. M., & McCormack, M. L. (2021). Filling gaps in our understanding of belowground plant traits across the world: an introduction to a Virtual Issue. New Phytologist, 231(6), 2097-2103.
- Jentsch, A., & Beierkuhnlein, C. (2008). Research frontiers in climate change: effects of extreme meteorological events on ecosystems. Comptes Rendus Geoscience, 340(9-10), 621-628.
- Jin, K., White, P. J., Whalley, W. R., Shen, J., & Shi, L. (2017). Shaping an optimal soil by root–soil interaction. Trends in Plant Science, 22(10), 823-829.
- Jones, P. D., Briffa, K. R., Osborn, T. J., Lough, J. M., van Ommen, T. D., Vinther, B. M., ... & Xoplaki, E. (2009). High-resolution palaeoclimatology of the last millennium: a review of current status and future prospects. The Holocene, 19(1), 3-49.
- Kaennel, M., & Schweingruber, F. H. (1995). Multilingual glossary of dendrochronology. WSL FNP, Haupt, 133, 162-184.
- Kaiser, C., Kilburn, M. R., Clode, P. L., Fuchslueger, L., Koranda, M., Cliff, J. B., ... & Murphy, D. V. (2015). Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New phytologist, 205(4), 1537-1551.
- Köppen, W. P. (1931). Grundriss der klimakunde. W. de Gruyter.
- Kreitler, C. W. (1979). Nitrogen-isotope ratio studies of soils and groundwater nitrate from alluvial fan aquifers in Texas. Journal of Hydrology, 42(1-2), 147-170.
- Laginha Pinto Correia, D., Bouchard, M., Filotas, É., & Raulier, F. (2019). Disentangling the effect of drought on stand mortality and productivity in northern temperate and boreal forests. Journal of Applied Ecology, 56(3), 758-768.
- La Mantia, T., Rühl, J., Massa, B., Pipitone, S., Lo Verde, G., & Bueno, R. S. (2019). Vertebrate-mediated seed rain and artificial perches contribute to overcome seed dispersal limitation in a Mediterranean old field. Restoration Ecology, 27(6), 1393-1400.
- La Provincia di Livorno 1962. Rivista bimestrale dell'amministrazione provinciale. September-October, 1962.
- Le Quéré, C., Andrew, R. M., Friedlingstein, P., Sitch, S., Hauck, J., Pongratz, J., ... & Zheng, B. (2018). Global carbon budget 2018. Earth System Science Data, 10(4), 2141-2194.
- Levanič, T. (2007). ATRICS–A new system for image acquisition in dendrochronology. Tree-Ring Research, 63(2), 117-122.
- Lévy, G., Bréchet, C., & Becker, M. (1996). Element analysis of tree rings in pedunculate oak heartwood: an indicator of historical trends in the soil chemistry, related to atmospheric deposition. In Annales des sciences forestières (Vol. 53, No. 2-3, pp. 685-696). EDP Sciences.
- Liu, Z., Dong, N., Zhang, H., Zhao, M., Ren, T., Liu, C., ... & He, N. (2021). Divergent long-and short-term responses to environmental gradients in specific leaf area of grassland species. Ecological Indicators, 130, 108058.
- Liu, L., Estiarte, M., Bengtson, P., Li, J., Asensio, D., Wallander, H., & Peñuelas, J. (2022). Drought legacies on soil respiration and microbial community in a Mediterranean forest soil under different soil moisture and carbon inputs. Geoderma, 405, 115425.
- López, R., Ramírez-Valiente, J. A., & Pita, P. (2022). How plants cope with heatwaves in a drier environment. Flora, 295, 152148.
- Majdi, H., & Andersson, P. (2005). Fine root production and turnover in a Norway spruce stand in northern Sweden: effects of nitrogen and water manipulation. Ecosystems, 8(2), 191-199.
- McCarroll, D., & Loader, N. J. (2004). Stable isotopes in tree rings. Quaternary Science Reviews, 23(7-8), 771-801.
- McCarthy-Neumann, S., & Kobe, R. K. (2010). Conspecific and heterospecific plant–soil feedbacks influence survivorship and growth of temperate tree seedlings. Journal of Ecology, 98(2), 408-418.

- McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., ... & Zadworny, M. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytologist, 207(3), 505-518.
- Médail, F., & Myers, N. (2004). Mediterranean basin. Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions, 144-147.
- Medrano, H., Flexas, J., & Galmés, J. (2009). Variability in water use efficiency at the leaf level among Mediterranean plants with different growth forms. Plant and Soil, 317(1), 17-29.
- Melillo, J. M., Richmond, T. T., & Yohe, G. (2014). Climate change impacts in the United States. Third national climate assessment, 52, 150-174.
- Metcalfe, D. B., Meir, P., Aragão, L. E. O., Da Costa, A. C., Braga, A. P., Gonçalves, P. H., ... & Williams, M. (2008). The effects of water availability on root growth and morphology in an Amazon rainforest. Plant and Soil, 311(1), 189-199.
- Milano, M., Ruelland, D., Fernandez, S., Dezetter, A., Fabre, J., Servat, E., ... & Thivet, G. (2013). Current state of Mediterranean water resources and future trends under climatic and anthropogenic changes. Hydrological Sciences Journal, 58(3), 498-518.
- Milly, P. C., Dunne, K. A., & Vecchia, A. V. (2005). Global pattern of trends in streamflow and water availability in a changing climate. Nature, 438(7066), 347-350.
- Montagnoli, A., Terzaghi, M., Di Iorio, A., Scippa, G. S., & Chiatante, D. (2012). Fine-root morphological and growth traits in a Turkey-oak stand in relation to seasonal changes in soil moisture in the Southern Apennines, Italy. Ecological Research, 27(6), 1015-1025.
- Montagnoli, A., Di Iorio, A., Terzaghi, M., Trupiano, D., Scippa, G. S., & Chiatante, D. (2014). Influence of soil temperature and water content on fine-root seasonal growth of European beech natural forest in Southern Alps, Italy. European Journal of Forest Research, 133(5), 957-968.
- Montagnoli, A., Terzaghi, M., Baesso, B., Santamaria, R., Scippa, G. S., & Chiatante, D. (2016). Drought and fire stress influence seedling competition in oak forests: fine-root dynamics as indicator of adaptation strategies to climate change. Reforesta, (1), 86-105.
- Montagnoli, A., Terzaghi, M., Giussani, B., Scippa, G. S., & Chiatante, D. (2018). An integrated method for high-resolution definition of new diameter-based fine root sub-classes of Fagus sylvatica L. Annals of Forest Science, 75(3), 76.
- Montagnoli, A., Dumroese, R. K., Terzaghi, M., Onelli, E., Scippa, G. S., & Chiatante, D. (2019). Seasonality of fine root dynamics and activity of root and shoot vascular cambium in a Quercus ilex L. forest (Italy). Forest Ecology and Management, 431, 26-34.
- Montagnoli, A., Terzaghi, M., Miali, A., Chiatante, D., & Dumroese, R. K. (2023). Unusual late-fall wildfire in a pre-Alpine Fagus sylvatica forest reduced fine roots in the shallower soil layer and shifted very fine-root growth to deeper soil depth. Scientific Reports, 13(1), 6380.
- Natali, S. (2023). Isotope hydrology of precipitation and cave waters in Tuscany (western Mediterranean) in relation to moisture origin and climatic variables.
- Newbold, T., Hudson, L. N., Hill, S. L., Contu, S., Lysenko, I., Senior, R. A., ... & Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. Nature, 520(7545), 45-50.
- Nicoll, B. C., & Ray, D. (1996). Adaptive growth of tree root systems in response to wind action and site conditions. Tree physiology, 16(11-12), 891-898.
- Nicotra, I., Vaccari, F. P., & Crisci, A. (2008). Analisi climatica dell'isola di Pianosa. CODICE ARMONICO, 94.
- Niklas, K. J., Cobb, E. D., Niinemets, Ü., Reich, P. B., Sellin, A., Shipley, B., & Wright, I. J. (2007). "Diminishing returns" in the scaling of functional leaf traits across and within species groups. Proceedings of the National Academy of Sciences, 104(21), 8891-8896.
- Odum, E. P. (1969). The Strategy of Ecosystem Development: An understanding of ecological succession provides a basis for resolving man's conflict with nature. science, 164(3877), 262-270.
- Oria-de-Rueda, J. A., Hernández-Rodríguez, M., Martín-Pinto, P., Pando, V., & Olaizola, J. (2010). Could artificial reforestations provide as much production and diversity of fungal species as natural forest stands in marginal Mediterranean areas?. Forest Ecology and Management, 260(2), 171-180.

- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lõhmus, K., ... & Brunner, I. (2007). Specific root length as an indicator of environmental change. Plant Biosystems, 141(3), 426-442.
- Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., ... & Hayes, D. (2011). A large and persistent carbon sink in the world's forests. science, 333(6045), 988-993.
- Pardo, L. H., Templer, P. H., Goodale, C. L., Duke, S., Groffman, P. M., Adams, M. B., ... & Wessel, W. (2006). Regional assessment of N saturation using foliar and root. Biogeochemistry, 80(2), 143-171.
- Pardo, L. H., & Nadelhoffer, K. J. (2009). Using nitrogen isotope ratios to assess terrestrial ecosystems at regional and global scales. In Isoscapes: understanding movement, pattern, and process on earth through isotope mapping (pp. 221-249). Dordrecht: Springer Netherlands.
- Parmesan, C., Root, T. L., & Willig, M. R. (2000). Impacts of extreme weather and climate on terrestrial biota. Bulletin of the American Meteorological Society, 81(3), 443-450.
- Pérez-Harguindeguy N, Diaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, et al. (2013) New handbook for standardized measurement of plant functional traits worldwide. Australian Journal of Botany 61(3),
- Pérez-Hernández, J., & Gavilán, R. G. (2021). Impacts of land-use changes on vegetation and ecosystem functioning: Old-field secondary succession. Plants, 10(5), 990.
- Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. Annual review of ecology and systematics, 293-320.
- Pierce, S., Negreiros, D., Cerabolini, B. E., Kattge, J., Díaz, S., Kleyer, M., ... & Tampucci, D. (2017). A global method for calculating plant CSR ecological strategies applied across biomes world-wide. Functional ecology, 31(2), 444-457.
- Piñol, J., Terradas, J., & Lloret, F. (1998). Climate warming, wildfire hazard, and wildfire occurrence in coastal eastern Spain. Climatic change, 38(3), 345-357.
- Plomion, C., Leprovost, G., & Stokes, A. (2001). Wood formation in trees. Plant physiology, 127(4), 1513-1523.
- Ponder, F., & Alley, D. E. (1997). Soil sampler for rocky soils.
- Rapetti E (1835) Dizionario geografico. Fisico, Storico della Toscana, pp 607–611
- Rathgeber, C. B., Cuny, H. E., & Fonti, P. (2016). Biological basis of tree-ring formation: a crash course. Frontiers in plant science, 7, 734.
- Reubens, B., Poesen, J., Danjon, F., Geudens, G., & Muys, B. (2007). The role of fine and coarse roots in shallow slope stability and soil erosion control with a focus on root system architecture: a review. Trees, 21(4), 385-402.
- Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA
- Roden, J. S., & Ehleringer, J. R. (2000). Hydrogen and oxygen isotope ratios of tree ring cellulose for field-grown riparian trees. Oecologia, 123(4), 481-489.
-Santonja, M., Foucault, Q., Rancon, A., Gauquelin, T., Fernandez, C., Baldy, V., & Mirleau, P. (2018). Contrasting responses of bacterial and fungal communities to plant litter diversity in a Mediterranean oak forest. Soil Biology and Biochemistry, 125, 27-36.
- Scartazza, A., Vaccari, F. P., Bertolini, T., Di Tommasi, P., Lauteri, M., Miglietta, F., & Brugnoli, E. (2014). Comparing integrated stable isotope and eddy covariance estimates of water-use efficiency on a Mediterranean successional sequence. Oecologia, 176(2), 581-594.
- Scheidegger, Y., Saurer, M., Bahn, M., & Siegwolf, R. (2000). Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. Oecologia, 125(3), 350-357.
- Schulze, E. D., Nicolle, D., Börner, A., Lauerer, M., Aas, G., & Schulze, I. (2014). Stable carbon and nitrogen isotope ratios of Eucalyptus and Acacia species along a seasonal rainfall gradient in Western Australia. Trees, 28(4), 1125-1135.
- Shah, N. H., & Paulsen, G. M. (2003). Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant and soil, 257(1), 219-226.

- Sillmann, J., Kharin, V. V., Zwiers, F. W., Zhang, X., & Bronaugh, D. (2013). Climate extremes indices in the CMIP5 multimodel ensemble: Part 2. Future climate projections. Journal of geophysical research: atmospheres, 118(6), 2473-2493.
- Smith, M. D. (2011). The ecological role of climate extremes: current understanding and future prospects. Journal of Ecology, 99(3), 651-655.
- Solomou, A., Proutsos, N., Karetsos, G., & Tsagari, C. (2017). Effects of climate change on vegetation in Mediterranean forests: A review.
- Sun, H., Jiang, S., Jiang, C., Wu, C., Gao, M., & Wang, Q. (2021). A review of root exudates and rhizosphere microbiome for crop production. Environmental Science and Pollution Research, 28(39), 54497-54510.
- Szymanowska-Pułka, J. (2013). Form matters: morphological aspects of lateral root development. Annals of Botany, 112(9), 1643-1654.
- Taibi, S., Messelmi, I., Meddi, M., & Feddal, M. A. (2018, November). Recent rainfall variability in the South-West Mediterranean Region and Links with Teleconnection patterns. In Conference of the Arabian Journal of Geosciences (pp. 111-114). Cham: Springer International Publishing.
- Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M. A., & Steppe, K. (2015). Responses of tree species to heat waves and extreme heat events. Plant, cell & environment, 38(9), 1699-1712.
- Tyree, M. T., & Zimmermann, M. H. (2002). Xylem structure and the ascent of sap. Springer Science & Business Media.
- Treydte, K. S., Schleser, G. H., Helle, G., Frank, D. C., Winiger, M., Haug, G. H., & Esper, J. (2006). The twentieth century was the wettest period in northern Pakistan over the past millennium. Nature, 440(7088), 1179-1182.
- Trenberth, K. E., Fasullo, J. T., & Shepherd, T. G. (2015). Attribution of climate extreme events. Nature climate change, 5(8), 725-730.
- Trouet, V., Esper, J., Graham, N. E., Baker, A., Scourse, J. D., & Frank, D. C. (2009). Persistent positive North Atlantic Oscillation mode dominated the medieval climate anomaly. science, 324(5923), 78-80.
- Ummenhofer, C. C., & Meehl, G. A. (2017). Extreme weather and climate events with ecological relevance: a review. Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1723), 20160135.
- Underwood, E. C., Viers, J. H., Klausmeyer, K. R., Cox, R. L., & Shaw, M. R. (2009). Threats and biodiversity in the mediterranean biome. Diversity and Distributions, 15(2), 188-197.
- Vaccari, F. P., Lugato, E., Gioli, B., D'Acqui, L., Genesio, L., Toscano, P., ... & Miglietta, F. (2012). Land use change and soil organic carbon dynamics in Mediterranean agro-ecosystems: The case study of Pianosa Island. Geoderma, 175, 29-36.
- Vicente-Serrano, S. M., Beguería, S., & López-Moreno, J. I. (2010). A multiscalar drought index sensitive to global warming: the standardized precipitation evapotranspiration index. Journal of climate, 23(7), 1696-1718.
- Vogt K A and Persson H 1991 Measuring growth and development of roots. In Eds J P Lassoie and T M Hinckley. Techniques and Approaches in Forest Tree Ecophysiology. pp 477-501. CRS,
Press, Boston.
- Vogt, K. A., Vogt, D. J., Asbjornsen, H., & Dahlgren, R. A. (1995). Roots, nutrients and their relationship to spatial patterns. Plant and Soil, 168(1), 113-123.
- Von Arx, G., Crivellaro, A., Prendin, A. L., Čufar, K., & Carrer, M. (2016). Quantitative wood anatomy—practical guidelines. Frontiers in plant science, 7, 781.
- Wassenaar, L. I. (1995). Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of15N and18O in NO3−. Applied geochemistry, 10(4), 391-405.
- Wilson JW. 1970. The Growing Tree. The University of Massachusetts Press, Amherst, MA.
- Withington, J. M., Reich, P. B., Oleksyn, J., & Eissenstat, D. M. (2006). Comparisons of structure and life span in roots and leaves among temperate trees. Ecological monographs, 76(3), 381-397.
- Wodzicki, T. J. (1971). Mechanism of xylem differentiation in Pinus silvestris L. Journal of Experimental Botany, 22(3), 670-687.

- Woodley, E. J., Loader, N. J., McCarroll, D., Young, G. H., Robertson, I., Heaton, T. H., ... & Warham, J. O. (2012). High-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry: simultaneous measurement of the stable isotopes of oxygen and carbon in cellulose. Rapid Communications in Mass Spectrometry, 26(2), 109-114.
- Wright, I. J., Groom, P. K., Lamont, B. B., Poot, P., Prior, L. D., Reich, P. B., ... & Westoby, M. (2004). Leaf trait relationships in Australian plant species. Functional plant biology, 31(5), 551-558.
- Yoneyama, M., Suhara, W., Fukuhara, Y., Sato, M., Ozato, K., & Fujita, T. (1996). Autocrine amplification of type I interferon gene expression mediated by interferon stimulated gene factor 3 (ISGF3). The journal of biochemistry, 120(1), 160-169.
- Zadworny, M., McCormack, M. L., Mucha, J., Reich, P. B., & Oleksyn, J. (2016). Scots pine fine roots adjust along a 2000-km latitudinal climatic gradient. New Phytologist, 212(2), 389-399.
- Zadworny, M., McCormack, M. L., Żytkowiak, R., Karolewski, P., Mucha, J., & Oleksyn, J. (2017). Patterns of structural and defense investments in fine roots of Scots pine (Pinus sylvestris L.) across a strong temperature and latitudinal gradient in Europe. Global Change Biology, 23(3), 1218-1231.
- Zech, M., Pedentchouk, N., Buggle, B., Leiber, K., Kalbitz, K., Marković, S. B., & Glaser, B. (2011). Effect of leaf litter degradation and seasonality on D/H isotope ratios of n-alkane biomarkers. Geochimica et Cosmochimica Acta, 75(17), 4917-4928.

OVERALL CONCLUSION

The analyses carried out in this dissertation have laid the foundations for a broader understanding of how environmental changes alter plant growth dynamics across different biological scales. A central outcome of this work is that it is not possible to fully capture the effects of climate and land-use change on plants by focusing solely on the aboveground compartment. Most research in plant sciences has traditionally concentrated on shoots, stems, and leaves, mainly because the study of the belowground compartment is technically challenging. However, the results presented here clearly demonstrate that roots often behave differently from shoots, and in some cases even display opposite trends, depending on environmental context and stress conditions. This highlights the necessity of adopting integrative approaches that include both above- and belowground perspectives.

At the cellular level, analyses of cambial activity under combined heat and drought stress revealed contrasting responses between stems and roots. In particular, cambial cells in roots maintained higher activity after 32 days of prolonged drought and showed a capacity for recovery after rewatering across all treatments. In contrast, at the same time point, stems exposed to high temperatures, alone or in combination with drought, failed to fully recover to control levels after rewatering. Additionally, the phloem response on the stem side did not demonstrate significant variations throughout the experimental period, as supported by multiple studies on this subject, whereas the roots displayed different behaviors depending on the treatment. These findings underscore that cambial plasticity is strongly organ-dependent and that belowground tissues can sustain growth and resilience even under severe environmental stress, whereas aboveground tissues may suffer irreversible damage.

At the individual level, experiments with biochar as an alternative to peat-based growing media provided further evidence of contrasting organ responses. Root morphology did not differ significantly between biochar treatments and peat controls, but leaves exhibited improved water potential values in biochar substrates. This indicates that biochar not only represents a sustainable substitute for peat but can also enhance plant performance under nursery conditions. Importantly, such positive effects would not have been evident if the analysis had been restricted to roots alone, reinforcing the importance of a holistic perspective.

At the ecosystem level, studies of *P. lentiscus* revealed greater complexity. In abandoned crops where climate conditions are harsh, competition is minimal, and secondary succession continues, roots and stems showed generally similar growth patterns, although roots usually grew slightly less than stems under similar conditions. Conversely, in Mediterranean shrubland, where plants face both climatic stress and intense competition, growth patterns between roots and stems were no longer comparable, with significant divergences emerging over the analyzed years. This suggests that interactions between environmental factors and community context strongly influence how plants grow above and below ground, with implications for long-term ecosystem functioning. Additionally, analysis of fine roots and leaves using a set of morpho-functional traits provided further insights into organ-specific strategies under different environmental conditions. While some traits, such as leaf dry matter content and root dry weight, responded similarly across above- and belowground parts, others showed clear differences. Specifically, traits related to resource acquisition and efficiency, like specific leaf area and specific root length, did not follow a linked trend.

Taken together, these results highlight the importance of considering multiple organization levels—cells, individuals, and ecosystems—to understand how plants respond to environmental stress. By linking belowground and aboveground tissues, this work shows that conclusions based on only one compartment may provide a biased or incomplete view of plant strategies. Ultimately, the integrative approach adopted in this thesis demonstrates that the resilience and vulnerability of plants under climate change cannot be understood without examining both aboveground and belowground processes at the same time. This perspective offers new insights into plant biology and has practical implications for sustainable land management, forestry, and agriculture. Future research should continue to develop this holistic framework, ensuring that the complexity of plant responses to global change is fully represented from the cellular to the ecosystem level.

REFERENCES FOR THE GENERAL INTRODUCTION AND THE OVERALL CONCLUSIONS

- Alexandrov, G. A., Brovkin, V. A., Kleinen, T., & Yu, Z. (2020). The capacity of northern peatlands for long-term carbon sequestration. Biogeosciences, 17(1), 47-54.
- Aloni, R. (1980). Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. Planta, 150(3), 255-263.
- Arend, M., Link, R. M., Zahnd, C., Hoch, G., Schuldt, B., & Kahmen, A. (2022). Lack of hydraulic recovery as a cause of post-drought foliage reduction and canopy decline in European beech. New Phytologist, 234(4), 1195-1205.
- Babst, F., Alexander, M. R., Szejner, P., Bouriaud, O., Klesse, S., Roden, J., ... & Trouet, V. (2014). A tree-ring perspective on the terrestrial carbon cycle. Oecologia, 176(2), 307-322.
- Babst, F., Bouriaud, O., Poulter, B., Trouet, V., Girardin, M. P., & Frank, D. C. (2019). Twentieth century redistribution in climatic drivers of global tree growth. Science advances, 5(1), eaat4313.
- Baesso, B., Chiatante, D., Terzaghi, M., Zenga, D., Nieminen, K., Mahonen, A. P., ... & Montagnoli, A. (2018). Transcription factors PRE 3 and WOX 11 are involved in the formation of new lateral roots from secondary growth taproot in A. thaliana. Plant Biology, 20(3), 426-432.
- Baesso, B., Terzaghi, M., Chiatante, D., Scippa, G. S., & Montagnoli, A. (2020). WOX genes expression during the formation of new lateral roots from secondary structures in Populus nigra (L.) taproot. Scientific Reports, 10(1), 18890.
- Bastos, A., Ciais, P., Friedlingstein, P., Sitch, S., Pongratz, J., Fan, L., ... & Zaehle, S. (2020). Direct and seasonal legacy effects of the 2018 heat wave and drought on European ecosystem productivity. Science advances, 6(24), eaba2724.
- Battipaglia, G., De Micco, V., Brand, W. A., Linke, P., Aronne, G., Saurer, M., & Cherubini, P. (2010). Variations of vessel diameter and δ13C in false rings of Arbutus unedo L. reflect different environmental conditions. New Phytologist, 188(4), 1099-1112.
- Belyea, L. R., & Malmer, N. (2004). Carbon sequestration in peatland: patterns and mechanisms of response to climate change. Global Change Biology, 10(7), 1043-1052.
- Beaulieu, J., Belayneh, B., Lea-Cox, J. D., & Swett, C. L. (2022). Improving Containerized Nursery Crop sustainability: effects of Conservation-driven adaptations in Soilless substrate and water use on plant growth and soil-borne Disease Development. HortScience, 57(6), 674-683.
- Blievernicht, A., Irrgang, S., Zander, M., & Ulrichs, C. (2013). Sphagnum biomass-the next generation of growing media. Peatlands International, 1(2013), 32-35.
- Brunner, I., Herzog, C., Galiano, L., & Gessler, A. (2019). Plasticity of fine-root traits under long-term irrigation of a water-limited scots pine forest. Frontiers in Plant Science, 10, 701.
- Buras, A., Rammig, A., & Zang, C. S. (2020). Quantifying impacts of the 2018 drought on European ecosystems in comparison to 2003. Biogeosciences, 17(6), 1655-1672.
- Byrne, K. A., Chojnicki, B., Christensen, T. R., Drosler, M., Frolking, S., Lindroth, A., ... & Zetterberg, L. (2004). EU peatlands: Current carbon stocks and trace gas fluxes.
- Chave, J., Coomes, D., Jansen, S., Lewis, S. L., Swenson, N. G., & Zanne, A. E. (2009). Towards a worldwide wood economics spectrum. Ecology letters, 12(4), 351-366.
- Chong, C. (1999). Experiences with the utilization of wastes in nursery potting mixes and as field soil amendments. Canadian Journal of Plant Science, 79(1), 139-148.
- Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., ... & Valentini, R. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature, 437(7058), 529-533.
- Clarke, D., & Rieley, J. (Eds.). (2010). Strategy for responsible peatland management (pp. 10-25). Finland: International Peat Society.
- Clarke, B., Otto, F., Stuart-Smith, R., & Harrington, L. (2022). Extreme weather impacts of climate change: an attribution perspective. Environmental Research: Climate, 1(1), 012001.
- Creber, G. T. (1977). Tree rings: a natural data-storage system. Biological Reviews, 52(3), 349-381.

- Cudlin, P., Kieliszewska-Rokicka, B., Rudawska, M., Grebenc, T., Alberton, O., Lehto, T., ... & Kuyper, T. W. (2007). Fine roots and ectomycorrhizas as indicators of environmental change. Plant Biosystems, 141(3), 406-425.
- Cuny, H. E., Rathgeber, C. B., Frank, D., Fonti, P., Mäkinen, H., Prislan, P., ... & Fournier, M. (2015). Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature plants, 1(11), 1-6.
- Cuny, H. E., & Rathgeber, C. B. (2016). Xylogenesis: coniferous trees of temperate forests are listening to the climate tale during the growing season but only remember the last words!. Plant physiology, 171(1), 306-317.
- Cusack, D. F., Chou, W. W., Yang, W. H., Harmon, M. E., Silver, W. L., & Lidet Team. (2009). Controls on long-term root and leaf litter decomposition in neotropical forests. Global Change Biology, 15(5), 1339-1355.
- Dawson, Q., Kechavarzi, C., Leeds-Harrison, P. B., & Burton, R. G. O. (2010). Subsidence and degradation of agricultural peatlands in the Fenlands of Norfolk, UK. Geoderma, 154(3-4), 181-187.
- De Micco, V., Carrer, M., Rathgeber, C. B., Camarero, J. J., Voltas, J., Cherubini, P., & Battipaglia, G. (2019). From xylogenesis to tree rings: wood traits to investigate tree response to environmental changes. IAWA journal, 40(2), 155-182.
- Evans, M. R., Taylor, M., & Kuehny, J. (2010). Physical properties of biocontainers for greenhouse crops production. HortTechnology, 20(3), 549-555.
- Fonti, P., & Jansen, S. (2012). Xylem plasticity in response to climate. New Phytologist, 195(4), 734-736.
- Fraixedas, S., Lindén, A., Meller, K., Lindström, Å., Keišs, O., Kålås, J. A., ... & Lehikoinen, A. (2017). Substantial decline of Northern European peatland bird populations: Consequences of drainage. Biological conservation, 214, 223-232.
- Freschet, G. T., Violle, C., Bourget, M. Y., Scherer-Lorenzen, M., & Fort, F. (2018). Allocation, morphology, physiology, architecture: The multiple facets of plant above-and below-ground responses to resource stress. New Phytologist, 219(4), 1338-1352.

- Gill, R. A., & Jackson, R. B. (2000). Global patterns of root turnover for terrestrial ecosystems. The New Phytologist, 147(1), 13-31.
- Giorgi, F. (2006). Climate change hot-spots. Geophysical research letters, 33(8).
- Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. Global and planetary change, 63(2-3), 90-104.
- Grime, J. P., Crick, J. C., & Rincon, J. E. (1986, January). The ecological significance of plasticity. In Symposia of the Society for Experimental Biology (Vol. 40, pp. 5-29).
- Gruda, N. S. (2019). Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. Agronomy, 9(6), 298.
- Fitter, A. (2002). Characteristics and functions of root systems. In Plant roots (pp. 49-78). CRC Press.
- Helmisaari, H. S., Makkonen, K., Kellomäki, S., Valtonen, E., & Mälkönen, E. (2002). Below-and above-ground biomass, production and nitrogen use in Scots pine stands in eastern Finland. Forest ecology and management, 165(1-3), 317-326.
- Hu, X., Wu, L., Zhao, F., Zhang, D., Li, N., Zhu, G., ... & Wang, W. (2015). Phosphoproteomic analysis of the response of maize leaves to drought, heat and their combination stress. Frontiers in plant science, 6, 298.
-IPCC, (2021): Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change[Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, In press

- IPCC (2023): Summary for Policymakers. In: Climate Change 2023: Synthesis Report.Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.
- Jin, K., White, P. J., Whalley, W. R., Shen, J., & Shi, L. (2017). Shaping an optimal soil by root–soil interaction. Trends in Plant Science, 22(10), 823-829.
- Joosten, H., & Clarke, D. (2002). Wise use of mires and peatlands. International mire conservation group and international peat society, 304.
- Joosten, H. (2016). Peatlands across the globe. Peatland restoration and ecosystem services: Science, policy and practice, 2016, 19-43.
- Keplin, B., & Hüttl, R. F. (2001). Decomposition of root litter in Pinus sylvestris L. and Pinus nigra stands on carboniferous substrates in the Lusatian lignite mining district. Ecological Engineering, 17(2-3), 285-296.
- Klesse, S., Peters, R. L., Alfaro-Sánchez, R., Badeau, V., Baittinger, C., Battipaglia, G., ... & Buras, A. (2024). No future growth enhancement expected at the northern edge for European beech due to continued water limitation. Global change biology, 30(10), e17546.
- Körner, C. (2015). Paradigm shift in plant growth control. Current opinion in plant biology, 25, 107-114.
- Knorr, W., Prentice, I. C., House, J. I., & Holland, E. A. (2005). Long-term sensitivity of soil carbon turnover to warming. Nature, 433(7023), 298-301.
- Kumar, A., Singh, S., Gaurav, A. K., Srivastava, S., & Verma, J. P. (2020). Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Frontiers in microbiology, 11, 1216.
- Leifeld, J., & Menichetti, L. (2018). The underappreciated potential of peatlands in global climate change mitigation strategies. Nature communications, 9(1), 1071.
- López, R., Ramírez-Valiente, J. A., & Pita, P. (2022). How plants cope with heatwaves in a drier environment. Flora, 295, 152148.
- Lynch, J. (1995). Root architecture and plant productivity. Plant physiology, 109(1), 7.
- Malamy, J. E. (2005). Intrinsic and environmental response pathways that regulate root system architecture. Plant, cell & environment, 28(1), 67-77.
- Mausolf, K., Wilm, P., Härdtle, W., Jansen, K., Schuldt, B., Sturm, K., ... & Fichtner, A. (2018). Higher drought sensitivity of radial growth of European beech in managed than in unmanaged forests. Science of the Total Environment, 642, 1201-1208.
- Mawphlang, O. I., & Kharshiing, E. V. (2017). Photoreceptor mediated plant growth responses: implications for photoreceptor engineering toward improved performance in crops. Frontiers in plant science, 8, 1181.
- McClaugherty, C. A., Aber, J. D., & Melillo, J. M. (1982). The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. Ecology, 63(5), 1481-1490.
- Medrano, H., Flexas, J., & Galmés, J. (2009). Variability in water use efficiency at the leaf level among Mediterranean plants with different growth forms. Plant and Soil, 317(1), 17-29.
- Montagnoli, A., Dumroese, R. K., Terzaghi, M., Onelli, E., Scippa, G. S., & Chiatante, D. (2019). Seasonality of fine root dynamics and activity of root and shoot vascular cambium in a Quercus ilex L. forest (Italy). Forest Ecology and Management, 431, 26-34.
- Montserrat-Marti, G., Camarero, J. J., Palacio, S., Pérez-Rontomé, C., Milla, R., Albuixech, J., & Maestro, M. (2009). Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees, 23(4), 787-799.
- Nichols, J. E., & Peteet, D. M. (2019). Rapid expansion of northern peatlands and doubled estimate of carbon storage. Nature Geoscience, 12(11), 917-921.
- Nicoll, B. C., & Ray, D. (1996). Adaptive growth of tree root systems in response to wind action and site conditions. Tree physiology, 16(11-12), 891-898.

- Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., ... & van Kleunen, M. (2010). Plant phenotypic plasticity in a changing climate. Trends in plant science, 15(12), 684-692.
- Olano, J. M., García-Cervigón, A. I., Arzac, A., & Rozas, V. (2015). Intra-annual wood density fluctuations and tree-ring width patterns are sex-and site-dependent in the dioecious conifer Juniperus thurifera L. Trees, 29(5), 1341-1353.
- Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., ... & Hayes, D. (2011). A large and persistent carbon sink in the world's forests. science, 333(6045), 988-993.
- Pérez-Ramos, I. M., Matías, L., Gómez-Aparicio, L., & Godoy, Ó. (2019). Functional traits and phenotypic plasticity modulate species coexistence across contrasting climatic conditions. Nature communications, 10(1), 2555.
- Piñol, J., Terradas, J., & Lloret, F. (1998). Climate warming, wildfire hazard, and wildfire occurrence in coastal eastern Spain. Climatic change, 38(3), 345-357.
- Plomion, C., Leprovost, G., & Stokes, A. (2001). Wood formation in trees. Plant physiology, 127(4), 1513-1523.
- Qiu, C., Zhu, D., Ciais, P., Guenet, B., & Peng, S. (2020). The role of northern peatlands in the global carbon cycle for the 21st century. Global Ecology and Biogeography, 29(5), 956-973.
- Reubens, B., Poesen, J., Danjon, F., Geudens, G., & Muys, B. (2007). The role of fine and coarse roots in shallow slope stability and soil erosion control with a focus on root system architecture: a review. Trees, 21(4), 385-402.
- Rossi, S., Morin, H., & Deslauriers, A. (2012). Causes and correlations in cambium phenology: towards an integrated framework of xylogenesis. Journal of experimental botany, 63(5), 2117-2126.
- Schiestl-Aalto, P., Kulmala, L., Mäkinen, H., Nikinmaa, E., & Mäkelä, A. (2015). CASSIA–a dynamic model for predicting intra-annual sink demand and interannual growth variation in S cots pine. New Phytologist, 206(2), 647-659.
- Schweingruber, F. H. (2007). Wood structure and environment. Berlin, Heidelberg: Springer Berlin Heidelberg.
- Silver, W. L., & Miya, R. K. (2001). Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia, 129(3), 407-419.
- Salomón, R. L., Peters, R. L., Zweifel, R., Sass-Klaassen, U. G., Stegehuis, A. I., Smiljanic, M., ... & Steppe, K. (2022). The 2018 European heatwave led to stem dehydration but not to consistent growth reductions in forests. Nature communications, 13(1), 28.
- Spinoni, J., Vogt, J. V., Naumann, G., Barbosa, P., & Dosio, A. (2018). Will drought events become more frequent and severe in Europe?. International Journal of Climatology, 38(4), 1718-1736.
- Steppe, K., Sterck, F., & Deslauriers, A. (2015). Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends in plant science, 20(6), 335-343.
- Swindles, G. T., Morris, P. J., Mullan, D. J., Payne, R. J., Roland, T. P., Amesbury, M. J., ... & Warner, B. (2019). Widespread drying of European peatlands in recent centuries. Nature Geoscience, 12(11), 922-928.
- Szymanowska-Pułka, J. (2013). Form matters: morphological aspects of lateral root development. Annals of Botany, 112(9), 1643-1654.
- Tanneberger, F., Moen, A., Barthelmes, A., Lewis, E., Miles, L., Sirin, A., ... & Joosten, H. (2021). Mires in Europe—Regional diversity, condition and protection. Diversity, 13(8), 381.
- Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M. A., & Steppe, K. (2015). Responses of tree species to heat waves and extreme heat events. Plant, cell & environment, 38(9), 1699-1712.
- Tobin, B., Čermák, J., Chiatante, D., Danjon, F., Di Iorio, A., Dupuy, L., ... & Spanos, I. (2007). Towards developmental modelling of tree root systems. Plant Biosystems, 141(3), 481-501.
- Tsakaldimi, M. (2004, June). Use of inorganic and organic solid wastes for container-seedlings production. In Proceedings of the International Conference 'Protection and Restoration of the Environment VII (Vol. 28).

- Tsakaldimi, M. (2006). Kenaf (Hibiscus cannabinus L.) core and rice hulls as components of container media for growing Pinus halepensis M. seedlings. Bioresource technology, 97(14), 1631-1639.
- Tsakaldimi, M., & Ganatsas, P. (2016). A synthesis of results on wastes as potting media substitutes for the production of native plant species. Reforesta, (1), 147-163.
- Tyree, M. T., & Zimmermann, M. H. (2002). Xylem structure and the ascent of sap. Springer Science & Business Media.
- United Nations. About the UN Decade | UN Decade on Restoration [Internet]. 2022. [cited 2023 June 25]. Available from: https://www.decadeonrestoration.org/about-un-decade. Accessed June 2025
- UNEP (2022) Global Peatlands Assessment—The State of the World's Peatlands: evidence for action toward the conservation, restoration, and sustainable management of peatlands. Main report. Global Peatlands Initiative. United Nations Environment Programme, Nairobi
- Urák, I., Hartel, T., Gallé, R., & Balog, A. (2017). Worldwide peatland degradations and the related carbon dioxide emissions: the importance of policy regulations. Environmental Science & Policy, 69, 57-64.
- Van Breemen, N. (1995). How Sphagnum bogs down other plants. Trends in ecology & evolution, 10(7), 270-275.
- Van Der Woude, A. M., Peters, W., Joetzjer, E., Lafont, S., Koren, G., Ciais, P., ... & Luijkx, I. T. (2023). Temperature extremes of 2022 reduced carbon uptake by forests in Europe. Nature Communications, 14(1), 6218.
- Vicente-Serrano, S. M., Beguería, S., & López-Moreno, J. I. (2010). A multiscalar drought index sensitive to global warming: the standardized precipitation evapotranspiration index. Journal of climate, 23(7), 1696-1718.
- Vieira, J., Campelo, F., Rossi, S., Carvalho, A., Freitas, H., & Nabais, C. (2015). Adjustment capacity of maritime pine cambial activity in drought-prone environments. PLoS One, 10(5), e0126223.
- Vile, D., Pervent, M., Belluau, M., Vasseur, F., Bresson, J., Muller, B., ... & Simonneau, T. (2012). Arabidopsis growth under prolonged high temperature and water deficit: independent or interactive effects?. Plant, cell & environment, 35(4), 702-718.
- Vogt, K. A., Vogt, D. J., Asbjornsen, H., & Dahlgren, R. A. (1995). Roots, nutrients and their relationship to spatial patterns. Plant and Soil, 168(1), 113-123.
- Yu, Z., Joos, F., Bauska, T. K., Stocker, B. D., Fischer, H., Loisel, J., ... & Schmitt, J. (2021). No support for carbon storage of> 1,000 GtC in northern peatlands. Nature Geoscience, 14(7), 465-467.
- Ziegler, R., Wichtmann, W., Abel, S., Kemp, R., Simard, M., & Joosten, H. (2021). Wet peatland utilisation for climate protection–An international survey of paludiculture innovation. Cleaner Engineering and Technology, 5, 100305.

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