Università
degli Studi
della Campania
Luigi Vanvitelli

Ph.D ENVIRONMENT DESIGN AND INNOVATION

Curriculum: Sustainable sciences and technologies
for the environment and the territory

SSD – AGR/05

Cycle 34°

DOCTOR EUROPAEUS

Climate change impacts and adaptation strategies in Mediterranean forests: a multidisciplinary approach

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A Giulia che mi è stata vicino e mi ha sostenuto
in questo difficile ma soddisfacente percorso.

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Abstract

Forests are a valuable resource: they regulate ecosystem services, safeguard biodiversity, and play an essential role in carbon and water cycles. However, increasing human pressure and recent climate change are threatening the integrity of forest heritage in many parts of the world, especially in the Mediterranean region. In this area, progressive global warming is causing an increase in the intensity and incidence of forest fires and droughts with serious consequences for the physiology, growth, and forests survival. Expanding knowledge on the vulnerability and sensitivity of Mediterranean species and understanding the links between climate and tree health is a crucial challenge to anticipate the impacts of climate change and to plan the most suitable forest management strategies. In this context, the following PhD thesis aims to understanding the responses of Mediterranean forests to the main forest disturbances linked to climate change, such as drought and fires, as well as evaluating forest management practices able to mitigate their effects. To achieve these important objectives, a multidisciplinary approach was applied by combining different methodologies: studies of dendrochronology and wood anatomy, analysis of stable isotopes in tree rings, xylogenesis investigations, xylem hydraulics analysis, as well as constant monitoring of eco-physiological and climatic parameters of trees using the innovative TreeTaleker system. The first part of the thesis evaluated the effects of forest fires and drought on a *Pinus Pinaster* Aiton population located in the Vesuvius National Park. Our results suggested that although maritime pine can survive severe forest fires in the short term, severe defoliation can compromise the photosynthetic and stomatal capacity of burnt trees over time, reducing the chances of plant recovery and increasing the possibility of death during extreme climatic episodes. The second part of the thesis focused on two case studies that evaluated two forest management practices, thinning and intercropping, capable of mitigating the effects of climate change on forest stands. The first case study evaluated the effects of three thinning treatments at different intensities applied in a *Quercus robur* L. plantation. The results obtained showed that a selective high intensity thinning guaranteed an increase in productivity and a limited water loss of the target species. Instead, the second case study compared different intercropping systems evaluating the effects on the two principal species *Populus alba* L. and *Juglans regia* L. intercropped with each other and with ancillary species. Our results showed that principal species had higher productivity and iWUE when growing with *Elaeagnus umbellate* Thunb, an N-fixing species that favors a non-competitive exploitation of soil water resources. In conclusion, the set of studies reported in this thesis, in addition to experimenting an innovative multidisciplinary approach, allowed to expand the knowledge on the eco-physiological processes triggered by forest fire and drought in *Pinus Pinaster* Aiton, as well as on forest management strategies able to improve adaptation and resistance of Mediterranean forests to climate change.

Chapter 1

1. General introduction

1.1 Forests under climate change

Forests are a valuable resource: they provide foods and goods, regulate ecosystem services, safeguard biodiversity, and play an essential role in the carbon and water cycles (Intergovernmental Panel on Climate Change, 2018; García-Valdés et al., 2020). However, increasing human pressure and recent climate change are threatening the integrity of forest heritage in many parts of the world (Allen et al., 2010; Hartmann et al., 2018). Progressive global warming is altering physiological rates, growth, phenology, and tree survival, with serious consequences for ecosystem functioning (Allen et al., 2015). Recent studies reported a considerable increase in forest mortality in several biomes (Neumann et al., 2017; Klein and Hartmann, 2018; Senf et al., 2018) and future climatic forecasts suggest an increase further of mortality in the future (Steinkamp and Hickler, 2015). According to recent observations, the global average temperature increased by 0.15-0.20 °C per decade since 1975 (NASA Earth Observatory, access to data 2021), and is expected to increase further by 1.5 °C between 2030 and 2050 (Intergovernmental Panel on Climate Change, 2018). Therefore, it is likely that future climate change will lead to increased frequency and intensity of extreme climate events, such as drought and heat waves (Christensen, 2007). In addition, higher temperatures and a lower humidity of wood fuels could cause an increase in the frequency and severity of forest fires (Michetti and Pinar, 2019; Halofsky et al., 2020). In Europe, the Mediterranean basin is one of the environments most affected by global warming (Schröter et al., 2005; Vitale et al., 2012; López-Tirado and Hidalgo, 2018). In this area the increase in temperatures and the variability of precipitation rate

(Intergovernmental Panel on Climate Change, 2018) combined with the expansion of secondary forests (Food and Agriculture Organisation of the United Nations, 2020) caused an increase in forest fires (Pausas and Fernández-Muñoz, 2012) and widespread episodes of forest decline due to drought (Carnicer et al., 2011). European forest mortality doubled in recent years (Senf et al., 2018) and in the coming decades there could be serious cascading effects on biodiversity and the functionality of Mediterranean forest ecosystems with important ecological and economic consequences (Peñuelas et al., 2017)

1.2 Impact of drought and forest fires on tree mortality

Despite climate change has been the focus of the scientific community since several decades, the mechanisms underlying the survival and death of trees are still much debated and partly unexplored (McDowell et al., 2008; Allen et al., 2010; McDowell and Sevanto, 2010; Sala et al., 2012; Williams et al., 2013; Allen et al., 2015; Hartmann et al., 2015; Anderegg et al., 2015; Adams et al., 2017). Generally, the forests decline is the result of the interaction of multiple disturbance factors related to climate change such as air pollution, competition, deforestation, pest outbreaks, forest fires, drought, diseases. Among these disturbances, droughts and fires can be considered as the main "triggers" and "contributors" factor to tree mortality on a large scale, respectively (Allen et al., 2010; Partelli-Feltrin et al., 2020).

In general, the prolonged shortage of water triggers to growth decline and trees death through two main mechanisms: carbon starvation and hydraulic failure (McDowell et al., 2008; Adams et al., 2017). Carbon starvation occurs when prolonged stomatal closure during drought limits photosynthetic assimilation, and the plant is forced to use non-structural carbohydrate reserves (NSC) to meet the metabolic demand for carbon. In case of persistent drought, the carbon reserves can run out and the plant is no longer able to maintain the metabolism (Sala et al., 2010; McDowell et al., 2008; McDowell and Sevanto, 2010; McDowell, 2011; Nardini et al., 2016). Hydraulic failure, on the other hand, occurs when poor water availability in the soil associated with high evaporative demand causes strong tensions in the xylem water column (Tyree and Zimmermann, 2002). If these tensions exceed the hydraulic safety margin of the plant, cavitation of the vessels happens, resulting in tissue desiccation (Urli et al., 2013; Nardini et al., 2016). Although hydraulic failure and carbon starvation are strongly interconnected processes that can lead to tree decline in a complementary way (Nardini et al., 2016; Savi et al., 2016; Tomasella et al., 2017; Petrucco et al., 2017), a recent review by Adams et al. (2017) established that hydraulic failure is the most dangerous mechanism: a widespread loss of xylem conductivity (>60%) leads the plant to death before carbon starvation occurs.

In addition to the effects on ecophysiology, drought increases the occurrence of large wildfire (Stavros et al., 2014; Westerling, 2016; Turco et al., 2017; Ruffault et al., 2020). This promotes tree mortality, which occurs either directly through the burning of the tissues or indirectly due to partial trunk and canopy damage (McHugh and Kolb, 2003; Sieg et al., 2006). The survival or death of trees after a fire is strictly dependent on the severity of injuries suffered (Hood et al., 2018). Usually, the direct death of the trees occurs due to extensive necrosis of the cribo-vascular cambium (Dickinson and Johnson, 2001; Michaletz and Johnson, 2007; Michaletz et al., 2012); severe destruction of the crown (Bär et al., 2019); and/or widespread xylem cavitation triggered by high flame temperatures (Bär et al., 2018). Although in most cases tree mortality is determined by the direct effects of fire, mortality from indirect effects currently accounts for a large percentage of forest biomass loss (van Mantgem et al., 2011; Hood et al., 2018). Partial lesions can impair plant physiology by amplifying the pre-existing stresses such as climatic stress and leading to a higher probability of delayed death in trees that would otherwise survive (van Mantgem et al., 2013; van Mantgem et al., 2018). Partial damage to the crown causes a reduced photosynthetic and stomatal capacity of the plant, fuelling the process of carbon starvation, and causing a decrease in growth (Ryan, 1993; Cernusak et al., 2006; Valor et al., 2018), and/or a reduced defensive capacity against insect and pathogen attacks (McHugh et al., 2003; Wallin et al., 2003). Even if the remaining crown can satisfy the metabolic demand for carbon, less evident lesions to the cribo-vascular cambium could compromise the downward translocation of sugars, and the cell division of the xylogenesis process (Midgley et al., 2011; Michaletz, 2018; Bär et al., 2019). Furthermore, recent studies suggested that high flame temperatures can induce deformation and destructuring of xylem vessels compromising hydraulic efficiency and causing greater susceptibility to hydraulic failure in the event of subsequent drought episodes (Michaletz et al., 2012; West et al., 2016; Bär et al., 2018).

1.3 Forest management to mitigate climate change

Expanding knowledge on the vulnerability and sensitivity of Mediterranean tree species and understanding the links between climate and tree health is a crucial challenge to anticipate the impacts of climate change and to plan the most suitable forest management strategies (Kolström et al., 2011; Keenan, 2015).

In recent decades, research produced new knowledge on forest management strategies aimed at improving the adaptation capacities of Mediterranean forests to climate change (Resco De Dios et al., 2007; Peterson et al., 2011; FAO, 2013; Fernandes, 2013; Corona et al., 2015; Doblas-Miranda et al., 2015; Keenan, 2015). Some strategies such as thinning and intercropping seem to play a significant role in improving the performance of trees because they are able to determine greater resistance to forest disturbances (Kelty, 2006; McDowell et al., 2007; Kohler et al., 2010; Brooks and Mitchell, 2011; Brooks and Mitchell, 2011; Booth and Williams, 2012; Hulvey et al., 2013) and an increase in the growth rates of the main species (Aussenac and Granier, 1988; Forrester et al., 2006; Sohn et al., 2016; Pelleri et al., 2020).

While the reduction of forest density through thinning leads to a notable decrease in competition for water, light and nutrients (Blanco et al., 2005; Sohn et al., 2016) with a substantial increase in the resistance and resilience of trees to drought (Aussenac, 2000; Giuggiola et al., 2013; Sohn et al., 2013); the use of mixed plantations makes it possible to increase the tolerance and resistance of tree stands to climate change through the partitioning of the ecological niches of the individual species or through the complementarity of resources, ensuring an improvement in tree productivity (Vandermeer, 1992; Kelty and Cameron, 1995; Loreau and Hector, 2001; Hulvey et al., 2013; Pretzsch, 2014).

Despite the great potential of these forest management practices, today there is a need to expand the information on how to maximize their success not only in relation to the characteristics of the different species but taking into consideration all the possible variables. The success of thinning, for example, may depend on its intensity (Duursma, 2011; Molina and del Campo, 2012; Guillemot et al., 2015; Primicia et al., 2016), the age of the stand (Juodvalkis et al., 2005), and the climatic conditions of the site (Sohn et al., 2016). Similarly, the success of intercropping depends on the density of the stand, the combination of the tree species characteristics, as well as the peculiarities of the soil and climate (Marron and Epron, 2019). Furthermore, it is important to carefully examine the consequences of any management practice on ecosystems: a forest management strategy can be advantageous for the achievement of a certain objective but, at the same time, it can undermine the pursuit of other objectives or induce negative impacts on the ecosystem (Bradford and D’Amato, 2012).

1.4 Main objectives

In this context, this PhD thesis aims to achieve the following main objectives:

1) Understanding Mediterranean forests responses to climate change and in particular to forest disturbances such as drought and fire.

2) Evaluating forest management practices able to mitigate the effects of global warming on tree productivity and water use efficiency of Mediterranean forests

To achieve these two important objectives, a multidisciplinary approach was applied by combining different methodologies: dendrochronology studies, analysis of stable isotopes in tree rings, xylogenesis investigations, xylem hydraulics analyses, as well as constant monitoring of eco-physiological parameters using the innovative TreeTaleker system.

1.5 Methods

Tree-rings are a valuable proxy in assessing the effects of climate change on forests. The dendrochronology, through the measurement of the annual tree-rings width, allows to reconstruct climatic and environmental influencing factors on tree growth (Fritts, 1972; F.H. Schweingruber, 1993; Anchukaitis, 2017). To improve the interpretation of the ecophysiological processes linked to variations in environmental conditions, dendrochronological studies are supported by the analysis of the isotopic composition in tree-rings (McCarroll and Loader, 2004). While stable carbon isotopes allow estimation of stomata activity, photosynthetic rate and intrinsic water use efficiency of plants (WUEi) (Farquhar et al., 1989; Di Matteo et al., 2010; Battipaglia et al., 2014), stable oxygen isotopes provide information on transpiration rates (Scheidegger et al., 2000; Gessler et al., 2014). However, dendrochronological studies are limited to interpreting plant ecophysiological responses at the annual or seasonal scale. Therefore, to evaluate the effects of climatic variability during the growing season and to identify the strategies used by the different forest species to overcome periods of stress, it is necessary to apply methodologies such as quantitative wood anatomy study (Fonti et al., 2010) and/or xylogenesis monitoring (Rossi et al., 2003). While in the first case, the quantitative wood anatomy analyses provide information on the functioning of the tree, for example in terms of carbon uptake and water use (Fonti et al., 2010; von Arx and Carrer, 2014), the study in continuum of the wood formation and lignification allows to examine the cambial phenology and activity and to correlate them with the seasonal climatic variations as well as physiological condition of plants (Deslauriers et al., 2003; Rossi et al., 2007). Although the studies on tree-rings and xylogenesis provide valuable information on forest growth dynamics, xylem hydraulics analyses allow to assess the hydraulic efficiency and safety of trees. In this study, to evaluate the hydraulic failure vulnerability of the trees analyses on the functional characteristics of the bordered pits were performed, as well as tests on xylem cavitation using the “Cavitron” technique (Cochard et al., 2010; Cochard et al., 2013).

Currently, the information collected from tree-ring studies can be supplemented with the help of recent forest monitoring technologies. In this research the multifunctional device "Treetalker" has been tested for the observation in real time of the physiological and environmental parameters of the trees amplifying the dataset useful for evaluating the functionality and resilience of trees under climate change (Valentini et al., 2019; Matasov et al., 2020).

1.6 Structure of thesis and specific objectives

This thesis is composed of a compendium of five scientific papers produced during the three years of the doctoral program. The studies, organized in different chapters, focused on specific goals linked to the general research line of this thesis. The first part of the dissertation (chapter 2,3,4) reports the studies aimed at understanding the effects of forest fires and drought on the growth and physiology of *Pinus Pinaster* Aiton, one of the most widespread and used species in the Mediterranean region. The second part of the thesis (chapter 5,6) reported two case studies relating to the forest management practices, thinning and intercropping, capable of mitigating the effects of climate change on forest stands.

Chapter 2 - Fire severity influences ecophysiological responses of *Pinus pinaster* Ait.

The effect of fire severity on the growth and ecophysiological responses of *Pinus pinaster* was evaluated in Vesuvio National Park to estimate its survival possibilities in the short term. After the wildfire of 2017 four sites were selected according to the different degree of fire severity and a multidisciplinary approach based on tree rings, stable isotopes and percentage of crown scorched was applied. Our findings demonstrated that *P. pinaster* growth reduction, recorded in all sites, was strictly linked to the percentage of crown scorch and that even trees with high level of crown scorched could survive. In all the burned sites the high temperatures and the time of exposure to the flames were not sufficient to determine the death of the cambium and all the trees were able to complete the 2017 seasonal wood formation.

Chapter 3 – Fire affects wood formation dynamics and ecophysiology of *Pinus pinaster* Ait. growing in a dry Mediterranean area

Inter-annual analyses of dendrochronology and stable isotopes of carbon and oxygen in tree-rings were combined with xylogenesis monitoring to investigate post-fire effects in medium term on the *Pinus pinaster* forest, which experienced an increase in the crown reduction in the years following the fire. Our data showed that trees with crown damage presented a reduction of photosynthetic capacity in comparison to control trees, resulting in a decrease of tree-growth in the medium term. Monitoring of cambial activity demonstrated not only the negative influence of warm and dry periods on wood formation dynamics of pine forests, but above all a lower xylem production, a delay in phenology and a poor xylem plasticity of burned trees.

Chapter 4 - Multiparametric approach to assess wildfire impacts on ecophysiological and hydraulic properties of *Pinus Pinaster* Aiton

The continuous monitoring of sap flow and micro-stationary climatic conditions trough TreeTalker system was paired with anatomical analyses of tree rings and xylem cavitation vulnerability studies to evaluate the effects of the wildfire on the ecophysiology and hydraulics of the burned pine forest of *Pinus pinaster* compared to the control population. Our results suggested that although the wildfire did not affect the hydraulic efficiency and safety of the burned plants, the severe defoliation triggered a grave derangement in stomatal control: the trade-off between carbon assimilation and water loss exposes trees to high risk of hydraulic failure, increasing the chances of die during extreme climatic episodes.

Chapter 5 - Effects of thinning intensity on productivity and water use efficiency of *Quercus robur* L.

The aimed of this study was to analyse how the different intensity of thinning affects, in the short and long term, the productivity and intrinsic water use efficiency (iWUE) of *Quercus robur* L., coupling the tree-ring analyses with the measurements of stable isotopes of carbon and oxygen. The findings highlighted that while the moderate and geometric thinning were not able to reduce the competition for resources among individuals, the high selective thinning (75% reduction of total biomass) was the most appropriate management practice resulting in plants studied improved photosynthesis and productivity, as well as limited water loss.

Chapter 6 - Tree species composition in mixed plantations influences plant growth, intrinsic water use efficiency and soil carbon stock

A combined approach of tree-ring analyses and carbon stable isotope was used to check tree growth and iWUE of two species, *Populus alba* L. and *Juglans regia* L., intercropped with each other and with N-fixing or competitive production species. Furthermore, soil analyses were performed to understand how the different intercropping systems can influence soil characteristics, in particular soil carbon stock. Dendrochronological data showed that during the first years, the growth of principal species was favoured by intercropping. This positive effect decreased in the following years due to light competition with the crown. Carbon isotope data showed that *P. alba* and *J. regia* had the highest iWUE when growing with *Elaeagnus umbellata* Thunb, a shrubby species with a shallow root system that favours a non-competitive exploitation of soil water resources. Finally, the intercropping of the principal species with *Corylus avellana* L. promoted the highest soil C stock.

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Chapter 2

2. Fire severity influences ecophysiological responses of *Pinus pinaster* Ait.

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Manuscript published in Frontiers in Plant science, 2019

doi: 10.3389/fpls.2019.00539

2.1 Introduction

Forest fires are a critical issue in the Mediterranean basin, experiencing increasing frequency and intensity in the last decades (IPCC, 2014). The impact of fire on trees can cause damage to the canopy, trunk, and root system (Ducrey et al., 1996). The single or combined effect of these damages can reduce the vigor of the plant and trigger a temporary reduction in growth (Battipaglia et al., 2014a) or can lead to tree mortality if the fire is particularly destructive (Brown, 2000). Understanding post-fire responses of trees is a crucial issue in planning forest management actions of burned area in the short term (Bovio et al., 2017) and fire risk reduction at the medium and long term (Battipaglia et al., 2017). It is well- known that the variation in plant responses to fire is linked to the species-specific heat sensitivity (Catry et al., 2010) and to fire regime. Indeed, several plant species are able to tolerate forest fires of medium and low severity, thanks to their adaptive traits that guarantee their survival: very thick bark and needles, deep root system, self-pruning capacity and particular structure of the crown (Brown, 2000; Madrigal et al., 2019). Fire sensitivity is most often studied in pine species due to their wide distribution range (Fernandes et al., 2008; Espinoza et al., 2019). An extensive literature deals with pine post-fire responses and mortality prediction after wildfire or prescribed burnings, mainly in North America (e.g., Peterson and Arbaugh, 1986a; Ryan and Reinhardt, 1988; Stephens and Finney, 2002; Beverly and Martell, 2003; McHugh and Kolb, 2003; Fowler and Sieg, 2004; Kobziar et al., 2006; Sieg et al., 2006; Breece et al., 2008; Hood et al., 2010), but only limited information is related to fire resistance of European pines (see Fernandes et al., 2008). In particular, several papers analyzed post-fire tree mortality or recruitment, while very few concerns the ecophysiological responses of pines (mainly *Pinus halepensis and Pinus sylvestris*) after fire (Beghin et al., 2011; De Micco et al., 2013; Battipaglia et al., 2014 a,b, 2016; Valor et al., 2018). However, little is known about the post-fire ecophysiological response of *Pinus pinaster* Ait., a widespread and fire-prone species which is also economically and ecologically important in Mediterranean region (Botelho et al., 1998; Vega et al., 2008, 2010; Catry et al., 2010). Previous studies addressed post-fire recruitment (Calvo et al., 2008; Vega et al., 2008, 2010), or post-fire regeneration (Maia et al., 2012) of *P. pinaster*, highlighting contrasting results for this species and its resistance to fire. Indeed, Fernandes et al. (2008) reported a considerable fire resistance of this species due to the bark depth and to the high temperature requested for needle necrosis in comparison to *Pinus pinea*. While Catry et al. (2010), using a mortality model, suggested that *P. pinaster* is more vulnerable to fire than other pines, such as *P. pinea* because of its crown architecture. Finally, Vega et al. (2010), found consistent differences in *P. pinaster* mortality both in relation to site and fire conditions. Thus, it becomes extremely important to better understand ecophysiological responses to fire of this species and its link with fire severity. In this study we used a multidisciplinary approach of dendrochronology, stable isotopes and percentage of crown scorch to assess the effect of a large wildfire, occurred in Italy during July 2017, when over 1300 hectares of vegetation, mainly *P. pinaster* woods, were destroyed and where different degrees of fire damage were recorded (Battipaglia et al., 2017). Dendrochronology allowed, not only, to reconstruct historical fire dynamics (Camarero et al., 2019) in the study area and involving sampled trees, but also to verify the growth related to the fire year 2017. Carbon and oxygen stable isotopes were used to understand the complex ecophysiological processes involved in post-fire responses, linking the possible reduction of carbon assimilation to crown damage or to reduction in stomatal activity (Battipaglia et al., 2014a,b, 2016). Fire severity, considered as a measure of the immediate impact of fire on trees, was evaluated in terms of the degree of crown scorch, cambium or root damage and height of burn on the trunk (Van Wagner, 1973; Moreno and Oechel, 1989; Lentile et al., 2006; Keeley, 2009). This research aims to improve our understanding of the wildfire effects on *P. pinaster*, with particular focus on the strategies and mechanisms that this species is able to adopt to face the passage of fire. We aim, not only, to verify the link between fire severity and *P. pinaster* ecophysiological responses, but also to understand the possibility of its survival in the short and long term. We hypothesize a tight relation between fire severity, in particular crown scorch, and tree damage in the short term, with needle defoliation triggering growth reduction and decrease in photosynthetic activity. Further, we hypothesize that stable isotopes can help assessing the ecophysiological processes activated by fire and can contribute to determine possible species survival. In particular, carbon stable isotope can be considered a proxy of changes in the gas exchange processes and can be used to estimate the ratio between photosynthetic activity (A) and stomatal conductance (gs) (Farquhar et al., 1982). Whereas $\delta^{18}O$ can help elucidating the independent effect of A and gs on $\delta^{13}C$ (Scheidegger et al., 2000). Indeed, high fire severity can damage the crown, reducing leaf surface and altering the plant’s photosynthetic rate and efficiency (Beghin et al., 2011). This research appeared extremely important from a forest management point of view, since the right assessment of actual tree damage and a better prediction of post-fire tree survival could avoid cutting down a scorched tree that could not be dead. Indeed harvesting trees, when is not necessary, could alter the post-fire germination and, generally, the carbon cycle and the ecosystem biodiversity.

2.2 Materials and methods

2.2.1 Study area

The study area is the "Tirone Alto Vesuvio Nature Reserve" within the Vesuvio National Park (Figure 1), which covers a total area of 8482 hectares and suffers a strong anthropic pressure: almost 400000 inhabitants live in the 13 municipalities around the protected area. The Park, is characterized by a large plantation forest: while the Northern slope is covered by tall deciduous trees, the Southern side presents a large plantation dominated by *P. pinaster* Aiton and *P. pinea* L, with the presence of *Pinus halepensis* Mill, *Pinus nigra* Arnold (Picariello et al., 2000) and scattered patches of typical Mediterranean macchia vegetation. The study area shows the typical Mediterranean climate, with hot and dry summers and rainy winters with mild temperatures. In the last 40 years the average temperatures have gradually increased from 14 to 16°C and the rainfall has been very erratic: some years (1980, 1984, 1996, 2005, 2009, 2010) have been characterized by heavy rains, others have been extremely dry (1977, 1999, 2003, 2012, 2016, 2017) (The Royal Netherlands Meteorological Institute, KNMI database Climate Explorer1, Trouet and Van Oldenborgh, 2013 and Supplementary Figure S1, data acquired on January 2018). The frequent drought seasons, the presence of strong winds and particularly flammable vegetation, associated with a strong anthropic pressure, make the area particularly vulnerable to forest fires (Sibilio et al., 2002). To evaluate the effects induced by the fire of July 2017 on *P. pinaster* plantation, four sampling sites (around 1 ha) were chosen within the Tirone Alto Vesuvio Nature Reserve, from areas burned with different fire severity (Table 1). Fire severity was evaluated based on parameters such as: presence and height of scorch on the trunk, presence of fire scars on the trunk, percentage of consumed crown and presence of damage to the roots related to 2017 fire. Fire severity was high in the high severity site (HSS), in which the individuals of *P. pinaster* showed important damages to the canopy (70% crown reduction) and scorch on the trunk (over four meters). The severity was medium in the medium severity site (MSS), where the plants showed partial damage of crown (10%) and a scorch on the trunk that not exceed three meters. In addition, low severity was estimated in the low severity site (LSS), where individuals presented mild damage to the needles (average reduction of 2%) and scorch of the trunk of two and a half meters. Finally, individuals were sampled in control site (CS): an area situated into the Nature Reserve but where individuals did not show any scorch or damage to the trunk roots or to the crown trees, since they were not affected by the fire event of 2017. Evidences of past fire events related to the four study sites were collected using the official fire reports, from 1988 to 2016, of the "Carabinieri Forestali per la Biodiversità (UTCB) of Caserta," a governmental agency, acting since 1974, as a park ranger force. In addition to the large fire of the 2017, two surface fires, in 2015 and 2007, occurred in the MSS. Further in August 1993, an important wildfire (more than 240 he was burned) occurred on the Southern slope of Vesuvio, including the MSS and LSS sites. This information was, subsequently, related to dendrochronological (especially to fire scars) and isotope data. Finally, the LSS site was subjected to prescribed fires in 2014 and 2016, promoted by the Campania Region (Battipaglia et al., 2017).

2.2.2 Sampling and processing of tree cores

In December 2017, for each study site, 15 dominant trees (trees with crowns extending above the general level of the main canopy and receiving full light from above and partly from the sides; Adams et al., 1994) were sampled with a 5 mm increment borer (Haglöfs, Långsele, Sweden), and from each individual two cores were collected, at 1.3 m above ground, at east and west direction. The total of 120 collected cores were fixed on specific wooden supports and, subsequently, subjected to a sanding process, to facilitate tree-ring identification. The measurement of the tree-ring growth was carried out through the LINTAB system: a stereo-microscope connected to a computer, in which a specific software called TSAP-Win is installed and allows to elaborate a series of representative curves of each individual plant growth trend. After measuring each sample, first a visual comparison, then a statistical synchronization of the curves, known as cross-dating, was performed using the Gleichaeufigkeit index (GLK), which evaluates the correlation between the different series (Eckstein and Bauch, 1969). Successively, the data were statistically analyzed using the COFECHA software, which allowed checking the quality of cross- dating, providing indications on the number of years to add or subtract to the chronologies. Finally, the elementary chronologies have been standardized using ARSTAN (Cook and Holmes, 1986). Standard chronologies were detrended using the smoothing spline function, with a 40-year step for the chronologies of MSS site and a 10-year step for those relating to the HSS, LSS, and CS sites. The mean chronology was calculated through an arithmetic mean, while the stabilize variance was determined through the Keith Briffa rbar-weighted method. Finally, a different running bar was used for each site, allowing to maximize the expressed population signal (EPS).

2.2.3 Sample pretreatment and isotopic analysis

For each site five cores were selected for isotope analysis using the Gleichaeufigkeit (GLK) which evaluates the correlation between the different series (Eckstein and Bauch, 1969). The five selected cores presented the best cross-dating (GLK > 0.70) with the corresponding average chronology. The annual rings of the last 30 years (1988–2017) were manually removed and divided into late-wood and early-wood. Only for the CS site, with the youngest trees, the isotopic analyzes were performed across a shorter time period (1994–2017). Further analyzes were performed on an individual of the MSS who had an obvious fire scar (see Supplementary Figure S2) belonging to a past fire event. Subsequently, the samples were milled using a pulverizing mill and were weighed precisely and encapsulated in a tin or silver capsules for the carbon or oxygen isotopic measurements, respectively.

The carbon and oxygen stable isotope composition was measured by continuous-flow isotope ratio mass spectrometry (Delta V Advantage, Thermo Scientific) at IRMS lab of University of Campania. The $\delta^{13}C$ series were corrected for the fossil fuel combustion effect (Francey et al., 1999).

2.2.3 Climate analysis

Climate data of minimum, maximum, and mean monthly temperature and total monthly precipitation for the period 1975–2017 were downloaded from the CRU TS3.23 gridded dataset at 0.5° resolution data (Harris et al., 2014) since Zalloni et al. (2018) demonstrated for the same area the high significant correlation with local meteo stations. A Pearson's linear correlation function analysis ($P<0.05$), performed using Excel, was implemented between climate data and tree-ring width and isotope data. Temperature and precipitation data were seasonally grouped from December of the previous year to February of the next year, in order to cover all the season which could influence tree-ring growth in Mediterranean species (Cherubini et al., 2003; Balzano et al., 2018). Those relationships help clarify the role of climate, namely temperature and precipitation, on past tree growth and to understand how much it influenced tree growth in CS site, not affected by fire.

2.3 Results

2.3.1 Dendrochronological data

The standardized average chronologies (Figure 2) of the individual study sites showed a discrete synchronization: of the 43 years analyzed, the growth trend was common for 25 years. The good synchronization of the chronologies of the study areas is confirmed by the EPS value > 0.85 (Table 2). Also, the average sensitivity values of the four sites are very similar (always < 0.25): the sampled trees can be considered compliant, even if it is necessary to underline that the plants of the control area have a particularly high MS. The HSS chronologies (Figure 2A), showed a good consistency in growth trend (EPS = 0.9; GLK > 60; MS = 0.12). In all individual chronologies, 1999 presented a simultaneous growth increase while a drastic growth reduction was observed in 2008, 2012, and 2017.

The MSS and LSS sites (Figures 2B,C) are very similar both in terms of age (mean age MSS = 38 years, mean age LSS = 37 years) and in terms of growth. Some years present a common increase of growth (e.s., in 1989, 1992, 1998, 2002) or a common decrease of growth (es., in 1993, 1997, 2000, 2003). In particular, a significant difference in growth between sites was observed in the period 2014–2016 (Figure 2C), in which there was an increase in tree growth in the individuals of the LSS site. Finally, the CS (Figure 2D) presented the youngest trees (average age = 16 years) with positive growth peaks during 1999, 2002, 2006, 2010, 2014, 2016 and negative growth in 2000, 2003, 2005, 2007, 2011, 2013, 2015. The tree-ring width showed that in 2017 all the sampled trees recorded a decrease of growth both in early-wood and latewood, in comparison to the previous year 2016 (Figure 3). In particular, in comparison to 2016, at HSS the total decrease in growth was 55,6% (early-wood decrease of 49,8%, late-wood 72%) while at the MSS of 55,7% (early-wood decrease of 65%, late-wood 38,5%) and LSS of 49,5% (early-wood decrease of 63,2%, late-wood 27,5). Finally, at CS the total decrease of the growth was 62,4% (early- wood of 66,4%, late-wood 50,8%). The tree-ring widths of 2017 of each individual of the LSS, MSS (see Supplementary Figure S3) and HSS sites (Figure 4), were related to the percentage of destroyed crown. In the HSS a strong negative correlation was found, suggesting that the decrease in growth is directly proportional to the amount of needles destroyed by the fire: the plants that have a 90% crown reduction, showed a strong decrease in growth. In particular, relating the percentage of scorched crown with the early-wood and late-wood ring-width, we observed that the highest negative correlation was found with late-wood. Indeed, the 2017 fire occurred in July, the period when the late-wood is normally formed.

2.3.2 Carbon isotopes

The HSS presented a $\delta^{13}C$ mean value, recorded between 1983 and 2017, of −26.3 ± 0,4‰ (Figure 5A), at MSS and LSS the average $\delta^{13}C$, measured between 1975 and 2017, was −26 ± 0,14‰ for MSS and −26.01 ± 0,3‰ for LSS (Figures 5B,C), finally in the CS the C isotope values, for the period 1994 and 2017, was −25.7 ± 0,05‰ (Figure 5D). The δ13C site-chronologies showed different trends with only MSS and LSS presenting a high correlation ($r = 0.26$, $P<0.05$). However, some years influenced the $\delta^{13}C$ values of the four sites in the same way: negatively during 1993, 2012, 2014, positively during 1994, 2006. While the 1993 fire drastically lowered the $\delta^{13}C$ in all sites, the fires of 2007 and 2015 caused the decrease only in MSS site. The different trends are also evident for $\delta^{13}C$ measured in the early-wood and in the late-wood of the four sites (Figure 5) with the exception of particular years (2000, 2001, 2012, 2014, 2016, and 2017 for early-wood and, 1990, 1993, 1994, 1996, 2005, 2006, 2013 for late-wood), in which the isotopic values showed a similar response (relationships rise or fall at the same time). During the 2017 the $\delta^{13}C$ values recorded in whole wood and particularly in late-wood resulted to increase at the HSS and MSS sites, compared to the previous year. At the LSS the δ13C of whole wood was almost unchanged in 2017 compared to 2016, with slightly decrease in late-wood while at CS we observed a decrease in $\delta^{13}C$.

2.3.3 Oxygen isotopes

The $\delta^{18}O$ values of the four sites varied in a range between 23.8 and 28.39‰ for the study period (Figure 6). The four chronologies showed a low synchronization among each other with only few years presenting the same trend, i.e., 2001, 2002, 2011, 2012, and 2017 both in whole wood and in each wood compartment (early-wood and late-wood). In particular $\delta^{18}O$ of 2017 strongly increased in all the sites.

2.3.4 Relationships among climate, growth, and isotopes

The mean temperature, especially during the summer (JJA = June, July, August), was negatively correlated with the annual growth of the plants of all the four sites (Table 3). While the mean temperature of spring (MAM = March, April, and May) and autumn (SO = September, October) led to an increase of $\delta^{13}C$ in the sampled individuals. The mean temperature of spring (MAM) and of summer period (JJA) triggered the increase of $\delta^{18}O$. A high positive correlation was found between total annual rainfall and tree growth of HSS, LSS and CS sites. While negative correlation resulted between total precipitation and the late-wood $\delta^{13}C$ of LSS site.

2.3.5 Isotopic response of an individual with 1993 fire scar

The analysis of stable isotopes, focused in the period 1992– 2001, showed how, due to the fire event of 1993, the $\delta^{13}C$ and the $\delta^{18}O$ increased sharply (Figures 7A,B). The carbon isotopic composition spanned from −28.24‰ of the spring ‘92 to −23.51‰ of summer ‘93, while oxygen ranged from 20.33‰ of 1992 to 23.39‰ of summer ‘93. After 1993, the δ^{13} C values were stabilized while the $\delta^{18}O$ continued to increase, reaching, in 1995, a value of 28‰. The normal trend was recovered only in 1998.

2.4 Discussion

2.4.1 Fire effect on tree growth

Pinus pinaster trees sampled in the three burned sites (HSS, MSS, and LSS) showed a decrease in tree growth in 2017, in particular in the late-wood at HSS. Indeed, the fire event occurred in July, when the late-wood is formed in several woody species growing on Vesuvio area (Balzano et al., 2018). Reduction in growth has been described as a short term effect of fire in many species and across different ecosystems (e.g., Hoffmann and Solbrig, 2003; Werner, 2005; Werner et al., 2006; Goldammer, 2007; Murphy et al., 2010; De Micco et al., 2013; Battipaglia et al., 2014b) and it has been interpreted as an abrupt narrowing of growth rings after fire (Schweingruber, 1993; Stahlea et al., 1999) mainly due to cambium or crown damage (Pausas et al., 2003). When the tree is seriously damaged, the formation of new cell can be interrupted in the injured sector of the trunk or branch and fire scars can be formed (De Micco et al., 2014). No fire scars have been observed after the 2017 fire in all the burned sites, further all the sampled trees, including the one growing in the HSS, were able to complete the 2017 seasonal wood formation. For *P. pinaster*, the bark is considered the main adaptive trait in response to forest fires (Ryan et al., 1994). As for *P. pinea*, the bark of the analyzed species is laminated, a peculiarity that allows to the outer layers of the trunk a gradual exfoliation during combustion, which contributes not only to the dispersion of heat, but also to an increase in the time necessary for killing the vascular cambium (Peterson and Ryan, 1986b; Rego and Rigolot, 1990; Battipaglia et al., 2016). The bark thickness is strongly dependent on tree age (Fernandes and Rigolot, 2007), indeed its diameter doubles when the plant reaches 10 years of age. The maritime pine (*P. pinaster*) growing at the study sites presented a DBH (diameter at breast height) > 20 cm, with a fairly thick bark (between 1.5 and 4 cm), which guarantees a good defense against fire (Burrows et al., 2000). This could explain why, even if the sampled individuals in the HSS, MSS, LSS presented a considerable bole char height, reaching the 4 m in the HSS, no fire scar were formed. For the majority of conifers crown volume damaged is considered the most important factor determining mortality (Sieg et al., 2006). Previous studies (Freitas, 1995) demonstrates that the needles of the *P. pinaster* are less susceptible to thermal trauma than those of *Pinus halepensis* and *P. pinea*. In fact, maritime pines are able to survive at temperatures between 55 and 65°C, for a time of exposure to the flame of 60 s. Also, the apical buds show a remarkable resistance to combustion: the protection offered by the surrounding long needles shield the gem from the connective heat of the flame (Michaletz and Johnson, 2006). Therefore, it seems evident that the burning of the needle in *P. pinaster* is not always a symptom of the destruction of the crown: even in the case of complete defoliation, the plant has been found able to survive (De Ronde, 1983). Experimental prescribed burning, carried out on the maritime pine by in McCormick (1976), have allowed to report the damage to the foliage with the respective growth rate, showing that, in adult individuals, a slight damage of the crown does not lead to a drastic decline in growth. On the contrary, when the crown damage exceeds 25%, it is possible to find a substantial decrease in growth. Our findings are in agreement with McCormick's studies: the plants of the MSS and LSS that showed a reduction of the crown of 10% did not show particular growth decreases, while the individuals sampled at HSS, which experienced a conspicuous damage to the crown (in some trees the defoliation was equal to 90%) showed a sharp decrease in growth. Further, the ability of *P. pinaster* to survive, although the presence of a large fire scar and a possible severe defoliation, is demonstrated by the individual sampled in the MSS presenting the 1993 fire scar. Dendrochronological analyses of that individual highlighted that the plant survived to the 1993 wildfire growing until nowadays. According to the tree-ring dating, that individual, similarly to the rest of the pines in the stand, in 1993 was more than 10 years old with a bark certainly greater than 1 cm. Ryan et al. (1994) applying a prescribed fire on *P. pinaster* stand showed that, with a bark greater than 1 cm of thickness, a scar cannot represent a significant contribution to the tree mortality. The reduction in growth recorded in 2017, in comparison to the 2016, was evident also in the individuals sampled in the CS and this could be due to the extreme high temperatures recorded in 2017. Indeed, this year has been considered as an extreme dry year (World Meteorological Organization, 2018). The climate-growth correlations reported in Table 3 showed the importance of temperature on the tree ring width for the whole period. The high temperatures can cause a change in the constant kinetics of the RuBisCo, leading to a consequent decrease in carboxylation rate (Medlyn et al., 2002) and thus to a reduction in tree growth. On the other hand, also precipitation affected the growth of the sampled trees: indeed, in all the four chronologies a reduction in growth has been found after particular dry years: for example, the low rainfall in 1999 (455 mm/year) determined a drastic decline in growth in 2000 in all the trees. In addition to the climatic factors, competition can also play an important role in the growth rate: dendrochronological analyzes have highlighted how the prescribed burning applications, carried out in 2014 and 2016 in the LSS, have led to a sharp increase in the growth of maritime pine. Prescribed burning, in addition to reducing in competition, through the biomass reduction of the herbaceous and shrubby species, has determined a large nutrients mineralization, ensuring to the dominant individuals to take advantage of the favorable conditions. Similar results were also observed in other prescribed burning studies performed on individuals of *Pinus halepensis* (Battipaglia et al., 2014b; Valor et al., 2018) and *P. pinea* (Battipaglia et al., 2016).

2.4.2 Ecophysiological responses of *P. pinaster* to fire

The isotopic analyses related to 2017 of the four sampled sites allowed to better understand the different processes triggering trees responses to fire. Indeed, the growth reduction found in all the trees was due to changes in ecophysiological mechanisms, mainly related to fire severity. Trees of HSS and MSS sites showed in 2017 a significant increase of $\delta^{18}O$ and a slight increase of $\delta^{13}C$ especially of late-wood, compared to previous years. The increase in the oxygen isotopic ratio may be associated with a decrease in stomatal activity, whilst the increase of $\delta^{13}C$ can indicate a lower partial pressure of CO_2 in the intracellular spaces of the leaf, due to both photosynthetic activity and stomatal conductance. Therefore, considering both isotopes, according to the dual-isotope approach (Scheidegger et al., 2000) we could hypothesize that the plants to protect themselves from strong stress condition, due to fire, and as a consequences of the serious observed crown damage, closed their stomata and lowered their photosynthetic activity. These conditions, added to the high temperatures and drought conditions through the growing season, explain the observed reduction in growth of 2017 tree ring. Those results are in agreement with the studies carried out by Battipaglia et al. (2014b) on of *Pinus halepensis*, in several forest fires in Southern France, that showed a simultaneous increase in $\delta^{13}C$ and $\delta^{18}O$ in occurrence of the fire events. In the same studies the authors demonstrated that the *Pinus halepensis*, considered a species highly vulnerable to fire, was able to survive and to recovery from fire damages, presenting a low mortality rate. To better understand the probability of death of maritime pine, exposed to high severity fire, the study carried out on the individual with the deep fire scar, was crucial. The study showed that, in the periods before the 1993 fire, the tree with the fire scar, located in the MSS, was not subjected to stressful climatic conditions since in the period between 1985 and 1992, precipitation and temperatures were rather constant with low anomalies. In the year of fire, the values of $\delta^{13}C$ and $\delta^{18}O$ appeared to drastically increase (Figure 7) and, therefore, the eco-physiological responses were similar to that recorded in the HSS individuals after the 2017 fire. In the following years from 1993 onwards, while the $\delta^{13}C$ decreased, returning to the average values, the $\delta^{18}O$ showed a progressive increase until 1995. This combination suggests that, although the stomatal conductance gradually stabilized over time, a severe defoliation of the plants, at least in the first years after fire, resulted in a lower photosynthetic capacity (Battipaglia et al., 2016), leading a lower growth rate (as recorded by the dendrochronological analyzes). After that period, the growth and the ecophysiological processes of that individual returned to the average values. The individuals sampled at MSS and LSS showed a strong synchronization in the $\delta^{13}C$ and $\delta^{18}O$ chronologies along all the time period ($R = 0.26$ and $R = 0.24$, respectively). However, between 2014 and 2016, the application of the prescribed burning in the LSS determined, in the plants of that site, an increase of $\delta^{13}C$ while the $\delta^{18}O$ remained unchanged. The variation of the $\delta^{13}C$ of the plants of the LSS can be connected to an increase of the photosynthetic activity, triggered by prescribed burning. A positive effect of fire was recorded also in the 2007 when a surface fire has affected individuals of the MSS and when a decrease of $\delta^{13}C$ and $\delta^{18}O$ was recorded until 2009, indicating a possible favorable effect of reduction in competition for water among survived plants, as found in a study by Beghin et al. (2011). On the other hand the August 2015 fire, which involved the MSS plants, did not result in significant changes of the isotopic response or drastic changes in growth rate. This allows us to hypothesize that the analyzed pinewood was only marginally affected by this event. Finally, the isotopic analyses of CS trees showed, in 2017, a moderate decrease of $\delta^{13}C$ and a drastic increase in $\delta^{18}O$. This variation, compared to the previous year, can indicate a lower photosynthetic activity and an unchanged stomatal conductance (Scheidegger et al., 2000) probably related to the extreme hot conditions experienced in that summer by all the plants. The climate-growth correlations demonstrated how the minimum temperature of the summer period ($r = -0.56^{**}$), and, in particular, of August ($r = -0.66^{***}$), has a negative influence on the growth of studied trees. The high temperatures, indeed, besides determining a reduction of the chlorophyll pigments and the denaturation of some thioacid proteins, can cause the inhibition of the photosynthetic process (Bussotti et al., 2012). When air temperature exceeds the compensation point (temperature at which the amount of CO_2 fixed by photosynthesis is equivalent to that released by respiration), the photosynthetic process is not able to replace the carbon used for respiration. This process implies a consumption of carbohydrates reserve that led the plant to a slowdown or a stop of its growth (Di Toppi et al., 2018). Climate conditions could also represent a cumulative factor of stress contributing to a delayed mortality. Catry et al. (2010) assessing the vegetative condition of 1040 burned trees from 11 different species during the first years after the 2003 wildfire, demonstrated how the tree mortality largely increased after the 2005 drought. Their results emphasize the importance of monitoring the tree health in the following years. The burned trees in the study sites showed, at the end of 2018, a very limited mortality rate, with only the 2–10% of plants reducing their vigor. We will continue monitoring the area and the analyzed trees since our work want to be a contribution on the fire ecology of the *P. pinaster* species, underling the importance of evaluating the ecophysiological responses of the species to fire severity in order to valuate the most suitable and effective silvicultural operations mainly in post-fire forest management. Indeed, *P. pinaster* trees affected by fire, with needles scorched or consumed, are not necessarily dead and before to remove every blackened trees, it is important to assess the real damage, waiting for the next growing season, in particular in Natural parks or in areas not close to roads or anthropic settlements. Savage logging could produce severe damage to soil stability and biodiversity, and in particular mechanical actions can largely increase seedling mortality (e.g., Martinez-Sanchez et al., 1995; Fernandes et al., 2008), negatively affecting the ratios final seedling density/initial seedling density and final seedling density/total viable seed dispersal (Vega et al., 2008).

2.5 Reference

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2.6 Figures

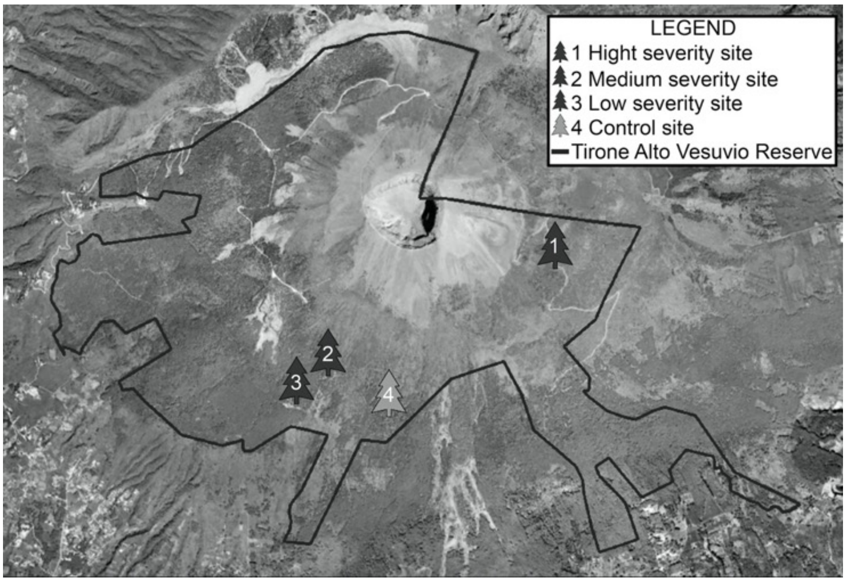


Figure 1 - Sampling sites in the Tirone Alto Vesuvio Nature Reserve of the Vesuvio National Park (Italy).

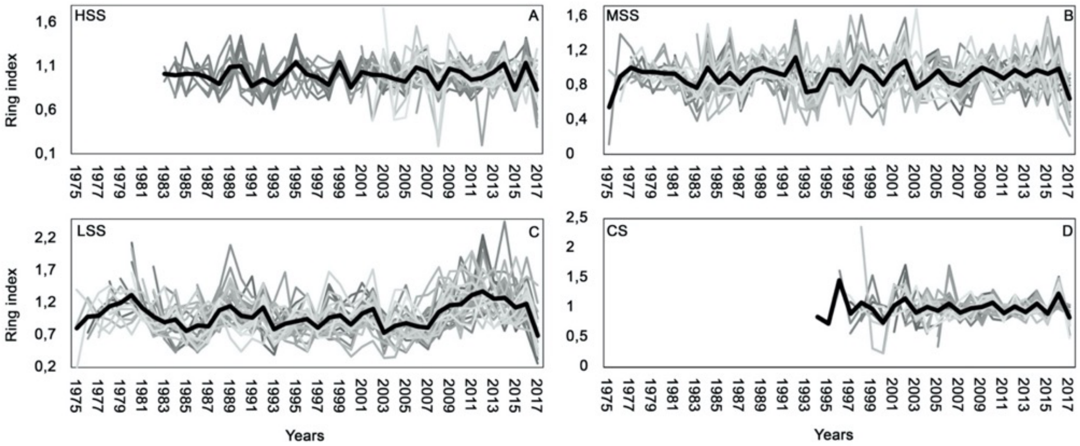


Figure 2 - Individual standardized chronologies in gray and average chronologies in black for (A) high severity site (HSS), (B) medium severity site (MSS), (C) low severity site (LSS), (D) control site (CS).

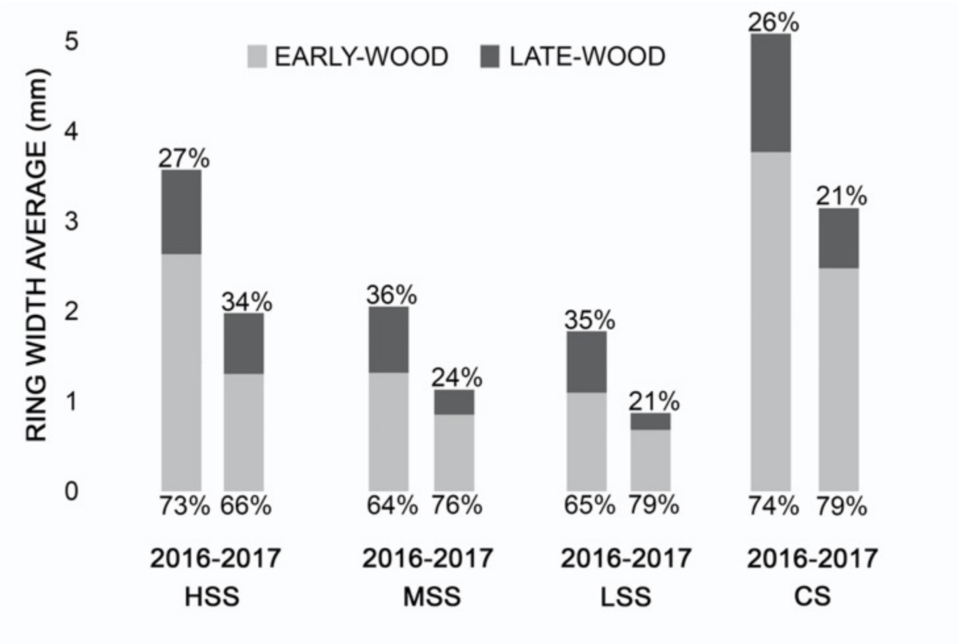


Figure 3 - Annual ring width of the 2016 and 2017 reported in each site. In gray the early-wood and in black the late-wood with the percentage of growth, respectively.

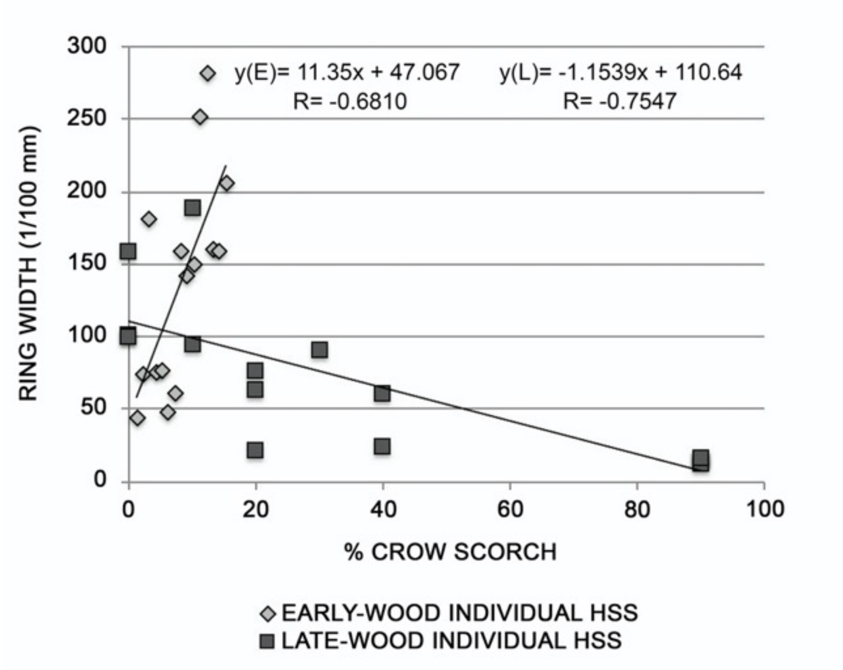


Figure 4 - Relationship between tree-ring widths of early-wood (E) and late-wood (L) of HSS site and the % of scorched crown.

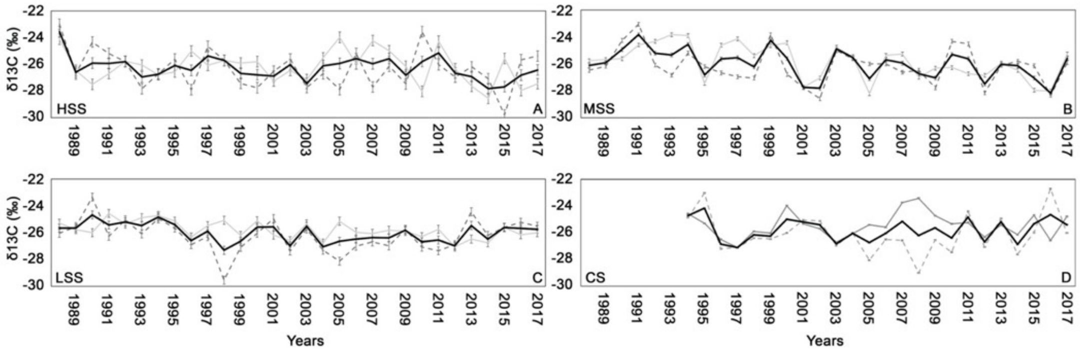


Figure 5 - $\delta^{13}C$ chronologies (± standard deviation) for early-wood (gray line), late-wood (dashed gray line), and for the whole wood (black line) for (A) high severity site (HSS), (B) medium severity site (MSS), (C) low severity site (LSS), and (D) control site (CS).

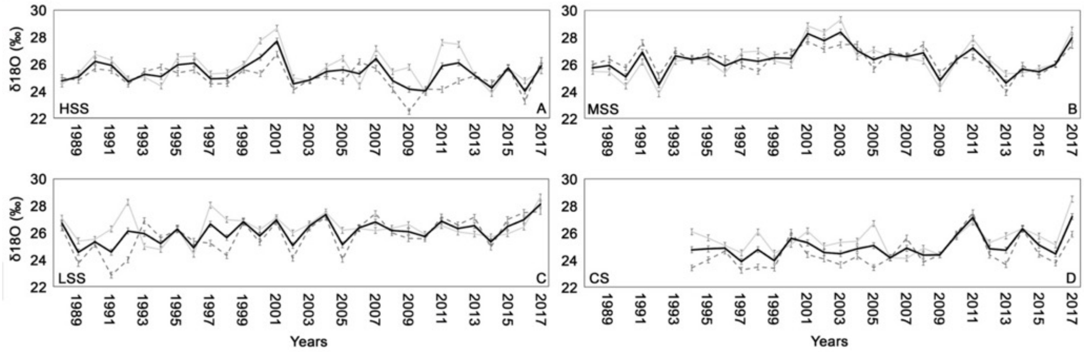


Figure 6 - $\delta^{18}O$ chronologies (± standard deviation) for early-wood (gray line), late-wood (dashed gray line), and for the whole wood (black line) for (A) high severity site (HSS), (B) medium severity site (MSS), (C) low severity site (LSS), and (D) control site (CS).

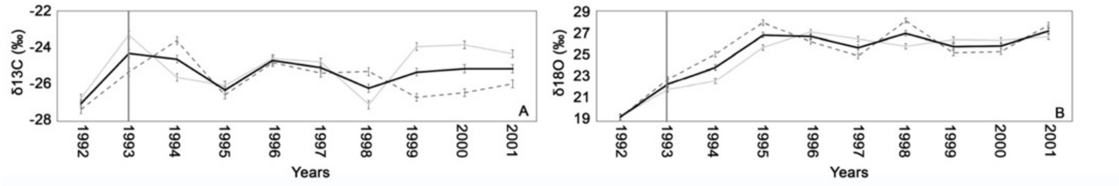
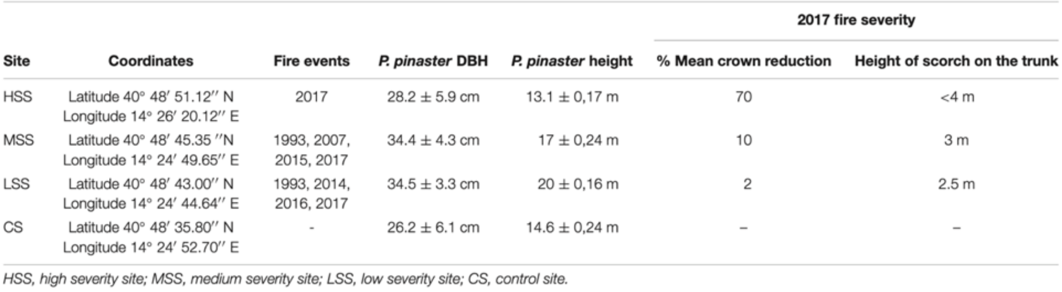


Figure 7 - Trend of the $\delta^{13}C$ (A) and the $\delta^{18}O$ (B) (± standard deviation) for early-wood (gray line), late-wood (dashed gray line), and for the whole wood (black line) recorded in the individual with a 1993 fire scar.

2.7 Tables

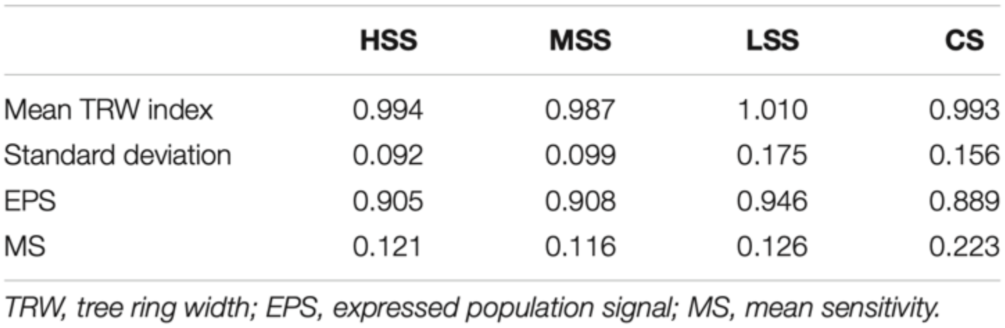
Table 1 - Sites coordinates, historical fire events, tree average diameter at breast height (DBH), tree height and severity parameters (percentage of crown reduction, height of scorch on the trunk) of 2017 wildfire.



					2017 fire severity	
Site	**Coordinates**	**Fire events**	***P. pinaster* DBH**	***P. pinaster* height**	**% Mean crown reduction**	**Height of scorch on the trunk**
HSS	Latitude 40° 48′ 51.12″ N Longitude 14° 26′ 20.12″ E	2017	28.2 ± 5.9 cm	13.1 ± 0,17 m	70	<4 m
MSS	Latitude 40° 48′ 45.35 ″N Longitude 14° 24′ 49.65″ E	1993, 2007, 2015, 2017	34.4 ± 4.3 cm	17 ± 0,24 m	10	3 m
LSS	Latitude 40° 48′ 43.00″ N Longitude 14° 24′ 44.64″ E	1993, 2014, 2016, 2017	34.5 ± 3.3 cm	20 ± 0,16 m	2	2.5 m
CS	Latitude 40° 48′ 35.80″ N Longitude 14° 24′ 52.70″ E	-	26.2 ± 6.1 cm	14.6 ± 0,24 m	–	–

HSS, high severity site; MSS, medium severity site; LSS, low severity site; CS, control site.

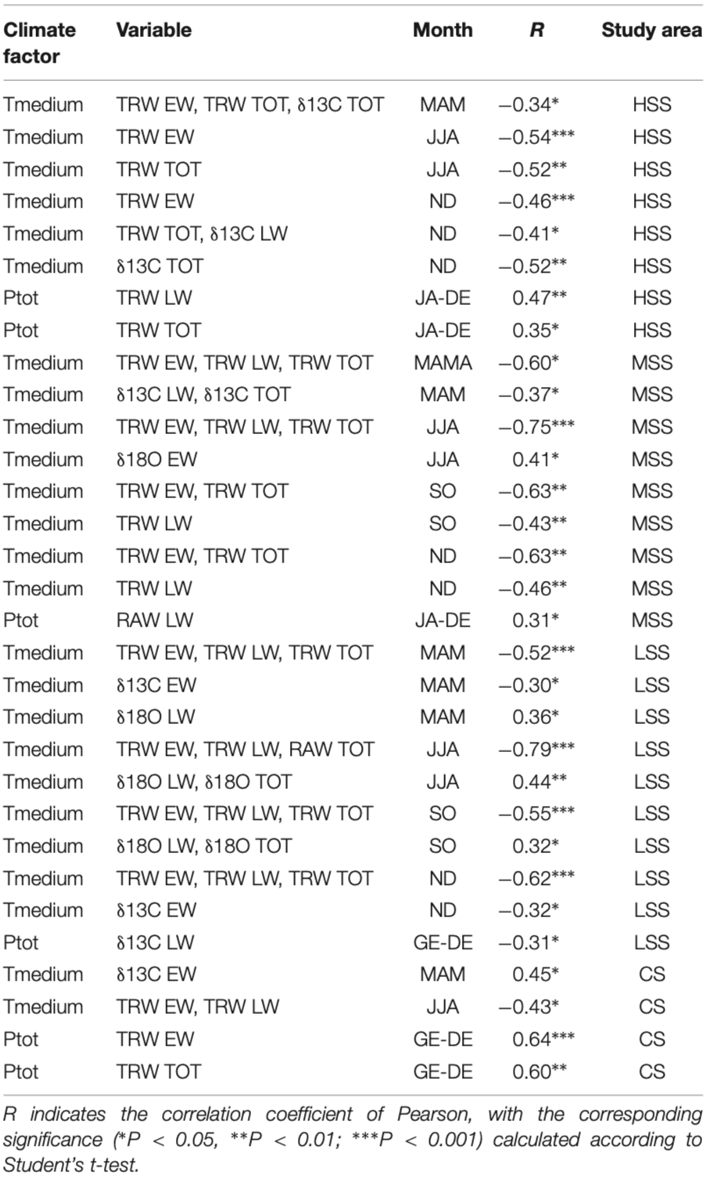
Table 2 - Statistical parameters of the indexed average chronologies of the four areas.



	HSS	MSS	LSS	CS
Mean TRW index	0.994	0.987	1.010	0.993
Standard deviation	0.092	0.099	0.175	0.156
EPS	0.905	0.908	0.946	0.889
MS	0.121	0.116	0.126	0.223

TRW, tree ring width; EPS, expressed population signal; MS, mean sensitivity.

Table 3 - Correlations between whole width (TRW), early-wood (TRW EW), late-wood (TRW LW), $\delta^{13}C$ of whole wood ($\delta^{13}C$ TOT), $\delta^{13}C$ of early-wood ($\delta^{13}C$ EW), $\delta^{13}C$ of late-wood ($\delta^{13}C$ LW), $\delta^{18}O$ of whole wood ($\delta^{18}O$ TOT), $\delta^{18}O$, of early-wood ($\delta^{18}O$ EW) and $\delta^{18}O$ of late-wood ($\delta^{18}O$ LW) of the four sites, with monthly mean temperature (Tmean) (MAM, March, April, and May; JJA, June, July, and August; SO, September and October; ND, November and December) and total annual precipitation (Ptot).



Climate factor	Variable	Month	*R*	Study area
Tmedium	TRW EW, TRW TOT, δ13C TOT	MAM	−0.34*	HSS
Tmedium	TRW EW	JJA	−0.54***	HSS
Tmedium	TRW TOT	JJA	−0.52**	HSS
Tmedium	TRW EW	ND	−0.46***	HSS
Tmedium	TRW TOT, δ13C LW	ND	−0.41*	HSS
Tmedium	δ13C TOT	ND	−0.52**	HSS
Ptot	TRW LW	JA-DE	0.47**	HSS
Ptot	TRW TOT	JA-DE	0.35*	HSS
Tmedium	TRW EW, TRW LW, TRW TOT	MAMA	−0.60*	MSS
Tmedium	δ13C LW, δ13C TOT	MAM	−0.37*	MSS
Tmedium	TRW EW, TRW LW, TRW TOT	JJA	−0.75***	MSS
Tmedium	δ18O EW	JJA	0.41*	MSS
Tmedium	TRW EW, TRW TOT	SO	−0.63**	MSS
Tmedium	TRW LW	SO	−0.43**	MSS
Tmedium	TRW EW, TRW TOT	ND	−0.63**	MSS
Tmedium	TRW LW	ND	−0.46**	MSS
Ptot	RAW LW	JA-DE	0.31*	MSS
Tmedium	TRW EW, TRW LW, TRW TOT	MAM	−0.52***	LSS
Tmedium	δ13C EW	MAM	−0.30*	LSS
Tmedium	δ18O LW	MAM	0.36*	LSS
Tmedium	TRW EW, TRW LW, RAW TOT	JJA	−0.79***	LSS
Tmedium	δ18O LW, δ18O TOT	JJA	0.44**	LSS
Tmedium	TRW EW, TRW LW, TRW TOT	SO	−0.55***	LSS
Tmedium	δ18O LW, δ18O TOT	SO	0.32*	LSS
Tmedium	TRW EW, TRW LW, TRW TOT	ND	−0.62***	LSS
Tmedium	δ13C EW	ND	−0.32*	LSS
Ptot	δ13C LW	GE-DE	−0.31*	LSS
Tmedium	δ13C EW	MAM	0.45*	CS
Tmedium	TRW EW, TRW LW	JJA	−0.43*	CS
Ptot	TRW EW	GE-DE	0.64***	CS
Ptot	TRW TOT	GE-DE	0.60**	CS

*R indicates the correlation coefficient of Pearson, with the corresponding significance (*P < 0.05, **P < 0.01; ***P < 0.001) calculated according to Student's t-test.*

2.8 Supplementary materials

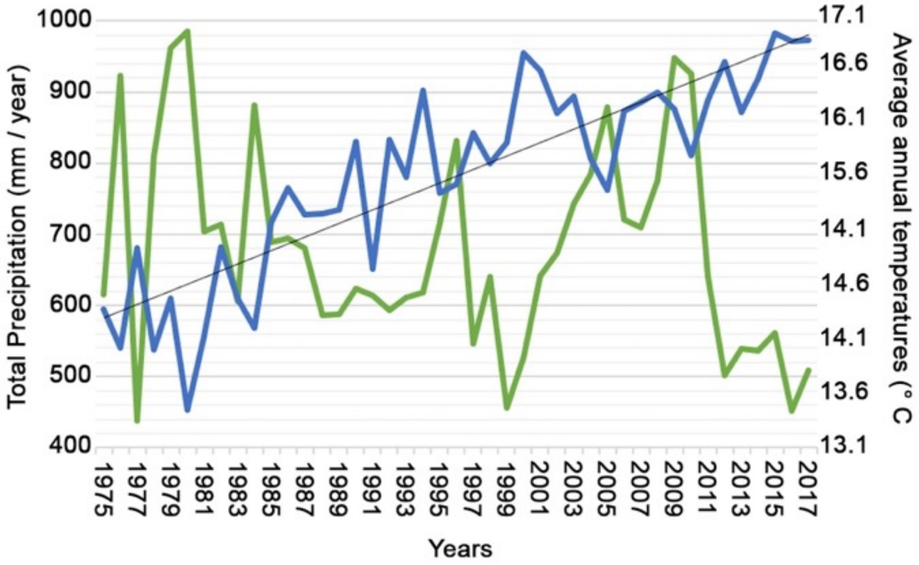


Figure S1 - Trend of total annual precipitation in green (expressed in millimetres/year) and average temperatures in blue (expressed in °C) recorded from 1975 to 2017 and coming from the KNMI Climate Explorer database.

Figure S2 - Individual of *P. pinaster* belonging to the MSS which has an important lesion on the trunk due to a previous fire, dated 1993.

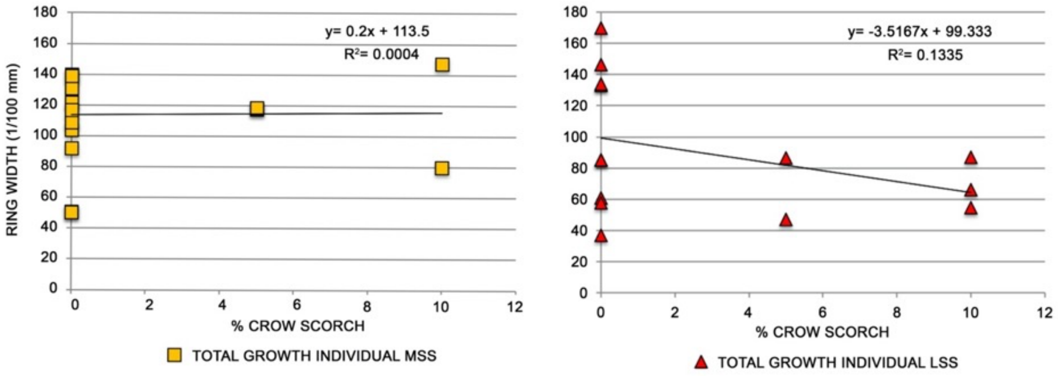


Figure S3 - Relationship between ring growth of 2017 and the percentage of crown scorch of the MSS trees (indicated with in yellow symbols) and LSS trees (indicated in red)

Chapter 3

3. Fire affects wood formation dynamics and ecophysiology of *Pinus pinaster* Aiton growing in a dry Mediterranean area

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Manuscript submitted in Tree Physiology

3.1 Introduction

In the Mediterranean basin, the occurrence of forest fires has increased significantly in recent decades (Intergovernmental Panel on Climate Change, 2018) and these events are expected to become even more severe and frequent in the future due to climate change (Stavros et al., 2014; Littell et al., 2018). Every year, millions of hectares of forest burn causing high rates of tree mortality with severe consequences for the forest ecosystem services such as loss of wood production, biodiversity, hydro-geological stability and carbon stock (Bowman et al., 2009). Tree mortality often occurs in the short term and is determined by the direct effects of fire on individual trees. These effects, defined as first-order, induce death through serious injury to the plant tissues (Bär et al., 2019). Several studies have shown that prolonged exposure to flames with temperatures around 60°C leads to necrosis of the cambium (Michaletz and Johnson, 2007; Kelsey and Westlind, 2017) due to a permanent interruption of the phloem and to a reduction of downward translocation of photosynthates (Midgley et al., 2011). However, in most cases, death occurs in the short term only if cambium is damaged for the large part of the trunk circumference (Dickinson and Johnson, 2001; Michaletz and Johnson, 2007) and/or if there is serious crown reduction (Varner et al., 2021). In fact, a total or almost total defoliation leads to a rapid death of the plant which, due to a reduced photosynthetic capacity, is unable to produce the non-structural carbohydrates necessary for the maintenance of cell survival (McDowell, 2011; Wiley et al., 2013). Forest fire resistance is closely linked to the adaptive traits of each individual species (Verma et al., 2017): for example, a very thick bark or a higher intersection of the canopy increase the probability of plant survival (Bova and Dickinson, 2005). However, some trees, while initially resisting a fire, can be compromised in their eco-physiological functionality by the indirect effects of fire (Bär et al., 2019). Plant's recovery capacity depends on the degree of damage of the tissues (McHugh and Kolb, 2003; Sieg et al., 2006) and can be influenced by further stressful conditions such as competition, disease and especially drought (van Mantgem et al., 2013, 2018). The single or combined effect of these conditions can trigger a reduction in carbohydrate reserves, important limitations in stomatal and photosynthetic activity as well as dysfunctions in the cambial production (Hood et al., 2018; Gričar et al., 2020), which lead to a reduction of tree vigor and growth (Schweingruber, 1993; Stahle et al., 1999) and in most serious cases to death even several years after the fire (Barlow et al., 2003; Baker et al., 2008; Barlow and Peres, 2008). Although several studies on the post-fire effects of forests are available (Kobziar et al., 2006; Sieg et al., 2006; Breece et al., 2008; Beghin et al., 2011; Valor et al., 2018), most of them refer to annual and/or multi-year time scales. The study of xylogenesis, i.e., the process of xylem formation during an entire growing season, allows assessing the intra-annual dynamics of tree growth and helps clarifying the ecophysiological strategies faced by species to cope with stress conditions (Deslauriers et al., 2003; Rossi et al., 2007). Indeed, cambium activity and cell differentiation are conditioned by genetic factors, but also by the physiological condition of plants (Vieira et al., 2015; Prislan et al., 2016). In recent years, several authors have studied the effects of climate on cambial activity, especially in conifers, showing that the inception, duration, and end of the various phenological phases of xylogenesis are strongly dependent by the temperature and water availability (Camarero et al., 2010; Vieira et al., 2014, 2015; Balzano et al., 2018). However, to date there is a lack of studies analysing how the cambial activity is influenced by fire, especially in drought prone ecosystems. In this context, our research aims to study the post-fire effects in the short and medium term on a population of *Pinus pinaster* Aiton, affected by severe crown damage and growing in a particularly hot and dry area of the Mediterranean region. To reach this goal, we combined inter-annual analyses of dendrochronology and isotope composition in tree rings with monitoring of xylogenesis. While the study of tree-rings allows to evaluate wood growth (Rozendaal and Zuidema, 2011), the combination of carbon and oxygen stable isotopes allows to estimate the eco-physiological processes involved in trees' responses to environmental fluctuations. Indeed, $\delta^{13}C$ reflects the balance between photosynthetic assimilation rate and stomatal conductance (Farquhar et al., 1982; Saurer et al., 2004), while the variation of $\delta^{18}O$, in addition to investigate the water source used by trees, helps to clarify the independent effect of photosynthesis process and stomatal activity on $\delta^{13}C$ (Scheidegger et al., 2000). Further, xylogenesis analysis allows to understand, with a high temporal resolution, the influence of exogenous disturbances on the cambium activity (Rossi et al., 2007). The aim of this research is twofold: 1) to understand how the forest fire influenced the recovery process of the *P. pinaster* stand in the post-fire period in terms of radial tree growth, eco-physiological processes (such as photosynthetic activity and stomatal conductance) and wood formation dynamic 2) to verify the climate effect on wood formation in a drought prone ecosystem. To reach those aims burned plants with serious damage to canopy due to fire (high defoliation) are compared to not defoliated trees (control). We hypothesize that burned plants have few chances of recovering the physiological and growing conditions of pre-fire period due to the crown damage and this will be reflected in wood formation dynamics. Further, climate conditions, in particular drought, will have a strong influence on wood formation especially in the defoliated trees compared to the control plants.

3.2 Materials and Methods

3.2.1 Study area

The study area is located in the integral reserve of the Vesuvius National Park, in southern Italy (Figure 1A). The examined forest of *Pinus pinaster* Aiton extend for about 2 ha along the southern slope of the volcano (40°48′45.35"N, 14°24′49.65"E). In July 2017 the Park was affected by a large wildfire (Battipaglia et al. 2017b) which severely damaged numerous individuals, causing partial defoliation but without leading trees to death in the short term (Niccoli et al. 2019). In fact, just after the fire, plants (Burned Trees - BT) showed scorch on the trunk between 2.5 and 3 m high, a partial defoliation of the crown (10%) and a very low mortality rate (10%) (Niccoli et al. 2019). However, in the following years the crown defoliation increased: in March 2020 the average stand defoliation was estimated around 50%. On the contrary, some individuals (Control Trees - CT), although located in the same area of the park, were not affected by fire and did not present defoliation or scorch on the trunk. Meteorological data in recent decades have shown a gradual rise in temperatures and a fluctuating rainfall trend in the study area (Figure S1) (KNMI database Climate Explorer) (Trouet and Van Oldenborgh, 2013). The daily climatic data recorded during the xylogenesis monitoring period [2 April 2019 and 3 March 2020; DOY 92_19 – 63_20 (day of the year_relative year)] (Figure 1B) from the nearby meteorological station, located 4.8 km from the study area (40°47'16.9"N; 14°27'50.4"E; www.agricoltura.regione.campania.it) showed how the pine forest of Vesuvius was subjected to average temperatures of 17.9 °C and total rainfall of 777.4 mm. During the spring months (2 April 2019 – 20 June 2019; DOY 92 – 171_19) the total precipitation was 36.4 mm while the average temperature was 17°C with maximum and minimum respectively of 21.3 °C and 12.6 °C. On the other hand, in summer (21 June 2019 – 22 September 2019, DOY 172 – 265_19) the total rain was 40.4 mm and the average temperature was 25.3 °C with maximum of 31.1 °C and minimum of 19.5 °C. In the autumn months (23 September - 21 December, DOY 266 – 355_19), while the total precipitation was 607 mm, the mean temperature was 16.7 °C, with maximum and minimum respectively of 21.2 °C and 12.2 °C. Finally, during the winter months (22 December 2019 - 3 March 2020, DOY 356_19 – 63_20), 93.6 mm of rain was recorded, and average temperatures of 10.7 °C with maximum and minimum temperatures of 14.6 °C and 6.8 °C respectively.

3.2.2 Dendrochronological and isotopic measurements

In winter 2020, 15 BT (average DBH 34.5 ± 3.3 cm, average H 20 ± 0.16 m) and 15 CT (average DBH 26.2 ± 6.1 m, average H 14.6 ± 0.24 m) of a comparable age were sampled using an increment borer (Haglöfs, Sweden) of 5 mm, allowing to update previously published data (Niccoli et al. 2019) which provided information up to 2017. Tree-ring width (TRW) of the samples were measured through the LINTAB system (Frank Rinn, Heidelberg, Germany). Subsequently, using the TSAP-Win software, the TRW of the individual plants were cross-dated through the Gleichaeufigkeit (GLK) parameter, which evaluates the concordance of trends between the different series (Eckstein and Bauch, 1969). Synchronization was considered acceptable at GLK>70. Subsequently, the raw chronologies were first statistically analyzed using the COFECHA software (Holmes, 1983), and then converted in basal area increment (BAI). This operation allows to minimize the effect of the size and age of trees on annual growth trends by maintaining the high and low frequency signals incorporated in tree rings (Biondi and Qeadan 2008, Battipaglia et al. 2017a, Niccoli et al. 2021). BAI (mm^2) was calculated as the average basal area increment following the equation:

$$BAI = \pi r^2_t - \pi r^2_{t-1}$$

where BAI per year t is equivalent to the area of the annual ring;
r_t is the radius of the stem at the end of the annual increment;
r_{t-1} is the radius of the stem at the beginning of the annual increment.

Finally, the wood-cores were used for the isotope analyses. The rings of the last 8 years (from 2013 to 2020) were first removed manually using a cutter and, subsequently, ground using a pulverizing mill (ZM 1000, Retsch, Germany). Subsequently, the samples were weighed and placed in tin capsules for $\delta^{13}C$ and silver for $\delta^{18}O$ measurements. The isotope composition was measured at the Icona Laboratory (DISTABIF, Caserta, Italy) by continuous flow isotope ratio mass spectrometry (Delta V Advantage, Thermo electron corporation, Bremen, Germany). Standard deviation for repeated analysis of an internal standard (commercial cellulose) was better than 0.1‰ for carbon and better than 0.3‰ for oxygen. The $\delta^{13}C$ series were corrected for the combustion effect of fossil fuels (Francey et al., 1999).

3.2.3 Monitoring of xylogenesis

Eight dominant trees were identified to perform the xylogeneis study. In particular, were selected 4 BT showing a crown defoliation of 50% and 4 CT not defoliated (Table 1) (Figure S2). Micro-cores with a diameter of 1.8 mm containing cambium and xylem were taken from the selected plants using the Trephor tool (Rossi et al., 2006). The samples were taken with a frequency of 14 days in the period between April 2019 and March 2020. To avoid damage to the trees, the micro-cores were taken along the circumference of the trunk, at a height of 1.5 m from the ground and with a distance of at least 5 cm from the previously drilled holes, avoiding the compression zones (Balzano et al., 2018). The microcores were immediately fixed in a 70% ethanol solution. Subsequently the samples were dehydrated in an ethanol series up to 95% and embedded in the JB4 acrylic resin (Polysciences, Germany). Samples were cut with a rotary microtome and the 5 µm-thick cross sections were mounted with mineral oil for fluorescence microscopy and observed through an epi-fluorescence microscopy (Olympus BX51, Olympus, Germany) with lamp and filters to detect the autofluorescence of lignin (Balzano et al. 2021). The sections were analysed to identify and count the cells in the different phases of xylogenesis: cambial cells (CCs), enlarging cells (ECs), cells with developing secondary walls (SWs) and mature xylem cells (MTs) (Figure 2). The cells in different stages of differentiation were identified based on visual criteria (De Luis et al. 2007, Čufar et al. 2008, 2011, De Micco et al. 2016a, Balzano et al. 2018): CCs appeared radially flattened; ECs were characterized by larger radial dimensions and thin cell walls; SWs showed thickening and lignifying (with autofluorescence stating at cell corners and middle lamellae) cell walls; MTs were characterized by completely thickened and lignified walls (i.e. secondary walls completely autofluorescent) and without any trace of protoplast in the lumen (Figure 2). For each microcore, the cells in the different phenological phases were counted along three radial lines and subsequently the average value was calculated (Balzano et al., 2018). To avoid the influence of variability around the circumference of the trunk, the number of cells in the previous ring was quantified and measurements were normalized following Rossi et al. (2003). Finally, for each sample, the possible formation of intra annual density fluctuations (IADFs) was identified according to De Micco et al. (2016b). Particular attention was paid to the possible formation IADFs of L-type, in which after the normal formation of earlywood (EW1) and latewood (LW1) there is the production of early wood-like mature cells (EW2), and the eventual subsequent generation of new latewood cells (LW2).

3.2.4 Data analysis

For all data, before applying the inferential statistic, normality (using the Shapiro-Wilk test) and homogeneous variance (using the Levene test) were checked. The differences between the pre and post fire years in terms of basal area increment, $\delta^{13}C$ and $\delta^{18}O$ in tree-rings were evaluated according to one-way ANOVA, using the Student-Newman-Keuls coefficient for comparison tests ($P<0.05$). Furthermore, the data of the BAI and the $\delta^{13}C$ and $\delta^{18}O$ grouped in the years before (2013-2016) and post fire (2017-2020) were correlated with the corresponding climatic data (precipitation, maximum and minimum temperatures). Finally, the number of CCs, ECs, SWs, and MTs of BT and CT, were correlated with precipitation and maximum and minimum temperatures applying moving averages of 60 days. All climatic correlations were performed applying Spearman function ($P<0.05$) and using RStudio.

3.3 Results

3.3.1 Analysis of the tree-rings

The analysis of the Basal Area Incremental (Figure 3) allowed to assess possible differences in tree growth between BT and CT. While in CT the BAI of the period before fire (2013-2016) was statistically comparable ($P>0.05$) with that after fire (2017-2020); the drastic decrease in BAI found in BT in the period after fire was significantly different compared to previous years ($P<0.05$).

Mean values of carbon isotope recorded in tree rings (Figure 4) showed that in BT the values of $\delta^{13}C$ decreased in the post-fire years ($P<0.05$). On the contrary in CT not statistically differences were found in the $\delta^{13}C$ between the two periods ($P>0.05$). Finally, $\delta^{18}O$ showed a significant increase ($P<0.05$) in the years following the fire in all analysed trees.

3.3.2 Wood formation dynamics

The xylogenesis monitoring highlighted a long period of cambium production in both groups of trees: the cambium was already active in April 2019 (DOY 92_19), continued its production throughout the study period and then slowed down drastically between the end of December 2019 and the mid-February 2020 (DOY 354_19 – 49_20). At the beginning of March

2020 (DOY 63_20) the cambium was no longer productive: in all trees the cambial area was characterized by the exclusive presence of CCs and MTs cells, suggesting a stop of cambial cells division.

In general, the comparison of xylogenetic curves of BT and CT (Figure 5) showed a good synchronization throughout the monitoring period with the maximum of CCs anticipated by 6 weeks compared to CT. Moreover, in almost all phases of xylem differentiation the number of cells was higher in the CT leading to the higher number of MTs throughout the period of xylem cell maturation. Although the average number of CCs during the growing season (Figure 5A) was quite similar [5.93 ± 0.50 (average cell number ± standard error) for CT; 5.23 ± 0.47 for BT], we found a significant difference ($P<0.05$) in the annual trend in the two groups of trees. In fact, in CT we observed a greater number of CCs in spring (DOY 92 – 162_19) (6.97 ± 0.80 for CT; 5.36 ± 0.61 for BT) and winter (DOY 354_19 - 49_20) (5.09 ± 0.34 for CT; 4.37 ± 0.33 for BT). In contrast, no significant differences ($P>0.05$) were found in the ECs curves (Figure 5B). Although the number of ECs cells was on average higher in CT (4.21 ± 0.53) than in BT (3.42 ± 0.76) the trend was comparable ($P>0.05$): all trees reached a peak in the ECs number between the end of April and May (DOY 120 - 148_19) and in October (DOY 288 - 302_19) with a decrease in the summer period (DOY 190 - 260_19). Regarding the SWs (Figure 5C) CT showed an average number of cells with secondary wall (4.1 ± 0.50) significantly higher ($P<0.05$) compared to BT ones (2.65 ± 0.67). Furthermore, both groups of trees reached peaks in the number of SWs in spring (DOY 148 - 162_19) and autumn (DOY 302 - 312_19). BT showed a decrease in the warmer and driest months of summer (DOY 190 - 260_19), while CT showed another peak in this period. Finally, significant differences ($P<0.05$) were found in the trend of MTs (Figure 5D) between BT and CT. Although all sampled trees reached the maximum number of mature cells in late December (DOY 354_19), the CT presented a greater number of mature cells (38.1 ± 2.95) than BT (24.4 ± 4.64).

Data on the cambial phenology calculated as first observation date in each cell differentiation phase (Table 1) suggested that in all sampled trees the cambium was active before the first sampling day (DOY 92_19) except for one BT, for which the cambial activity started on 30 April 2019 (DOY 120_19). Similarly, two CT and one BT already showed ECs in the microcores at the first sampling date, while in the remaining individuals the formation of ECs started between 17 (DOY 107_19) and 30 (DOY 120_19) April 2019. Although the onset of ECs was observed simultaneously in the two group of trees independently of fire damage, the first formation of SWs cells in some BT was recorded only at the end of May 2019 (DOY 148_19) with a delay of some weeks compared to CT, which formed SWs cells between late April and mid-May 2019 (DOY 120 – 134_19). Important differences in the MTs cell formation were observed between BT and CT. Indeed, while CT formed the first mature cells (EW1) in May 2019 (DOY 134 – 148_19), some BT formed the first MTs cells (EW1) only in June 2019 (DOY 162 – 176_19). Furthermore, the BT showed a contemporary and premature onset of first latewood cells (LW1) on 23 July (DOY 204_19), while in only one CT we observed the onset of LW1 on 23 July (DOY 204_19), the other trees formed LW1 in August (DOY 218-232_19). Finally, the data on cambial phenology showed that all CT, except one, formed intra-annual density fluctuations (IADFs), while only 1 BT showed the formation of IADF. All the intra-annual density fluctuations observed were classified as L-type characterized by completely mature earlywood-like cells (EW2) in the second half of the tree-rings (De Micco et al. 2016b). While the onset of EW2 were recorded between October and November (DOY 302 - 326_19), new latewood cells completely differentiated (LW2) were found at the end of December 2019 and the beginning of January 2020 (DOY 354_19 – 7_20).

3.3.3 Climatic influences

The correlations between BAI, $\delta^{13}C$ and $\delta^{18}O$ data measured in both groups of trees and corresponding climatic variables for pre-fire period (2013-2016) showed a positive correlation in CT between $\delta^{18}O$ and precipitation. On the other hand, in the post-fire years (2017-2020) in CT positive correlations ($P<0.05$) were found between BAI and minimum temperatures and $\delta^{13}C$ and precipitation. In BT there was a positive correlation of $\delta^{13}C$ with precipitation and a negative correlation between $\delta^{18}O$ and minimum temperatures.

Furthermore, several significant correlations were found between the xylogenesis data and the climatic conditions of the study area (Figure 6). BT showed positive correlations ($P<0.05$) between the maximum and minimum temperatures and the number of CCs (in all time windows, especially in the shortest one) and ECs (in the 15 days time window). Furthermore, positive correlations ($P<0.05$) between temperatures and SWs number of two groups were found in all time-windows. Regarding precipitation, BT and CT showed negative correlations ($P<0.05$) with the formation of CCs and ECs in the long time -window. Finally, positive correlations ($P<0.05$) between precipitation and the maturation of xylem cells emerged in BT and CT in all time -windows, however, the strongest correlations were found in the long-time windows (60 days).

3.4 Discussion

3.4.1 Forest-fire effects on tree growth and ecophysiology

Tree-ring width measurements showed that while the CT did not experience any difference in basal area increment between the period before and after the fire ($P>0.05$), BT suffered a strong reduction ($P<0.05$) in tree growth in the years following the fire (2017-2020). Niccoli et al (2019) already reported a growth decrease of the burned stands in the Vesuvius National Park in the months after the fire event and our data confirmed that this reduction occurred also in the mid-term (2018-2020). The growth decrease of BT cannot be attributed to climatic factors, as the conditions of precipitation and temperature are the same experienced in the CT trees (located in the same area). Indeed, we found no correlation between growth parameters and climatic conditions in both group of trees in the pre-fire period (Table 2). More realistically, the low growth may be linked to crown damage that burned plants have experienced (Hood et al., 2018). Indeed, decrease of tree-rings width in the years after a fire was observed by several authors (Werner, 2005; Werner et al., 2006; Goldammer et al., 2007; Murphy et al., 2010; De Micco et al., 2013; Battipaglia et al., 2014), and has been related to crown damage (Brown and Swetnam, 1994; Schmid and Schweingruber, 1997; Pausas et al., 2003; Schweingruber, 2007; Valor et al., 2018). In BT, the damage to the crown was rather limited (10%) in 2017 immediately after the fire (Niccoli et al. 2019). However, starting from the following vegetative seasons an important increase in defoliation was observed: in March 2020 the average crown defoliation reached around 50%. Generally, the degree of crown damage is influenced by the period in which a fire occurs (Harrington, 1987). Harrington (1993) showed that mortality in *Pinus poderosa* Douglas was higher in spring and summer fires than in autumn fires for trees with similar levels of crown burn. In the Vesuvio area, the fire hit the pine forest in the middle of the growing season (Battipaglia et al. 2017b), exposing the BT to a high mortality of the buds and leading to an increase in defoliation in the following years (Wallin et al., 2003; Varner et al., 2021). The combined analysis of $\delta^{13}C$ and $\delta^{18}O$ in the tree-rings (Scheidegger et al., 2000) allowed to infer important ecophysiological mechanisms development in the BT and to compare them with the CT. In BT, $\delta^{13}C$ decreased ($P<0.05$) in the the period post-fire (2017-2020) compared to pre-fire (2013-2016), while the $\delta^{18}O$ showed a significant increase ($P<0.05$) in the years following fire (2017-2020). The post-fire decline in carbon isotopes could be attributable to an increase in stomatal conductance rather than to an improvement in photosynthetic assimilation (Farquhar et al., 1982; Scheidegger et al., 2000). This is suggested by the simultaneous increase in $\delta^{18}O$. Indeed, as is known, the variation in $\delta^{18}O$ is independent from photosynthetic activity (Barbour, 2007) and it is often used to clarify the influence of stomatal conductance on changes in $\delta^{13}C$, especially in the dry environment and where no change in water source occurred (Battipaglia et al. 2014, 2016, Gessler et al. 2014, Battipaglia et al. 2017a). All the sampled trees were growing in the same area and experiencing similar environmental conditions over time; further they were similar in age and size at the time of the study. Therefore, it is unlikely that CT and BT were accessing water at different depths (Brooks et al., 2006).

Therefore, it is plausible to hypothesize that the reduction of the leaf area of BT led to a smaller leaf transpiration, resulting in a greater availability of water and light for the remaining leaves (Reich et al., 1990; Wallin et al., 2003; Bär et al., 2019). This increase in water and light resource however, did not lead to an increase in radial growth over the period 2018-2020, as showed by tree ring data and as it was reported by previous studies related to fire effects on tree growth (Battipaglia et al., 2014; Valor et al., 2018). On the contrary, the CT showed a significant increase ($P<0.05$) in $\delta^{18}O$ in the post-fire period, and no change in $\delta^{13}C$ ($P>0.05$) between the years before and after the fire. This could suggest that CT experienced an increase in stomatal and photosynthetic activity (Scheidegger et al., 2000) in the post-fire years which resulted in greater radial growth compared to BT. These results would confirm that the reduction in radial growth of BT could be related to crown damage rather than to climatic conditions.

Additional evidence supporting this hypothesis was found in xylogenesis studies. First, the monitoring of xylogenesis in the vegetative season 2019-2020 allowed to exclude that the reduction in growth of BT was connected to a direct damage of the cambium due to fire: none of the trees of this stand showed fire scars and in all individuals the cambium was active. Secondly, the monitoring highlighted important difference in the cambial activity: compared to CT, the BT not only showed a delay in the achievement of the maximum CCs and phenology of formation of SWs and MTs cells (Table 1), but also a lower average number ($P<0.05$) of CCs, SWs and above all of mature xylem cells during the growing season. As known, in conifers the formation of new xylem cells is fueled by the photosynthates of the current year rather than by the stored carbon (Hansen and Beck 1994, Carbone et al. 2013, Deslauriers et al. 2015b). Therefore, the post-fire decline in photosynthetic activity found in BT may have compromised the ability to support the requirement of carbohydrates (Ryan, 1993; Valor et al., 2018) causing severe limitations in the productivity of the cambium

(Hood et al., 2018; Gričar et al., 2020) and in the regrowth of destroyed leaves (Sayer et al., 2020). In fact, in case of severe defoliation, only a small part of the carbohydrate reserves is allocated to growth and foliage, because the majority is used primarily to ensure cell respiration (McDowell, 2011; Jacquet et al., 2014; Varner et al., 2021). Although we sampled a limited number of trees, 4 per each group, the xylogenesis monitoring was consistent among trees, showing a small intra species variability and allowing to infer common responses to the fire effect (De Micco et al., 2019). Further, BT showed a limited xylogenetic plasticity: BT not only formed premature LW1 cells but presented a lack in intra-annual density fluctuations formation in comparison to CT (table 1). Intra annual density fluctuations has been found as an important functional trait to indicate a good capability of the trees to exploit the favourable conditions occurring in the autumn period in such an environment (De Micco et al. 2016b, Zalloni et al. 2016, 2018, 2019). Their occurrence, in several tree species, growing on the Vesuvio area was related to tree high plasticity in xylem formation and as a sign of tree climate adaptation (Balzano et al., 2019).

3.4.2 Influence of climate on xylogenesis

The inter-annual study of xylogenesis allowed to evaluate the influence of the climate conditions on wood formation and dynamics. The vegetative activity in all the sampled trees was active for a long period of time: the xylogenesis was already active in April 2019 (DOY 92_19) and lasted until February 2020 (DOY 49_20). Although many researchers have often assumed a complete interruption of xylogenesis in the cold periods (De Luis et al., 2007; Čufar et al., 2011; del Castillo et al., 2016), the long duration of cambial activity found in our study is a very common condition for conifers living in the Mediterranean coastal areas (Cherubini et al., 2003). In fact, autumn and winter temperatures in these environments may not be low enough to induce cambial dormancy (Sperlich et al., 2014): a study by Prislan et al. (2016) reported that temperatures of 10 °C in January did not induce cambial dormancy in *Pinus halepensis* Mill. Furthermore, Balzano et al. (2018) in a stand of *Pinus pinea* L. located in the Vesuvius National Park, observed active xylogenesis activity for a whole calendar year, linking this process to mild temperatures of the area that prevented cambium stop.

In our study case, the wood formation dynamics in BT and CT perfectly reflected the evolution of the climatic conditions of the experimental area demonstrating that climatic conditions had similar effect in the two groups of trees: the xylogenesis curves of BT and CT followed a bimodal growth pattern with two periods of greater activity, in spring and autumn (Camarero et al., 2010; Campelo et al., 2018; Garcia-Forner et al., 2019). In most analysed plants, the cambial division was already fully active in spring (DOY 92_19) when there was an increase in temperatures (the average temperature was 17 °C) (Figure 1B) reaching peak between April and May (DOY 120-148_19). The positive correlations found in all climatic windows between the temperatures and the number of CCs in BT, as well as with SWs in all trees (Table 2) confirmed that the warmer conditions are the predominant factor that triggers and favours xylogenesis (Körner, 1998; Deslauriers and Morin, 2005; Rossi et al., 2008; Vieira et al., 2014). The positive correlation found between temperatures and CCs and ECs in BT could be attributed to the lower leaf coverage of this stand which involves a greater heating of the trunk determining, as demonstrated by artificial stem heating experiments, a positive effect on the division of the cambial cells (Gričar et al., 2006; Begum et al., 2010). On the contrary, during the summer period (DOY 172 - 265_19), the strong increase in temperature (the average maximum temperature was 31.1 °C) and the scarce precipitation (40.4 mm total) determined at first, the formation of late-wood (LW1) - in July for BT (DOY 204_19) and between July and August for CT (DOY 204-232_19) - and, subsequently, a slowdown in cell division rates (DOY 190–260_19). The negative correlations found in CT and BT between precipitation and the number of CCs and ECs in the long climatic windows (Figure 6) suggested a strong sensitivity of *Pinus pinaster* to water availability. As it is known, in warm and dry condition the conifers tend to avoid hydraulic failure limiting the stomatal activity affecting carbon assimilation (Ripullone et al., 2007; McDowell et al., 2008). Therefore, during a prolonged water deficit the sugars reserves are more likely to be sequestered for osmoregulation and they may not be fully available for growth (Pantin et al. 2013, Deslauriers et al. 2015a). Consequently, it is plausible that *P. pinaster* may not be able to maintain high rates of cell division and this was particularly evident in the BT. In fact, the trees, in order to mitigate the negative impact of drought on the xylem structure, extended the duration of the xylogenesis process: the cells of new formation remain blocked in the phases of differentiation of expansion and thickening (Balducci et al., 2016). This strategy ensures complete cell development when optimal water and temperature conditions are re-proposed (Lachenbruch and Mcculloh, 2014; Balducci et al., 2016). In fact, during the autumn period, the increase in precipitation (607 mm total) and the establishment of optimal temperatures (average temperature was 16.7) induced cambial activity resumption in October (DOY 288 – 302_19) in all sampled trees. The positive correlations found in all trees between mature xylem cell (Figure 6) and $\delta^{13}C$ (Table 2) with precipitations confirm that the availability of water is an important factor for the physiology of this species (Camarero et al., 2010). However, the positive correlations found in CT in the post-fire years between BAI and minimum temperatures (Table 2) suggested that unburned plants took advantage from the favourable climatic conditions of autumn period. On the contrary, in BT we found a negative correlation between the $\delta^{18}O$ and minimum temperatures, this suggested a greater sensitivity of the stomatal closure of these trees which could have influenced their xylem plasticity. Indeed, while three out of the four CT increased their productivity by forming intra-annual density fluctuations with onset of earlywood-like cells (EW2) among October and November (DOY 302 – 326_19), only one BT showed this capacity (Table 1). This finding, in addition to suggesting the greater xylem plasticity of CT, as we previously stated, confirmed previous studies where the formation of earlywood-like cells in the latewood had been correlated with the occurrence of precipitation in autumn after a summer drought (Battipaglia et al. 2010, De Luis et al. 2011a, 2011b, Novak et al. 2013a, 2013b, Zalloni et al. 2016). Subsequently, at the end of December with the onset of winter, the climatic conditions became less advantageous, and the trees that showed IADFs formed again late-wood cells (LW2) (DOY 354_19 – 7_20) (Table 1). In this period, we observed in all studied trees a drastic decline in xylem formation due to the rigid temperatures: between the end of December 2019 and the beginning of March 2020 (DOY 356_19 – 63_20) the average minimum temperature was 6.8 °C, below the threshold temperature for cambial xylem cell production (Rossi et al., 2007; Prislan et al., 2016). This condition led to a stop of the cellular differentiation of trees: on 3 March 2020 (DOY 63_20) cambium was no longer productive in all sampled trees.

3.5 Conclusion

The inter-annual study of dendrochronology showed that, although the trees initially survived to 2017 wildfire, they experienced a medium-term radial growth decrease compared to the unburned pines. At the same time, the isotope analyses suggested that the partial damage to the canopy, which became more severe in the following years, compromised the photosynthetic capacity of the burned stand. On the other hand, intra-annual studies on xylogenesis excluded a direct damage to the cambium and, allowed to highlighted important cambial differences in BT, which showed a delay in the cambial phenology and a clearly lower number of mature xylem cells during the growing season compared to CT. Furthermore, the different xylem plasticity was evident: the CT were able to exploit the favourable autumn conditions forming IADFs and consequently increasing their productivity. Finally, it was possible to explore the dynamics of wood formation related to seasonal climatic variations. Although the temperatures of spring and autumn were very favourable and this allowed a long period of xylogenesis, the hot and dry months of summer drastically influenced the cambial activity causing a strong slowdown of growth especially in BT. In conclusion, our findings allowed to hypothesize that the BT have less chances of recovery; in fact, the strong photosynthetic limitations caused by the crown defoliation and the recurrent drought events could lead these individuals to severe growth decrease and to a reduction of their ability to regain the pre-disturbance productivity rates. However, further monitoring studies are needed:

analyzing the subsequent growing seasons will be essential to understand and evaluate the evolution of future dynamics.

3.6 Reference

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3.7 Figures

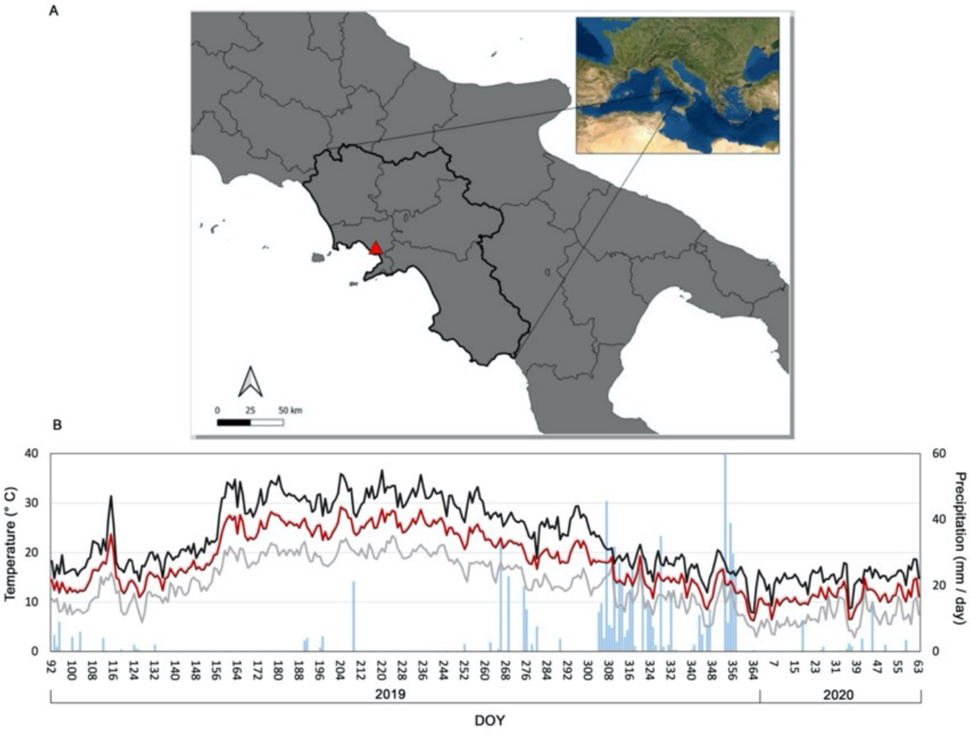


Figure 1 - (A) Location of the study area. (B) Daily trend of maximum (black line), mean (red line) and minimum temperature (gray line) and precipitation (blue histograms) of the study site recorded during the xylogenesis monitoring period 2 April 2019 (DOY 92_19) 3 March 2020 (DOY 63_20).

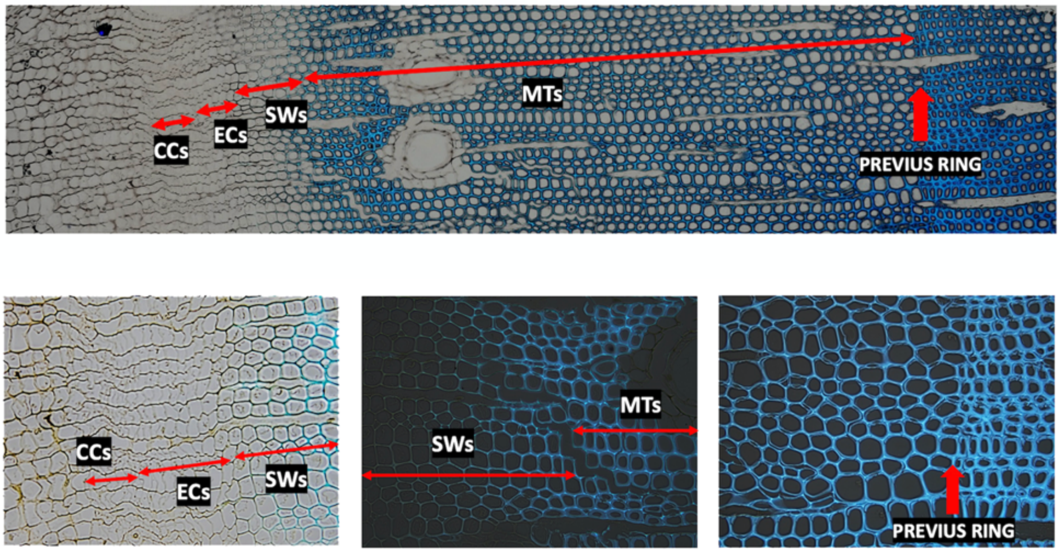


Figure 2 - Development of the xylem in the various phases of cellular differentiation in *Pinus pinaster* Aiton: Cambial cells (CCs); Enlarging cells (ECs); Cells with developing and lignifying secondary walls (SWs); and mature xylem cells (MTs).

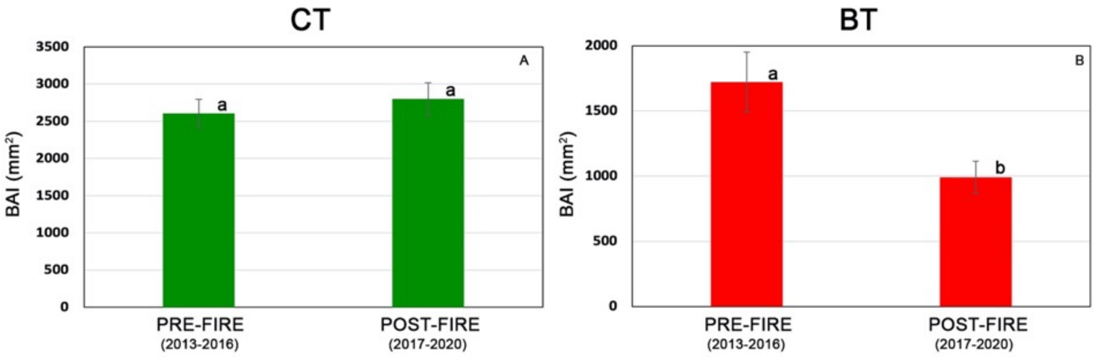


Figure 3 - Average BAI recorded in BT (red histogram) and CT (green histogram) in the years pre (2013-2016) and post (2017-2020) fire. The bars indicate the standard error while different letters indicate significant differences ($P<0.05$) according to one-way ANOVA using Student–Newman–Keuls coefficient for comparison tests.

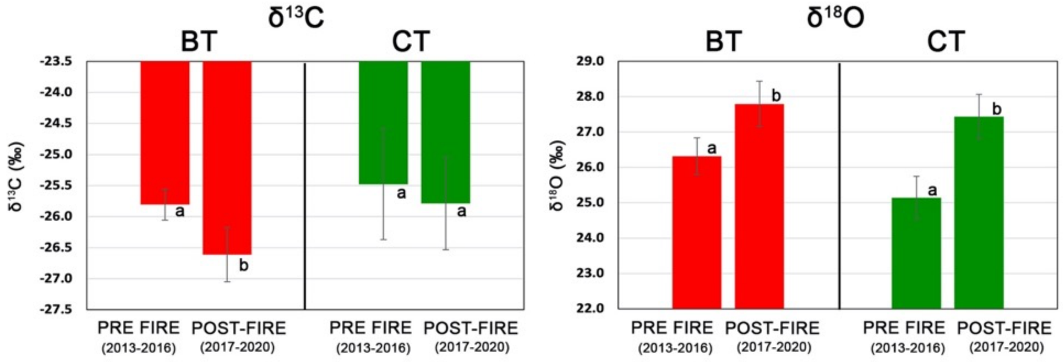


Figure 4 - Average values of the isotope ratio of carbon and oxygen recorded in BT (red histogram) and CT (green histogram) in the years pre (2013-2016) and post (2017-2020) fire. The bars indicate the standard error while the letters indicate statistically significant differences ($P<0.05$) according to one-way ANOVA, using Student–Newman–Keuls coefficient for comparison tests.

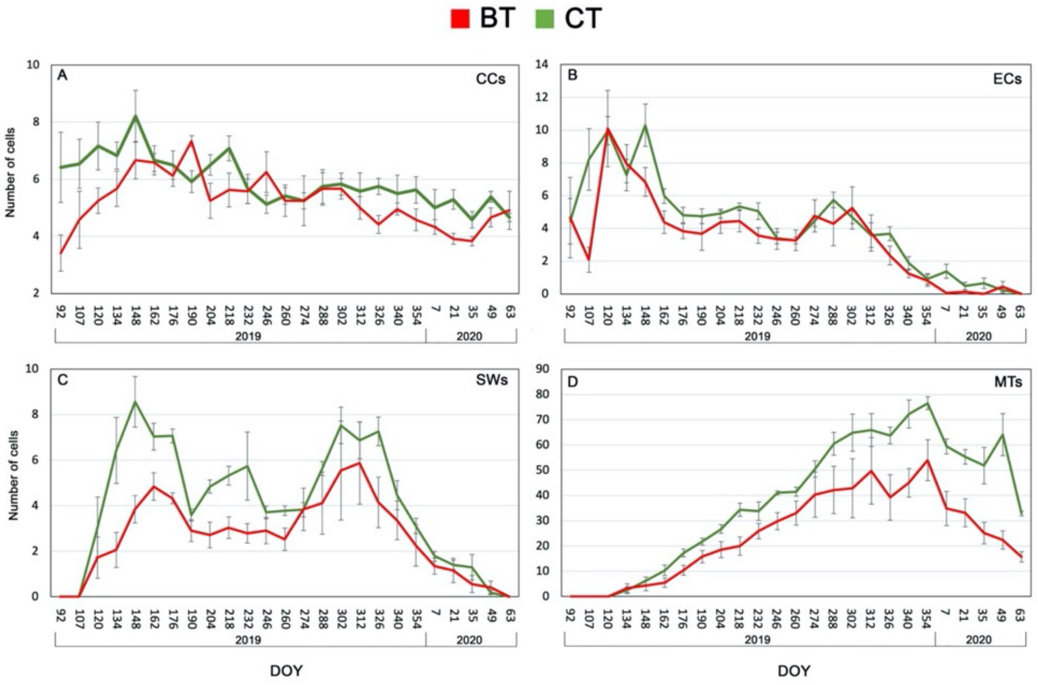


Figure 5 - Mean number of cells in the various phases of xylem formation observed in the BT (red line) and CT (green line) during the period between 2 April 2019 (DOY 92_19) and 3 March 2020 (DOY 63_20). A - cambial cells (CCs), B - enlarging cells (ECs), C - cells with development and lignifying of secondary walls (SWs), D - mature cells (MTs). Bars indicate standard errors. The day of the year and corresponding year is reported on the x-axis.

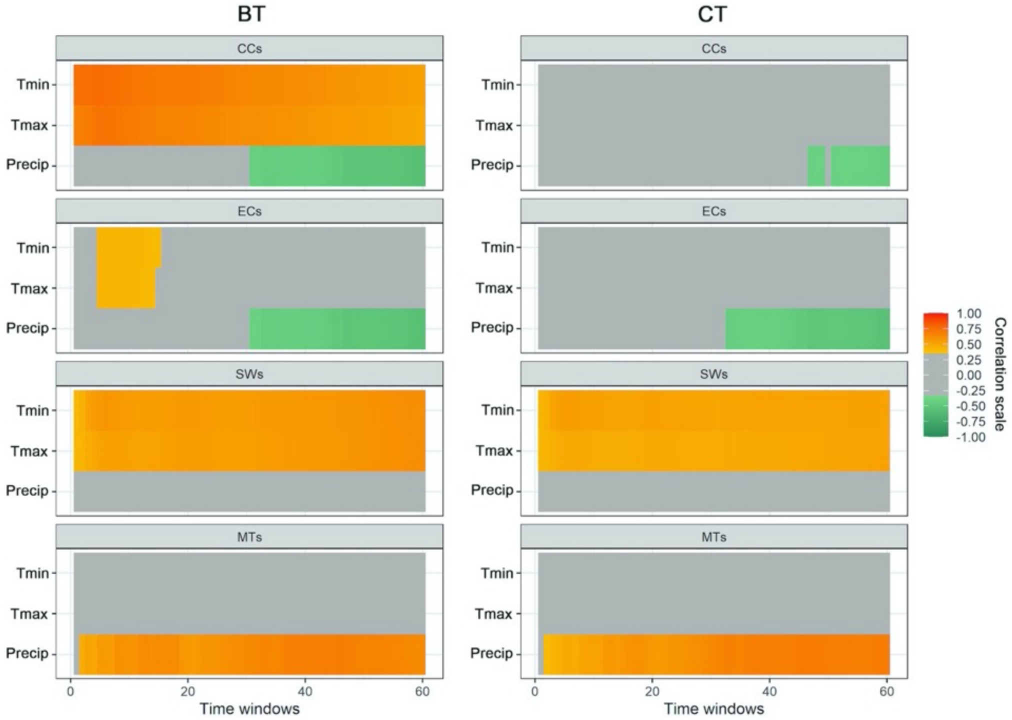


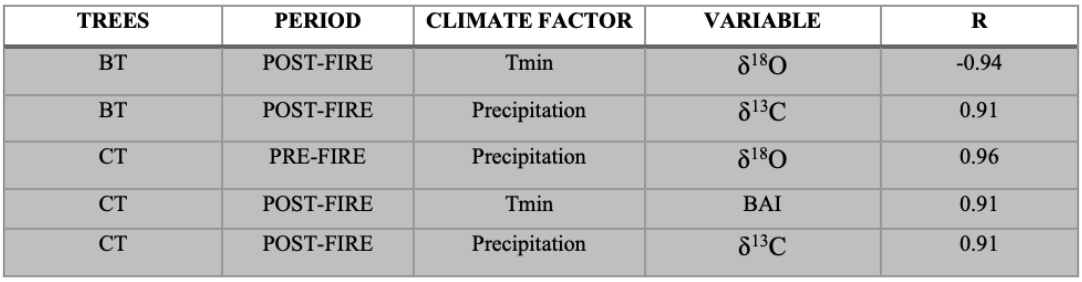
Figure 6 - Correlations between cambial cells (CCs), enlarging cells (ECs), cells with development and lignifying of secondary walls (SWs), and mature cells (MTs) observed in the BT and CT with absolute maximum and minimum temperatures and daily precipitation applying moving averages: the number of cells observed in each sampling day was correlated with the climatic data of the day before, with the average of the two previous days, three previous days and so on up to 60 days. Spearman correlation coefficient above │0.38│ are significant at P<0.05, n=25.

3.8 Tables

Table 1 - Cambial phenology in the in BT and CT. The dates indicate the onset of the specific cell-type. "-" in the crow defoliation indicates that the tree did not showed canopy damage. "*np*" in CCs and ECs indicates that the tree already presented CCs and ECs at the first sampling day. "*na*" in EW2 and LW2 indicated that the tree did not form IADFs.

	BT				**CT**			
Tree	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Crown defoliation	**50%**	**50%**	**50%**	**50%**	**-**	**-**	**-**	**-**
CCs	*np*	30 April 2019 **DOY 120_19**	*np*	*np*	*np*	*np*	*np*	*np*
ECs	*np*	30 April 2019 **DOY 120_19**	17 April 2019 **DOY 107_19**	30 April 2019 **DOY 120_19**	17 April 2019 **DOY 107_19**	30 April 2019 **DOY 120_19**	*np*	*np*
SWs	30 April 2019 **DOY 120_19**	28 May 2019 **DOY 148_19**	14 May 2019 **DOY 134_19**	28 May 2019 **DOY 148_19**	30 April 2019 **DOY 120_19**	30 April 2019 **DOY 120_19**	14 May 2019 **DOY 134_19**	14 May 2019 **DOY 134_19**
MTs (EW1)	14 May 2019 **DOY 134_19**	28 May 2019 **DOY 148_19**	11 June 2019 **DOY 162_19**	25 June 2019 **DOY 176_19**	14 May 2019 **DOY 134_19**	28 May 2019 **DOY 148_19**	28 May 2019 **DOY 148_19**	28 May 2019 **DOY 148_19**
MTs (LW1)	23 July 2019 **DOY 204_19**	23 July 2019 **DOY 204_19**	23 July 2019 **DOY 204_19**	23 July 2019 **DOY 204_19**	20 August 2019 **DOY 232_19**	6 August 2019 **DOY 218_19**	6 August 2019 **DOY 218_19**	23 July 2019 **DOY 204_19**
MTs (EW2)	*na*	*na*	22 November 2019 **DOY 326_19**	*na*	*na*	22 November 2019 **DOY 326_19**	29 October 2019 **DOY 302_19**	29 October 2019 **DOY 302_19**
MTs (LW2)	*na*	*na*	20 December 2019 **DOY 354_19**	*na*	*na*	20 December 2019 **DOY 354_19**	7 January 2020 **DOY 7_20**	20 December 2019 **DOY 354_19**

Table 2 - Correlations between BAI $\delta^{13}C$ and $\delta^{18}O$ recorded in BT and CT during the pre (2013-2016) and post fire (2017-2020) years with the corresponding minimum (Tmin) and maximum (Tmax) temperature, and precipitation. Spearman correlation coefficient above $|0.88|$ are significant at $P<0.05$, $n=4$.



TREES	PERIOD	CLIMATE FACTOR	VARIABLE	R
BT	POST-FIRE	Tmin	$\delta^{18}O$	-0.94
BT	POST-FIRE	Precipitation	$\delta^{13}C$	0.91
CT	PRE-FIRE	Precipitation	$\delta^{18}O$	0.96
CT	POST-FIRE	Tmin	BAI	0.91
CT	POST-FIRE	Precipitation	$\delta^{13}C$	0.91

3.9 Supplementary materials

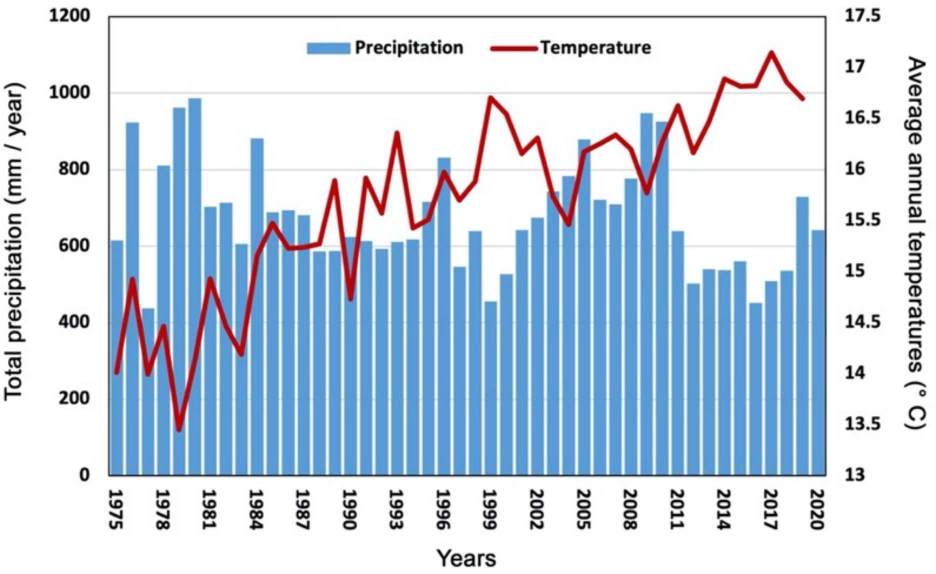


Figure S1 - Pluvio-thermal diagram of the study area from 1975 to 2020 (KNMI database Climate Explorer).

BT

CT

Figure S2 - BT with high defoliation (on the left), CT not defoliated (on the right).

Chapter 4

4. Multiparametric approach to assess wildfire impacts on ecophysiological and hydraulic properties of *Pinus Pinaster* Aiton

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Manuscript submitted in Agricultural Forest and Meteorology

4.1 Introduction

Forests play an essential role in mitigating climate change effects, and in maintaining ecosystem services (Bonan, 2008; Intergovernmental Panel on Climate Change, 2018). However, forest stresses such as drought and forest fires negatively affect their integrity, leading to a reduction of productivity and high mortality rates (Bond et al., 2005; Allen et al., 2010; Yi et al., 2015). Fire and water shortage are strongly interconnected: trees subject to chronic drought are more likely to die if struck by a fire, on the other hand, burnt plants, although they survive, are more susceptible to subsequent episodes of water stress (van Mantgem et al., 2013, 2018). The physiological mechanisms underlying tree survival and death have been at the centre of scientific debate for years but are still poorly understood (McDowell et al., 2008; Choat et al., 2012; Hartmann et al., 2013, 2015; Adams et al., 2017; Cailleret et al., 2017; Kono et al., 2019). However, several studies agree that xylem hydraulic failure is the main mechanism leading to forest decline and death (McDowell et al., 2008; Kursar et al., 2009; Anderegg et al., 2016; Adams et al., 2017). In general, tree survival is closely related to the ability to control water loss during prolonged periods of drought (McDowell et al., 2008). Low soil water availability and strong evaporative demand can put under stress the transport system triggering the formation of air bubbles in the xylem vessels that break the continuity of the water column (Tyree and Zimmermann, 2002). Therefore, in extreme drought conditions, widespread xylem cavitation can result in the total arrest of the sap flow with the consequent drying of the tissues and cells death (Urli et al., 2013). Several studies showed that hydraulic failure is often coupled with carbon starvation, induced by stomata closure due to extreme climate conditions (Adams et al., 2017; Puchi et al., 2021). Drought stresses can be amplified in plants affected by fire. The high temperatures reached during fires, apart from causing important cavitation events, can trigger structural alterations of the xylem vessels (Balfour and Midgley, 2006; Michaletz et al., 2012; West et al., 2016; Bär et al., 2018), influencing the hydraulic efficiency and safety of plants and increasing the chances of post-fire mortality by cavitation (Zwieniecki and Holbrook, 2009; Nardini et al., 2011; Bär et al., 2019). Alterations in the xylem can also affect the architecture and functionality of bordered pits (Hacke et al., 2006; Plavcová et al., 2013; Li et al., 2016), i.e. the structures capable of blocking the air spreading from embolized vessels towards functional ones (Zimmermann, 1982), reducing its sealing reliability during drought stress and its resistance to hydraulic failure (Delzon et al., 2010; Nardini et al., 2011; Brodersen and McElrone, 2013; Bär et al., 2019). Furthermore, fire events can produce large canopy defoliation, inducing temporarily or permanently changes in stomatal and photosynthetic activity and thus leading to impaired carbon uptake (Battipaglia et al., 2014, 2016; Niccoli et al., 2019). While the hydraulic efficiency of trees can be evaluated through the study of the xylem anatomical traits, the resistance to embolism can be estimated not only by the study of the functional characteristics of the bordered pits but also through the cavitation vulnerability curves which allow to evaluate the percentage loss of sapwood conductivity as the xylem pressure decreases (Cochard et al., 2010, 2013). The P50 (i.e. the water potential at which 50% of the hydraulic conductivity is lost) is universally used to compare the plant drought resistance at both inter-specific (Delzon et al., 2010; Bouche et al., 2014; Torres-Ruiz et al., 2017) and at intra-specific levels (Lamy et al., 2014; David-Schwartz et al., 2016). Although cavitation vulnerability curves provide valuable information on tree plant tolerance limits to water stress (Brodribb and Cochard, 2009; Brodribb et al., 2010), understanding the strategies species adopt to overcome stress events is very limited due to the lack of direct and continuous tests (McDowell and Sevanto, 2010; Sala et al., 2010). Most of the research currently available on drought effect on trees has considered only single events, as the severe drought of 2003 (Ciais et al., 2005; Czajkowski et al., 2005; Leuzinger et al., 2005; van der Werf et al., 2007; Schuldt et al., 2020). In recent years, the development of thermoelectric technologies for monitoring the sap flux density has made it possible to estimate the transpiration and hydraulic dynamics of plants with a very high temporal resolution (Granier, 1985; Andrade et al., 1998; Braun and Schmid, 1999; Do and Rocheteau, 2002; Čermák et al., 2004; Lu et al., 2004; Poyatos et al., 2016). In this context, our research aims to study the xylem hydraulics of *Pinus pinaster* Aiton which grows in an area of southern Italy subject to decennial drought stress and affected by a severe forest fire in the summer of 2017. We combined the continuous monitoring of sap flow and micro-stationary climatic conditions with anatomical analyses of tree rings and cavitation vulnerability studies to evaluate the effects of fire on the ecophysiology and hydraulics of a burned pine forest with severe defoliation compared to a nearby control stand where no fire occurred. The main objectives of this study were to evaluate the impact of the wildfire on the xylem vessels in terms of hydraulic efficiency and safety, and to analyze the survival strategies of burnt plants growing in a dry area. We hypothesize that *Pinus pinaster* damaged by fire and with serious reduction of crown are more affected by water shortage in comparison to control plants in terms of vulnerability and sap flow.

4.2 Materials and Methods

4.2.1 Study area

The two *Pinus pinaster* stands, (mean age 35±10), cover about 1 ha each and are located along the southern slope of the Vesuvio volcano in the integral reserve of the Vesuvius National Park, in southern Italy, at about 650 m asl (Figure 1). The pines in the burned site (BS - 40°48'43.00"N; 14°24'44.64"E) were hit by a low-medium severity fire in July 2017 (Battipaglia et al., 2017). Immediately after fire, trees presented scorch on the trunk between 2.5 and 3 m from the ground (Niccoli et al., 2019), as well as a mild defoliation (around 10%). However, in the following years the crown damage increased: the average site defoliation was estimated around 50% in 2020 and 60% in 2021. The trees in control site (CS) (40°48'35.80'N; 14°24'52.70'E), used as reference site for unburned conditions were characterized by the absence of damage to the canopy and trunk from fire (Niccoli et al., 2019).

4.2.2 Installation of TreeTalker system

At the end of June 2020, 10 dominant trees in BS (mean DBH 34.5 ± 3.3, mean height 20 ± 0.16 m) and 10 dominant trees in CS (mean DBH 26.2 ± 6.1 m, height average 14.6 ± 0.24 m) were randomly selected and a TreeTalker ® device (TT +) was installed on each tree. Each TT+ (oriented to the North to avoid direct solar heating of the sensors) and its corresponding battery pack (oriented to the South to facilitate recharging by solar panel mounted on its case) were attached to the trunk of the plants by a belt and housed respectively on two support plates placed at a height of about 230 cm from the soil in order to reduce the risk of voluntary or accidental manipulation by strangers. In addition, a further device, the TT-Reference (TT-R), was housed in an area without vegetation and in the proximity of the study areas to allows the monitoring of the reference environmental parameters, such as air temperature and humidity, and the total light incident on the study area.

4.2.3 Technical characteristics and measured parameters

The TT + is a multiparameter device, designed and developed by an Italian research team (Valentini et al., 2019) and marketed by the start-up Nature 4.0 (www.nature4.org), consisting of a microcontroller with an ATMega 328 processor chip enclosed in a rectangular case (11.5x6.5x6 cm) powered by high efficiency lithium batteries (3.7 V). The device is able, thanks to the presence of specific sensors (Figure 2), to continuously monitor various environmental and physiological parameters of the tree. In particular, it measures radial growth through an IR proximity sensor positioned few centimetres from the surface of the trunk using two carbon fiber sticks; the tree stability through an accelerometer and the amount of light that penetrates through the canopy, divided into 12 spectral bands, using two spectrometers (VIS and NIR) mounted on the top of the case. Furthermore, it is possible to monitor the information of air temperatures and humidity thanks to a thermo hygrometric sensor covered by a membrane in Goretex incorporated in the microcontroller (Valentini et al., 2019; Matasov et al., 2020) allowing to evaluate the microclimatic conditions of the forest and to calculate the trend of the Vapor Pressure Deficit (VPD). This parameter is essential for evaluating the evaporative demand of the surrounding environment, and consequently allows for the interpretation of the stomatal activity of the tree population as a function of varying environmental conditions (Verbeeck et al., 2007). The VPD allowed to estimate the difference between the vapor pressure of the air in the stomata (es) and the vapor pressure of the air outside the leaf (ea):

$$\text{VPD (kPa)} = \text{es} - \text{ea} \qquad \text{equ. 1}$$

Where *es* = 0.6108 * Exp (17.27 * T / (T + 234.175),
ea = (RH / 100 * es),
T is the temperature expressed in ° C,
RH is the relative humidity percentage.

In addition to the above-mentioned parameters, the TT+ is provided also with a sensor to measure continuous real-time data of the sap flux density based on the transient thermal dissipation (TTD) technique (Do and Rocheteau, 2002; Do et al., 2011, 2018) with a cyclic heating system of 10 minutes of heating - 50 minutes of cooling. The technique is based on the temperature difference between two paired probes, a reference probe and heater probe which are inserted into the xylem to a depth of 20 mm and placed at 10 cm distance along the vertical axis of the trunk. While the probe in the higher position is heated, the lower one provides the reference temperature of sapwood. In the TTD approach, the transient signal (dT) is the relative change in temperature over the heating period, calculated as the temperature difference between the probes reached at the end of the heating phase (ΔT_{on}) and the one just before the heating phase (ΔT_{off}):

$$\boldsymbol{dT\ (°C) = \Delta T_{on} - \Delta T_{off}} \quad \text{equ. 2}$$

To estimate the sap flux density (SFD), a non-species-specific calibration equation is used by applying dT_0 , which is the maximum transient signal obtained under zero flow conditions and dT_u as the measured signal at a given sap flux density, *SFD* (l/m^2h) (Do and Rocheteau, 2002; Do et al., 2011, 2018).

$$SFD = 12.95 \left[\frac{dT_0}{dT_u} - 1\right] * 27.\overline{7} * \left(\frac{3600}{1000}\right) \quad \text{equ. 3}$$

Finally, to calculate the total sap flow (*SF*, l/h) of each tree, the sap flux density (l/m^2h) was multiplied with the relative cross-section of sapwood area (*SA*, m^2). *SA* was estimated using the translucence method (Quiñonez-Piñón and Valeo, 2018): from each tree with the TT+, 4 wood-cores were sampled in the N, S, E and W directions using a 5 mm increment Borer (Haglöf Sweden). The samples were labelled, sealed in silver paper, and placed in the refrigerator. At the same time the diameter at breast height (*DBH*) was measured. In laboratory, each sample was first left for two hours at room temperature and then immersed in distilled water for 20 minutes. The wood-core was then exposed to an artificial light source and the sapwood depth (*sd*) was estimated as the length of the portion lighter in colour, while the darker part was considered heartwood (Světlík et al., 2013; Børja et al., 2016). The sapwood area was estimated through the following equation (Quiñonez-Piñón and Valeo, 2018):

$$SA = (sd * DBH - sd^2) * \pi \quad \text{equ. 4}$$

Where *SA* is the sapwood area (m^2), *sd* is the average of the sapwood depth calculated in the 4 cores extracted from the single tree (m), *DBH* is the diameter calculated at breast height (m).

4.2.4 Data transmission

Each TT+ device of the TreeTalker network is set to record all measurements every 60 minutes, which are stored in the internal memory and also transmitted via radio, through the supplied LoRa chipset, to a gateway device called TT-Cloud, installed in each experimental stand. Once the data of all nearby TT+ devices are collected, the TT-Cloud transmits via GSM/GPRS connection the data to a central hub at Unicampania, on an hourly basis, allowing real time observation of physiological and environmental parameters of the plants measured remotely. All data generated by a single device are marked with a univocal serial code which allows direct association of data string to TT+ device and hence individual tree performance.

4.2.5 Tree growth and wood anatomy

To evaluate possible differences in productivity between the pre-fire (2015-2016) and post-fire (2017-2020) years, in spring 2021 trees with TT devices were sampled in BS and CS sites at a height of 1.3 m from the ground (NS and EW tree quadrants) with an increment borer (Haglöfs, Sweden) of 5 mm, containing at list 6 tree-rings. The samples were sanded and the tree rings width (TRW) was measured through the LINTAB system (Frank Rinn, Heidelberg, Germany). The chronologies of the plants were cross-dated through TSAP-Win using the Gleichaeufigkeit (GLK) parameter, which evaluates the concordance of trends between the different series (Eckstein and Bauch, 1969). Synchronization was considered acceptable at GLK> 70. Subsequently, the data were first statistically analyzed using the COFECHA software and then standardized using the ARSTAN software (Cook and Holmes, 1986).

At the same time, to investigate possible xylem structural and hydraulic alterations between the years pre-fire and post-fire, 12 mm diameter wood cores containing the last 6 tree-rings were also taken from the same trees in CS and BS sites. In the laboratory, the wood-cores were first boiled in water to soften the wood and remove excess resin and then cut into transversal sections of about 10-12 µm using a rotary microtome. Subsequently, the sections were stained using a safranin solution (1%) and rinsed with water and two increasing concentration solution of ethanol (50% and 95%). Finally, the sections were permanently fixed on a glass-slide using Eukitt mounting solution (Bioptica, Milan, Italy) and photographed using a high-resolution color camera (JVC TK-C701EG) connected to an optical microscope (Nikon Eclipse E200) with a 40x magnification. Therefore, the images were analysed using ROXAS v3.0.31 (Von Arx and Dietz, 2005; von Arx and Carrer, 2014; Prendin et al., 2017) to quantify lumen area (La) (μm^2), theoretical hydraulic conductivity (Kh) (m3/MPa s), and cell wall thickness (CWT) (µm).

4.2.6 Xylem vulnerability to cavitation

Contextually at sampling for tree-rings analysis, branches with a diameter of about 0.7 cm under bark were collected on 20 trees in both the BS and CS site to estimate vulnerability curves using the Cavitron technique (Cochard, 2002; Cochard et al., 2005). Each sample collected was immediately stripped of the leaves and was subsequently wrapped in wet paper, sealed in bags, and shipped to the PHENOBOIS platform (University of Bordeaux, Talence, France; http://sylvain-delzon.com/caviplace). In the laboratory, the branches were debarked and cut underwater to 27 cm in length. The samples were then installed in a

Cavitron equipped with a 28 cm diameter honeycomb rotor (DGMeca, Gradignan, France) and a camera (Scout Sc640gm, Basler, Germany). The Cavitron was connected to a dedicated computer provided with a specific software (Cavisoft v.4.0, University of Bordeaux) for the control of the experiment parameters and for the data acquisition. Initially, the maximum hydraulic conductance of the stem (*Kmax*) was measured by subjecting the sample to xylem pressure close to zero (low speed). Subsequently, the speed rotation of the centrifuge was gradually increased to lower the xylem pressure with steps of –0.5 or –1 MPa. The change in the hydraulic conductance of the samples was measured by injecting into the Cavitron a reference ionic solution of 10 mM KCl and 1 mM $CaCl_2$ that flowed through the stem. The percentage loss of hydraulic conductivity (*PLC*) was calculated at each pressure step according to the following equation to obtain a vulnerability curve per sample:

$$PLC = 100 * (1 - \frac{K}{Kmax}) \quad \text{equ. 5}$$

where *Kmax* is the maximum conductance of the stem,
K is the conductance associated with each pressure step.

The vulnerability curves were fitted using a sigmoidal function (Pammenter and Willigen, 1998) with SAS 9.4 software (SAS Institute, Cary NC) using the following equation:

$$PLC = \frac{100}{[1 + \exp(S * (P - P50))]} \quad \text{equ. 6}$$

where *S* (% MPa-1) is the slope of the vulnerability curve at the inflection point,
P is the xylem pressure value used at each step,
P50 is the xylem pressure that induces a 50% loss of hydraulic conductivity.

Finally, to characterize morphological and functional traits of bordered pits in both CS and BS trees, 12 branches for each site were randomly selected for xylem anatomy analyses. The samples were cut tangentially with a razor blade, coated with a layer of gold in high vacuum by an automatic sputter coater (Cressington 108auto, Watford, UK) and dehydrated in oven for 48 hours at 70°C. Subsequently, the sections were observed and photographed using a scanning electron microscope (SEM, PhenomG2 pro; FEI, Netherlands). Torus overlap against the pit aperture (*O*), Margo flexibility index (*F*), and valve effect (V_{ef}) were calculated following Delzon et al. (2010):

$$O = \frac{(Dt-Da)}{Dt}\ ;\ F = \frac{(Dm-Dt)}{Dm}\ ;\ V_{ef} = F\ x\ O \quad \text{equ. 7}$$

Where *Dm*, *Dt* and *Da* are respectively, pit membrane diameter, torus diameter and pit aperture diameter of each bordered pits. Measurements were performed on 40 bordered pits per sample using ImageJ software.

4.2.7 Statistical analysis

For all data, before applying the inferential statistic, normality was checked using the Shapiro-Wilk test while Levene's mean test was used to assess equal variance. To compare the different data of the TreeTalkers, the results of the analysis on tree rings, and the parameters of vulnerability to embolism of the two pine forests a one-way ANOVA, using the Student-Newman-Keuls coefficient for multiple comparison ($P<0.05$). Data analyses were carried out using the Sigmaplot software.

4.3 Results

4.3.1 Structural and hydraulic analysis of the xylem

4.3.1.1 Xylem traits

Xylem hydraulic and productivity traits in the pre-fire (2015-2016) and post-fire (2017-2020) years were compared. Data showed no significant difference ($P>0.05$) in lumen Area (LA) and theoretical hydraulic conductivity (Kh) between the two analysed periods for BS stand, whereas we found a significant increase ($P<0.05$) of these parameters for the wood sampled in CS stands in the period 2017-2020 (Figure 3A, B). Conversely, the traits related to the wood carbon sink, such as cell wall thickness (CWT) and tree-rings width (TRW - Index) (Figure 3C, D) did not show statistical differences in CS plants ($P>0.05$) between the two periods, while both traits significantly decreased in the post-fire period in the wood of the BS stand.

4.3.1.2 Xylem vulnerability

The loss of hydraulic conductivity (*PLC*) estimated over a range of applied pressures in the cavitron provided a clear sigmoidal relationship for all the tested pine branches allowing to define average vulnerability curves for both BS and CS stands (Figure 4).

No significant differences were found between samples of BS and CS stands for any of the hydraulic traits that are most commonly used to define and describe the vulnerability curve, P12, P50, P88, which represent the the xylem pressure that induces a 12%, 50% and 88% loss of hydraulic conductivity, respectively and *S* (% MPa-1), which is the slope of the vulnerability curve at the inflection point (Table 1).

The micro-morphological study of bordered pits did not show significant differences in the traits linked to the resistance to the embolism between the plants of BS and CS stands (Figure 5). In fact, Torus Overlap (*O*), Margo Flexibility Index (*F*), and Valve Effect (V_{ef}) showed similar values in the two sample groups (P>0.05).

4.3.2 Monitoring in *continuum* through the TreeTalker system

4.3.2.1 Meteorological data of the study area

The combination of the maximum minimum and average daily temperature data recorded by the TT-Reference with the daily rainfall data from the nearby meteorological station (located 4.8 km away from the pine forests examined; 40°47'16.9"N; 14°27'50.4"E; www.agricoltura.regione.campania.it) allowed to characterize the climatic conditions of the study area with high resolution (Figure 6). During the examined period (20 June 2020 - 22 September 2021) the total rainfall was 958 mm, while the average temperature was 18.5°C, with maximum and minimum of 32°C and 11°C respectively. In the summer of 2020 (20 June 2020 - 21 September 2020) the total rainfall was 51 mm and the average temperature was 26.1°C with maximum of 42.7°C and minimum of 16.6°C. In autumn 2020 (22 September 2020 - 21 December 2020), while the total precipitation was 356.2 mm, the average temperature was 12°C, with maximum and minimum respectively of 22.3°C and 7.5°C. On the other hand, during the winter months (22 December 2020 - 21 March 2021), 401 mm of rain and average temperature of 9.2°C was recorded with maximum and minimum temperature of 16.1°C and 4.9°C respectively. In the spring of 2021 (22 March 2021 - 21 June 2021) the total rainfall was 47.2 mm and the average temperature was 17.3°C with maximum of 32.8°C and minimum of 8.5°C. Finally, during the summer of 2021 (22 June 2021 - 22 September 2021), while the total rainfall was 65 mm, the average temperature was 27.5°C, with maximum and minimum respectively of 45°C and 17.4°C.

4.3.2.2 Seasonal monitoring of sap flux and environmental parameters

The data of sap flow (*SF*) and vapor pressure deficit (VPD) measured by the TT+ in the BS and CS stands and incoming radiation measured by the TT-Reference in the clear area outside the forest stands are reported in Figure 7 A, B and C, respectively, for the period June 2020-September 2021, with reference to the daily hours from 6 am to 8 pm considered the most relevant for the monitored tree activities. In summer 2020 (20 June 2020 - 22 September 2020) the TT + devices recorded a rather stable *SF* in both stands, without any clear peak of transpiration, throughout the day. However, the *SF* values in BS were significantly ($P<0.05$) higher than in CS, with mean values equal to 5.93 ± 0.81 l/h and 4.33 ± 0.31 l/h (mean ± SE), respectively. Statistical differences were also found in the values of VPD ($P <0.05$), which was on average higher in the BS stand (1.4 ± 0.09 kPa) than in the CS stand (1.1 ± 0.06 kPa). During the autumn period (23 September 2020 - 21 December 2020) no statistical differences were found between stands ($P >0.05$): CS showed a similar *SF* (4.53 ± 0.54 l/h) than

BS (4.03 ± 0.30 l/h), as well as the VPD was comparable ($P>0.05$) in both stands (0.25 ± 0.01 kPa in the CS, 0.30 ± 0.01 kPa in the BS). Similarly, in the winter period (22 December 2020 - 20 March 2021) and spring (21 March 2021 - 21 June 2021), no significant differences were found in *SF* ($P>0.50$). Both pine stands showed the lowest *SF* in the colder months of winter (4.94 ± 0.42 l/h in BS, 4.15 ± 0.44 l/h in CS) and the highest *SF* values during spring (8.91 ± 1.33 l/h in BS, 7.64 ± 0.86 l/h in the CS). As observed in autumn, also in winter and spring there was no difference in the VPD between BS and CS stands ($P>0.50$), which VPD reaching the lowest values during winter (0.19 ± 0.01 kPa in the CS; 0.23 ± 0.01 kPa in the BS) and higher values in spring (0.54 ± 0.04 kPa in the CS; 0.52 ± 0.01 kPa in the BS), although not as high as in summer. During the summer 2021 (22 June 2021 - 22 September 2021) the trend of *SF* was very similar to that recorded in the previous summer, with a quite flattened trend of sap flow during the day. However, in summer 2021 *SF* was much higher ($P<0.05$) in BS (8.87 ± 1.75 l/h) compared with the previous year and compared with the *SF* recorded in the CS stand in the same summer 2021 (4.28 ± 0.82 l/h), although also in this case no statistical difference ($P>0.05$) was found between VPD values in the two stands (1.19 ± 0.08 in the CS, 1.45 ± 0.10 in the BS). The VPD seasonal trend followed the incoming radiation trend with a summer and a spring peak value 3 and 2 times the peak value recorded in winter. Data of total incident light from the first summer are not shown due to a problem excessive out of scale data which did not allow for a robust evaluation. The problem was solved by introducing a different scaling factor in the TT-Reference software in the following seasons.

Figure 8 shows the transmitted radiance measured by the TT+ below canopy and is reported as season mean value of the daily average of all the TT+ devices in the hours of maximum light (11 am - 14 pm) in each stand, calculated for each wavelengths of the TT+ spectrophotometer. Data show that the transmitted radiance was higher in the BS stand compared with the CS stand in all the seasons, with significant differences measured in summer 2020, 2021 and spring 2021.

4.4 Discussion

4.4.1 Effect of wildfire on xylem hydraulic traits

Data on anatomical traits showed that 2017 fire event did not induced any clear alteration of xylem vessels in the burned pine. Indeed, no significant changes ($P>0.05$) of lumen Area (LA) and in theoretical hydraulic conductivity (Kh) was found between the years before (2015-2016) and after (2017-2020) fire. Several studies reported that wildfires can induce severe hydraulic dysfunctions in plants: the heat released by flames can trigger structural alterations of the xylem that affect the hydraulic efficiency and safety of trees, increasing the risk of embolism (Balfour and Midgley, 2006; Midgley et al., 2011; Michaletz et al., 2012; West et al., 2016; Thompson et al., 2017; Bär et al., 2019). Hydraulic efficiency can be reduced if plant tissues are exposed for a prolonged time at temperatures around 60°C or higher (Michaletz and Johnson, 2007; Kelsey and Westlind, 2017). If these conditions are reached, the polymers that make up the wood (lignin, cellulose, and hemicellulose) can soften inducing deformations in the xylem vessels which become permanent when the wood cools (Michaletz et al., 2012; Bär et al., 2019). However, our results allow to hypothesize that the temperature and/or the exposure time of flames of

2017 wildfire were not high enough to trigger damage to the xylem. Those findings agree with a xylogenesis study carried out in the BS by Niccoli et al. (Tree Physiology – under review) where no xylem injury or cambial damage in the short and medium period after fire (2017-2020) were reported. On the other hand, it is well known that the thick bark of the maritime pine guarantees a lower exposure of xylem tissues to heat, consequently increasing the chances of tree survival (Fernandes et al., 2008).

Wildfires can also influence xylem hydraulic safety by inducing xylem structural alterations which modifying the xylem vulnerability to cavitation. For example, Bär et al. (2018) provided important experimental evidence by observing a 49% reduction in the xylem hydraulic conductivity due to cavitation in branches of *Pinus sylvestris* L. and *Fagus sylvatica* L. subjected to temperatures of 90°C. The vulnerability curves, obtained in this study, showed that hydraulic safety traits were comparable in pine growing in both BS and CS stands with a P50 values around - 3.7 MPa, which is in line with previously research conducted on *Pinus pinaster* (Corcuera et al., 2011; Lamy et al., 2011, 2014). This suggested that the wildfire did not induce a change in long term cavitation resistance in the tree exposed to fire (BS). Moreover, the micro-morphological analyses on the bordered pits of tree from both stands showed that fire did not induce any difference in the functional properties of the torus-margo structure, which plays a fundamental role in cavitation spread, preventing air bubble to spread within the vascular system (air-seeding) (Sperry and Hacke, 2004; Delzon et al., 2010; Bouche et al., 2014). A better torus overlap against the pit aperture (O) assisted by a high margo flexibility (F) determines a better valve effect (V_{ef}) which allows to seal the pit aperture more effectively during episodes of drought stress (Pittermann et al., 2005; Delzon et al., 2010; Bouche et al., 2014; Roskilly et al., 2019). In the analyzed *P. pinaster* trees from BS and CS, the values of *O*, *F*, and V_{ef} were utterly comparable. Thus a similar functional capacity of the bordered pits might be expected in CS and BS trees, corroborating the evidence that fire in BS did not influence the hydraulic safety of burnt plants. These finding are also in agreement with data of Battipaglia et al. (2016) who described the absence of alterations in the xylem structure and in the hydraulic safety in a stand of *Pinus pinea* L. subjected to a prescribed fire compared to a control pine forest, concluding that the temperature of the fire was not sufficient to cause internal damage.

On the other hand, while the trees in the BS stand showed no significant reduction of the lumen Area (LA) and theoretical hydraulic conductivity (Kh) in pre and post fire period, the tree of the CS stand which did not experience fire showed a significant increase of both parameters in the year 2017-2020. Moreover, a reduction in cell wall thickness and tree ring width was observed after fire in the BS stand, while this reduction was not observed in the CS stand. These data suggest an indirect effect of fire on C sequestration and hence C allocation rather than a physical effect on anatomical hydraulic structures. Indeed, the canopy cover in the burned stand (BS) was found to be between 50 and 60% lower than in the CS stand as one of the main consequences of fire. Such canopy reduction might translate into lower overall gross primary productivity of the tree and hence less C to be allocated to non-photosynthetic organs.

4.4.2 Sap flow *continuum* monitoring

The monitoring of sap flow (*SF*) with the TreeTalker system allowed to investigate the effect of the wildfire on the eco-physiology dynamics of the plants. Our data showed a strong influence of VPD on the seasonal water use. VPD can be considered a key driving force of tree transpiration due its ability to influence water potential (Will et al., 2013) and stomatal conductance of plants (Verbeeck et al., 2007). An increase in VPD usually leads to an increase in tree transpiration causing greater water movement in the soil-plant-atmosphere compartment as long as there is enough available water for the plant to avoid water stress and stomata closure (Wieser, 2012). In this regard the seasonal dynamics of VPD and sap flux (*SF*) measured in the different seasons are very interesting. In autumn and winter, with plenty of available water coming from abundant rainfall (757.5 mm in total), the low VPD (0.1 - 0.3 kPa, average air temperature 10.6 °C) corresponded to a minimum *SF* in both BS and CS stands. At such low transpiration driving force trees of both stands did not show significant difference in *SF*. In the spring an increase in VPD (0.3 - 0.7 kPa, average temperature 17.3°C with peaks of average daily temperature in June up to 32.8 °C) resulted in much higher *SF* rates. VPD and water availability conditions were still not so limiting to determine a clear different in *SF* between the two stands (BS and CS), while the higher *SF* value in spring allowed to better appreciate the diurnal variations of *SF* which gradually increased during the first hours of light, reaching its maximum peak around mid-day and then decreased at sunset. In more extreme Mediterranean summer conditions with VPD values 10 times higher than in winter and from 3 to 5 times higher than in spring (1.5 - 2 kPa) were coupled with extremely high temperature for almost the whole period and very little rainfall (Figure 6) which most probably induced sufficient stress condition to limit the stomatal conductance thus limiting the *SF* over the whole day. This resulted in a flattening of the diurnal *SF* trend. At very high VPD levels the stomata tend to partially close to counteract water loss and reduce the risk of embolism (Katul et al., 2009; Allen et al., 2010; Medlyn et al., 2011; Williams et al., 2013; Rigden and Salvucci, 2017). McAdam and Brodribb (2015) observed in a controlled experiment a reduction of stomatal conductance in conifers between 50% and 70% when VPD increases from 0.7 to 1.5 kPa. The strongest VPD in the BS stand could trigger the higher *SF* values observed in BS compared to CS stands. Such difference was statistically significant in both summer 2020 and 2021and could be explained as a result of trade-off strategy between water loss and C assimilation. While CS trees might reduced stomatal conductance to limit water loss and avoid dangerous water tensions in the xylem (Zweifel et al., 2007; Duursma et al., 2008; Hentschel et al., 2014), the plants of the BS might need to keep a higher stomata conductance to compensate the lower capacity in photosynthetic carbon assimilation linked to the severe canopy reduction and hence to counteract the possible reduction in carbon assimilation. The clear canopy reduction was also proved by the light transmittance data (up to 4 times higher), that were always higher in the BS stand in all the seasons, most probably due mainly to a direct effect of the reduced canopy cover. Similarly to what observed in our sites, Salmon et al. (2015) reported during drought stress periods a higher sap flow and stomatal conductance in defoliated tress of *Pinus sylvestris* compared to control trees, linking this mechanism to the necessity for these individuals to maintain greater assimilation of carbon at the expense of greater water loss and exposure to the risk of embolism. In our BS site, the even higher *SF* reached in summer 2021 compared to summer 2020 coincides with increased defoliation from 50% in 2020 to 60% in 2021. These data would be coherent with the post-fire decrease in the TRW-Index ($P<0.05$)

compared to CS plants, as well as a decline in cell wall thickness (CWT) in the years following the fire ($P<0.05$), which overall indicated a lower capacity in assimilation and availability of carbon useful for growth and formation of thicker xylem walls in BS tress. In fact, although tree growth is not completely dependent on recent photosynthates, but also on carbon reserves (Kagawa et al., 2006; Kuptz et al., 2011; von Arx et al., 2017), recent studies confirmed that conifers use very little of the carbon fixed in previous years for new xylem formation (Zweifel et al., 2006; Soudant et al., 2016; Castagneri et al., 2018; Puchi et al., 2021). On the contrary, CS plants did not show carbon deficiency (TRW-index and CWT remained unchanged), rather they were able to increase ($P<0.05$) their LA and Kh in the period 2017-2020, with an improvement in water supply and a more efficient water transport capacity (Wilkinson et al., 2015; Hietz et al., 2017).

4.5 Conclusion

Overall structural and dynamic data allowed us to define a coherent picture of the post fire effects on the analysed *P. pinaster* trees on Vesuvius. *Pinus pinaster* is a species particularly adapted to Mediterranean conditions and to fire as an ecological factor. Our results showed, in fact, that fire did not induce dramatic effect of structural alteration of the xylem vessels or an increase in the cavitation vulnerability in burned plants. However, data also suggested that fire could represent a serious risk for plant health and survival in the mid-long term, by altering the site conditions and by reducing e tree canopy to such an extent to affect tree capacity to store carbon in the woody tissues. Our continuous monitoring system allowed to evidence that during drought stress periods BS trees increased their sap flow rates compared to CS. This indicated that fire did not affect directly the hydraulic efficiency and safety of the burned plants, but the severe defoliation triggered a grave derangement in stomatal control, modifying the trade-off between carbon assimilation and water loss and exposing trees to high risk of hydraulic failure. Under the current rates of climate change in Mediterranean areas, characterized by warmer and drier summers and more frequent fires, the risk to reach tipping points of hydraulic failure in forests exposed to fire events might strongly increase, undermining the C sink potential of forests.

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4.7 Figures

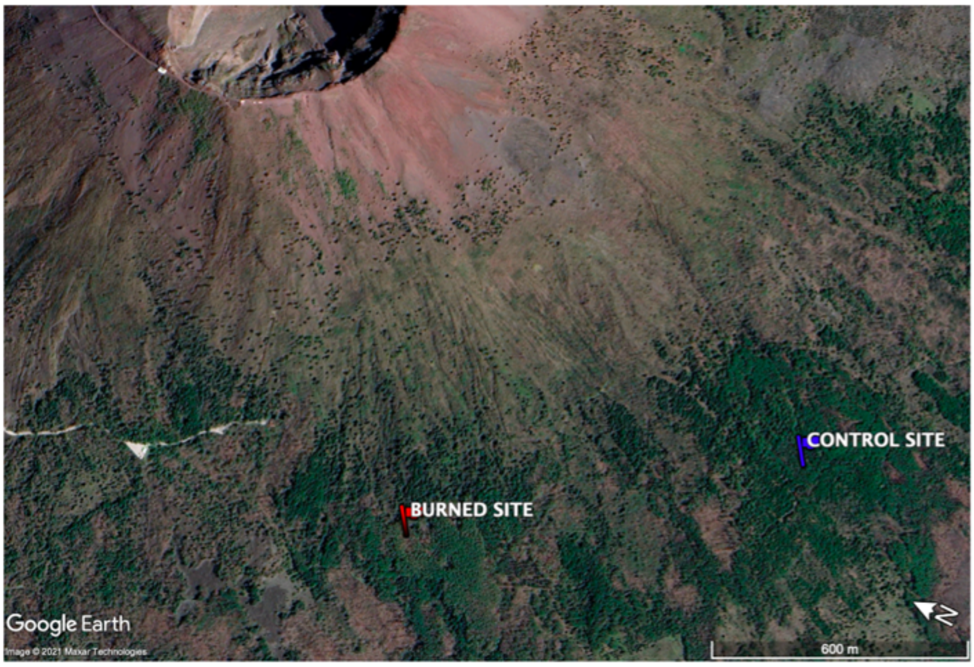

Figure 1 - Location of the Burned site (BS), and of the Control Site (CS) within the Vesuvius National Park.

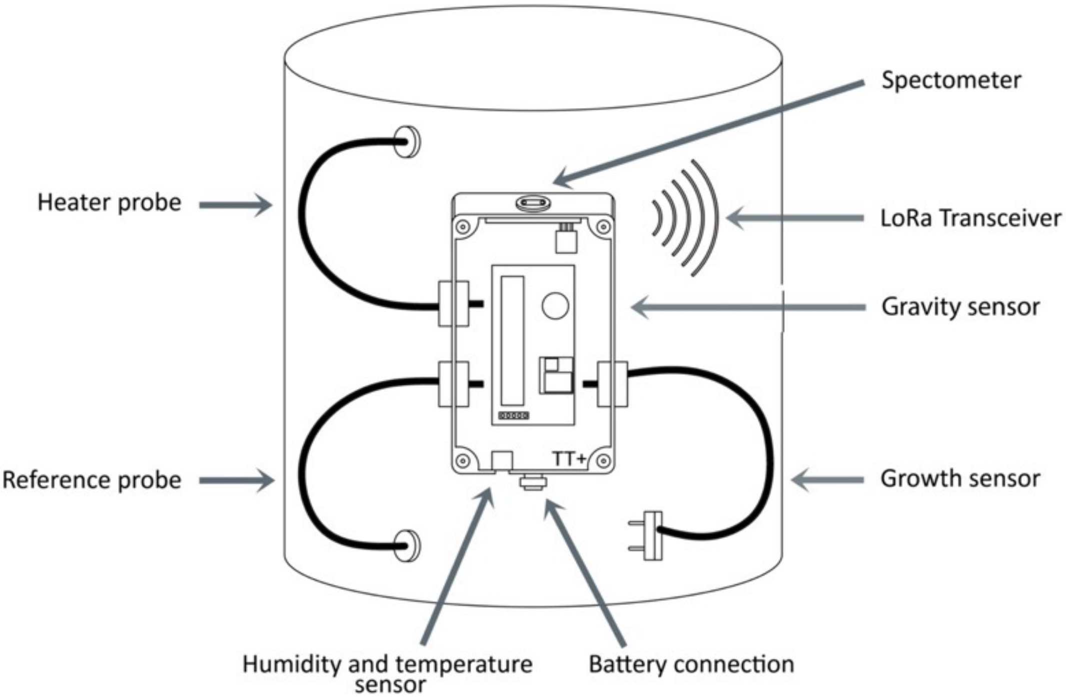

Figure 2 - Layout of the sensors of the Tree Talker + device

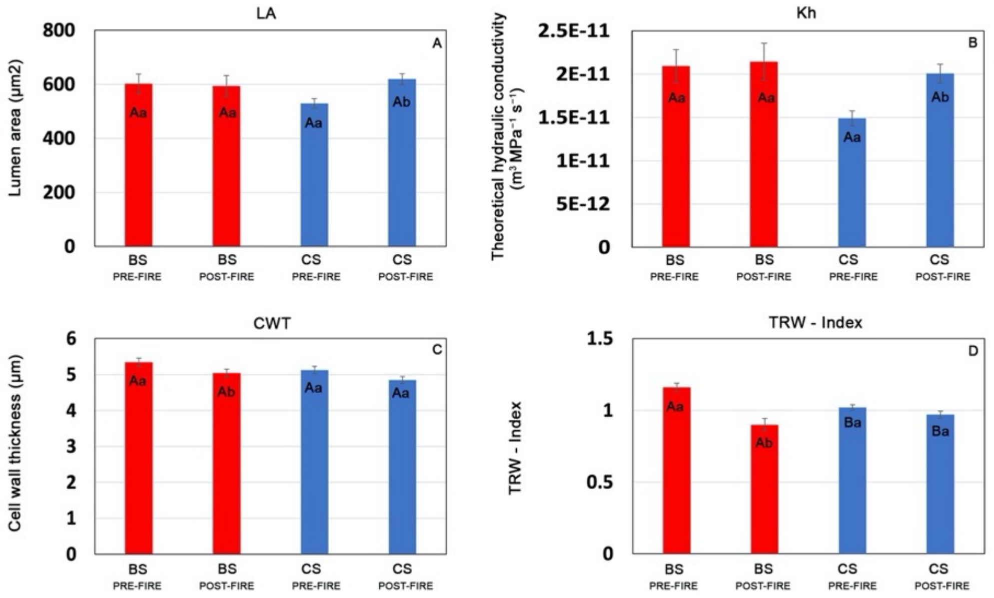

Figure 3 - (A) Average lumen area (LA), (B) theoretical hydraulic conductivity (Kh), (C) cell wall thickness (CWT) and (D) tree-rings width (TRW - Index) measured on wood samples of the BS stands (red histogram) and CS stands (blue histogram) referring to the years 2015-2016 (pre-fire) and 2017-2020 (post-fire). The capital letters indicate a statistical difference ($P<0.05$) between stand referred to the same time frame period (pre-fire or post fire), whereas lowercase letters indicate statistical differences between pre and post fire periods within the same pine stand. The bars indicate standard errors.

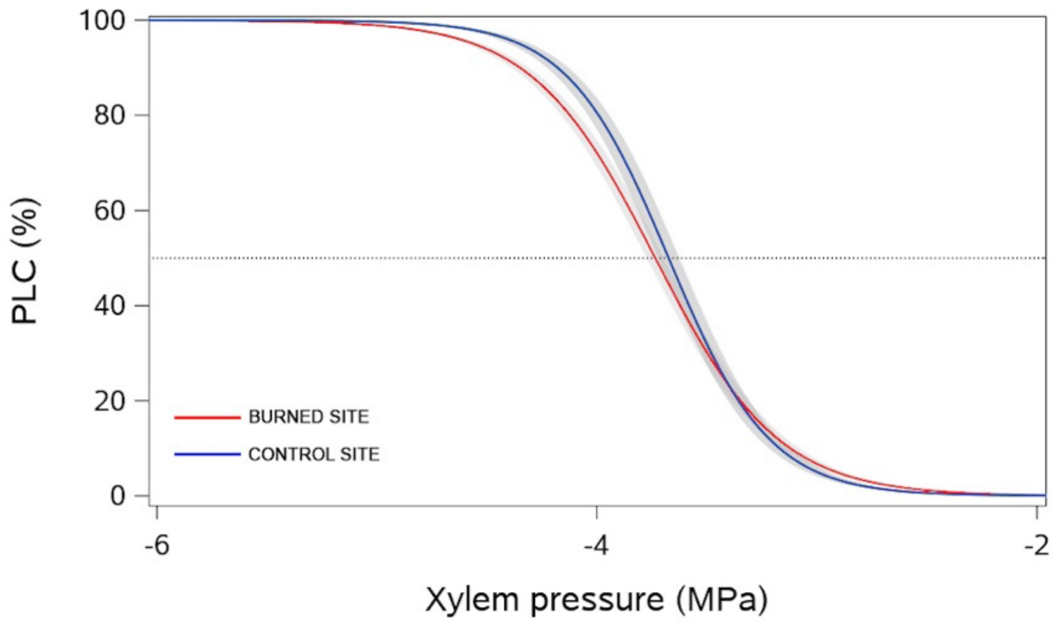

Figure 4 - Mean vulnerability curves for CS (in blue) and BS (in red) showing the percentage loss of hydraulic conductance in the xylem (%) as a function of the applied xylem pressure (MPa). The shaded bands represent standard errors. The P50 is indicated by a horizontal dashed line.

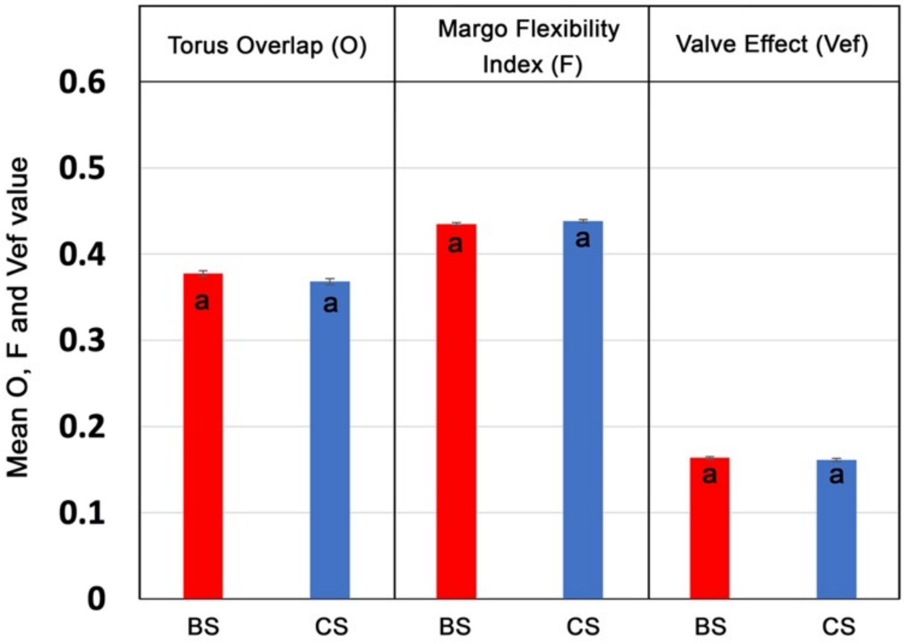

Figure 5 - Average value of Torus Overlap (*O*), Margo flexibility index (*F*), and Valve Effect (*Vef*) measured in the plants of the BS (red histogram) and CS (blue histogram) stands. The lowercase letters indicate a statistical difference between the sites. The bars indicate one standard error.

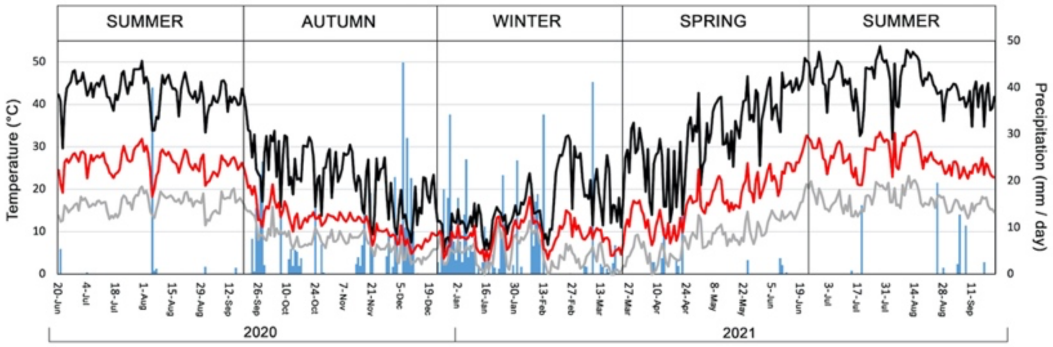

Figure 6 - Daily trend of the maximum (black line), average (red line) and minimum (gray line) temperature recorded by TT-Reference, and the precipitation (blue histograms) collected from the nearby meteorological station. Reference period 20 June 2020 - 22 September 2021.

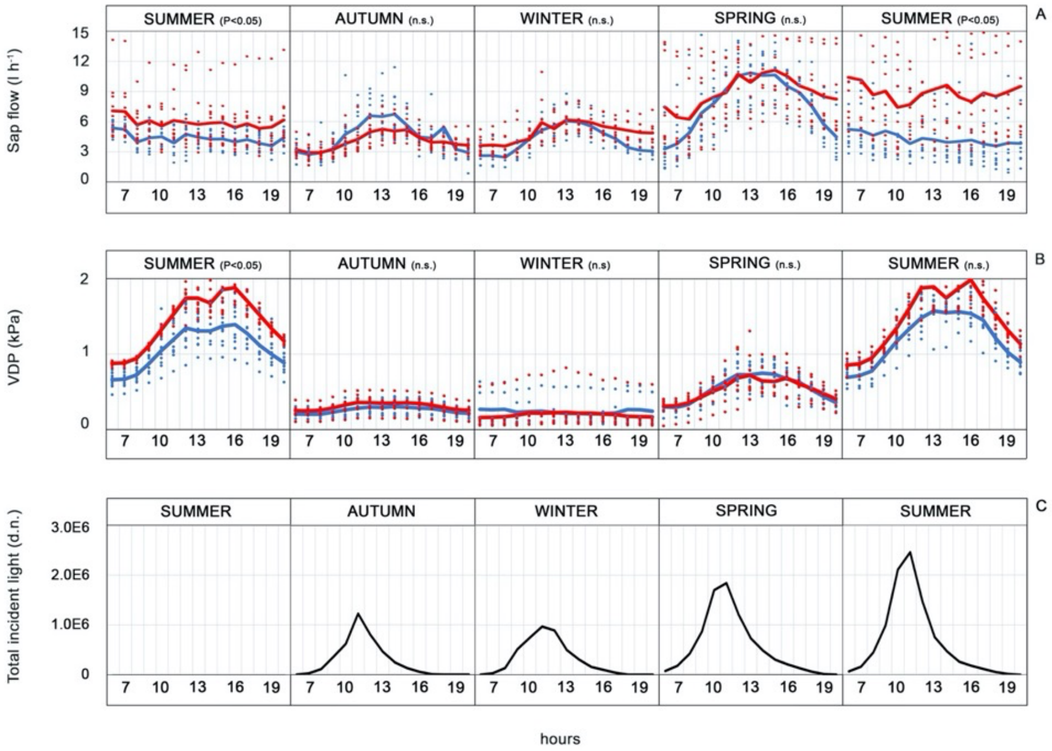

Figure 7 - Continuous lines represent average hourly values of Sap flow (A) and VPD (B) calculated from TT+ measurements in BS (red line) and in CS (blue line) stands during the 5 analysed seasons from 6 am to 8 pm. Each dot represents the seasonal average hourly value recorded by each TT + installed in BS (red points) and CS (blue points) in the specific season. (C) Average hourly amount of income radiation measured as the sum of counts recorded by spectroradiometer of TT-Reference system at different wavelengths (450, 500, 550, 570, 600, 610, 650, 680, 730, 780, 810, 860). Reference period 20 June 2020 - 22 September 2021.

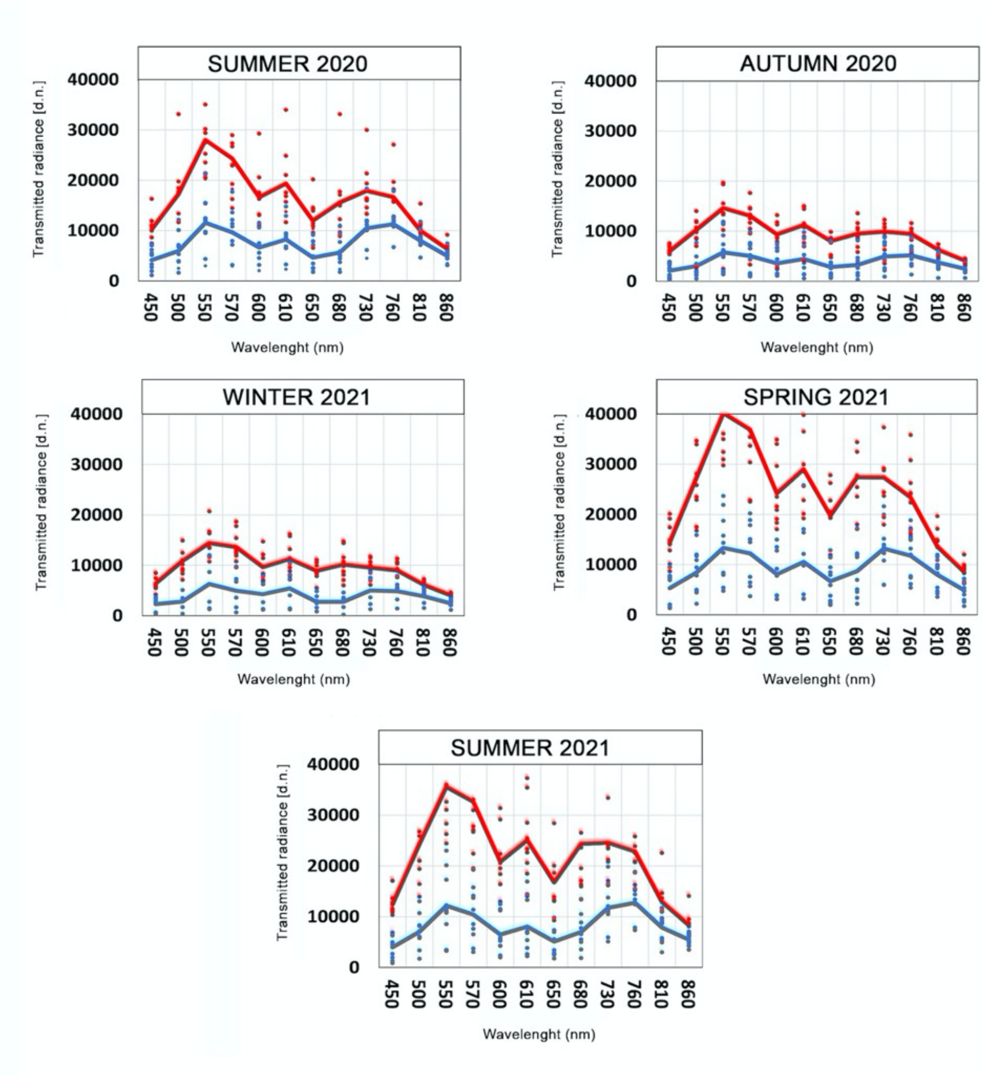

Figure 8 - Seasonal average value of transmitted light measured by the TT+ below canopy at BS (red line) and in the CS (blue line) sites reported for the different wavelengths (450, 500, 550, 570, 600, 610, 650, 680, 730, 760, 810, 860) analysed by the the TT+ spectrometers in the hours of maximum light (11 am – 14 pm). Data are reported as absolute counts measured by the spectrophotometer (gain 64x). Reference period 20 June 2020 – 22 September 2021.

4.8 Tables

Table 1 - Vulnerability parameters of xylem embolism estimated on the branches sampled from the CS and BS stands; "n" is the number of samples; "P50, P12 and P88" are water potential that induce 50%, 12% and 88% loss of hydraulic conductivity; "S" is the slope of vulnerability curve at inflection point (equ.7).

SITE	n	P50	P12	P88	S
CONTROL SITE	20	-3.67 ± 0.04 MPa (a)	-3.1 ± 0.05 MPa (a)	-4.18 ± 0.06 Mpa (a)	108.97 ± 37.3 (a)
BURNED SITE	20	-3.73 ± 0.03 MPa (a)	-3.1 ± 0.06 MPa (a)	-4.35 ± 0.06 MPa (a)	89.43 ± 25.72 (a)

Chapter 5

5. Effects of thinning intensity on productivity and water use efficiency of *Quercus robur* L

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Manuscript published in Forest Ecology and Management, 2020

doi: 10.1016/j.foreco.2020.118282

5.1 Introduction

The Mediterranean basin is one of the regions most affected by global warming (Schörter et al., 2005): the increasing frequency of drought events is triggering a progressive decrease in forest productivity and an enhancement in tree mortality (Allen et al., 2010; Chmura et al., 2011), with negative economic and environmental consequences. In this context, forest management strategies, such as thinning seems to have a significant role to improve tree performance, determining a greater resistance of forests to drought stress (McDowell et al., 2006; Battles et al., 2007; Voelker et al., 2008; Sohn et al., 2016) and a consequent increase in tree growth rates (Black et al., 1980; Morikawa et al., 1986; Aussenac and Granier, 1988; Piussi, 1994; Brèda et al., 1995). Indeed, the reduction of stand density involves not only an increase of the nutrient availability and incident light (Blanco et al., 2005), but also a considerable increase in water supply, due to the lower competition between the roots and the lower interception of rainwater (Sohn et al., 2016). These conditions can have, on the remaining plants, positive effects on transpiration and photosynthetic capacity, with a consequent increase in tree growth and efficiency of water use (Lagergren et al., 2008; Magruder et al., 2013; Hawthorne et al., 2013; Giuggiola et al., 2013). However, thinning intensity is an important factor affecting whole forest structure and density and influencing variations in microclimatic conditions (Duursma, 2011). A very intense thinning (40–50% of volume was removed) has been demonstrated to have negative effects on the productivity and water availability (Macfarlane et al., 2010) especially of the old stands (Juodvalkis et al., 2005), due to the increasing in temperature and quantity of light inside the stand and/or the increase of evaporation rate of water from the ground. In recent years, several studies investigated the relationships between the intensity of thinning and tree growth in ecosystems, such the Mediterranean basin, affected by water stress, with contrasting results (Aussenac and Granier, 1988; Brèda et al., 1995; Cutini and Mascia, 1998; Di Matteo et al., 2010). For example, some studies stated that a slight thinning action can stimulate the individual tree growth and that, conversely, a high intensity thinning (70–90% reduction of basal area) can negatively affect the total basal area (Harrington and Reukema, 1983; Misson et al., 2003; Jimènez et al., 2011). On the other hand, Kohler et al. (2010) and Sohn et al. (2013) demonstrated, using a dendrochronological approach, that a basal area reduction of more than 40% in *Picea abies* stand, allows an increase in tree growth, mostly after particularly dry years. These results are confirmed by studies conducted on *Cedrus atlantica*, which highlighted, how a high intensity thinning (50% reduction of basal area) reduced drought stress and increased growth rates (Guillemot et al., 2015). Tree age is an important aspect in determining the effects of thinning on growth: an increase in growth resulted to be directly proportional to the increase in the intensity of thinning for young stands of coniferous and deciduous trees, while in older stands this trend turns out to be reversed (Juodvalkis et al., 2005). Plants intrinsic water use efficiency (WUE_i), which is the ratio between net carbon assimilation in photosynthesis and stomatal conductance (Farquhar et al., 1982) seems to be affected by the intensity of thinning, even if few and contradictory information can be found in the literature related to positive or negative effects on water use (Fotelli et al., 2003; D'Alessandro et al., 2006; Martìn-Benito et al., 2010; Park et al., 2018). Martìn-Benito et al. (2010), evaluated the effects of a moderate thinning action (removing around 25% of initial basal area) on growth and WUE_i of *Pinus nigra* stand using tree rings and $\delta^{13}C$ and $\delta^{18}O$. The authors found an increase of tree growth in the thinned plots with no modification of WUE_i, probably due to an increase of resources availability (i.e. light, nutrients and water) for each individual rather to an optimization on the use of these resources. On the opposite, the work of Park et al. (2018) observed an increase in growth and in WUE_i as a consequence of an intensive thinning action (removing the 30% of the basal area) carried out on *Pinus koraiensis*. The increase seemed to be correlated to the higher quantity of light, allowing highest photosynthetic activity and therefore, a more efficient assimilation of carbon. Related to *Quercus* species, D'Alessandro et al. (2006) examined the WUE_i responses, using the $\delta^{13}C$ as a proxy, in *Quercus cerris* L. and *Fraxinus ornus* L. stands subjected to different thinning intensity and to different relative irradiance (IR). The results showed, for both species, a lower WUE_i inside the stands subjected to high intensity thinning (where half of the total trees were removed) and greater IR compared to the stands subject to low intensity thinning (where the one third of total trees were removed) and to less IR. Other studies, instead, have investigated the effect on growth and on WUE_i following intensive thinning, combined with the intercropping with nitrogen-fixing species: Corazzesi et al. (2010) found positive effects, after a high intensity thinning (48% of basal area was removed), on the productivity in *Q. robur*, when this species was associated with *Alnus cordata*. Subsequently, further studies carried out by Battipaglia et al. (2017) showed that the greater availability of nitrogen, determined by the intercropping with *A. cordata*, had positive effects on the assimilation of carbon and, consequently, also on the WUE_i of *Q. robur*. In this context, the present study aimed to analyze how the different intensity of thinning affects, in the short and long term, tree growth and the WUE_i of *Q. robur*, a widespread species in Italy and in Europe. Three thinning types of different intensity were applied in 14-year mixed plantation of *Q. robur* intercropped with a nitrogen-fixing species (*Alnus glutinosa*): (1) a light selective thinning, preserving all the N-fixing species, (2) a heavy selective thinning removing all the trees around the selected *Q. robur* individuals, including the N-fixing species, and (3) a geometric thinning, removing half of total trees and releasing the half of N-fixing species. To reach our aims we used a multiproxy approach where the dendrochronological analyzes were associated with the analysis of stable isotopes of carbon and oxygen. Indeed, $\delta^{13}C$ is influenced by the irradiance level, that change according to thinning intensity (Yakir and Israeli, 1995; D'Alessandro et al., 2006) and it can be used as a proxy of WUE_i. Further, change in $\delta^{18}O$, that is independent from photosynthetic activity (Barbour, 2007) can be used to disentangle the influence of stomatal conductance on WUE_i modification, in water limited environments (Gessler et al., 2014; Battipaglia et al., 2014, 2016, 2017). Since Battipaglia et al. (2017) demonstrated a good influence of the nitrogen fixing species on *Q. robur* we hypothesized not only different responses in terms of internal competition and resources availability (e.g. light and water) proportionally to the thinning intensity, but also an increase in growth for the treatment where the nitrogen fixing individuals were not completely removed by the thinning action. The information gathered in this work will allow the expansion of scientific knowledge on the effects of thinning and will provide a tool to improve tree growth and water use of this kind of mixed plantations.

5.2 Materials and methods

5.2.1 Study area

The investigated plantation is located in Empoli (43° 40′46″ N, 10° 55′12″E), in Tuscany, central Italy, on deep and fertile flat lands of alluvial nature previously used for agriculture. The plantation, which extends over about 4 ha, was planted in the December 1996. The soils are deep, fresh, well drained, with a predominantly sandy-loam texture, low presence of clay, rich in active limestone and organic substance; the pH is 7.8–8.0 (Ciardi, 1997). The climate of the area is characterized by a typically Mediterranean climate with hot, dry summers and mild winters: average annual rainfall is 790 mm, while average annual temperature is 15 °C (Royal Netherlands Meteorological Institute, KNMI Climate Explorer database, period 1996–2016, Trouet and Van Oldenborgh, 2013). The plantation is a mixed plantation with nurse trees where valuable broadleaves species, common oak (*Quercus robur* L.), European walnut (*Juglans regia* L.) and narrow-leafed ash (*Fraxinus angustifolia* Vahl.), were alternated with nurse tree black alder (*Alnus glutinosa* (L.) Gaertn.). The trees were arranged with a regular square layout with a distance of 4 m among the trees (planting density: 625 trees per hectare). The plantation is divided in two experimental replications (block A and block B), close 50 m each other, where the alder represents the 50% of the planted trees. In December 2009 the 1st inventory was carried out measuring diameters of each tree and a sample of heights to build up an isometric curve. Afterwards the selection of the best 70–80 crop trees per hectare (common oak and other valuable tree species) was carried out in the whole plantation. The best valuable trees were selected and marked, considering tree vigor (evaluated ac- cording to the diameter dimension and crown development, Wilhelm and Rieger 2017), stem quality (according to CEN standard, Nosenzo et al., 2012) and potential timber value (according to Pelleri et al., 2020) as well as the ground distribution. In general, common oak was favored as species most suitable for the local site condition. On these crop trees specific measurements were done: total height, crown insertion height, crown area (calculated measuring four radius along the cardinal directions), crown symmetry (ratio between minimum and maximum diameter of the crown), stem quality. The main characteristics of the *Q. robur* trees are reported in Table 1. In the two blocks three treatments with random distribution (T1, T2, T3) were carried out in the December 2010, according to three different thinning methods (Fig. 1), aimed at favoring the productivity of *Q. robur*:

- In the T1 treatment a selective thinning of moderate intensity was performed (Fig. 1), removing 31% of the total biomass. This action involved the cutting of 4 surrounding trees around each selected crop tree preserving the *A. glutinosa* trees.
- In the T2 treatment, a selective thinning of very high intensity was performed (Fig. 1) removing 75% of the total biomass. In this case, 8 competitor trees (4 valuable trees and 4 *A. glutinosa*) were removed around each selected crop tree.
- In the T3 treatment a geometric-selective thinning of high intensity was performed (Fig. 1) removing 62% of total biomass. This method involved the cutting of *J. regia* and *A. glutinosa* growing in the same row and the preservation of the adjacent lines with the other two valuable broadleaved alternates with black alder. Only some individuals of European walnut, characterized by an excellent

stem quality class (only class A, suitable for veneer) the higher diameter dimension and the best crown development, were preserved. To favour the preserved *J. regia*, two trees located in the adjacent rows were eliminated.

5.2.2 Stand and crop trees characteristics

5.2.2.1 Monitoring

All trees were measured and mapped according to the planting design. In both blocks, in the 2009 and 2017 the total height, crown insertion, crown area, crown symmetry index and stem quality of the crop trees were estimated. The crown asymmetry was calculated through the ratio of the minor diameter of the crown to the major diameter of the crown measured along the North-South and East-West directions. The index assumes values equal to 1 when the crowns are perfectly symmetrical and regular, on the contrary when the crowns appear unbalanced and developed mainly in one direction the value tends towards 0. The quality of the stem of the cultivated trees has been defined using a stem quality classification recognized at European level (Nosenzo et al., 2012), based on a visual analysis of the first log (up to 2.6 m), and considering 4 classes: A = timber suitable for veneer; B = first saw timber quality; C = second saw timber quality; D = wood for biomass. ANOVA analysis and HSD di Tukey were performed among treatments, taking into consideration the block effect and were computed by STAT 7.1 Statsoft software.

5.2.2.2 Competition index

To verify the effect of the different intensity of thinning, the Hegyi competition index was estimated after thinning occurrence. This competition index, commonly applied in plantation forestry, is related to the distances among a specific tree and its surrounding competitors. In our case, Hegyi competition index (Hegyi, 1974; Daniels, 1976; Liotto and Tabacchi, 1993; Biging and Dobbertin, 1995) was calculated considering all the trees inside a circular area of 8 m radius from the crop trees. This dimension was chosen considering both the potential crown area of the crop trees at the end of the rotation period, and the planting layout (Fig. 2).

5.2.2.3 Canopy cover characteristics

The effect of thinning on canopy cover was also estimated by the measurement of transmittance and leaf area index for three years (2017, 2018 and 2019), selecting three crop trees of *Q. robur* for each treatment. The measurements were done in points placed every 3 m along a cross-section of 18 m length, under the canopy of the crop trees, following the direction N-S and E-W. Leaf area index was estimated indirectly using the LAI 2000 Plant Canopy Analyzer (PCA; Li-Cor Inc., Lincoln, NE, USA). PCA measurements were taken on 11 positions per tree, during summer, close to sunset, under diffuse and uniform sky conditions. One above-canopy reference measurement for each crop trees was recorded in clearings near the study area. Direct canopy transmitted light in the Photosynthetically active radiation (PAR) waveband was measured on 11 position per tree, during summer using the AccuPAR ceptometer (Decagon Devices, Pullman, WA, USA) at midday on sunny days. Incident radiation was measured in open areas near the experimental blocks. Transmittance was calculated as percent fractions of below-canopy light, divided by the incident radiation (Chianucci and Cutini, 2013; Cutini et al., 2015). The mean data for thinning types were calculated averaging all the sampled trees and years per treatment.

5.2.3 Sampling and analysis of wood samples

In September 2017, 10 *Q. robur* individuals were sampled for each plot. Two cores per tree were sampled through an increment borer (Haglöfs, Langsele, Sweden) of 5 mm, at 1.3 m from the ground, to- wards east-west and north-south directions. The 120 total samples were fixed on a wooden support and were sanded in order to facilitate the identification of ring boundary. The dendrochronological measurements were performed through the LINTAB system (Frank Rinn, Heidelberg, Germany): a stereomicroscope connected to a computer in which a specific software called TSAP-Win (FrankRinn, Heidelberg, Germany) is installed. Tree-ring width (TRW) of each core was measured and the raw TRW series of each treatment were visually and statistically crossdated by the Gleichaeufigkeit (GLK), which evaluates the correlation between the different series (Eckstein and Bauch, 1969). Synchronization was considered acceptable with a GLK > 60. Annual Basal Area increment (BAI) was calculated from raw ring width, considering concentrically distributed tree-rings (Martìn-Benito et al., 2010). BAI was used because it is considered less dependent on age and thus it avoids detrending procedure (Biondi, 1999) which could remove also low frequency variability, important component in our short time span series. The cumulative BAI was calculated according to Battipaglia et al. (2015, 2011).

5.2.4 Preparation of samples for isotopic analyses

For each block (A and B), five samples per treatment presenting the best cross-dating (GLK > 0.70) with the corresponding average treatment chronology, were selected. Subsequently the 18 rings relative to the period 1997–2016 were annually divided with a razor, pulverized with a pulverizing mill (ZM 1000, Retsch, Germany) and were weighed in a tin capsule or silver for isotopic measurements of carbon or oxygen, respectively. The isotopic composition was measured at the IRMS laboratory of the University of Campania "Luigi Vanvitelli" by mass spectrometry with continuous flow isotope ratio (Delta V Advantage, Thermo Scientific). Standard deviation for repeated analysis of an internal standard (commercial cellulose) was better than 0.1‰ for carbon and better than 0.3‰ for oxygen. The $\delta^{13}C$ series were corrected for the fossil fuel combustion effect (Francey et al., 1999).

5.2.5 Estimation of the WUE_i

Once the $\delta^{13}C$ was measured, this was used to calculate the WUE_i of each series per each treatment. The WUE_i is defined as the ratio between the net photosynthesis (A) and the stomatal conductance to water vapor (gH_2O) (Ehleringer et al., 1993). From here:

$$WUE_i = A/gH_2O = (ca - ci)/1.6. \qquad (1)$$

Where is it:

ca = concentration of CO_2 in the atmosphere;

ci = concentration of CO_2 in the intercellular spaces.

For *Q. robur* plants, the WUE_i can be calculated starting from the $\delta^{13}C$ of the plant material ($\delta^{13}C$ sample), which is related to atmospheric $\delta^{13}C$ ($\delta^{13}C$ atmosphere) and to the ratio ci/ca, according to the equation of Farquhar et al. (1989):

$$\delta^{13}C \text{ sample} = \delta^{13}Ca - a - [(b - a)\, ci]/ca \qquad (2)$$

where

a = fractionation of $13CO_2$ by diffusion in the atmosphere (4.4%);

b = fractionation during the biochemical process of carboxylation (27%).

$$ci = ca\, (\delta^{13}Ca - \delta^{13}C \text{ sample} - a)/(b - a) \qquad (3)$$

Replacing the (3) equation in (2), we get:

$$WUE^i = (ca\, b - \delta^{13}Ca + \delta^{13}C \text{ sample})/1.6(b - a) \; (4)$$

$\delta^{13}ca$ was estimated for the period 1997–2004 from McCarroll and Loader (2004), and measured values for the period 2005–2016 available online (http://www.esrl.noaa.gov/gmd/), while a is the concentration of CO_2 in the atmosphere, estimated for each year and obtained by NOAA (http://www.esrl.noaa.gov/, Mauna Loa station).

5.2.6 Climate analysis

To assess the influence of the main climatic factors on the productivity and physiology of the trees studied, the climate data of the minimum, maximum and average monthly temperatures were used, as well as those related to the total monthly precipitation for the period 1996–2016 coming from the dataset CRU TS3.23 grid with 0.5° resolution (Harris et al., 2014). The climatic data were grouped by single or multiple months (i.e. mean maximum temperature registered in May or May and June or May, June July or total monthly precipitation of May or May and June or May, June, July), following standard methods used in climate-growth relationships (Fritts, 1976) and were subjected, through the aid of the software Microsoft Excel, to the analysis of the linear correlation function of Pearson ($P<0.05$), implemented among the data of amplitude of the rings of the trees, the data of the Cumulative Basal Area and the values of the isotopic analyses, for the period between 1996 and 2016, in order to identify the climatic factors and the months that could have most influenced the growth of *Q. robur* .

5.3 Results

5.3.1 Stand and crop trees characteristics

5.3.1.1 Monitoring

The average height values of the crop trees in block A were higher and significant ($P<0.01$) different compared to block B, both in 2009 (14.5 m in A and 13 m in B) and in 2017 (22.5 m in A and 20 m in B). The crown diameter (Fig. 3) did not show significant differences for the two blocks and thinning treatments, neither in 2009 (6.0–6.5 m in A and B) or 2017 (10.0–10.5 m in A and B). On the contrary, the crown symmetry was influenced by thinning type: in 2009, before thinning occurrence, the crown symmetry values were similar in the *Q. robur* trees of three treatments (0.88–0.90). In 2017, after thinning, the differences among treatments were significant (Table 2). In the T3, characterized by geometric thinning, a significant ($P<0.05$) reduction of crown symmetry (0.90 vs. 0.83) was observed, indicating an irregular development of the crown. In the other two thinning treatments, the values remained stable (in T2 = 0.89) or increased (in T1 0.88 vs. 0.92). In 2009, before thinning, the majority of the crop trees were classified in class B (first saw timber quality) for the stem quality (Fig. 4); the stem in class A (timber suitable for veneer) were 6% in block A and 11% in block B. After 8 years and thinning occurrence, the percentage of stem in class A increased in both blocks, but the values were higher in the T2 treatment in comparison to T1 and T3. In T3, where the geometric thinning was applied, the percentage of stem in class C (second saw timber quality) was 22% in block A and 17% in block B.

5.3.1.2 Competition index

Hegyi competition index (H), recorded 8 years after thinning around the oak crop trees, reported no significant difference between the two blocks (Fig. 5). For both blocks, H was higher (mean value 0.96H) in T1 (moderate intensity thinning) where *Alnus glutinosa* was preserved in comparison to the other treatments. In T3 (geometric thinning) the H index of the crop trees presented mean values of 0.54 while, in T2 (high intensity thinning) the mean value was 0.47.

5.3.1.3 Canopy properties

The annual data of LAI and transmittance were averaged since no significant differences were recorded in the three treatments during the three years (2017–2019) of monitoring. The LAI values presented significant differences only between T1 and T2 (Table 3). The values are placed in the lower part of the LAI range (from 1.6 to 18.0 $m^2\ m^{-2}$) found for wood plantations (Asner et al., 2003) and indicate a simple structure. The average transmittance data showed that the availability of light was lower in T1, characterized by a low thinning intensity, and significantly different in comparison to T2 and T3 that presented similar and no significant different values.

5.3.2 Dendrochronological analyses

The dendrochronological analyses carried out on the *Q. robur* plants of blocks A and B (Fig. 6) showed, for each treatment, a good coherence in the growth trend of the single chronologies respect to the relative average growth curve. Indeed, the measured GLK was always very high: the plants of block A had an average GLK of 77, 76, 80 respectively calculated in the treatments T1, T2, T3; in the same way, the plants of block B showed an average GLK of 75, 72, 75 measured in the treat- ments T1, T2, T3, respectively. Comparing the average growth curves of each treatment of blocks A and B, it is possible to observe a good synchronization: all the trees of the plantation showed a strong reduction in growth in 2002, 2003, 2010 and 2012 while they showed an increase in growth in 1999, 2000, 2001, 2004, 2007, 2011, 2013 and 2016. Further in all the treatments, there was a significant increase in productivity in the year after the thinning actions. In particular, in both blocks, the T2 treatment recorded the highest growth after the thinning treatment. Results were confirmed observing the Cumulative basal area (CBA – Fig. 7) where the T2 treatment presented a significant growth increase in both blocks A and B in comparison to the treatments T1 and T3.

5.3.3 Isotope analyses

The carbon isotope analysis, carried out on the tree rings and performed for the period between 1997 and 2016 on the plants showed an average value of $\delta^{13}C$ of –26.88 ± 0.08‰ for T1 block A and $\delta^{13}C$ of –27.06 ± 0.07‰ for T1 block B; for the treatment T2 an average value of $\delta^{13}C$ equal to −26.75 ± 0.06‰ for block A and −26.89 ± 0.04‰ for block B; for T3 an average value of −26.81 ± 0.07‰ for block A and −26.85 ± 0.07‰ for block B. No significant differences were found between the two blocks, thus we report the mean $\delta^{13}C$ chronology related to both blocks (Fig. 8) for each treatment. The chronologies of the $\delta^{13}C$ of the different treatments presented high variability among each other. However, a reduction in the average value of $\delta^{13}C$ in the post-thinning period was found in all the treatments. Similarly, the isotopic analysis of oxygen (Fig. 8) showed no significant difference between the two blocks with an average value of $\delta^{18}O$ of: 25.45 ± 0.45‰ for T1, 25.58 ± 0.45‰ for T2 and 25.40 ± 0.45‰ for T3. Also, in this case, the average curves of $\delta^{18}O$ related to the three treatments were different from each another: the chronologies presented the same visual trend only in 2000, 2002, 2006, 2011, 2013 and 2014. However, it is possible to observe how, in all treatments, there was a net increase in $\delta^{18}O$ in the years following the thinning treatments.

5.3.4 Analysis of the WUE$_i$

The WUE$_i$ data, derived by the $\delta^{13}C$ estimated for the period 1997–2016, of the *Q. robur* trees (Fig. 9a) showed an average value of 82.18 ± 8.02 μmol mol^{-1} for T1, 83.39 ± 9.27 μmol mol^{-1} for T2 and 83.69 ± 7.62 μmol mol^{-1} for T3. It is evident that the average values recorded in the different treatments were very similar, in fact there were no statistically significant differences. However, comparing the average WUE_i values recorded in the pre-thinning period with the average WUE_i values measured in the post-thinning years, we observed significant differences (Fig. 9b). In the T1 the average value of the WUE_i recorded from 1997 to 2010 was 83.15 ± 8.78 $\mu mol\ mol^{-1}$, while in the period from 2011 to 2016 the average value decreased to 80.06 ± 6.21 $\mu mol\ mol^{-1}$. Similarly, in T2 the mean WUE_i measured in the pre-thinning period of 84.75 ± 9.58 $\mu mol\ mol^{-1}$ decreased in the years following the thinning to 80.21 ± 8.41 $\mu mol\ mol^{-1}$. Differently, the average value of WUE_i recorded in T3 from 1997 to 2010 equal to 83.22 ± 8.06 μmol^{-1} increased, from 2011 onwards, to 84.72 ± 7.18 $\mu mol\ mol^{-1}$. Therefore, this comparation showed a statistically significant difference in the years following the thinning action between the WUE_i values registered in the T3 treatment compared to those measured in the T1 and T2 treatments.

5.3.5 Relationship between climate, growth and isotopes

From the climate-growth correlations, it emerged that the minimum average temperatures had an extremely positive effect on the *Quercus robur* trees growing into both blocks (Table 4). In particular, the data concerning the minimum temperatures positively correlated, during the entire vegetative season (March-October), not only with the CBA data, but also with the values of $\delta^{13}C$ and $\delta^{18}O$. Similarly, even the maximum temperatures had a positive influence on the trees, especially in terms of growth. Nevertheless, in the T1 treatment, trees showed in the spring season (MAM = March, April, May), negative correlations between maximum temperature data and growth data. Instead, regarding the average temperatures, the statistical analyses showed their positive correlation with the growth especially in the period from September to October (SO). Finally, no statistically significant correlations were found between precipitation and isotopic composition and growth data.

5.4 Discussion

5.4.1 Effects of thinning on the growth of *Quercus robur*

The growth of *Q. robur* in the two blocks had a similar trend, as expected since they are repetitions located in the same geographic area and characterized by the same soil composition, thus the environmental variability is low. Climate-growth correlations showed that precipitations seemed to have no decisive weight on tree growth while the temperatures, especially the minimum one, had positively influenced the growth. Those results demonstrated that the studied *Q. robur* trees do not have particular limitations in the availability of water: the soil where the plantation was planted is characterized by an excellent ability to accumulate water (Soil map 1: 250,000 of the Tuscany Region - UniSi-CGT laboratory) and thus their growth is not limited by the precipitation water. The chronologies of the TRW and CBA showed a very rapid growth of trees in the juvenile phase (1996–2000, the first 5 years): the age effect, the lower competition, the greater availability of nutrients and the considerable availability of light determined, at this stage, a more intense photosynthetic activity with a resulting higher productivity (Fritts, 1976). On the contrary, in the intermediate phase (until 2009, from 5 to 14 years), the individuals of *Q. robur* showed a decline in growth, which can be linked to the increase in competition and the mutual shadowing of the canopy with a consequent lower availability of light. In the following years, the analysis on tree-rings showed that, following the different intensity of thinning, in all the treatments, there was an increase in tree growth starting from 2011. These results proved the favorable effects of thinning actions: the reduction of competition (Kerr et al., 2011), the increase in the availability of light (Park et al., 2018), the greater accessibility to water present in the soil (Brèda et al., 1995; Aussenac, 2000; Ganatsios et al., 2010) and the increase in the water potential of the leaves that could have determined a better photosynthetic capacity (Donner and Running, 1986; Gauthier and Jacobs, 2009) and a consequent increase in growth (Aussenac and Granier, 1988; McDowell et al., 2003; Anning and McCarthy, 2013). However, the growth of the individuals, although increasing, showed a different yield within the different treatments depending on the intensity of the thinning. The dendrochronological and CBA data highlighted the greater growth of the plants belonging to the T2 treatment of both blocks, emphasizing the better effectiveness of selective thinning at high intensity. These results, in accordance with the studies carried out by Kohler et al. (2010) and Sohn et al. (2013) on *P. abies* and by Guillemot et al. (2015) on *C. atlantica*, indicated that this treatment has determined, through the strong modification of the structural characteristics of the forest population, very favorable microclimatic conditions, especially in terms of light, which have favored the overall functionality of the trees. The analysis of post-thinning transmittance showed how the improvement of the light conditions, triggered by the significant decrease in the density of T2 treatment, has determined a higher productivity: the greater interception of light by the remaining individuals may have resulted, compared to the other treatments, in a better stomatal conductance and a more effective assimilation of the carbon, with a consequent improvement in the photosynthetic activity. This hypothesis is in agreement with findings of Park et al. (2018) on *Pinus* *koraiensis*, and is confirmed by the values of $\delta^{13}C$ and $\delta^{18}O$ recorded in the tree-rings of the T2 treatment. In fact, in the post-thinning period, the value of the isotopic carbon ratio decreased, especially in 2015. The decrease suggested a greater discrimination of ^{13}C by the carboxylating enzymes of the plant (McCarroll and Loader, 2004). Regarding the $\delta^{18}O$ values, although increasing in the post-thinning years, turn out to be on average lower than those measured in the other treatments. This condition denoted a lower stomatal conductance of T2 compared to the pre-thinning years (Farquhar et al., 1989), which was however slightly greater than that recorded in the T1 treatment and strongly greater than the T3 treatment. The dendrochronological and CBA curves recorded in T1 treatment showed a lower tree growth. The analysis of post-thinning transmittance carried out in the T1 treatment suggested that the low intensity thinning, did not lead to an increase of the available light, maybe because of the high stand density, triggering, a lower productivity of *Q. robur* trees compared to T2, in the post- thinning period. Indeed, the low light availability in T1 may have caused in *Q. robur* trees a reduced rate of photosynthesis and a lower assimilation of CO2 compared to T2, with a consequent reduction in growth (Apel and Hirt, 2004). This hypothesis was confirmed by $\delta^{13}C$ and $\delta^{18}O$ data. Indeed, $\delta^{13}C$ and $\delta^{18}O$ were, in the post-thinning years, higher than those found in the T2, suggesting lowest photosynthetic activity (McCarroll and Loader, 2004) and lowest post-thinning stomatal conductance. The combination of these results allowed us to hypothesize how the micro-climatic conditions generated by the applied treatment determined the lowest growth rate in T1 in comparison to the other treatments (Scheidegger et al., 2000). In both blocks, in T3 treatment, the dendrochronological analyzes and the CBA data showed a productivity similar to that recorded in the T1 treatment. The post-thinning transmittance analysis performed in the T3 suggests that the trees experienced almost the same light condition of the T2 treatment, but the crown symmetry analyses revealed that the geometric thinning influenced the crown development. Indeed, it has been demonstrated that the crown symmetry can influence the light interception and can determine photosynthetic carbon gain of the plant canopy (Cannell et al., 1987; Stenberg et al., 1994, Seidel et al., 2011). In the row where all the trees were preserved, the crowns can experience a reduction of growth capacity due to the lateral compression, while in the other di- rection, the trees can have more space for crown development (Ishii and Asano, 2010). Although light is the primary resource for photosynthesis and growth, crown architecture reflects other important functions related to plant survival, growth, and reproduction, including hydraulic architecture and mechanical stability (Farnsworth and Niklas 1995; Kennedy, 2010).

5.4.2. Influence of the different intensity of thinning on the WUE$_i$ of *Q. Robur*

The mean value of WUE$_i$ across the study period (1996–2016) was similar in all the examined treatments. However, the comparison of the WUE$_i$ mean values in the pre and post-thinning years provided indications on the effects induced by the intensity of the different treatments. Indeed, while in the first years (1996–2010) the WUE$_i$ was practically equal in all the treatments, the data changed in the years following the thinning (2011–2016): the values decreased in the T1 and T2 and increased in the T3. The different results of WUE$_i$ were connected to the different light and competition conditions determined by the different type of thinning. The temperature and the transmittance appeared to be, in the studied plantation, a more important limiting factor than water stress, as confirmed by climate-growth relations. Further, the fact that the three treatments presented no statistically significant differences in the $\delta^{18}O$ chronologies, can indicate a possible common source water (Battipaglia et al., 2009; Treydte et al., 2014). Thus, the reported increase in WUE_i in T3 could be due to competition induced by crown asymmetry. The crown symmetry is important, since it helps trees to adjust to the heterogeneity of light availability and to intercept light more efficiently (Ishizuka, 1984; Franco, 1986; Sorrensen-Cothern et al., 1993). After the geometric thinning, plant crown lost the symmetry and while one side could grow with no limitation, the other side was partially limited by the neighbour trees. The high values of $\delta^{13}C$ and of $\delta^{18}O$ measured in post-thinning period in both blocks, suggested a condition of stress in the individuals thinned with geometric approach: the trees to counteract the competition effect were forced to limit photosynthetic activity and to reduce their stomata conductance and, consequently, this affects their WUE_i (McCarroll and Loader, 2004). Finally, the decrease in WUE_i, found within the T1, showed how the selective thinning at moderate intensity had a negative influence on the photosynthetic rate due essentially to the high light competition. The understory light environment is one of the primary factors influencing photosynthesis rate and the amount of carbon sequestered by trees (Lieffers et al., 1999). The proportion of incident radiation that is transmitted through the canopy (canopy transmit- tance) depends on the size, distribution and density of the tree crowns (Brunner, 1998; Jennings, 1999). Thus, the thinning activity carried out in the T1 was not able to reduce the trees competition, especially due to the presence of the N-fixing species that was preserved during thinning. Several studies demonstrated that high density of stand can produce a stronger competition for resources (e.g. water, nutrient, light), affecting the growth and biomass allocation of trees (Newman, 1973; Caldwell, 1987; Tilman, 1987). The effect of competition was higher of the favorable benefits found for *Q. robur* intercropped with a N fixing species in a different study (Battipaglia et al., 2017). Indeed in that study, the authors indicated a considerable increase in cumulative basal area and in WUE_i in two mixed stands of *Q. robur* and *A. cordata*, largely resulting from an increase in N availability, due to the N-fixing species that induced an enhancement of photosynthesis. In both blocks of our study site we didn't observe an increase in growth for the stands where the nitrogen fixing species were preserved by the thinning action, suggesting that *A. Cordata* at this age-stage of plantation has finished its role of nurse trees (Corazzesi et al. 2010; Battipaglia et al. 2017), and is acting as a light competitor (Loewe et al., 2019). Finally, the post-treatment decreases in the WUE_i value, found in the T2 trees, demonstrates how the application of selective thinning at high intensity did not generate any type of water stress: the trees did not need to increase their water use efficiency (Farquhar et al., 1989). This confirmed how the conditions established following the treatment have determined a good ability of plants to absorb higher concentrations of carbon, implying not only the maintenance of high photosynthetic rates, with a consequent higher productivity, but also a limited water loss, thanks to the best control of stomatal opening and closure (Flexas et al., 2013; de Santana et al., 2015). This result is in agreement with finding from McDowell et al. (2003, 2006), Di Matteo et al. (2010), Brooks and Mitchell (2011), Sohn et al. (2013) where a decrease in WUE_i was related to an increase in soil water availability, through reduced root competition and canopy rainfall interception, thus decreasing drought stress (Fernández-de-Uña et al., 2016). Therefore, our results suggested that the response in WUE_i in this plantation is mainly due to the environmental conditions (such as light and water) created by thinning intensity and that influenced tree physiology and competition among species.

5.5 Conclusion

This study highlighted how the different intensity of thinning treatments influenced tree growth and their WUE_i. Indeed, tree growth responses and variation of WUE_i in the tested thinning with different intensities indicated that light and competition conditions could be the main drivers of the thinning effect. The moderate thinning activity, removing the 31% of biomass, was not sufficient to win the high competition for resources among tree individuals and produced a low tree growth. Microclimatic conditions of the stand (such as water and light availability) had an important role on determining competition among species. Indeed, in our study site we did not find positive effects on growth due to the presence of N-fixing species reported in other water-limited sites. On the other hand, the geometric thinning activity (T3 treatment) had a negative effect as well on the growth, due to crown asymmetry and to the limited carbon gain. Finally, the high intensity selective thinning resulted the more appropriate management practice for this plantation, in terms of tree growth and water use efficiency, since it improved the photosynthetic activity of the trees that in any case where not limited by water availability.

5.6 Reference

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5.7 Figures

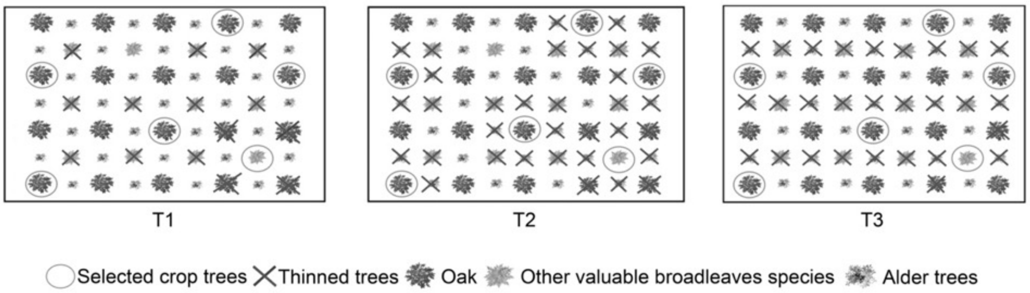


Figure 1 - Treatments reporting thinning actions: T1 = Selective thinning of moderate intensity (removed 31% of total biomass); T2 = Selective thinning of high intensity (removed 75% of total biomass); T3 = Geometric thinning (removed 62% of total biomass).

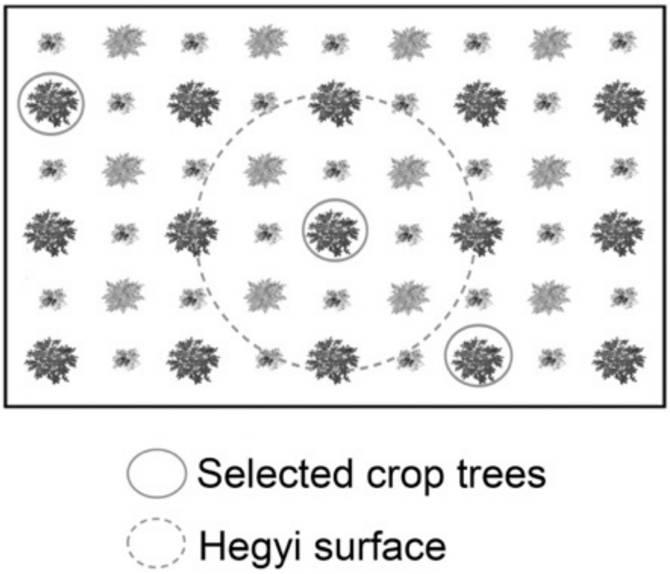


Figure 2 - Hegyi competition index (distance -weighted size ratio index) H = $\sum^{n}_{j=1} (dj/di) * 1/Dij$). dj = DBH competitor; di = DBH crop tree, Dij = distance between competitor and crop tree; circle radius = 8 m.

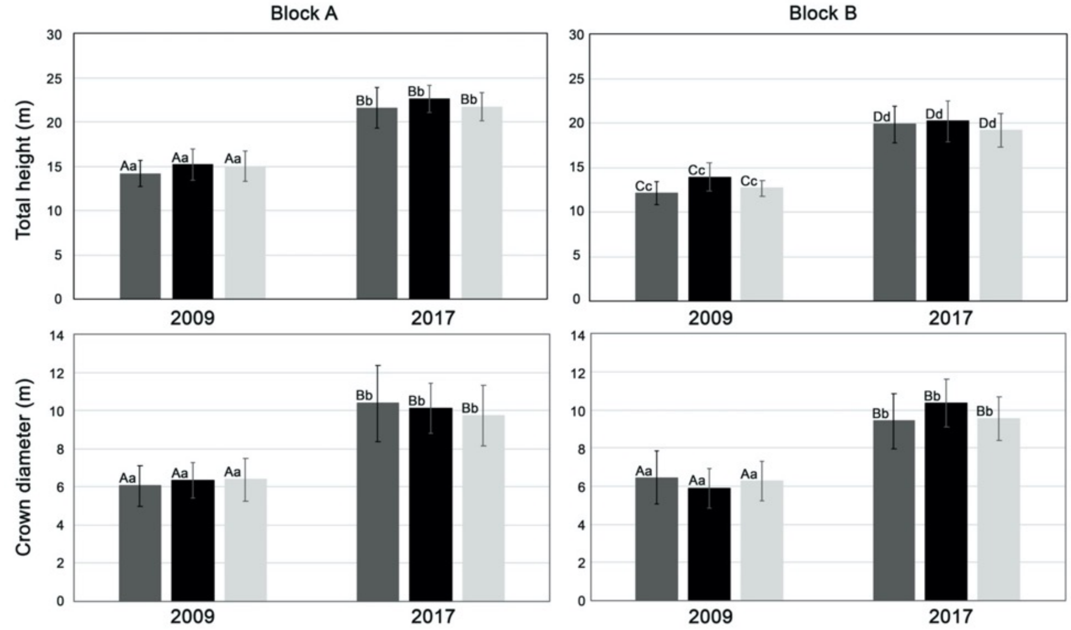


Figure 3 - Main values of Total height and Crown diameter of the common oak crop trees in 2009 and 2017, in the two blocks (A on the right, B on the left) and in three treatments (T1, T2 and T3). Different lower-case letters indicate significant differences among treatments while different capital letters indicate difference among the two blocks.

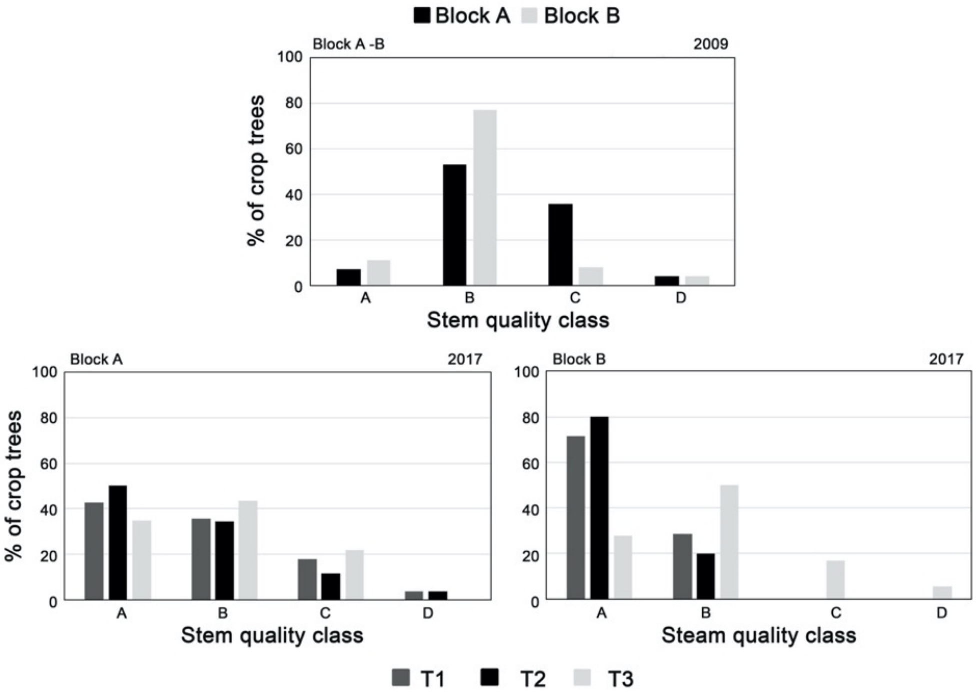


Figure 4 - Stem quality at the two blocks in 2009 and 2017.

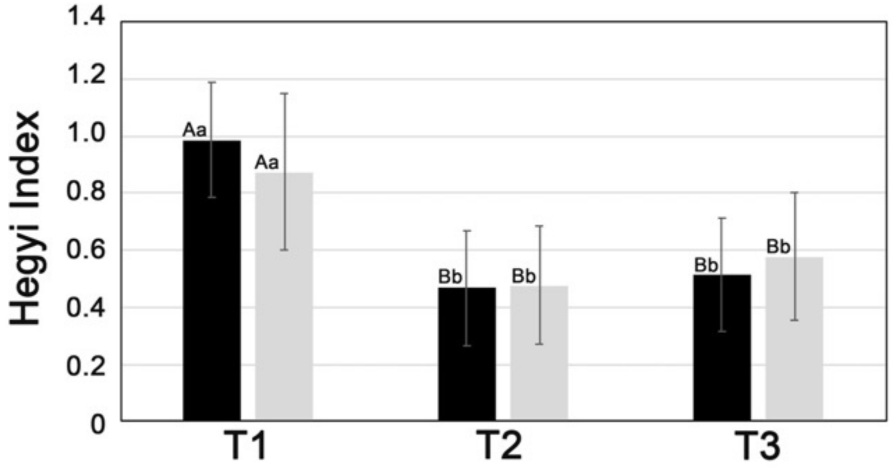


Figure 5 - Hegyi competition index 8 years after thinning occurrence. Different lower-case letters indicate significant differences among treatments while different capital letters indicate difference among the two blocks.

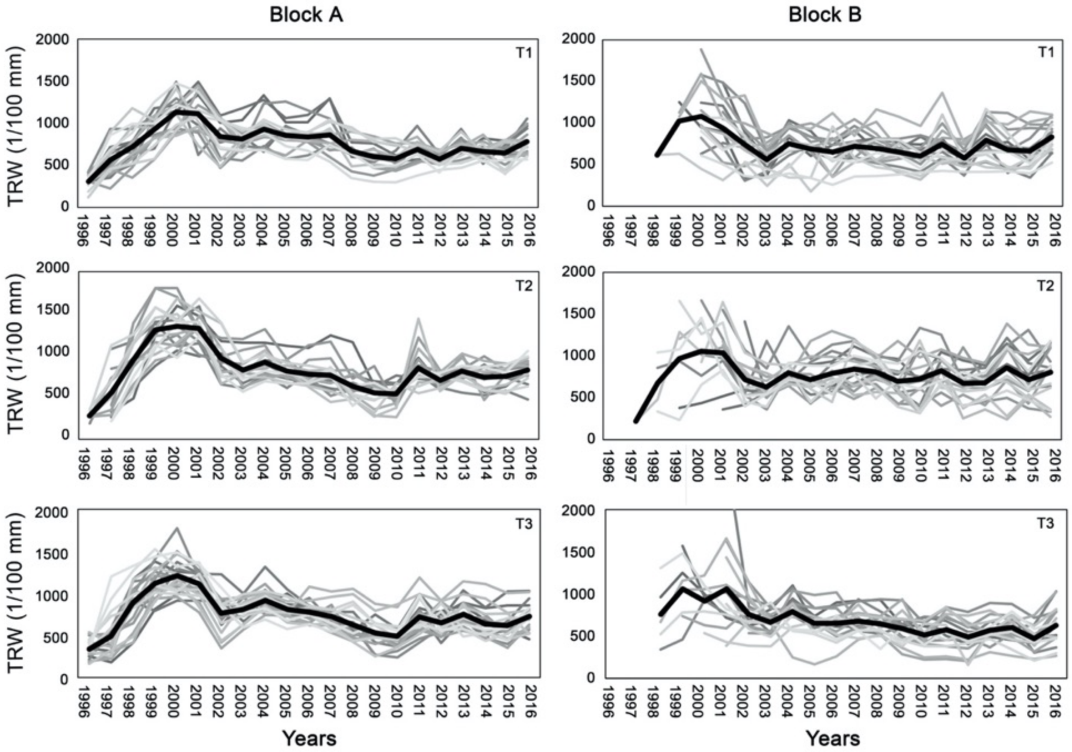


Figure 6 - Individual tree-ring chronologies (in grey) and mean chronologies (in black) for block A and block B. In each block we reported the chronologies referring to T1 (A), T2 (B) and T3 treatments (C).

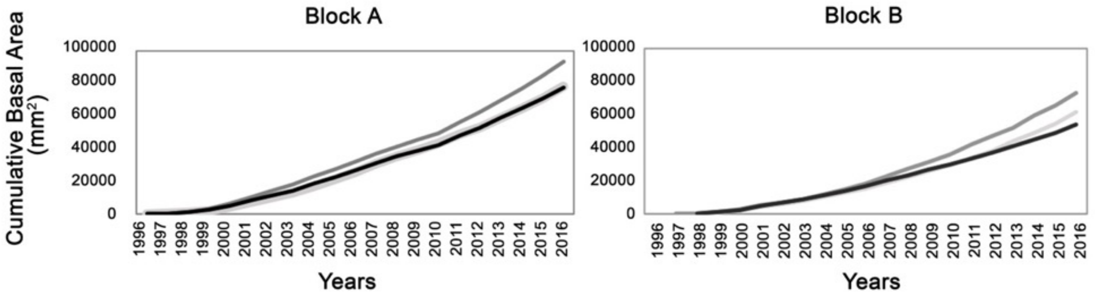


Figure 7 - Cumulative Basal area of each treatment in the block A and B. Light grey indicate T1, grey T2 and black T3.

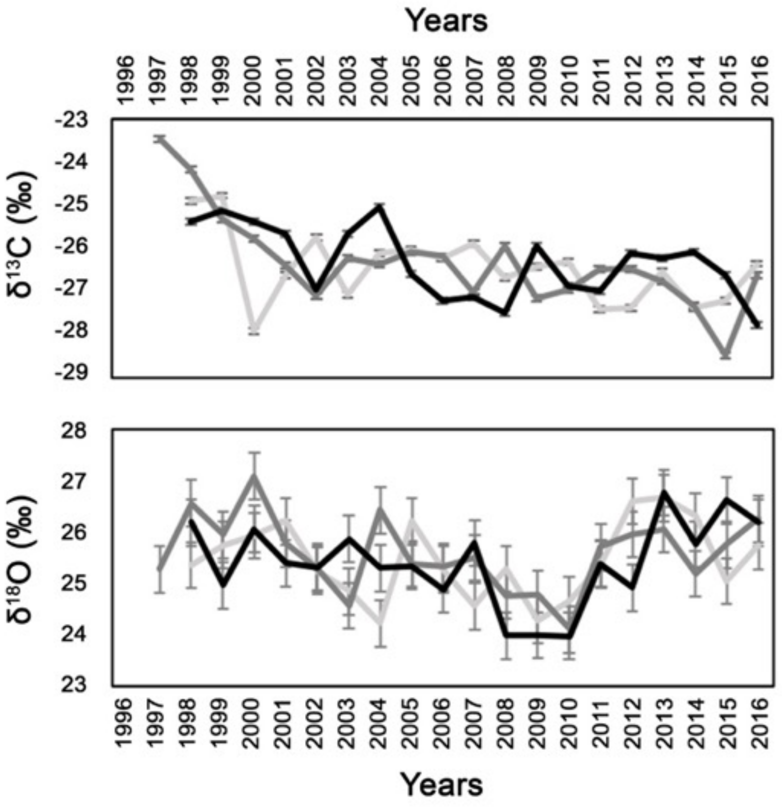


Figure 8 - $\delta^{13}C$ and $\delta^{18}O$ mean chronologies for the three treatments. Light gray indicates T1, grey T2 s and black T3.

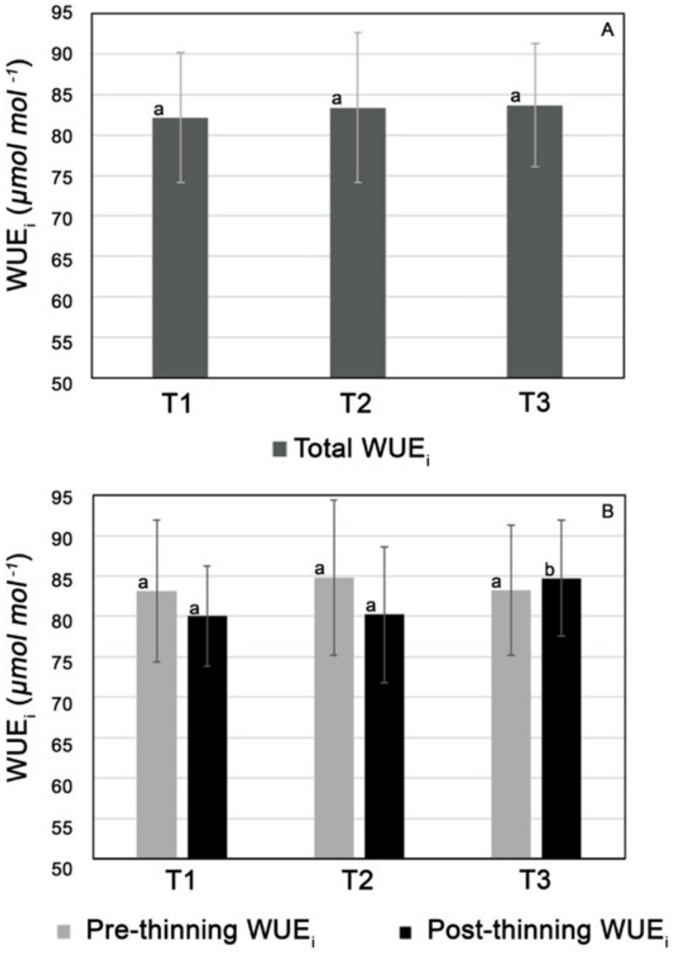
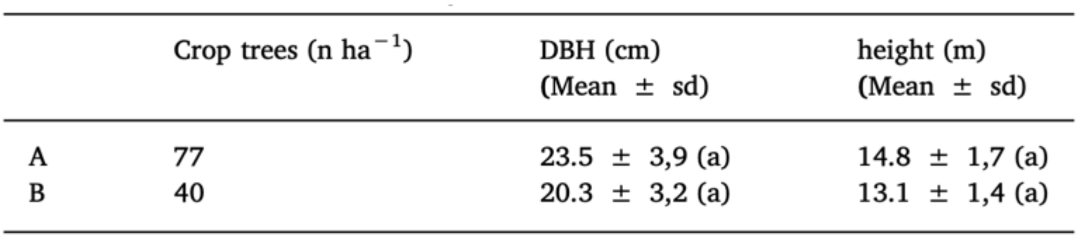


Figure 9 - Mean value of WUE$_i$ (above) in the whole period (1997–2016) where letters indicate difference among blocks. Mean value of WUE$_i$ (below) in the two pre-thinning (1997–2011) and post thinning (2012–2016) periods where different letters indicated statistical difference between the two periods.

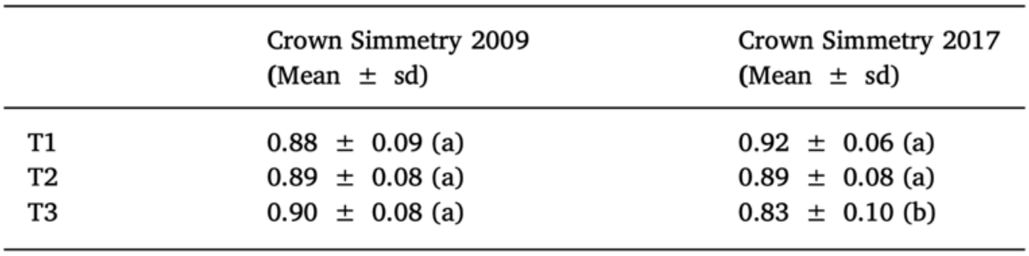
5.8 Tables

Table 1 - DBH and height of the common oak crop trees, at the time of the 1st inventory (December 2009, age 14 year, before thinning) in the two blocks (A and B). Letters indicate difference among blocks.



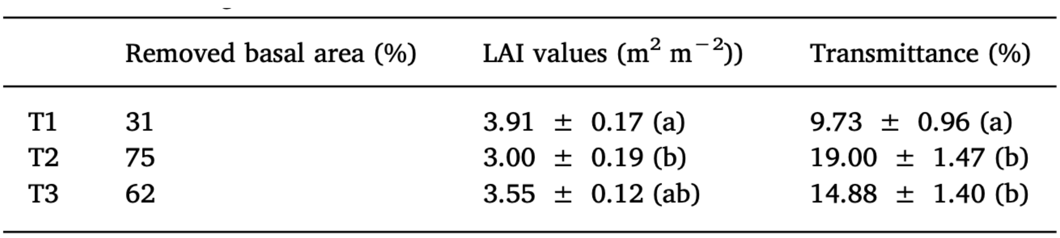
	Crop trees (n ha^{-1})	DBH (cm) (Mean ± sd)	height (m) (Mean ± sd)
A	77	23.5 ± 3,9 (a)	14.8 ± 1,7 (a)
B	40	20.3 ± 3,2 (a)	13.1 ± 1,4 (a)

Table 2 - Mean Crown symmetry in 2009 and 2017 ± standard deviation (sd) of crop trees in the three treatments (T1, T2, T3). The values of the two blocks were averaged. Letters indicate difference among treatments.



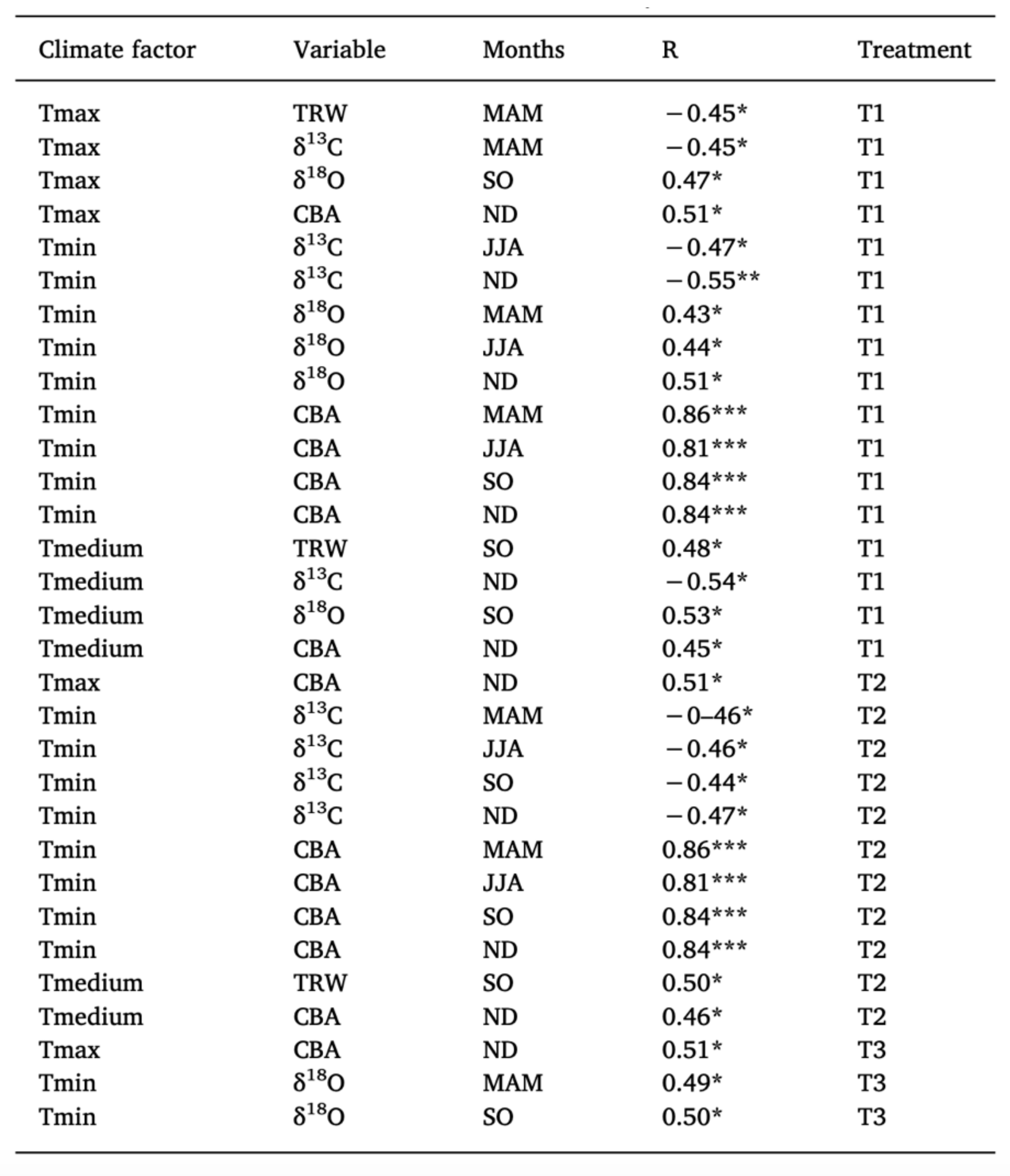
	Crown Simmetry 2009 (Mean ± sd)	Crown Simmetry 2017 (Mean ± sd)
T1	0.88 ± 0.09 (a)	0.92 ± 0.06 (a)
T2	0.89 ± 0.08 (a)	0.89 ± 0.08 (a)
T3	0.90 ± 0.08 (a)	0.83 ± 0.10 (b)

Table 3 - LAI and transmittance values ± standard error recorded in the three treatments (T1, T2, T3). The values of the two blocks were averaged. Letters indicate difference among treatments.



	Removed basal area (%)	LAI values (m^2 m^{-2}))	Transmittance (%)
T1	31	3.91 ± 0.17 (a)	9.73 ± 0.96 (a)
T2	75	3.00 ± 0.19 (b)	19.00 ± 1.47 (b)
T3	62	3.55 ± 0.12 (ab)	14.88 ± 1.40 (b)

Table 4 - Correlations between tree ring width (TRW), $\delta^{13}C$, and $\delta^{18}O$ of the three treatments, with the medium (Tmedium) maximum (Tmax) and minimum (Tmin) seasonal temperatures registered during combination of months (MAM = March, April and May; JJA = June, July and August; SW = September and October; ND = November and December). R indicates the Pearson correlation coefficient, with the corresponding statistical significance (* 0.05, ** 0.01, *** 0.001) calculated by the t-test.



Climate factor	Variable	Months	R	Treatment
Tmax	TRW	MAM	−0.45*	T1
Tmax	$\delta^{13}C$	MAM	−0.45*	T1
Tmax	$\delta^{18}O$	SO	0.47*	T1
Tmax	CBA	ND	0.51*	T1
Tmin	$\delta^{13}C$	JJA	−0.47*	T1
Tmin	$\delta^{13}C$	ND	−0.55**	T1
Tmin	$\delta^{18}O$	MAM	0.43*	T1
Tmin	$\delta^{18}O$	JJA	0.44*	T1
Tmin	$\delta^{18}O$	ND	0.51*	T1
Tmin	CBA	MAM	0.86***	T1
Tmin	CBA	JJA	0.81***	T1
Tmin	CBA	SO	0.84***	T1
Tmin	CBA	ND	0.84***	T1
Tmedium	TRW	SO	0.48*	T1
Tmedium	$\delta^{13}C$	ND	−0.54*	T1
Tmedium	$\delta^{18}O$	SO	0.53*	T1
Tmedium	CBA	ND	0.45*	T1
Tmax	CBA	ND	0.51*	T2
Tmin	$\delta^{13}C$	MAM	−0–46*	T2
Tmin	$\delta^{13}C$	JJA	−0.46*	T2
Tmin	$\delta^{13}C$	SO	−0.44*	T2
Tmin	$\delta^{13}C$	ND	−0.47*	T2
Tmin	CBA	MAM	0.86***	T2
Tmin	CBA	JJA	0.81***	T2
Tmin	CBA	SO	0.84***	T2
Tmin	CBA	ND	0.84***	T2
Tmedium	TRW	SO	0.50*	T2
Tmedium	CBA	ND	0.46*	T2
Tmax	CBA	ND	0.51*	T3
Tmin	$\delta^{18}O$	MAM	0.49*	T3
Tmin	$\delta^{18}O$	SO	0.50*	T3

Chapter 6

6. Tree species composition in mixed plantations influences plant growth, intrinsic water use efficiency and soil carbon stock

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Manuscript published in Forests, 2021

doi: 10.3390/f12091251

6.1 Introduction

The wide spreading of secondary forests worldwide is connected to the huge depopulation of the countryside and mountain marginal lands in favor of big cities and to the abandonment of the agricultural and pastoral activities (FAO, 2020). This land use change has certainly caused a series of positive effects, such as contributing to mitigate global warming through a higher absorption and storage capacity of atmospheric carbon (Zhao et al., 2019). There are, however, also some downside effects such as increase of fire frequency and the closure of open areas with the homogenization of the landscape (Agnoletti, 2007). To mitigate these negative effects and to safeguard the economic and ecological functions of forests, it is necessary to implement sustainable management of secondary forests (Ferretti et al., 2018). In the last decades huge afforestation plans have been financially supported to enhance marginal agricultural areas. Compared to the past, planted forests have globally increased due to the growing demand for timber, pulp, energy and other goods (Evans, 2009; FAO, 2015). In particular, in the European Union, under the support of European Commission Regulation 2080/92, approximately 1,000,000 ha were afforested between 1992 and 1999 (IFD, 2001). Most planted forests were monocultures (Kelty, 2006; Piotto, 2008; Richards et al., 2010; Alem et al., 2015), the main advantages of which being the ease of implementation and management and the possibility of orienting production towards high valued woody assortments. On the other hand, the main disadvantages of monocultures are the high sensitivity to pest and disease attacks, a progressive reduction of soil fertility, the excessively simple plantation structure and the poor biodiversity which lead to low profits in the timber market (Khanna, 1997; Liu et al., 2006; Altieri, 2009). Mixed plantations are increasing globally and various studies have demonstrated their superiority in providing various ecosystem services such as greater biodiversity, climate change mitigation, protection from hydrogeological instability, as well as, in some cases, an increasing in tree productivity (Forrester et al., 2006; Liu et al., 2018; Pelleri et al., 2020). Indeed, mixed-tree plantations in comparison to monocultures could promoted a reduction in management costs (Pelleri et al., 2013), an excellent adaptability of plants to extreme climatic events (Booth and Williams, 2012; Hulvey et al., 2013), a better resistance to pests and pathogens outbreaks (Kelty, 2006; Bauhus et al., 2017), improved nutrients cycle and soil fertility (Vandermeer, 1992; Butterfield, 1993). Several studies have highlighted the positive effects on soil fertility of intercropping with N-fixing species (Crews and Peoples, 2004; Vidal et al., 2019; Danise et al., 2021). This type of mixed plantation implies increase in soil organic matter (SOM) (Torbert, J.L., Burger, 2000; Chiti et al., 2007; Voigtlaender et al., 2012; Hoogmoed et al., 2014). A meta-analysis study demonstrated statistically significant increase in the concentrations of C and N in soil of mixed plantations enriched with a N - fixing species (Johnson and Curtis, 2001). However, the success of mixed plantations is variable (Marron and Epron, 2019). As stated by the stress gradient theory (Bertness and Callaway, 1994) and confirmed by subsequent studies (Forrester, 2014), the positive effects determined by competitive production and facilitation species cannot prevail over negative effects (e.g., competition) in stands where stressful abiotic conditions, such as drought, occur. In this context, the study of intrinsic water use efficiency ($_{i}$WUE), defined as the relationship between the net assimilation of C in photosynthesis (A) and stomatal conductance (gs) (Farquhar et al., 1982), allows to evaluate how water competition affects tree growth (Kelty, 2006; Vanclay, 2009) as well as the positive influence of intercropped species on the photosynthetic activity and nitrogen supply of the principal species (Siegwolf et al., 2001; Ripullone et al., 2004; Guerrieri et al., 2010; Battipaglia et al., 2017). Currently, information on how to match different species to increase the chances of success of mixed plantations and maximize site conditions is scarce (Nichols et al., 2007; Manson et al., 2013; Nguyen et al., 2014; Liu et al., 2018). Therefore, to maximize the chances of success of mixed plantations it is necessary to deepen knowledge on their dynamics and the ecophysiological processes that are established among the different tree species, as well as to understand the effects of intercropping on biomass productivity and soil fertility (Vidal et al., 2019). Our research aimed to evaluate the wood formation and tree productivity, trees intrinsic water use efficiency and impact on soil fertility of an experimental tree plantation varied in composition. We examined distinct stands in which two valuable species widely used for the production of timber, white poplar (*Populus alba* L., Salicaceae) and common walnut (*Juglans regia* L., Juglandaceae), are intercropped with each other and with a shade tolerant shrub species such as hazel (*Corylus avellana* L., Betulaceae) and with N-fixing species such as Italian alder (*Alnus cordata* (Loisel.) Duby, Betulaceae) and autumn-olive (*Elaeagnus umbellate* (Thunb.), Elaeagnaceae). We hypothesize that species mixtures, especially N-fixing, could positively affect wood growth and water use efficiency of the studied tree species. Further, the intercropped species could positively affect soil N fertility and increase C stock in soil.

6.2 Materials and Methods

6.2.1 Study area

The experimental area is located nearby Brusciana, 40 km east of Florence, Central Italy (43°40′31″ N, 10°55′22″ E). The area has a Mediterranean climate, with mild and rainy winters and hot and dry summers. The average annual temperature measured in the period between 2008 and 2019 at a climatic station located 5 km from our study site was 15.4 °C, while the average annual rainfall was 878 mm (Servizio Idrologico Regione Toscana (SIR), http://www.sir.toscana.it/consistenza-rete, access on 15 March 2020). The soils developed on recent (Holocene) fluvial deposits and are *Fluventic Haplustepts coarse-loamy, mixed, thermic* of the USDA Soil Taxonomy, according to the 1:250,000 soil map by Regione Toscana (http://sit.lamma.rete.toscana.it/websuoli, access on 15 March 2020). The study site was a mixed tree plantation (7.44 ha) established in 1996 on formerly cultivated land. The tree plantation comprises different and neighboring stands/mixed intercropping where poplar and walnut were planted together using a triangular layout with a distance of 8 m (179 trees per ha), and intercropped with different nurse trees and shrubs, using a rectangular layout of 3.5 × 4 m (715 trees per ha) and maintaining the same layout and density of the two main tree species (Figure S1). The individuals of the same species were all the same genotypes. In this study, the experimental design is the same used by a previous study (Danise et al., 2021). Four types of intercropped systems were examined and compared using a randomized blocks design with three replications:

- Poplar and walnut (plot PJ).
- Poplar, walnut intercropped with hazel (plot PJC).

- Poplar, walnut intercropped with autumn-olive (plot PJE).
- Poplar, walnut intercropped with Italian alder (plot PJA).

6.2.2 Sampling and dendrochronological processing

In December 2018, 10 dominant trees of *P. alba* (mean diameter at breast height 43.5 ± 6.2 cm, mean height 22.4 ± 2.6 m) and 10 dominant trees of *J. regia* (mean diameter at breast height 17.5 ± 2.6 cm and mean heigh 12.7 ± 1.8 m) were randomly selected in each replicate of each mixture. For each tree, two wood-cores were collected by an incremental borer (Haglöfs Langsele, Sweden). The cores were taken at a height of 130 cm from the ground and in east-west direction. The collected cores were fixed on specific wooden supports and subjected to a sanding process, to facilitate tree-rings identification. Subsequently, dendrochronological measurements were made to the nearest 10 µm using a semiautomatic device (LintabTM, Rinntech, Heidelberg, Germany). Through this system, it was possible to measure the width of the tree-rings and elaborate the representative curves of the growth trend of each individual plant (tree-ring width - TRW). In a second step, the obtained TRW series were visually and statistically crossed with each other through the “Gleichaeufigkeit” (GLK), a specific parameter that assesses the correlation between the different series (Eckstein and Bauch, 1969). Synchronization was considered acceptable with a GLK > 0.70. Finally, to understand the effect of the different intercropping on the diametrical increase, the mean tree-ring width chronology of each replicate and the annual Basal Area Increment (BAI) have been calculated for each intercropping systems and species, J. regia and P. alba. BAI was calculated from raw ring width, considering concentrically distributed tree-rings (Martín-Benito et al., 2010). The use of BAI avoids detrending procedure (Biondi, 1999) allowing to not lose information on low frequency variability. Then average of Cumulative Basal Area (CBA) was determined by summing the average BAI and propagating uncertainties (Battipaglia et al., 2015; Niccoli et al., 2020). To distinguish the measurements of the two studied tree species in each intercropping systems, hereafter we refer to intercropping and then P (for poplar) and the J (for walnut) after an underscore, e.g., PJ_P refers to P. alba sampled in the PJ intercropping and PJ_J refers to *J. regia* sampled in the PJ intercropping.

6.2.3 Soil sampling and laboratory analysis

In winter of 2018, eight soil cores (10 cm thick) were sampled by a steel cylinder of known volume (so as to determine the bulk density, BD) in each replication of the four intercropping systems, following a regular net approximately centered in the centroid of the stand, from the surface and after litter removal. Once in the laboratory, soil moisture was measured with standard gravimetric method, oven-drying the soil samples (60 °C) to constant weight, and then passing them through a 2 mm sieve to remove rock fragments and large roots. The fine earth was analyzed for particle size distribution (by the hydrometer method (Gee, G.W., Bauder, 1986)), pH (potentiometrically, in 0.01 M $CaCl_2$, using a liquid to soil ratio of 2.5:1), total C (TC) and N (TN) (by dry combustion at a C/N/S Carlo Erba NA1500 Analyzer). Organic C was measured by pre-treating the samples with 6 M HCl at 80 °C to eliminate carbonates (Santi et al., 2006). Total inorganic carbon (TIC) was the difference between total carbon (TC) and total organic carbon (TOC). For estimating the recalcitrant organic carbon - which is the fraction of TOC assumed to be more stable in soil- the soil was submitted to a chemical oxidation aimed at removing the more labile soil organic matter and isolate an older, chemically resistant fraction of it (Zimmermann et al., 2007). Briefly, 10 g of soil were mixed with 20 mL of 1 M HCl for removing the inorganic carbon. The residue was then washed with 40 mL of distilled water and then added with 100 mL of 1 M NaOCl adjusted to pH 8 and put on a mechanical shaker for six hours at 25 °C. Finally, it was centrifuged at 2000× g for 15 min and the solution discarded. This treatment was repeated four times. Thereafter the sample was washed four times with 50 mL distilled water and oven-dried at 60 °C to constant weight. The C content of the residue was measured by dry combustion and accounted for the mass balance, *i.e.*, subtracting the mass of C in the residue from the mass of TOC in the untreated sample.

6.2.4 Intrinsic water use efficiency determination

From each of the four-intercropping systems, five tree cores that presented the best cross-dating (GLK > 0.80) were selected for carbon isotopic analyses. Tree rings were very narrow, preventing annual resolution, thus groups of three-years rings were separated using a blade cutter. Then the three-year samples were ground into a powder using a centrifugal mill (ZM 1000, Retsch, Germany), using a mesh size of 0.5 mm to ensure homogeneity. The C isotope composition was measured at the Icona Laboratory (DISTABIF, Caserta, Italy) by continuous-flow isotope ratio mass spectrometry (Delta V Advantage, Thermo electron corporation, Bremen Germany), using 0.06 mg of dry matter for $\delta^{13}C$ measurements. The standard deviation for the repeated analysis of an internal standard was less than 0.1% for $\delta^{13}C$ measurements, whereas the standard deviation for Sigma-Aldrich α-cellulose (item C8002) were less than 0.2‰ for $\delta^{13}C$.

The results from the isotope ratio deviations are presented using the common δ notation:

$$\delta = \left(\frac{Rs}{Rr} - 1\right) \text{x } 1000‰ \quad (1)$$

where *R* refers to the ratio of the ^{13}C to ^{12}C isotopes in the sample (‘*s*’) and the reference (‘*r*’), compared to the PDB (Pee Dee Belemnite) standard. Therefore, tree Δ^{13}C was calculated as follows:

$$\Delta^{13}\text{C} = \frac{\delta 13\text{ca} - \delta 13cp}{1 + \delta 13cp} \quad (2)$$

where $\delta^{13}c_a$ and $\delta^{13}c_p$ are the ratios in atmospheric CO_2 and tree-ring, respectively. The relative rates of carbon fixation and stomatal conductance are the primary factors that determine Δ (Farquhar et al., 1989).

$$\Delta^{13}\text{C} = \text{a} + (\text{b} - \text{a})\,\frac{ci}{ca} \quad (3)$$

where a is the discrimination against $^{13}CO_2$ during CO_2 diffusion through the stomata (a = 4.4‰), *b* is the discrimination associated with carboxylation (*b* = 27‰), and *ci* and *ca* are the intercellular and ambient CO_2 concentrations, respectively. The intrinsic water-use efficiency ($_i$WUE) can be defined as the ratio of the fluxes of net photosynthesis and conductance for water vapor, which indicates the cost of assimilation per unit of water. Using the simplified, linear relationship (Farquhar et al., 1982), the intrinsic water use efficiency is calculated as:

$$_i\text{WUE} = \frac{A}{gs} = \frac{ca - ci}{1.6} = ca\,\frac{1 - \frac{ci}{ca}}{1.6} \quad (4)$$

where *A i*s the rate of CO_2 assimilation, *gs* is the rate of leaf stomatal conductance, and 1.6 is the ratio of diffusivities of water and CO_2 in the atmosphere. Eq. 4 is the "basic" form of isotopic discrimination that does not include effects due to mesophyll conductance and photorespiration (*i.e.*, assuming an infinite internal conductance), which were not available for the species here. δ^{13}ca can be estimated for the period 1995–2004 according to a reference study (McCarroll and Loader, 2004) and the measured values for the period 2005-2018 available online (http://www.esrl.noaa.gov/gmd/), while ca is the concentration of CO_2 in the atmosphere, estimated for each year and obtained by NOAA (http://www.esrl.noaa.gov/, Mauna Loa station).

6.2.5 Climate analysis

To evaluate how climatic factors affected the productivity of the studied tree species, the climatic information recorded in the study area in the period between 1995 and 2018, available from the CRU TS3.23 gridded dataset at 0.5° resolution data (Harris et al., 2014), were examined (Figure 1). In particular, we correlated minimum, maximum, and average monthly temperatures and monthly and total rainfall with CBA data and water use efficiency of the two species, *J. regia* and *P. alba*. Climate data, after being grouped by months, were correlated using the software Microsoft XLSTAT, with Spearman correlation function ($P<0.05$).

6.2.6 Data analyses

All data were checked for normality (using the Shapiro–Wilk test) and homogeneous variance (by means of Levene's test) before applying inferential statistics. Differences between intercropping systems in terms of soil parameters, tree growth and $_{i}$WUE, were evaluated according to one-way ANOVA, using Student–Newman–Keuls coefficient for comparison tests ($P<0.05$). Cumulative basal area data were compared with one way ANOVA, during the whole period and comparing the juvenile phase (8 years) and the adult one. To explore multivariate patterns between the intercropping systems, a principal component analysis (PCA) (Cook and Kairiukstis, 1990) was applied using the package XLSTAT. Data matrices considering the study period and eight variables ($_{i}$WUE, CBA, TN, TOC, TIC, C/N, recalcitrant C and standing tree density) of each intercropping system were processed as ordination method for indirect gradient analysis (Podani, 2000). This analysis allows to reduce the number of independent variables by eliminating those accounting for only a few percent of the total variance (Podani, 1994).

6.3 Results

6.3.1 Dendrochronological analysis of *Populus alba*

The dendrochronological analyses carried out on *P. alba* showed that the growth curves in all intercropping systems had a similar trend: in 2008, 2010, 2012 and 2014 there was a simultaneous decrease in growth, while in 2009, 2011, 2013 and 2015 an increase in growth was observed in all the mixtures (Figure 2). The GLK was very high in all intercropping systems: 75, 78, 82, and 75 in PJ_P, PJC_P, PJA_P, and PJE_P, respectively. In some cases, the wood-cores did not have the first rings because it was not possible to sample the pith, which explains why the curves had different starting points. Anyhow, all *P. alba* trees showed a sustained and regular growth trend in the first years. Subsequently, from 2010 onwards, a substantial reduction in growth occurred. In addition, the collected data showed different growth rates of *P. alba* according to the type of intercropping: intercropping with a third species, such as hazel (PJC_P), Italian alder (PJA_P), or autumn-olive (PJE_P), resulted in higher productivity compared to the simple association with *J. regia* (PJ_P). Looking at the mean cumulative basal area (CBA, Figure 3, referring to the whole ANOVA model testing the overall effect), the greatest mean growth of *P. alba* was found in the PJE_P system, i.e., when it was intercropped with the N-fixing *E. umbellata* ($p = 0.03$). On the contrary, there were no statistical differences ($p > 0.05$) in the growth trends in the case of the intercropping with *A. cordata* (PJA_P) and *C. avellana* (PJC_P). Finally, *P. alba* showed the lowest growth when intercropped with *J. regia* alone (PJ_P, $p = 0.02$).

6.3.2 Dendrochronological analysis of *Juglans regia*

The study of the tree rings carried out suggested an absolute growth lower for *J. regia* than for *P. alba* (Figure 4, $P<0.05$). The average GLK measured in the different intercropping systems was high: 76, 77, 77, and 82 in PJ_J, PJC_J, PJA_J, PJE_J, respectively. The mean chronologies of *J. regia* presented in all intercropping systems a reduction in tree growth in 2003, 2005, 2011, 2012, 2015, and 2017 and a homogeneous increase in tree growth in 2000, 2004, 2014 and 2018. The mean cumulative basal area showed a similar mean growth trend for walnut in the juvenile phase and then a substantial increase in tree growth in the PJE_J (Figure 5, $p = 0.02$). Furthermore, the cumulative curves showed that the plants from PJA_J and PJ_J had low and similar productivity, while that of PJC_J showed the lowest growth (Figure 5).

6.3.3 Relationship between growth, $_{i}$WUE and climate

The comparison between the average values of $_{i}$WUE and mean TRW (Figure 6), calculated for each intercropping systems, showed that both parameters had the highest value when the studied species were intercropped with *E. umbellata* (PJE_J, PJE_P, $p = 0.008$ and $p = 0.006$ respectively). Climate analysis, on the other hand, suggested a positive influence of temperature on the growth of *P. alba* (Table 1). In all intercropping systems the CBA values correlated positively ($p = 0.043$) with the maximum and medium temperatures of autumn (SON = September October and November), as well as with the maximum, minimum and medium temperature of the summer period (JJA = June, July, August). On the contrary, no significant correlations ($p > 0.05$) were observed between growth and precipitation. However, positive correlation ($r = 0.95$ *) between $_{i}$WUE and the precipitation in the June-July-August trimester was observed in the PJE_p plot. Finally, negative correlations with the minimum ($r = -0.80$ *) and medium temperatures ($r = -0.82$ *) of autumn were highlighted with $_{i}$WUE in PJ_P (Table 2). For *J. regia* we observed for all intercropping systems a positive correlation ($p = 0.033$) between CBA values and summer and autumn temperatures (Table 1). There were no significant correlations ($p > 0.05$) between precipitation and CBA. Finally, the correlations between the values of the intrinsic water use efficiency and the climatic data showed positive correlations just in PJE_J with the maximum ($r = 0.86$ *) and medium temperatures ($r = 0.89$ *) of the spring period and whit summer rainfall ($r = 0.84$ *) (Table 2).

6.3.4 Soil properties

The soil analyses showed significant differences between intercropping systems (Table 3). As regards to soil texture (a variable that is actually independent from the tree cover, but with major repercussions on it), PJA showed the lowest sand and the highest silt contents, contrary to what happened in PJC. These differences were most likely antecedent to the establishment of trees and the used randomized blocks design approach did not completely solve the problem. On the other hand, all the tree systems had a rather homogeneous contribution from the clay fraction, which is the pedological driving factor of SOC accumulation (McLauchlan, 2006; Matus, 2021) and water holding capacity of soil. Actually, no major differences in soil moisture (28% ± 7) were found between intercropping systems. Soil bulk density and pH were similar, no significant differences being found between plots. The C concentration in soil showed the most remarkable differences. PJC had the highest soil C content (18.19 g C kg^{-1}), followed by far by PJA, PJ, and PJE (15.2, 13.4, and 13.2 g C kg^{-1}, respectively). No significant difference in soil N was observed between the intercropping systems, despite the presence of N-fixer trees in two of them, i.e., PJA and PJE. The resulting C/N ratio was significantly higher in PJC (15.7), while in all the other plots it ranged between 11.5 and 13. The recalcitrant soil C fraction did not vary much between intercropping systems, ranging from 2.5% and 2.9% of TOC, with the only exception of PJ, where its contribution to TOC was even 8.4%.

6.3.5 Multivariate analysis

The PCA identified the weight of the different variables calculated for the different systems (Figure 7). The first and second components were used for the biplot, so both objects (years) and variables were represented in the same best plane explaining the maximum of variance present in the data. The first two components together explained 60.4% of the variance, with the first component (F1) explaining the higher variance (38.1%). The multivariate analysis showed a clear separation between the different mixtures, with PJ showing a strong positive correlation with recalcitrant C and stand density, and a negative correlation with TIC. Studied tree species in PJC showed a good relationship with TOC and the C/N ratio, while the PJA intercropping was positively related to TN. Finally, the distribution of PJE seemed to be positively influenced by $_{i}$WUE and CBA.

6.4 Discussion

6.4.1 Influence of the Intercropping on Productivity

Climate–growth correlations proved that temperature was an important factor for the growth of the studied tree species, showing a positive influence in summer and, especially, autumn, when Mediterranean species can reactivate the cambium because the temperature and water are not limiting factors (Balzano et al., 2018; Balzano et al., 2020). The mean annual and cumulative basal area of the studied tree species in all intercropping systems seemed to be linked to the ecological relationships between those species and the ancillary ones. Multi-species forest crops determine favorable interactions among species through a mechanism known as "principle of competitive production", triggering highest productivity (Vandermeer, 1992; Loreau et al., 2001; Tobner et al., 2014). This effect is based on niche separation among species, thus interspecific competition can be lower than intraspecific competition for a given environmental factor (such as light, water, nutrient), determining a more efficient use of resources with consequent greater production of biomass of studied tree species species (Vandermeer, 1992; Kelty and Cameron, 1995). Further positive effects can arise from the “facilitative production principle” (Vandermeer, 1992; Loreau et al., 2001; Tobner et al., 2014), which works when the studied tree species are intercropped with facilitation plants; these are plants capable of positively influencing tree growth in terms of height and diameter of the principal species, despite the increase of stand density, through an advantageous alteration of the environmental conditions, such an increase of light availability for the crown (Pretzsch et al., 2014). In the PJ system the two pivotal tree species, in particular *P. alba*, showed low productivity despite the low stand density. Here, soil organic matter was relatively richer in the recalcitrant fraction, i.e., the one that best resists decomposition because of its intrinsic chemical composition (Fang et al., 2005; von Lützow et al., 2007), and this may indicate slow recycling of nutrients and their inadequate availability in the vegetative period. However, we cannot speculate further, as we explored neither the litter decay rate nor the association between SOM and the mineral fraction, which are controlling factors of nutrient availability to plants. Also, the moderate differences in soil texture we found between the various intercropping systems may have somehow affected the different growth rate. In the PJC stands, the intercropping with *C. avellana* led to a positive effect on the growth of the studied tree species, at least in the first years; indeed, hazel has an excellent ability to rapidly colonize the soil with roots and reduce its competition with weed species (Becquey, J., Vidal, 1997), exploiting different resources. Furthermore, this ancillary species can influence the structure and the height of the canopy of the principal species, in particular of walnut (Bianchetto et al., 2013).. However, the positive effect on growth decreased after few years. When the ancillary species become competitive, equaling or exceeding the principal species in height, thinning is necessary in order to favor the canopy development of the valuable species (Bianchetto et al., 2013). The intercropping with hazel promoted soil C sequestration. Indeed, it has been demonstrated that hazel positively affects forest floor mass (as is evident in our case), soil nutrients and microbial properties in mixed forests when N is not a limiting factor (Mohr and Topp, 2005). The introduction of N-fixing species is generally considered a good forest practice to increase soil fertility and foster the growth of principal species (Frouz et al., 2001; Forrester et al., 2006). In fact, some studies found higher productivity in principal species when they were intercropped with *Alnus glutinosa* L., *Robinia pseudoacacia* L., or *E. umbellata* (Becciolini, R., Pelleri, 2006; Tani et al., 2006; Corazzesi et al., 2010). In our case, the mixed plantations with a N-fixing species, *A. cordata* and *E. umbellata*, determined a high productivity of the studied tree species with some noticeable differences. In fact, especially *P. alba* seemed to be better influenced by *A. cordata* during the juvenile phase (8 years), while in the longer-term the average tree growth was higher in the PJE stands. Italian alder, thanks to its ability to supply nitrogen, guarantees benefits to the growth and development of the canopy (Buresti et al., 1991) and determines greater availability of light and space for the principal plants with its pyramidal conformation (Tani et al., 2006). However, the absence of thinning interventions, before the onset of negative competition phenomena, in the long-term triggered a reduction in tree growth of the studied tree species in PJA compared to PJE. As demonstrated by a previous study (Cutini and Giannini, 2009) on the management of walnut-Italian alder plantations, after a certain period of time thinning of both species have positive effects on the growth of walnuts, stimulating their radial development. The PJE stands presented the highest productivity of the studied tree species, confirming results from several previous studies (Schlesinger and Williams, 1984; Tani et al., 2006, Clark et al., 2008; Mohni et al., 2009). In fact, in addition to being a N-fixing plant, *E. umbellata* has a dense canopy able to reduce competition with weeds (Ponder, 1988; Hemery, 2001), forcing the valuable plants to take on a more slender shape thus positively influencing their height and diameter (Clark et al., 2008; Becciolini and Pelleri, 2006). In the absence of any management intervention, the PJE intercropping offered greater benefits than PJA, most probably because Italian alder is more competitive than *E. umbellata* for light, water, and nutrients, especially towards the *J. regia* (Bianchetto et al., 2013).

6.4.2 Influence on the $_{i}$WUE

As *P. alba* and *J. regia* are very susceptible to water stress (Loewenstein and Pallardy, 1998) (Monclus et al., 2006), the analysis of intrinsic water use efficiency may be the key to explore their different growth responses and productivity in the four systems (Palandrani et al., 2020). *Populus alba* showed almost everywhere higher $_{i}$WUE than *J. regia*. Poplar resorts to various drought resistance strategies that may impact productivity differently: decreased leaf area, leaf abscission, enhanced root growth, increased $_{i}$WUE, stomatal closure, and osmotic adjustment, among others (Roden et al., 1990; Monclus et al., 2006; DesRochers et al., 2007). It has been observed that poplar genotypes with high water use efficiency were less susceptible to water stress than genotypes with low $_{i}$WUE. Thus, poplars with high $_{i}$WUE could be identified and selected for the purpose of ensuring high biomass productivity (Edwards et al., 2012). As for walnut, its growth is strongly affected by water deficit, which results in decreased yield (Lampinen et al., 2003; Buchner et al., 2008) and in a strong competition for soil water when the rooting zones of neighboring plants overlap (Caldwell and Richards, 1989). This could account for the lower tree growth and $_{i}$WUE compared to *P. alba*. There were no significant differences of $_{i}$WUE of the studied tree species in the PJ, PJA and PJC stands, while there was in the $_{i}$WUE of both studied species growing with *E. umbellata*. The PCA analysis showed that the higher productivity of the studied trees in the PJE stand was linked to an increase in their $_{i}$WUE. Such an increase can be attributed to inherent differential physiological responses of different species [explained following (Farquhar et al., 1982) by either an increase assimilation (A) at constant stomatal conductance (gs) or by a reduction in gs at constant A] or by interaction effects of other environmental or climatic factors (Battipaglia et al., 2013; Walker et al., 2021). As the environmental variables were the same in all intercropping systems, we suggest that the increase in $_{i}$WUE and productivity found in PJE could be connected to a major resource availability compared to the other stands. A previous research (Battipaglia et al., 2017) highlighted an improvement in $_{i}$WUE in the principal species *Quercus robur* growing together with *A. cordata* to be essentially due to the increase in N availability (inferred by the increase in N% in tree-rings) that induces an increase in the photosynthetic activity. Indeed, N fertilization has been frequently reported to increase tree $_{i}$WUE (Siegwolf et al., 2001; Ripullone et al., 2004; Guerrieri et al., 2010). *Alnus cordata* and *E. umbellata* are both N fixing species, but the latter appears to have favored more the studied tree species in terms of $_{i}$WUE and productivity. This could be due to the different strategies for water supply implemented by growing in the mixed stands. To minimize water competition, *J. regia* is able to opportunistically exploit the different available water sources (Lauteri et al., 2006), thanks to a very deep and plastic root system (Boudru, 1986). In fact, in the first years of growth, the roots reach a depth of about 3–5 m (Becquey, 1997; Bernetti, 1995), while later they can even deepen up to 10 m (Stanescu et al., 1997). However, intercropping with *A. cordata* can limit roots development in *J. regia* (Salbitano et al., 2001). Therefore, one can hypothesize that in the PJA, a lower root development of the walnut intercropped with an extensive and shallow root system of *P. alba* and *A. cordata* (Lauteri et al., 2006), forced the three species to compete for water supply. Such a competition may have resulted in an increase in $_{i}$WUE and lower productivity of the studied tree species in comparison to the PJE system. On the other hand, intercropping with *E. umbellata* (PJE), an ancillary tree with a superficial root system, could have favored a non-competitive exploitation of the soil water resources with the principal plants. The positive correlations found between the summer precipitations and the $_{i}$WUE values of the studied tree species (Table 2) confirmed greater availability of water in those systems. Indeed, studied species improved their water use efficiency during the June–July–August period. This likely resulted in an increase in photosynthetic activity (A) in the studied tree species, then translated into higher productivity (Ehleringer et al., 1993). Furthermore, the shrubby conformation of *E. umbellata*, unlike Italian alder, implies lower evaporation of water thanks to a greater soil cover (Clark et al., 2008) ensuring large water availability and providing shelter and protection to the main species, particularly in the juvenile stages (Aussenac, 2000).

6.5 Conclusion

We found that *Populus alba* and *Juglans regia* increased their tree growth when growing with ancillary species, especially with N-fixing species, such as *A. cordata* and *E. umbellata.* Further, in all the intercropping systems, the mixture with hazel enhanced soil C stock. *Populus alba* productivity increased especially in the juvenile phase (first 8 years), while in the adult phase we could speculate that resources competition among species became limiting (both aboveground and belowground), in particular with *C. avellana*. Management practices, such as thinning and pruning, as well as scheduling a gradual removal of fast-growing species are thus crucial to favor the development of the principal species in these mixed plantations. Of the two intercropping systems with N-fixing species, the one with *E. umbellata* promoted water use efficiency better in both studied tree species, in particular in poplar. Our study confirms our research hypothesis that companion species, especially with N-fixing species, can influence substantially wood formation and growth as well as soil C stock and highlights the importance of carefully considering the species dynamics in planning forest management.

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6.7 Figures

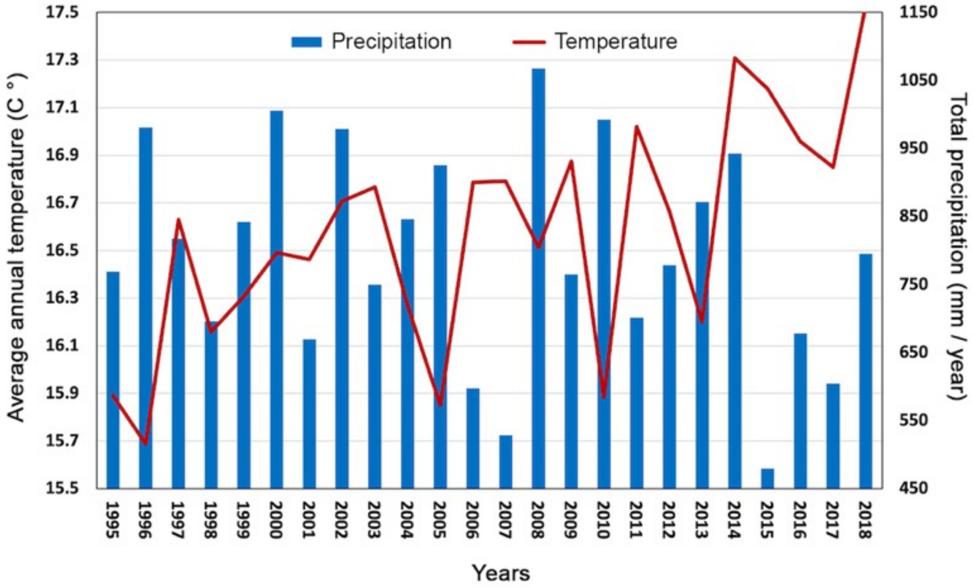


Figure 1 - Trend of total annual precipitation in blue (expressed in millimeters/year) and average temperatures in red (expressed in °C) recorded from 1995 to 2018 and coming from the KNMI Climate Explorer database.

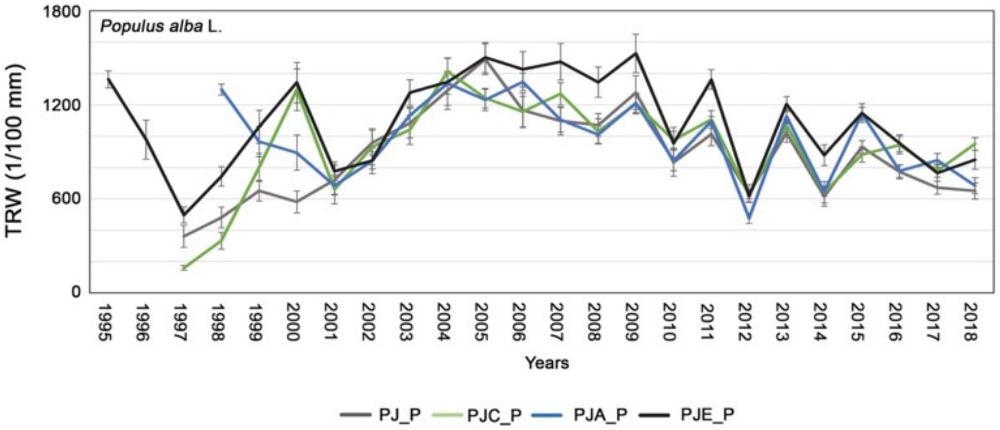


Figure 2 - Average tree-ring chronologies of *Populus alba* in the different systems: PJ_P in gray; PJC_P in green; PJA_P in blue; PJE_P in black. The bars indicate the standard error.

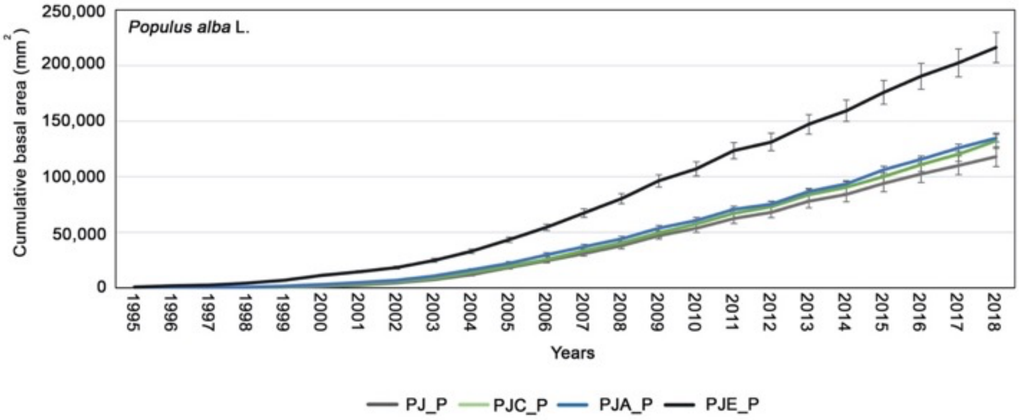


Figure 3 - Cumulative basal area (CBA) calculated for *Populus alba* in the different systems: PJ_P in gray; PJC_P in green; PJA_P in blue; PJE_P in black. The bars indicate the standard error. Differences between intercropping systems were evaluated according to one-way ANOVA, using Student–Newman–Keuls coefficient for comparison tests ($P<0.05$).

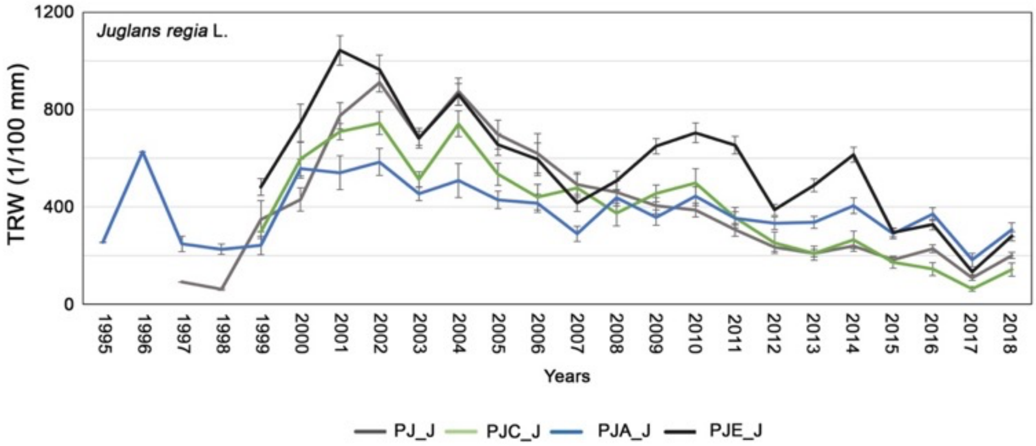


Figure 4 - Average tree-ring chronologies of *Juglans regia* in the different systems: PJ_J in gray, PJC_J in green, PJA_J in blue, and PJE_J in black. The bars indicate the standard error.

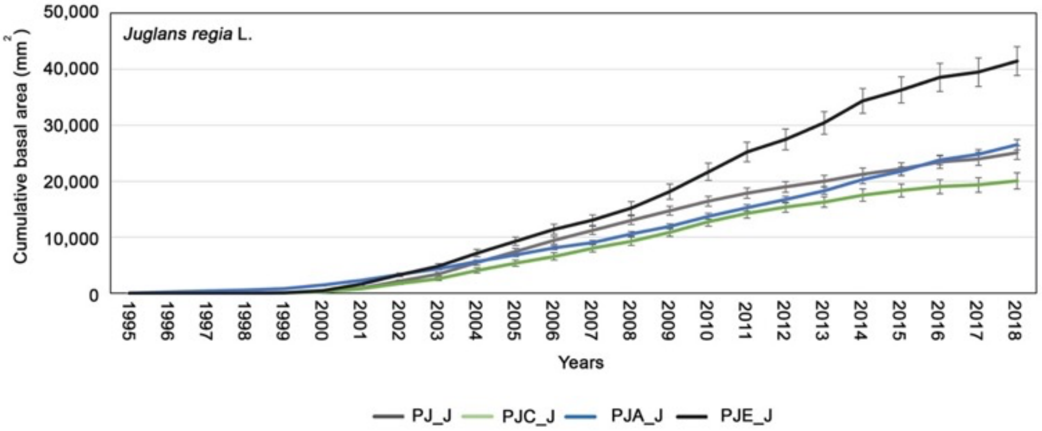


Figure 5 - Cumulative basal area (CBA) calculated for *Juglans regia* in the different systems: PJ_J in gray, PJC_J in green, PJA_J in blue, and PJE_J in black. The bars indicate the standard error. Differences between intercropping systems were evaluated according to one-way ANOVA, using Student–Newman–Keuls coefficient for comparison tests (P<0.05).

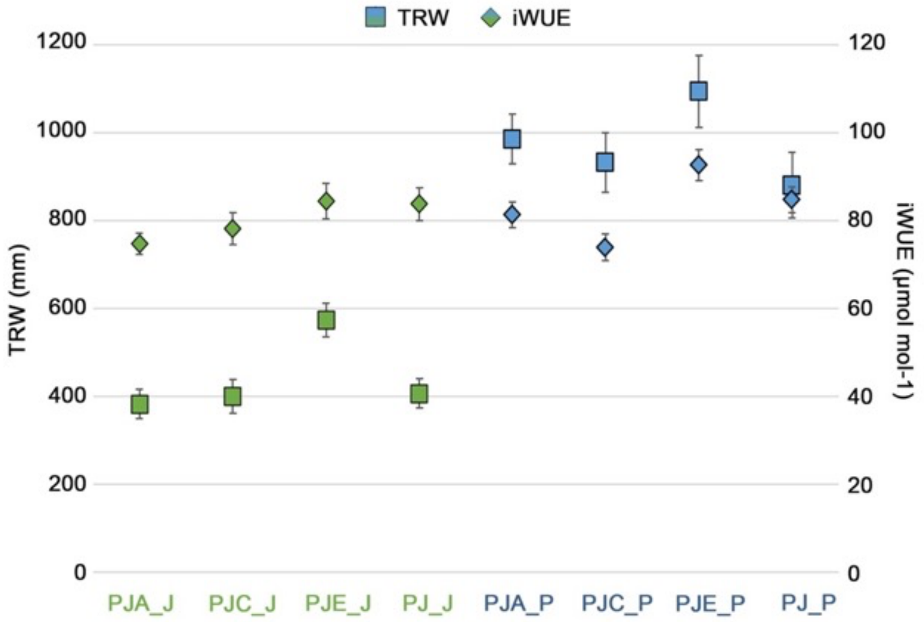


Figure 6 - Comparison between the average values of TRW (square indicator) and the average values of iWUE (rhombus indicator) calculated for *Juglans regia* (in green) and *Populus alba* (in blue) in the different plots. The bars indicate the standard error. Differences between intercropping systems were evaluated according to one-way ANOVA, using Student–Newman–Keuls coefficient for comparison tests ($P<0.05$).

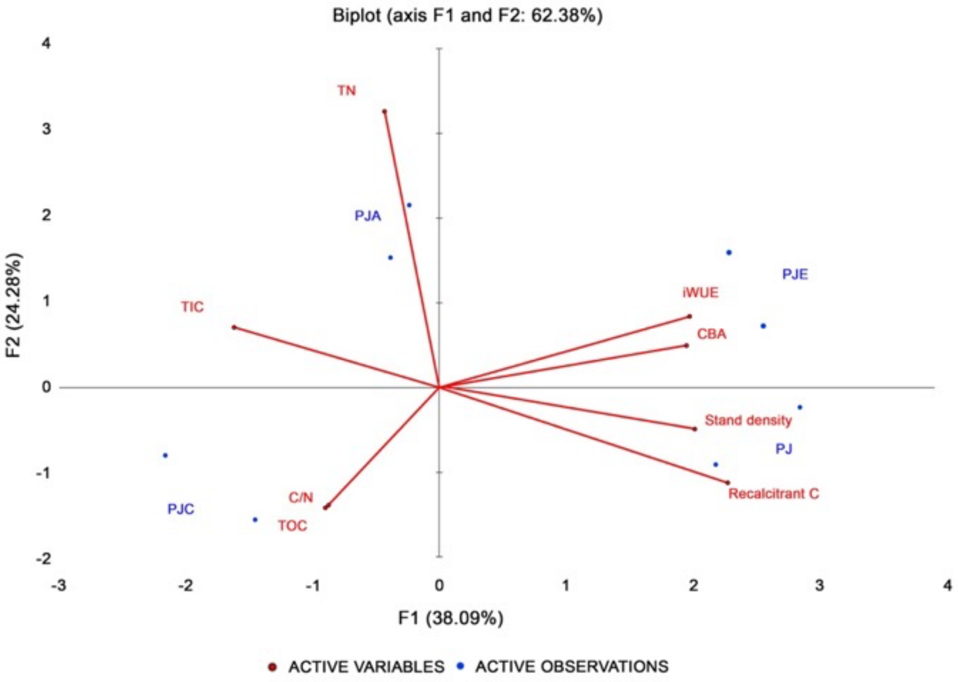
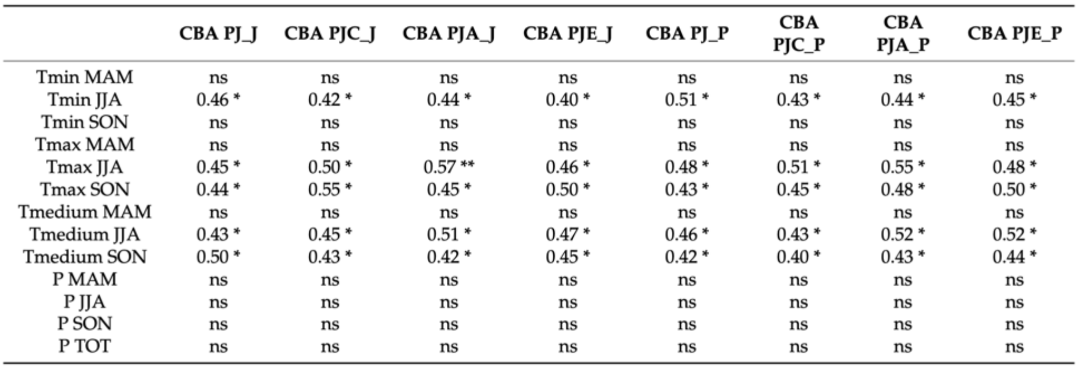


Figure 7 - Biplot of the first two principal components, which accounted for the total variance of 38.1% and 22.3%, respectively. The length of the vectors (in red) indicates the strength of the correlations with respect to the active observations (intercropping systems, in blue). A strong positive correlation is indicated by vectors pointing in approximately the same direction as the active observations. Conversely, vectors pointing in opposite directions suggest a strong negative correlation.

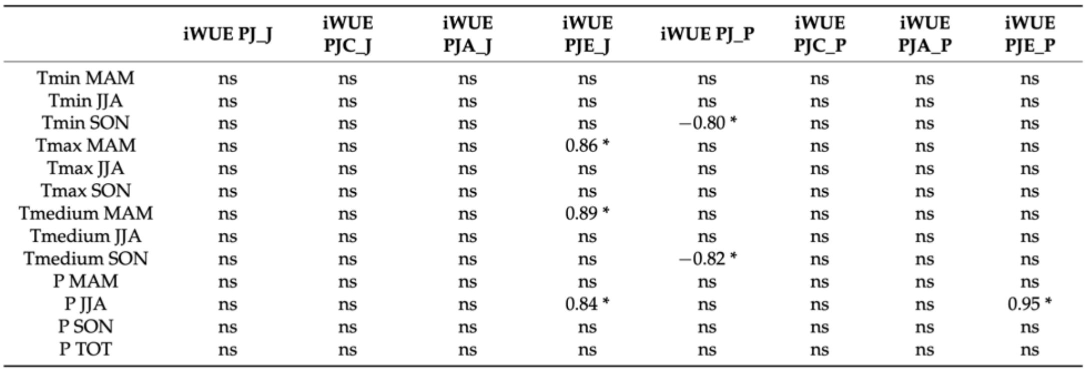
6.8 Tables

Table 1 - Correlations between cumulative basal area (CBA) of each intercropping systems with average temperature (Tmean), minimum temperature (Tmin), maximum temperature (Tmax), and total precipitation (Ptot) grouped seasonally (MAM = March, April and May; JJA = June, July and August; SON = September, October and November). Spearman correlation coefficients are reported (r), with the corresponding statistical significance (* 0.05, ** 0.01) n = 25.



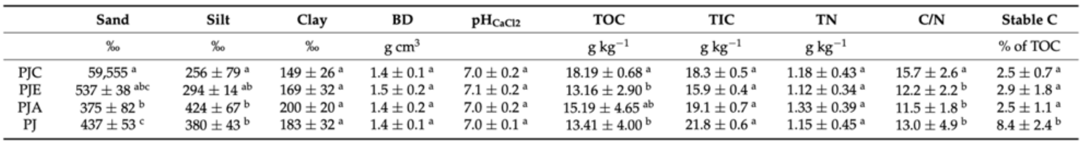
	CBA PJ_J	CBA PJC_J	CBA PJA_J	CBA PJE_J	CBA PJ_P	CBA PJC_P	CBA PJA_P	CBA PJE_P
Tmin MAM	ns	ns	ns	ns	ns	ns	ns	ns
Tmin JJA	0.46 *	0.42 *	0.44 *	0.40 *	0.51 *	0.43 *	0.44 *	0.45 *
Tmin SON	ns	ns	ns	ns	ns	ns	ns	ns
Tmax MAM	ns	ns	ns	ns	ns	ns	ns	ns
Tmax JJA	0.45 *	0.50 *	0.57 **	0.46 *	0.48 *	0.51 *	0.55 *	0.48 *
Tmax SON	0.44 *	0.55 *	0.45 *	0.50 *	0.43 *	0.45 *	0.48 *	0.50 *
Tmedium MAM	ns	ns	ns	ns	ns	ns	ns	ns
Tmedium JJA	0.43 *	0.45 *	0.51 *	0.47 *	0.46 *	0.43 *	0.52 *	0.52 *
Tmedium SON	0.50 *	0.43 *	0.42 *	0.45 *	0.42 *	0.40 *	0.43 *	0.44 *
P MAM	ns	ns	ns	ns	ns	ns	ns	ns
P JJA	ns	ns	ns	ns	ns	ns	ns	ns
P SON	ns	ns	ns	ns	ns	ns	ns	ns
P TOT	ns	ns	ns	ns	ns	ns	ns	ns

Table 2 - Correlations between water use efficiency (iWUE) of each intercropping systems with average temperature (Tmean), minimum temperature (Tmin), maximum temperature (Tmax), and total precipitation (Ptot) grouped seasonally (MAM = March, April and May; JJA = June, July and August; SON = September, October and November). Spearman correlation coefficients are reported (r), with the corresponding statistical significance (* 0.05), n = 8.



	iWUE PJ_J	iWUE PJC_J	iWUE PJA_J	iWUE PJE_J	iWUE PJ_P	iWUE PJC_P	iWUE PJA_P	iWUE PJE_P
Tmin MAM	ns	ns	ns	ns	ns	ns	ns	ns
Tmin JJA	ns	ns	ns	ns	ns	ns	ns	ns
Tmin SON	ns	ns	ns	ns	−0.80 *	ns	ns	ns
Tmax MAM	ns	ns	ns	0.86 *	ns	ns	ns	ns
Tmax JJA	ns	ns	ns	ns	ns	ns	ns	ns
Tmax SON	ns	ns	ns	ns	ns	ns	ns	ns
Tmedium MAM	ns	ns	ns	0.89 *	ns	ns	ns	ns
Tmedium JJA	ns	ns	ns	ns	ns	ns	ns	ns
Tmedium SON	ns	ns	ns	ns	−0.82 *	ns	ns	ns
P MAM	ns	ns	ns	ns	ns	ns	ns	ns
P JJA	ns	ns	ns	0.84 *	ns	ns	ns	0.95 *
P SON	ns	ns	ns	ns	ns	ns	ns	ns
P TOT	ns	ns	ns	ns	ns	ns	ns	ns

Table 3 - Soil data of the different intercropping systems. Values are mean and standard deviation of 24 replicates, with the exception of sand, silt, clay and recalcitrant C that are mean and standard deviation of three samples. BD = bulk density; TOC = total organic carbon, TIC = Total inorganic Carbon; TN = total nitrogen. The superscript letters (^{a,b,c}) indicate significant differences between means in the different column and for each system, according to One-way ANOVA, followed by Student–Newman–Keuls coefficient for comparison tests (P<0.05). Systems that do not share at least one letter are significantly different.



	Sand	Silt	Clay	BD	pH_{CaCl2}	TOC	TIC	TN	C/N	Stable C
	‰	‰	‰	g cm^3		g kg^{-1}	g kg^{-1}	g kg^{-1}		% of TOC
PJC	59,555 ^{a}	256 ± 79 ^{a}	149 ± 26 ^{a}	1.4 ± 0.1 ^{a}	7.0 ± 0.2 ^{a}	18.19 ± 0.68 ^{a}	18.3 ± 0.5 ^{a}	1.18 ± 0.43 ^{a}	15.7 ± 2.6 ^{a}	2.5 ± 0.7 ^{a}
PJE	537 ± 38 ^{abc}	294 ± 14 ^{ab}	169 ± 32 ^{a}	1.5 ± 0.2 ^{a}	7.1 ± 0.2 ^{a}	13.16 ± 2.90 ^{b}	15.9 ± 0.4 ^{a}	1.12 ± 0.34 ^{a}	12.2 ± 2.2 ^{b}	2.9 ± 1.8 ^{a}
PJA	375 ± 82 ^{b}	424 ± 67 ^{b}	200 ± 20 ^{a}	1.4 ± 0.2 ^{a}	7.0 ± 0.2 ^{a}	15.19 ± 4.65 ^{ab}	19.1 ± 0.7 ^{a}	1.33 ± 0.39 ^{a}	11.5 ± 1.8 ^{b}	2.5 ± 1.1 ^{a}
PJ	437 ± 53 ^{c}	380 ± 43 ^{b}	183 ± 32 ^{a}	1.4 ± 0.1 ^{a}	7.0 ± 0.1 ^{a}	13.41 ± 4.00 ^{b}	21.8 ± 0.6 ^{a}	1.15 ± 0.45 ^{a}	13.0 ± 4.9 ^{b}	8.4 ± 2.4 ^{b}

6.9 Supplementary materials

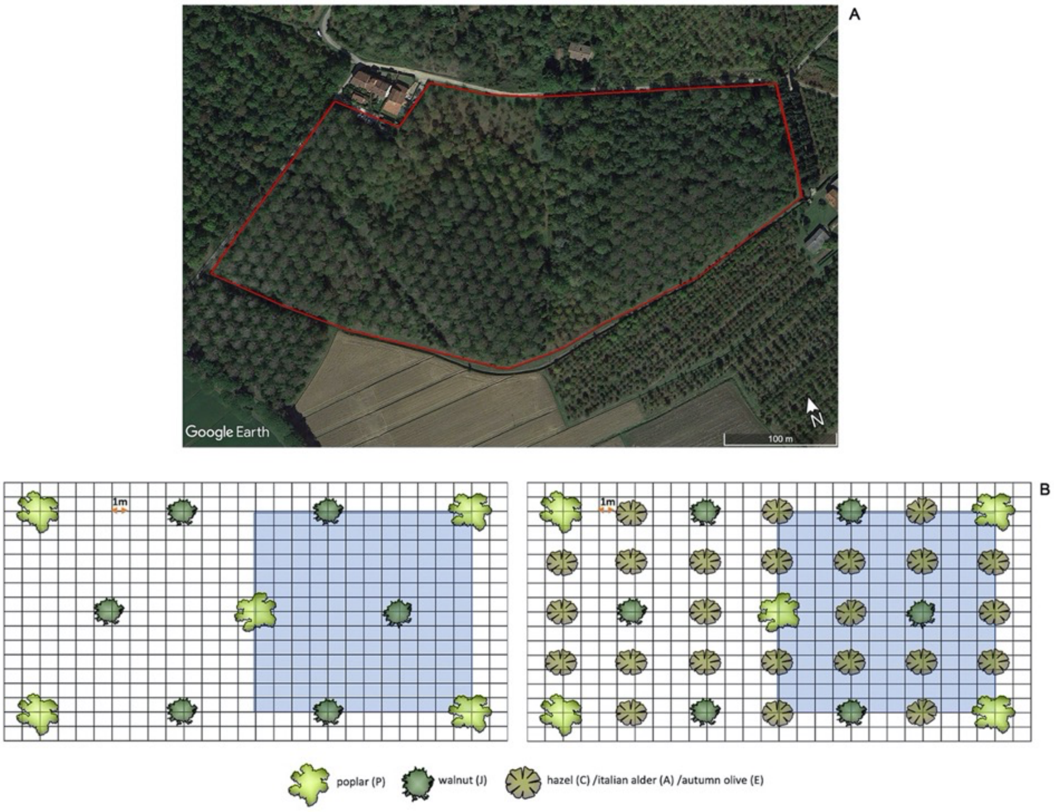


Figure S1 - (A) Study area in which four intercropping systems were compared using a randomized blocks design with three replications. Photo modified by Google Earth, (B) Planting layout of the different intercropping. On the left poplar and walnut intercropping, on the right poplar and walnut intercropped respectively with hazel, italian alder and autumn-olive

Chapter 7

7. General conclusions

The first part of this PhD thesis (chapter 2, 3, 4) evaluated the effects of forest fires and drought on the growth and physiology of a *Pinus Pinaster* Aiton population situated in the Vesuvius National Park. The study carried out in the short-term showed that pine growth reduction was directly proportional to the percentage of crown scorch and that even trees with a high level of defoliation were able to survive in the year of the wildfire thanks to the adaptive traits typical of this species, such as very thick bark and needles. However, medium-term studies showed that the increase of defoliation triggered important limitations in photosynthetic capacity, with severe reduction in productivity compared to the unburned pines. The xylogenesis monitoring, although it excluded direct fire damage to the cambial tissues, underlined a negative influence of hot and dry periods on wood formation dynamics, and above all showed a lower xylem production, a delay in phenology and a poor xylem plasticity in the burned trees. On the other hand, the study of xylem hydraulics, despite allowed to exclude a possible fire influence on the hydraulic efficiency and safety of plants, highlighted problems in stomatal control of burned trees. During severe drought episodes, the burned plants in order to compensate the lower photosynthetic capacity and to counteract the possible reduction in carbon assimilation, decreased the stomatal control of the remaining leaves at the cost of exposing themselves to a greater risk of hydraulic failure. Overall, our results suggested that although maritime pine can survive severe forest fires in the short term, severe defoliation could compromise the species' eco-physiological functions reducing the chances of recovery and increasing the possibility of death during extreme climatic episodes.

The second part of PhD thesis (chapter 5,6) focused on the evaluation of forest management practices able to mitigate the global warming effects on productivity and intrinsic water use efficiency in Mediterranean forests. The first case study analysed the effects of three thinning treatments with different intensity applied in a *Quercus robur* plantation. The results obtained showed that selective thinning of high intensity, with a 75% reduction in biomass, was the most appropriate management practice compared to other treatments: the reduction in competition for light and resources led the examined plants to an increase of productivity and a limited water loss. Instead, the second case study compared different intercropping system evaluating the effects on the two principal species *Populus alba* and *Juglans regia* intercropped with each other and with N-fixing or competitive production species. The results of this research showed not only that the principal species had higher productivity and iWUE when growing with *Elaeagnus umbellata*, an N-fixing species that favours a non-competitive exploitation of soil water resources, but also that the mixture with *Corylus avellana* improved the carbon stock in the soil.

In conclusion, the set of studies reported here, in addition to experimenting an innovative multidisciplinary approach through the combination of intra and inter-annual studies in tree rings with the most modern forest monitoring technologies, allowed to expand the information on general understanding of the mechanisms underlying the survival and death of Mediterranean forests under climate change effects. The results contained in this compendium provided new knowledge on the eco-physiological processes triggered by forest fire and drought in *Pinus Pinaster* Aiton, as well as on forest management strategies aimed at improving adaptation and resistance of Mediterranean forests to forest disturbances.

Summary (in Italian)

Le foreste sono una risorsa preziosa: regolano i servizi eco-sistemici, salvaguardano la biodiversità e svolgono un ruolo essenziale nei cicli del carbonio e dell'acqua. Tuttavia, la crescente pressione antropica ed i recenti cambiamenti climatici stanno minacciando l'integrità del nostro patrimonio forestale in molte parti del mondo. Infatti, il progressivo surriscaldamento globale sta alterando i tassi fisiologici, la crescita, la fenologia e la sopravvivenza degli alberi, determinando gravi conseguenze sul funzionamento degli ecosistemi. Recenti studi hanno riportato un considerevole aumento della mortalità delle foreste in diversi biomi e le previsioni future suggeriscono scenari pessimistici. Secondo le recenti osservazioni la temperatura media globale è aumentata di 0.15-0.20 °C per decennio dal 1975, e si prevede che possa aumentare ulteriormente di 1.5°C tra il 2030 e il 2050. Pertanto, anche in scenari conservativi, è probabile che i futuri cambiamenti climatici determineranno un aumento della frequenza e della gravità di eventi climatici estremi, come la siccità e le ondate di calore, oltre che un aumento della frequenza e della severità degli incendi boschivi. Tra le regioni europee, il bacino del Mediterraneo è uno degli ambienti più colpiti dagli effetti del surriscaldamento globale. In quest'area l'aumento delle temperature e l'alterazione dei tassi di precipitazione hanno provocato diffusi episodi di declino forestale. Negli ultimi anni la mortalità delle foreste europee è raddoppiata e nei prossimi decenni potrebbero verificarsi gravi effetti a cascata sulla biodiversità e sulla funzionalità degli ecosistemi forestali mediterranei con importanti conseguenze ecologiche ed economiche.

Nonostante i cambiamenti climatici siano da decenni al centro dell'interesse della comunità scientifica, i meccanismi alla base della sopravvivenza e della morte degli alberi rimangono ancora molto dibattuti e parzialmente inesplorati. Spesso, il declino delle foreste è il risultato dell'interazioni di più fattori di disturbo legati al cambiamento climatico come l'inquinamento atmosferico, la competizione, il disboscamento, le epidemie di parassiti, gli incendi boschivi, la siccità, e le malattie. Tra questi la siccità e gli incendi sono i più importanti e diffusi a livello globale e possono essere considerati rispettivamente come i principali fattori "scatenanti" e "contribuenti" alla mortalità degli alberi su larga scala.

In generale, anche in assenza del fattore fuoco, la prolungata carenza di acqua comporta il declino e la morte delle piante attraverso due principali meccanismi: la carbon strarvation e il fallimento idraulico. La carbon starvation si manifesta quando la prolungata chiusura stomatica durante la siccità limita l'assimilazione fotosintetica e la pianta è costretta ad utilizzare le riserve dei carboidrati non strutturali per soddisfare la richiesta metabolica del carbonio. Il fallimento idraulico, invece, si verifica quando la scarsa disponibilità idrica nel suolo associata ad un'elevata domanda evaporativa determina forti tensioni nella colonna d'acqua xilematica. Se tali tensioni superano il margine di sicurezza idraulico della pianta si verifica la cavitazione dei vasi con il conseguente disseccamento dei tessuti. Oltre agli effetti sull'ecofisiologia, la siccità aumenta le probabilità che grandi incendi si verifichino. Nonostante nella maggior parte dei casi la mortalità arborea sia determinata dagli effetti diretti del fuoco, lesioni parziali possono compromettere la fisiologia delle piante amplificando gli eventuali stress preesistenti come quelli climatici e determinando maggiori probabilità di morte ritardata in alberi che altrimenti sarebbero sopravvissuti. Danneggiamenti parziali alla corona causano una ridotta capacità fotosintetica e stomatica della pianta alimentando il processo della carbon starvation, e provocando una diminuzione della crescita. Anche se il fogliame rimanente riuscisse a soddisfare la richiesta metabolica del carbonio, lesioni meno evidenti al cambio cribo-vascolare potrebbero compromettere la traslocazione degli zuccheri verso il basso, e la divisione cellulare del processo di xilogenesi. Inoltre, le alte temperature delle fiamme possono indurre la deformazione e la destrutturazione dei vasi xilematici compromettendo l'efficienza idraulica e causando una maggiore suscettibilità al fallimento idraulico nel caso di successivi episodi siccitosi.

Pertanto, ampliare le conoscenze sulla vulnerabilità e sulla sensibilità delle specie arboree mediterranee, e comprendere i collegamenti tra clima e salute degli alberi rappresenta una sfida cruciale per anticipare gli impatti del cambiamento climatico e per pianificare le strategie di gestione forestale più adatte. Negli ultimi decenni la ricerca ha prodotto nuove e importanti conoscenze che hanno consentito di sviluppare innovative strategie di gestione forestale finalizzate a migliorare le capacità di adattamento delle foreste mediterranee al cambiamento climatico. Alcune strategie come il diradamento e la consociazione sembrano avere un ruolo significativo per migliorare le prestazioni degli alberi in quanto sono in grado di determinare una maggiore resistenza ai disturbi forestali e un aumento dei tassi di crescita delle specie principali. Tuttavia, nonostante le grandi potenzialità di queste pratiche, ad oggi esiste la necessità di ampliare le informazioni su come massimizzare le loro possibilità di successo, non solo in relazione delle caratteristiche delle diverse specie, ma prendendo in considerazione tutte le possibili variabili influenti.

In questo contesto, questa tesi di dottorato mira a comprendere e valutare le risposte delle foreste mediterranee ai principali disturbi forestali legati ai cambiamenti climatici, come siccità e incendi, nonché valutare le pratiche di gestione forestale in grado di mitigarne gli effetti. Per raggiungere questi importanti obiettivi è stato applicato un approccio multidisciplinare combinando diverse metodologie: studi di dendro-anatomia, analisi degli isotopi stabili negli anelli degli alberi, indagini di xilogenesi e idraulica xilematica, nonché una costante attività di monitoraggio dei parametri eco-fisiologici delle piante arboree utilizzando l'innovativo sistema TreeTalker.

La tesi è composta da una raccolta di cinque articoli scientifici prodotti durante i tre anni del dottorato ed organizzati in diversi capitoli che focalizzano l'attenzione su obiettivi specifici. La prima parte della tesi (capitolo 2, 3, 4) riporta tre studi volti a comprendere gli effetti degli incendi boschivi e della siccità sulla crescita e sulla fisiologia del *Pinus Pinaster* Aiton, una delle specie più diffuse e utilizzate nell'area mediterranea.

Nel capitolo 2 (*Fire severity influences ecophysiological responses of Pinus pinaster Ait.*) è riportata una ricerca che mirava a valutare le possibilità di sopravvivenza nel breve termine di diversi popolamenti di pino marittimo interessati da un grave incendio che ha colpito il Parco Nazionale del Vesuvio nel 2017. Immediatamente dopo l'incendio sono state selezionate quattro aree di studio sulla base del diverso grado di danneggiamento da fuoco riscontrato nelle piante. Al fine di comprendere l'impatto della severità dell'incendio sui processi ecofisiologici e la crescita della specie sono state effettuate analisi dendrocronologiche ed isotopiche sugli anelli di accrescimento. I risultati conseguiti hanno mostrato una diminuzione della crescita negli alberi bruciati direttamente proporzionale al grado di defogliazione subito. Le analisi degli isotopi stabili del carbonio e dell'ossigeno hanno permesso di collegare la minore produttività riscontrata ad una drastica riduzione dell'attività fotosintetica e della conduttanza stomatica nell'anno dell'incendio. Inoltre, lo studio ha evidenziato che, nonostante le gravi limitazioni ecofisiologiche innescate dal fuoco, le alte temperature e il tempo di esposizione delle fiamme non sono state sufficienti a determinare la morte del cambio cribo-vascolare. Infatti, tutti gli alberi sono stati in grado di completare la formazione dell'anello 2017 confermando l'eccellente capacità di adattamento e resistenza al fuoco a del pino marittimo.

Nel capitolo 3 (*Fire affects wood formation dynamics and ecophysiology of Pinus pinaster Ait. growing in a dry Mediterranean area*) analisi inter-annuali di dendrocronologia e degli isotopi stabili negli anelli di accrescimento sono state combinate con il monitoraggio intra-annuale della xilogenesi per studiare gli effetti dell'incendio nel medio termine sulla pineta del Vesuvio. Le indagini dendrocronologiche hanno mostrato che, sebbene gli alberi bruciati siano sopravvissuti all'incendio del 2017, hanno subito nei successivi anni successivi una netta diminuzione della crescita rispetto ai pini incombusti. Allo stesso tempo, le analisi isotopiche hanno suggerito che il danno alla chioma, divenuto più grave nel medio termine, aveva compromesso irrimediabilmente la capacità fotosintetica del popolamento bruciato con gravi ripercussioni sulla fisiologia delle piante. Ciò ha trovato conferma nel monitoraggio della xilogenesi che ha evidenziato non solo un chiaro ritardo nella fenologia cambiale e una minore produttività di cellule xilematiche delle piante bruciate, ma anche messo in luce la maggiore plasticità xilematica delle piante controllo. Queste ultime, infatti, erano in grado di formare fluttuazioni di densità intra-annuali sfruttando il favorevole periodo autunnale e ampliando la loro stagione di crescita rispetto agli alberi defogliati. Infine, l'elevata risoluzione temporale del monitoraggio di xilogenesi ha consentito di esplorare le dinamiche di formazione del legno legate alle variazioni climatiche stagionali. Nonostante le temperature primaverili ed autunnali abbiano consentito una lunga attività cambiale, i mesi caldi e secchi dell'estate hanno influenzato drasticamente la xilogensi provocando un forte rallentamento della crescita soprattutto nelle piante bruciate. Nel complesso i risultati ottenuti da questa ricerca hanno consentito di ipotizzare che le forti limitazioni fotosintetiche e gli eventi ricorrenti di siccità possono determinare negli individui defogliati una incapacità di recuperare lo stato fisiologico ed i tassi di produttività pre-incendio.

Nel capitolo 4 (*Multiparametric approach to assess wildfire impacts on ecophysiological and hydraulic properties of Pinus Pinaster Aiton*) il monitoraggio in continuo del flusso di linfa degli alberi attraverso l'innovativo dispositivo TreeTalker è stato combinato con analisi dendro-anatomiche e studi di vulnerabilità alla cavitazione xilematica per valutare l'impatto del fuoco sui processi fisiologi e idraulici della foresta bruciata di *Pinus pinaster* rispetto al popolamento di controllo. Le analisi di idraulica xilematica hanno mostrato che l'incendio boschivo non ha provocato negli alberi un'alterazione strutturale dei vasi né tantomeno un aumento del rischio di embolia. Tuttavia, le piante bruciate con un alto tasso di defogliazione presentavano una minore capacità di assimilazione e disponibilità di carbonio utile per la crescita e la formazione di pareti xilematiche più spesse rispetto agli alberi di controllo. A questo si aggiunge che il monitoraggio in continuo con il sistema TreeTalker ha evidenziato un inusuale aumento del flusso di linfa degli alberi defogliati rispetto a quelli di controllo durante i mesi più caldi e più secchi dell'anno indicando un'incapacità nel controllo stomatico. Nel complesso, i risultati ottenuti da questa ricerca hanno suggerito che, sebbene il fuoco non abbia influito sull'idraulica xilematica delle piante, il grave danno alla chioma ha innescato nel corso del tempo un grave squilibrio nella conduttanza stomatica, modificando il compromesso tra assimilazione di carbonio e perdita d'acqua ed esponendo gli alberi bruciati a un alto rischio di guasto idraulico durante i periodi di siccità.

La seconda parte della tesi (capitolo 5,6) ha riportato due casi studio relativi alle pratiche di gestione forestale, diradamento e consociazione, in grado di mitigare gli effetti del cambiamento climatico sui popolamenti forestali.

Nel capitolo 5 (*Effects of thinning intensity on productivity and water use efficiency of Quercus robur L.*) è riportato uno studio in cui analisi dendrocronologiche ed isotopiche sono state combinate per analizzare l'influenza della diversa intensità di diradamento sulla produttività e l'efficienza di utilizzo dell'acqua di *Quercus robur* L. Tre tipi di diradamento a diversa intensità sono stati effettuati in una piantagione mista, in cui la specie principale cresceva in consociazione con *Alnus glutinosa* (L) Gaertn., una specie azotofissatrice. I dati hanno suggerito che il diradamento selettivo moderato (riduzione del 31% della biomassa totale) non è stato in grado di ridurre l'elevata competizione per le risorse tra gli individui influenzando negativamente la crescita degli alberi target. La presenza della specie azotofissatrice, rimasta dopo il diradamento, non è stata in grado di contrastare l'effetto negativo della competizione. Un effetto negativo sulla produttività è stato registrato anche con il diradamento geometrico (riduzione del 62% della biomassa totale). Tale trattamento, infatti, ha comportato un aumento dell'asimmetria della chioma della specie principale con una conseguente limitazione dell'attività fotosintetica e della crescita. Infine, il diradamento selettivo ad alta intensità (riduzione del 75% della biomassa totale) è risultata essere la pratica gestionale più adeguata, in quanto ha garantito alla specie principale effetti positivi sia in termini di accrescimento che di efficienza intrinseca di utilizzo dell'acqua.

Nel capitolo 6 (*Tree species composition in mixed plantations influences plant growth, intrinsic water use efficiency and soil carbon stock*) è riportata una ricerca che ha confrontato diversi sistemi di consociazione esaminandone gli effetti sulla crescita e l'efficienza nell'uso dell'acqua di due specie principali *Populus alba* L. e *Juglans regia* L. consociate tra loro e con differenti specie accessorie. Le analisi dendrocronologiche hanno mostrato che sebbene la crescita delle specie principali fosse favorita dalle consociazioni durante i primi anni, nella fase adulta l’effetto positivo diminuiva nella maggior parte dei casi a causa di una maggiore competizione per le risorse tra le specie principali e le specie accessorie.

Tuttavia, contrariamente a quanto riscontrato negli altri stand, *Populus alba* e *Juglans regia* aumentavano notevolmente la loro produttività quando crescevano in consociazione con *Elaeagnus umbellate*, una specie azoto fissatrice. I dati sugli isotopi del carbonio hanno evidenziato che l'Eleagno determinava una più alta efficienza nell'utilizzo dell'acqua delle specie principali grazie ad un apparato radicale poco profondo in grado di favorire uno sfruttamento non competitivo delle risorse idriche del suolo. Infine, le analisi eseguite per comprendere l'influenza dei diversi sistemi di consociazione sulle caratteristiche del suolo hanno evidenziato un maggiore contenuto di carbonio organico nel suolo quando le specie principali crescevano con *Corylus avellana* L.

In conclusione, l'insieme degli studi riportati in questa tesi, oltre ad aver consentito di sperimentare un innovativo approccio multidisciplinare combinando studi intra e inter-annuali negli anelli degli alberi con le più moderne tecnologie di rilevamento forestale, ha permesso di ampliare le informazioni esistenti sulla comprensione generale dei meccanismi alla base della sopravvivenza e della morte delle foreste mediterranee sotto gli effetti dei cambiamenti climatici. I risultati contenuti in questo *compendium* forniscono nuove conoscenze sui processi eco-fisiologici innescati dalla siccità e dagli incendi nel *Pinus Pinaster* Aiton, nonché sulle strategie di gestione forestale volte a migliorare le capacità di adattamento e resistenza delle foreste mediterranee ai disturbi forestali.