



Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und  
Umwelt

Professur für Ökoklimatologie

**Physiological and anatomical acclimation of contrasting tree  
functional types under climate change along their southern  
range limits in Europe**

Elisabet Martínez Sancho

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan  
für Ernährung, Landnutzung und Umwelt der Technischen Universität München  
zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften  
genehmigten Dissertation.

Vorsitzender: Prof. Dr. Axel Göttlein

Prüfer der Dissertation:

1. Prof. Dr. Annette Menzel
2. Prof. Dr. Emilia Gutiérrez Merino (Universitat de Barcelona/Spain)
3. apl. Prof. Dr. Thorsten Grams

Die Dissertation wurde am 14/09/2017 bei der Technischen Universität München  
eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für  
Ernährung, Landnutzung und Umwelt am 05/12/2017 angenommen.



*“For me, trees have always been the most penetrating preachers. In their highest boughs the world rustles, their roots rest in infinity; but they do not lose themselves there, they struggle with all the force of their lives for one thing only: to fulfil themselves according to their own laws, to build up their own form, to represent themselves. Whoever knows how to speak to them, whoever knows how to listen to them, can learn the truth. They do not preach learning and precepts, they preach, undeterred by particulars, the ancient law of life.”*

Bäume: Betrachtungen und Gedichte, Hermann Hesse





## Acknowledgements

This PhD thesis is the final destination of a long four-year journey. I learned so many new things during this experience in a scientific, social and personal level. No one said that it was easy to start from zero in a new place but, with time, I learned to appreciate the new landscapes that this place offered me and I ended up surrounded by wonderful people. This journey has also changed me, resulting, for instance, in a combination of the Mediterranean lifestyle with lots of *leberkäse* and Bavarian beer.

I want to specially thank Prof. Annette Menzel and Dr. Isabel Dorado Liñán for giving me the opportunity to work on this project. I want to thank Annette for her contributions, advice and for teaching me how to be more practical. Thank you Isabel for our nice field campaigns, your unconditional support and advice; and specially for accompanying me on this journey even despite the distance.

However, to be honest, this journey began much earlier, when I started working with Prof. Emilia Gutiérrez Merino from the University of Barcelona. Nothing would have ever happened if she would had not “intoxicate” me with the virus of the dendrochronology. Thank you for our never-ending scientific and personal discussions and all your help and contributions during these years.

I also want to thank all my colleagues along the way, Dr. Anna Bock, Dr. Steffen Taeger, Prof. Dr. Michael Leuchner, Prof. Dr. Susanne Jochner, Dr. Nicole Estrella, Dr. Christian Zang, Dr. Allan Buras, Dr. Christian Schunk, Dr. Julia Laube and Stephan Jung for the excellent working atmosphere and their help, as well as, Nik Hofmann, Anton Knötig, and specially Brigitte Fleischner for their technical and administrative support during these four years. I am especially grateful to Renée for her warm welcome to the group and all the time we spent together, to Michael Matiu for improving *significantly* my statistical and R skills as well as my mood with his relax life style, to Marvin for finding the positive part of everything, to Gourav for explaining me so many stories about documentaries and providing me with all kind of ArcGIS maps, to Wael and Ye for being always so optimistic and friendly, to Upsee for being my friend in the good and in the bad moments, to the bad boys (they know who they are) for the amount cigarettes that I will never be able to pay back, to Nils for having the solution of all kind of problems, to Homa

for having always a smile and to Hannes for sharing with me the joy and the craziness of this job.

Moreover, this PhD thesis would have never been possible without the help of all the students and friends who contributed in the field campaigns, sample processing and analyses: Linar Akhmetzyanov, Ke Chen, Lizeth Vásconez, Kidan Patanant, Hieronymus Jäger and Teresa Schalingner.

I also want to thank Dr. Gerhard Helle, and his team from GFZ-Potsdam, for allowing me to work in his lab and made me feel part of the group, as well as for struggling and fighting with the mass-spectrometer when it was required. I am also thankful to Dr. Ingo Heinrich for his advice and contributions in this PhD thesis, to Heiko for his advice in all the technical-related issues and to Laura, Jenny, Franziska, Katrin and Camilla for their help and friendship during my long journeys in the lab.

I am also thankful to Dr. Uwe Hacke and his team in Edmonton (Canada). He introduced me into the plant hydraulic field and showed me how “challenging” but also rewarding is to work with ring-porous species. He has always been so kind and helpful especially during the review process of my second publication included in this thesis. I also want to thank to all the members of his team (Calya Brocious, Stephan Schreiber, Ryan Stanfield, Rachel Hillabrand and Jaime Sebastian Azcona) for the nice atmosphere and for showing me the charming of the Rocky Mountains.

I also want to thank my friends from Granollers “The Zorris” (Xuxi, Montxifli, Lafilu, Isiflisi, Tanieta and Puigito; and the new generation of mini-zorris Clara, Ivan, Enric and Martí) and also the ones from Barcelona (Joselito, Aurora, Neus, Joan, Ana, Pepo, Manu Marta, Diana, Roberto, Irenini ...) for being there all these four years and made me feel that time has never passed. I am also grateful to the friends I met in Bayern, my little Catalonia in Munich (Laia and Nai and also Martin, David and the “cigronet”) for all the events, dinners, calçotades, telephone calls and hikings. Thanks to Laia for sharing with me her completely different way to see the world (full of colours and shapes) and for asking me so many questions about things that I do not have a clue even being a biologist. Thanks to Nai for showing me the Alps, enjoy them with me! and talking for hours (like “lloques”) on the way to all the summits. Thank you for being my shoulder when it was required. I am also thankful to the *latin*-crew from Freising, and especially to Dr. Picardi

Avocato, Willy Fox and Diego. You bothered me so much but you also made me laugh so much. Ricardo, no one will ever forget how comfortable your guitar is!

I would like to give my special thanks to my parents, my sister and my brother-in-law for their unconditional love and support. They taught me how important is to be honest, to fight hard for the things I want to and to respect my work and myself no matter what the others think. I definitely know where I am from and where I belong to. ¡Os quiero! To my nice and nephews: Martina, Nico and Leo. For asking me funny questions like: How do you go to Munich? Do you have your own airplane? Will you show it to us? Us estimo!

And last but not least to my partner on this journey. Carlos, I might be crazy, but *mein lieber*, you are not in a better shape than me. If we were normal, life would be so boring! Thanks for waiting, standing, coming, trying, learning German, leaving, forgetting German, again waiting, getting angry, sometimes understanding some others not, skyping for hours, cycling, climbing, coring trees; but specially for being there no matter what (time zone or country). I am sure that we will find some new exciting adventures in our paths. I just hope that this time, we find them together.

*“A l'atzar agraeixo tres dons:  
haver nascut dona, de classe baixa i nació oprimida.  
I el tèrbol atzur de ser tres voltes rebel.”*

Divisa, Maria-Mercè Marçal



## Summary

The last report from the Intergovernmental Panel on Climate Change projected relevant climate changes, which comprised an increase of the global annual temperature and heterogeneous changes in the global precipitation by the end of the 21<sup>st</sup> century. Since drought has been described as the main driver of long-term changes and shifts in tree species distributions, its effects would be exacerbated in drought-prone areas such the Mediterranean Basin due to climate change. This fact is crucial for many temperate tree species that reach their southernmost distribution limits in southern Europe. Thus, the persistence of temperate tree species in this area will depend upon their ability to acclimatize to the new climate conditions.

In order to improve our ability to predict future changes in forest composition and coverage, the aim of this doctoral thesis was to gain a better mechanistic understanding of drought-induced responses of trees from different functional types at their rear-edge distribution limits in the Mediterranean Basin. This PhD thesis was focused on two of the most important tree species in Europe in terms of wood production, natural conservation and ecosystem services, Scots pine (*Pinus sylvestris* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.). A multidisciplinary approach was chosen in order to evaluate how drought affects the hydraulic performance, wood anatomical structures and indirect carbon allocation estimators (i.e., tree rings and stable carbon isotopes). A greenhouse experiment was set up to better recognize the species' strategies, particularly for those variables not possible to measure under field conditions. In addition, this investigation also sought to assess the spatial variability of these responses along a west-east gradient in the Mediterranean Basin.

The drought-avoidance strategy displayed by Scots pine mainly relied on a strict stomatal control, which maintained moderately high water potentials during drought. Such a strategy was directly observed in the seedling experiment. At the hydraulic level, this resulted in a reduced number of embolisms within xylem conduits, preserving the xylem functionality for longer periods. A lower plasticity of Scots pine hydraulic traits across rear-edge populations was found, as well as low percentages of loss of conductivity, even during one of the harshest dry spells in the region. This strict control of the stomatal conductance led to an overall increase in water-use efficiency in all the studied populations. However, Scots pines from the studied populations did not benefit from the

increase in atmospheric CO<sub>2</sub> concentrations over the last 50 years, particularly in western populations of the Mediterranean Basin located in Spain, France and Italy, where the ratio between intercellular and atmospheric CO<sub>2</sub> concentrations was tightly limited by climatic conditions. Thus, the increase in water-use efficiency was negatively linked to radial growth in the five studied Scots pine populations, showing negative growing trends.

In contrast, sessile oak as a drought-tolerant species showed a relaxed stomatal control, allowing water potentials fluctuating according to climatic conditions. The high tensions that this feature imposed on the hydraulic system resulted in high percentages of loss of conductivity under dry conditions and lower shares of functional xylem. However, sessile oak also adjusted its wood anatomy after recurrent droughts in order to avoid the impairment of the water transport through smaller but more numerous earlywood vessels. Such an adjustment aimed at better optimizing hydraulic conductivity towards aridity. At the assimilation level, climatic conditions did not restrict intercellular CO<sub>2</sub> of most studied populations, following proportionally the increase in atmospheric CO<sub>2</sub>. Moreover, intercellular CO<sub>2</sub> of populations with higher water availability located in Italy and Slovenia followed the atmospheric CO<sub>2</sub> increase at the same rate, and was exacerbated by high temperatures. Radial growth displayed high site-specific variability, showing negative trends in the most western studied population located in Spain, most likely due to the harsher climatic conditions; whereas central and eastern populations located in Italy, Bulgaria and Romania displayed positive growth trends during the last 50 years. Moreover, the radial growth from the most eastern stand located in Romania seemed to benefit from the increase in water-use efficiency.

Overall, this thesis compiles new evidence in the responses to drought of contrasting functional tree types such as Scots pine and sessile oak. Under the projected drying of the Mediterranean region, the strategy displayed by Scots pine might suggest that possible mortality events triggered by carbon starvation may occur, whereas sessile oak might be more prone to suffer hydraulic failure. Nevertheless, populations located in the west of the Mediterranean Basin of both species may show higher vulnerability. Therefore, the intensity and duration of the forthcoming dry spells will be the key factor reshaping the distribution area of the studied species in the Mediterranean Basin.

## Zusammenfassung

Der letzte Bericht des Intergovernmental Panel on Climate Change prognostizierte relevante Klimaveränderungen, die von einer Erhöhung der globalen jährlichen Temperatur und einer heterogenen Verteilung der globalen Niederschläge bis zum Ende des 21. Jahrhunderts ausgehen. Als einer der wichtigsten Antriebsfaktoren von Langzeitveränderungen und Verschiebungen in Baumartenarealen gilt Dürre, deshalb könnten sich die Auswirkungen in dürreanfälligen Gebieten wie dem Mittelmeerraum aufgrund des Klimawandels verschärfen. Diese Tatsache ist vor allem für viele Baumarten aus dem gemäßigten europäischen Bereich, die ihre südlichsten Verbreitungsgrenzen in Südeuropa erreichen, entscheidend. Denn in diesen Regionen wird das Fortbestehen von den Baumarten der gemäßigten Breiten von ihrer Anpassungsfähigkeit an die neuen klimatischen Bedingungen abhängen.

Das Ziel dieser Doktorarbeit war ein tieferes Verständnis für mechanistische, trockenheits-induzierte Reaktionen von verschiedenen funktionalen Baumtypen an ihren südlichen Verbreitungsgrenzen im mediterranen Bereich zu entwickeln, um die zukünftigen Veränderungen in der Walddzusammensetzung und -bedeckung besser vorhersagen zu können. Der Fokus lag hierbei auf den zwei wichtigsten Baumarten in Bezug auf Holzproduktion, Naturschutz- und Ökosystemdienstleistungen, der Waldkiefer (*Pinus sylvestris* L.) und der Traubeneiche (*Quercus petraea* (Matt.) Liebl.). Es wurde ein multidisziplinärer Ansatz gewählt, um den Einfluss von Dürre auf die hydraulische Leitfähigkeit, holzanatomische Strukturen und indirekte Abschätzungen der Kohlenstoffallokationen (Baumringe und stabile Isotope) zu bewerten. Außerdem wurde ein Gewächshaus-Experiment durchgeführt, um die Anpassungsstrategien der Arten besser zu erfassen zu können, insbesondere für die Variablen, die unter Freilandbedingungen nicht zu messen sind. Darüber hinaus versuchte diese Untersuchung auch die räumliche Variabilität der Reaktionen entlang eines ost-west Gradienten im Mittelmeerraum zu beurteilen.

Die Strategie der Waldkiefer zur Dürrevermeidung beruht vor allem auf der strikten Stomata-Kontrolle, die mäßig hohe Werte des Wasserpotentials während der Dürre halten konnte. Diese Strategie wurde direkt in einem Sämlingsexperiment beobachtet. Unter dem hydraulischen Gesichtspunkt führte dies zu einer reduzierten Anzahl von Embolien innerhalb der Xylemgefäße wobei die Xylem-Funktionalität über längere Zeiträume

aufrechterhalten werden konnte. Deshalb wurde eine geringere Plastizität in den hydraulischen Eigenschaften der Waldkiefer über die Populationen am südlichen Arealrand beobachtet sowie ein prozentual geringerer Verlust der Leitfähigkeit, sogar während einer der stärksten Trockenperioden in dieser Region. Bezüglich der Assimilation führte diese rigorose Kontrolle der stomatalen Leitfähigkeit zu einer Gesamtzunahme der Wassernutzungseffizienz bei allen untersuchten Populationen. Trotzdem profitierten diese Bäume nicht von der Zunahme der atmosphärischen CO<sub>2</sub>-Konzentration in den letzten 50 Jahren, insbesondere bei westlichen Populationen (Spanien, Frankreich und Italien) war das Verhältnis zwischen interzellulären und atmosphärischen CO<sub>2</sub>-Konzentrationen eng durch die klimatischen Bedingungen limitiert. Somit war die Zunahme der Wassernutzungseffizienz negativ mit dem Radialzuwachs der untersuchten Populationen verbunden, dies bestätigte auch der beobachtete Zuwachsrückgang.

Im Gegensatz dazu zeigte die trockenheitstolerante Traubeneiche eine angepasste Stomata-Kontrolle, so dass die Wasserpotentiale je nach klimatischen Bedingungen schwankten. Die hohen Spannungen, die dadurch auf das hydraulische System einwirkten, führten zu hohen prozentualen Verlusten der Leitfähigkeit und geringeren Anteilen des funktionalen Xylems unter Trockenheit. Die Traubeneiche hatte sogar ihre Holzanatomie durch kleinere, aber zahlreichere Frühholzgefäße nach wiederkehrenden Dürren angepasst, um eine Verminderung des Wassertransports zu vermeiden. Diese Anpassung zielte darauf ab, die hydraulische Leitfähigkeit während der Trockenheit zu optimieren. Bei der Assimilation führten klimatische Bedingungen nicht zu Einschränkungen der interzellulären CO<sub>2</sub>-Konzentration bei den meisten untersuchten Populationen sondern folgten dem proportionalen Anstieg der CO<sub>2</sub>-Konzentration. Zudem folgte die interzellulare CO<sub>2</sub>-Konzentration der Populationen bei höherer Wasserverfügbarkeit im selben Verhältnis wie der atmosphärischen CO<sub>2</sub>-Konzentration und erhöhte sich noch bei höheren Temperaturen. Außerdem zeigte der Radialzuwachs hohe standortspezifische Variabilität und negative Trends in den meisten westlichen Populationen, sehr wahrscheinlich aufgrund der trockeneren klimatischen Bedingungen dort, wohingegen zentrale und östliche Populationen positive Zuwächse während der letzten 50 Jahre aufwiesen. Der Radialzuwachs der östlichsten Population scheint von der gestiegenen Wassernutzungseffizienz profitiert zu haben.



Diese Doktorarbeit erarbeitet neue Belege zu den Reaktionen von Waldkiefer und Traubeneiche, zwei gegensätzlichen funktionalen Baumtypen. Während der projizierten Austrocknung der Mediterranregion könnte die Strategie, die von der Waldkiefer verwendet wird zu möglichen Absterbeereignissen durch Kohlenstoffmangel führen, während die Traubeneiche eher unter hydraulischen Versagen leiden wird. Dennoch zeigen westliche Populationen beider Arten eine höhere Vulnerabilität. Deshalb wird die Intensität und Dauer der kommenden Trockenperioden der zentrale Faktor, bei der Neuordnung der Verbreitungsregionen der untersuchten Arten im Mediterrangebiet.



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## 1. Introduction

### 1.1. Climate change

#### 1.1.1. Observations and projections

According to the Intergovernmental Panel on Climate Change (IPCC, 2013) and the Food and Agriculture Organization of the United Nations (FAO, 2010), climate change is one of the greatest challenges of the 21<sup>st</sup> century for the environment, people and economies. Although climate has always been subjected to fluctuations, the scientific community achieved three main consensuses during the last decades: (i) climate is changing, (ii) these changes are predominantly caused by human activities and, (iii) these changes are largely irreversible (IPCC, 2013).

Warming of the climate system is unequivocal, as now evident from observations of the increase in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level. Since the late 19<sup>th</sup> century, the mean global air temperature increased by 0.85°C (period 1880 – 2012) resulting in the late 20<sup>th</sup>- and early 21<sup>st</sup>-centuries being the warmest period of the last 800 years in the Northern Hemisphere (Luterbacher, 2004; Hansen *et al.*, 2010; Luterbacher *et al.*, 2016). Regarding precipitation changes, an irregular annual rainfall distribution across the globe was observed during the last century. Moreover, climate change has also led to changes in the likelihood of the occurrence of extreme weather and climate events such as extreme precipitation or warm spells.

It is generally accepted that the anthropogenically-induced increase in greenhouse gas concentrations along with other internal and external forcings have been the dominant cause of the observed warming (IPCC, 2013). Carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) concentrations have increased to unprecedented levels in at least the last 800,000 years due to human activities (IPCC, 2013). Specifically, carbon dioxide concentration has increased by 40% since pre-industrial times, primarily due to fossil fuel emissions and secondarily due to net land use change emissions (IPCC, 2013). However, the internal variability of the climate system may also play a relevant role according to some studies (Deser *et al.*, 2012).

Climate projections forecast that global warming will continue. Even when assuming the absence of future greenhouse gas emissions, a rise of temperature would be expected due to the inertia of the system (Solomon *et al.*, 2009). However, atmospheric CO<sub>2</sub> concentration in 2100 is projected to increase to at least 486 ppm (in some scenarios even beyond 1000 ppm) compared to the pre-industrial concentration of 280 ppm (Nakicenovic *et al.*, 2010). Thus, by the end of the 21<sup>st</sup> century, global surface temperature is expected to exceed 1.5°C relative to 1850 to 1900 in most of the projected scenarios (IPCC, 2013). Warming will continue to exhibit inter-annual-to-decadal variability and will not be regionally uniform.

#### 1.1.2. Climate change impacts on forest ecosystems

Forests play an essential role for human populations all over the globe, being a crucial natural resource that provides multiple benefits and services. In forests, climate change processes are certainly affecting ecosystem interactions at various temporal and spatial scales. Some of the expected consequences of projected climatic changes in forest ecosystems are, for instance, loss of ecosystem services (Anderegg *et al.*, 2013a); reduction of the terrestrial net productivity (Zhao & Running, 2010) and changes in sink–source carbon dynamics (Jones *et al.*, 2009). Indeed, forests are particularly sensitive to climate change, because the long life-span of trees does not allow for rapid adaptation to environmental changes (Lindner *et al.*, 2010). The CO<sub>2</sub>-induced climate change affects forests in both indirect and direct ways (Figure 1).

The steady CO<sub>2</sub> enrichment of the atmosphere has direct implications for forest ecosystems, modifying the carbon-water relationships within the plants (Körner, 2000; Körner *et al.*, 2007). Theoretically, photosynthesis in most of the C3 plants remains below saturation levels even at twice-current concentrations of atmospheric CO<sub>2</sub>. The increase of atmospheric CO<sub>2</sub> concentration also induces lower stomatal conductance reducing water loss by transpiration. This results in increased water-use efficiency (the amount of carbon uptake per unit of water loss; Farquhar *et al.*, 1982) at leaf and at stand level (Field *et al.*, 1995; Leuzinger & Körner, 2007). Furthermore, an increase of CO<sub>2</sub> might also stimulate the storage of non-structural carbohydrates enhancing tree reserves, for example in form of starch (Handa *et al.*, 2005). However, this potential “fertilization”

effect might not be proportionally translated into tree growth since it could be overridden by other limiting factors such as nutrients (Norby *et al.*, 2010) and must counterbalance the negative effects of the CO<sub>2</sub>-induced global climate change (Andreu-Hayles *et al.*, 2011; Franks *et al.*, 2013). In fact, a whole generation of experiments, the so-called Free Air CO<sub>2</sub> Enrichment studies (FACE), exploring plant responses to future atmospheric CO<sub>2</sub> concentrations found little or non-significant growth increments in both conifers (Klein *et al.*, 2016) and deciduous stands (Körner *et al.*, 2005; Norby *et al.*, 2010; Bader *et al.*, 2013), pointing to a complex interaction between diverse limiting factors (Norby & Zak, 2011).

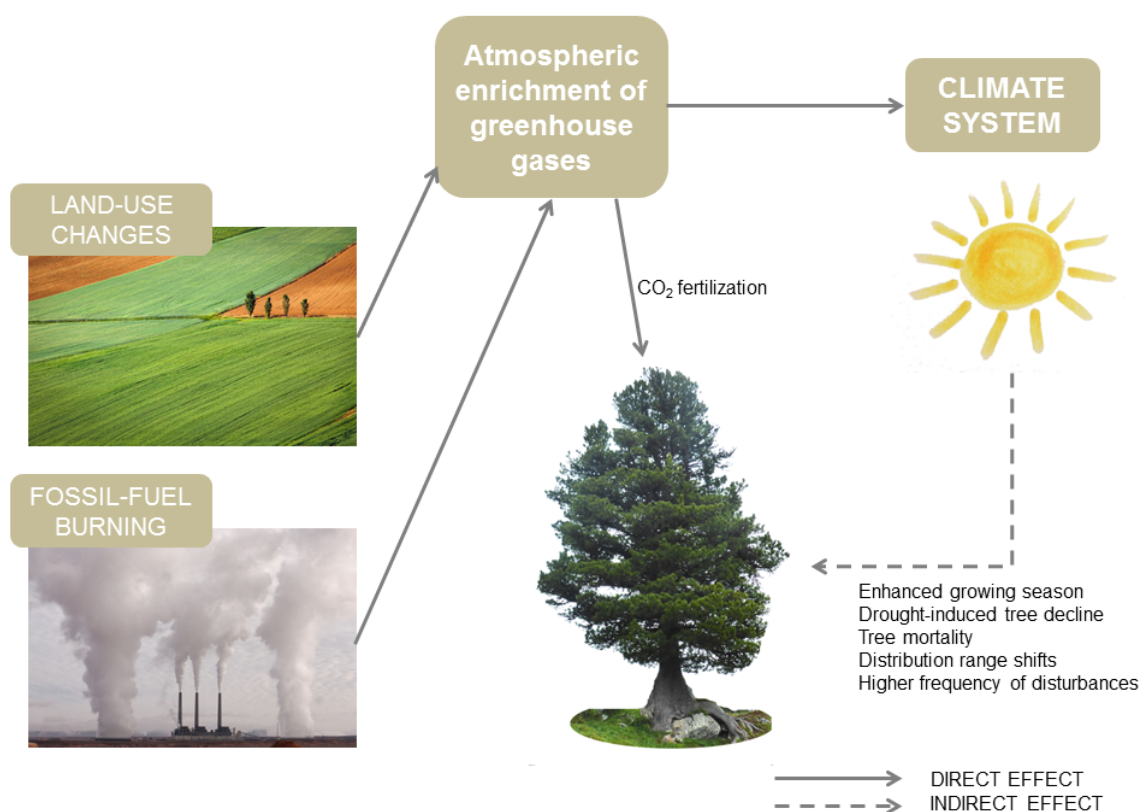


Figure 1. Direct and indirect effects of the increase in atmospheric CO<sub>2</sub>.

Phenology is one of the most obvious processes suitable for tracking changes in the ecology of tree species in response to CO<sub>2</sub>-indirect effects (Walther *et al.*, 2002). The CO<sub>2</sub>-indirect rise of temperature has not only promoted a global earlier timing of spring events such as leaf unfolding or flowering but also an extension of the growing season (Menzel & Fabian, 1999; Menzel *et al.*, 2006; Gordo & Sanz, 2010; Beaubien & Hamann, 2011). Some evidence also indicates a later onset of autumnal phenological

events, but these shifts are less pronounced and show a more heterogeneous pattern (Menzel, 2000).

The rise of temperature and the increase in precipitation variability and extreme events in some areas of the globe also pose additional stress on tree physiology. Plant responses to these novel conditions depend on species-specific characteristics and on the magnitude and rates of the environmental changes (Jackson & Overpeck, 2000; Nicotra *et al.*, 2010). Most species tolerate short-term climatic variations through phenotypic plasticity. However, beyond the point at which individuals (and therefore species) are able to tolerate changes in climate, distributional and evolutionary changes are expected (Jump & Peñuelas, 2005). The accumulating evidence indicates that such thresholds could have already been surpassed for some species. Forest decline and tree mortality have increased all over the world, likely triggered by impacts of global warming and extreme drought events (Allen *et al.*, 2010). Differential phenotypic plasticity in the face of such events is, among other factors, crucial to predict plant mortality and species migrations (Allen & Breshears, 1998; Mueller *et al.*, 2005). These migrations are generated by widespread establishment of new populations at the “leading edge” of species distributions and extinction of populations at the “retreating edge” (Hampe & Jump, 2011). Over the last decades, several studies have reported climate change-induced shifts in the range and abundance of species (Walther *et al.*, 2002; Parmesan & Yohe, 2003), for instance, following elevation gradients in the Alaskan treeline (Lloyd & Fastie, 2003), in southern California (Kelly & Goulden, 2008), in the Swiss Alps (Walther *et al.*, 2005) and in the Mediterranean (Peñuelas & Boada, 2003; Sanz-Elorza *et al.*, 2003) and also following latitudinal gradients (Pearson *et al.*, 2013).

Disturbances, both human-induced and natural, also shape forest systems by influencing their composition, structure and functional process (Dale *et al.*, 2001). Climate change will also alter the frequency and intensity of forest disturbances such as insect outbreaks, invasive species, wildfires and storms, directly impacting ecosystem dynamics. For instance, rising temperatures expand the suitable climatic habitat for insect species and in combination with land-use changes and drought-impacts, results in large areas of forests affected by outbreaks (Kurz *et al.*, 2008). Climate change also strongly influences wildfire activity, since warmer spring and summer temperatures, coupled with decreases



in water availability and dry out wood material in forests, resulted in an increase of the forest fire season by 18.7% from 1970 to 2013 worldwide (Jolly *et al.*, 2015).

### 1.1.3. Vulnerability of rear-edge forests

There is no doubt that climate plays a major role in limiting ranges of terrestrial species (Parmesan, 2006). Individuals growing at the limit of the species distribution are exposed, *per se*, to suboptimal climatic conditions compared to the centre of the distribution range (Kawecki, 2008). These populations residing at the current low-latitude margins of the species distribution range where climate is warmer and drier are called “rear-edge” (Hampe & Petit, 2005). Moreover, these populations are disproportionately important for the long-term conservation of genetic diversity, phylogenetic history and evolutionary potential of species (Hampe & Petit, 2005).

The on-going climate change is reshaping species ranges at high rates, as mentioned in the previous section, and adding an extra pressure on rear-edge populations, which are supposed to have reduced tolerance to climatic changes (Jump & Peñuelas, 2005). In Europe, most of the boreal and temperate tree species reach their southern distribution limits in the Mediterranean Basin. This region is expected to be more strongly affected by climate change than most other regions on earth (Mariotti, 2010; IPCC, 2013), consisting of a combination of a pronounced warming with a decrease in precipitation, especially in summer (Giorgi & Lionello, 2008; Bartolini *et al.*, 2012), which is already the hottest season in the region. By the end of the 21<sup>st</sup> century, climate projections forecast an increase of  $\sim 7^{\circ}\text{C}$  in summer temperature and a decrease of  $\sim 30\%$  in April-September precipitation, resulting in an extraordinary drying of the area (IPCC, 2013). Although the Mediterranean Basin is usually modelled as a homogeneous climatic domain, recent studies have described west-east geographical differences, particularly in precipitation patterns, which are linked to North Atlantic Oscillation (Xoplaki *et al.*, 2003; Bladé *et al.*, 2012; Barkhordarian *et al.*, 2013). Negative Summer North Atlantic Oscillation increases the dryness in the western Mediterranean, which recent studies found significant relationships with rear-edge population growth decline (Chen *et al.*, 2015; Dorado-Liñán *et al.*, 2017). Therefore, not only the strategy followed by the species to cope with drought but also the location of the rear-edge populations in the Mediterranean Basin will

be crucial for tree survival and species persistence, and thus, will be a determinant feature shaping boreal and temperate tree species distributions in the area (Thuiller *et al.*, 2005; Lindner *et al.*, 2010).

In fact, the Mediterranean Basin has been considered as one of the twenty-five global biodiversity hotspots defined as areas in the world biologically rich but highly threatened by climate change effects (Myers *et al.*, 2000). Thus, the greatest vegetation changes in Europe are expected in the transition between the Mediterranean and Euro-Siberian regions (Thuiller *et al.*, 2005), largely affecting vegetation dynamics and, rear-edge populations. Plant species growing at rear-edge populations are limited not only by drought but also through competition by other species, which might perform better in this climate (Thuiller *et al.*, 2008). Plasticity patterns of populations at the distribution margins will be important in determining distributions under novel climates (Valladares *et al.*, 2014), which is considered as a fundamental challenge of climate change ecology.

## 1.2. Above ground tree-water relations

Water is vital for all the living forms we know to-date. For autotrophic organisms such as plants, water is an essential substrate, together with CO<sub>2</sub>, for photosynthesis. Evolution has shaped numerous vascular systems, from simple capillarity to 100-metre pipe networks of *Sequoia sempervirens*. Water transport within plants can be explained by the soil-plant-atmosphere continuum (SPAC), which is the pathway for water moving from soil through plants into the atmosphere (Sperry, 2003).

The water movement is initiated at the plant-atmosphere interface located at the stomata. The water evaporation of the mesophyll cells generates a surface tension, resulting in a negative hydrostatic pressure. According to the cohesion-tension theory (Dixon, 1914; Tyree & Zimmermann, 2002), water uptake occurs when the water potential of the roots is lower than the water potential of the soil, and this ascends from roots to leaves via a continuous water column in a metastable state under tension through the xylem (Tyree & Ewees, 1991). This mechanism allows plants to move large quantities of water from the soil to the transpiring leaf surface with little input of metabolic energy (Tyree & Zimmermann, 2002). However, about 90% of all the water taken up by a plant is not used

directly for growth or photosynthesis, but is simply lost by transpiration since plants take up CO<sub>2</sub> through their stomata (Kramer & Boyer, 1995).

Evolution resulted in highly diversified types of xylem structures in both temporal and geographical scales (Carlquist, 2012). Several functions such as long-distance transport of water and nutrients, mechanical support, and storage rely on the xylem tissue, which originates from the vascular cambium. Conifers xylem is mostly formed by tracheids (Figure 2), which rarely exceed 2 mm in length (Brodribb *et al.*, 2012). These water-conducting cells must play a dual role, transporting water to the canopy and physically supporting it (Hacke *et al.*, 2015). By contrast, angiosperm wood is more complex showing a separation of water conduction from mechanical support, vessels and fibres, respectively (Tyree & Zimmermann, 2002). Vessels are multicellular tubes composed of vessel elements that can reach several metres in length and up to 500 µm in width (Figure 2) (Brodribb *et al.*, 2012). Within angiosperms, diffuse-porous structure is defined when vessels show little variation in diameter along a growth ring, whereas ring-porous structure shows a sharp decrease in vessels diameter along the growth ring (McCulloh *et al.*, 2010). The two types of conduits (tracheids and vessels) are lignified and include a thick secondary wall layer. Their rigidity and strength are necessary to withstand the compression caused by negative pressures.

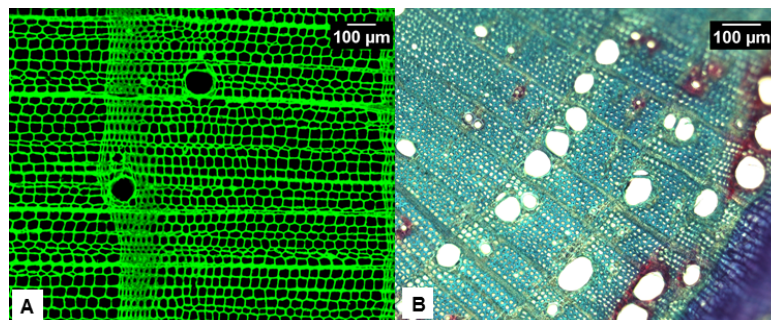


Figure 2. Water-conducting cells. A) Scots pine ring-structure mainly formed by tracheids. B) Ring-porous wood-structure of ash composed by vessels and fibres.

### 1.2.1. Plant hydraulic system under drought stress

Plant resistance to unfavourable climatic conditions, such as drought, is conferred by a complex set of interacting traits. Trees can partly cope with water limitations, for instance, through physiological regulations in different parts of the SPAC (Sperry *et al.*,

2002; Martínez-Vilalta *et al.*, 2014), including above-ground structural and physiological adjustments.

Within short timescales, stomatal closure is the main mechanism by which plants limit water losses, and thus, maintain water potentials within species-specific safety limits. Although stomatal closure can be reversed, it will definitely leave a footprint in the molar ratio of carbon isotope abundances ( $^{13}\text{C}/^{12}\text{C}$ ) in the cellulose produced, since discrimination against  $^{13}\text{C}$  decreases as stomata close (Francey & Farquhar, 1982; Farquhar & Richards, 1984; Farquhar *et al.*, 1989). The regulation of the plant water potential is critical, not only because of its function in plant responses to drought, but also because of the impact on metabolic processes, including plant growth through turgor-driven cell expansion (Kramer, 1988).

As drought continues, stomatal closure slows down but does not completely stop the decline of xylem pressure and hydraulic capacity (Choat *et al.*, 2012). The water column inside the xylem may break as a consequence of cavitation when the negative hydrostatic pressure exceeds a species-specific threshold (Sperry *et al.*, 1988). According to the air-seeding hypothesis (Tyree & Zimmermann, 2002), air will be pulled into the xylem conduit and the affected conduit element will become embolised and dysfunctional (Hacke & Sperry, 2001). This air-filled xylem element could act as a source of further air spreading along the xylem pathway. Since the xylem conduits are interconnected, a single cavitation event will not significantly impair overall conductivity. However, if a large proportion of xylem conduits contain air rather than water; hydraulic transport would be significantly blocked, disrupting the water transport and resulting in a reduced water uptake (Sperry *et al.*, 2002).

Within long timescales, structural adjustments in the hydraulic architecture of the tree (xylem, leaves and roots) play a critical role in determining plant responses to drought. Xylem is one of the plant tissues in which the largest hydraulic conductivity losses occur during drought, and is certainly the tissue in which these losses have been most thoroughly studied. Several studies have highlighted how climate directly modulates structural changes in the xylem architecture of trees (i.e., number and/or size of the conduits) in broadleaf species (García González & Eckstein, 2003; Fonti & Garcia-Gonzalez, 2004; Sass-Klaassen *et al.*, 2011; González-González *et al.*, 2013) and in conifers (Martín *et al.*, 2010; Bryukhanova & Fonti, 2012; Olano *et al.*, 2012; Liang *et*

*al.*, 2013). According to the Hagen-Poiseuille law (Tyree & Zimmermann, 2002), hydraulic conductivity rises by the power of four with increasing lumen-pipe diameters, so even minimal increases in conduit size translate into major gains in flow. However, wider conduits are more prone to suffer cavitation (Hacke & Sperry, 2001; Sperry *et al.*, 2006). Thus, there is a well-known trade-off between efficiency and safety in relation to the diameter/size of the conduit element (Sperry *et al.*, 1994, 2008; Hacke *et al.*, 2006; Venturas *et al.*, 2013). A global study analysing trade-offs across the woody plant species worldwide found weak but significant results that support this concept (Gleason *et al.*, 2016). Although xylem structure is able to plastically acclimate to environmental variations during growth and development, subsequent acclimation of embolism resistance to environmental stress is not possible because xylem conduits are dead at maturity (Choat *et al.*, 2012). Recent studies support the potential capacity of some tree species to refill embolised vessels by releasing sugars from the phloem into the disrupted conduits despite negative pressures in the surrounding transpiration stream (Nardini *et al.*, 2011). However, the frequency and conditions of refilling still remain unresolved (Sperry, 2013).

Long-term acclimation also occurs in other tree compartments, modifying structural properties of the tree canopy or the root system (Bréda *et al.*, 2006). For instance, smaller total leaf areas (Kubiske & Abrams, 1992; Günthardt-Goerg *et al.*, 2013) or massive leaf-shedding (Wolfe *et al.*, 2016) reduce the potential transpiration losses associated with drought events. However, this strategy is usually linked to modifications in the hydraulic pathway such as reductions in the leaf/sapwood ratio and the leaf-specific hydraulic conductivity (Delucia *et al.*, 2000; Martínez-Vilalta *et al.*, 2009). Moreover, changes in the below and above biomass allocations have also been commonly reported under drought conditions (Schall *et al.*, 2012; Taeger *et al.*, 2015; Seidel *et al.*, 2016). These changes in allocation are usually related to the exploitation of new water resources in deeper soil layers (Hendrick & Pregitzer, 1996).

### 1.2.2. Strategy-related consequences for tree performance

Drought impacts on the tree physiology describe a circle that starts by affecting water transport and assimilation and continues with allocation processes (Bréda *et al.*, 2006), since leaf and xylem water potentials directly influence cell turgor pressure, affecting in

turn cell division and expansion, and thus, radial growth (Fichot *et al.*, 2009). A hydraulic-based framework was developed to disentangle the consequences of these physiological adjustments underlying drought-induced tree decline and mortality (McDowell *et al.*, 2008). This study suggested that the stomatal-related strategy performed by a tree under drought conditions would have different consequences in tree vitality and fitness.

In general, angiosperms display a lower sensitivity of stomatal-conductance to vapour pressure deficits (VPD) compared to conifers (Johnson *et al.*, 2012). In turn, this key characteristic seems to be tightly connected to plant hydraulic safety margins. These are calculated as a function of the minimum xylem pressure measured in the field in relation to the xylem pressure causing 50% loss of hydraulic conductivity, which is commonly reported as the threshold to xylem dysfunction. Choat *et al.* (2012) found a global pattern across species showing that conifers operate with wider safety margins, having higher resistance to drought-induced cavitation compared to angiosperms. However, recent studies proved that loss of conductivity associated with tree decline in angiosperms is closer to the 88% than the 50% commonly reported (Urli *et al.*, 2013). This results in a potential underestimation of the angiosperm safety margins in other studies, e.g. (Choat *et al.*, 2012).

Moreover, plants have been classified into two broad groups regarding their ability of stomata to regulate leaf water potentials (Buckley, 2005). Isohydric species are those that maintain relatively stable leaf water potentials due to a strict stomata control whereas anisohydric species would show a looser stomatal regulation allowing leaf water potential to track environmental fluctuations (Martínez-Vilalta *et al.*, 2014). Although plenty of literature has followed this categorical distribution of isohydric and anisohydric species, new studies claim that should rather be continuous since plant responses to drought involve several interrelated traits, and stomata behaviour is just one of them (Klein, 2014; Martínez-Vilalta & Garcia-Forner, 2016). Nevertheless, according to McDowell *et al.* (2008), relatively isohydric species would be more prone to suffer carbon starvation during long-lasting dry spells since their strict stomatal control would imbalance carbon supplies and demands to meet osmotic, metabolic and defence carbon requirements (Sevanto *et al.*, 2014). This continued demand for carbohydrates may deplete the carbon reserves, leading to tree mortality. On the contrary, relatively anisohydric species are

more disposed to suffer cavitation events under extreme droughts. If the resulting embolisms impede the transport of water to the canopy, this situation can ultimately result in desiccation of tissues and cellular death. Moreover, biotic agents may play an important role amplifying the aforementioned drought-induced impacts (Desprez-Loustau *et al.*, 2006).

In the last decade, there is no doubt that this framework has been hugely influential in shaping the research agenda on physiological mechanisms behind drought-induced tree decline and mortality. Although many studies have been published since McDowell *et al.* (2008), the physiological details of both hypotheses are not completely understood yet (Sala *et al.*, 2012). Carbon starvation has been typically assessed via changes in tissue non-structural carbohydrate concentrations. However, literature reports contradictory findings across publications. Whereas some studies analysing drought-responses of isohydric species provided evidence of the relation between non-structural carbon depletion and tree decline (Galiano *et al.*, 2011), others observed an increase in non-structural carbohydrate reserves during drought (Muller *et al.*, 2011; Bachofen *et al.*, 2017), indicating that a complete knowledge of the topic is still lacking. In fact, carbon dynamics under drought stress seem to be highly variable within the same genus (Piper, 2011) and across different tree tissues (Hartmann *et al.*, 2013). The drought-induced increase in non-structural carbohydrates reported by some authors has been linked to carbon sink limitations rather than an inhibited photosynthesis (Körner, 2003). This hypothesis suggests that carbohydrates are actively stored (Dietze *et al.*, 2014), which would enable faster recovery after the dry period (Piper, 2011). However, other authors highlighted that a tree could die without depleting its carbon reserves, since the stored carbohydrate mobilization may be restricted under drought conditions due to phloem failure (Sala *et al.*, 2010; Sevanto *et al.*, 2014).

In contrast to carbon starvation, there is a higher consensus regarding the relation between hydraulic failure and tree decline (Anderegg *et al.*, 2013a). Indeed, a recent publication assessing the mechanisms in drought-induced tree mortality across multiple taxa and biomes found solid evidence that tree mortality was universally associated with losses of conductivity, whereas there was no clear pattern regarding non-structural carbohydrates (Adams *et al.*, 2017). All the studied species included in Adams *et al.* (2017) displayed more than 60% of loss of hydraulic conductivity, suggesting hydraulic failure as the main

cause of tree decay. However, the capacity of refill embolisms already mentioned (Nardini *et al.*, 2011) and the lethal loss of conductivity thresholds (Urli *et al.*, 2013) remain still unclear. Moreover, the same publication highlighted the connection between both physiological mechanisms underlying tree mortality, also pointed by other authors (McDowell, 2011). They found, for instance, high losses of hydraulic conductivity combined with reduced non-structural carbohydrates, which have been usually thought to represent mutually exclusive mechanisms. Therefore, more studies assessing the combination of both mechanisms and the potential impairment of the phloem transport are urgently needed to obtain a complete picture of the processes involved in tree decline.

## **2. Objectives and thesis outline**

### **2.1. Research gaps**

Differences in morphological and physiological features across tree species are important for understanding responses to the on-going climate change (McDowell *et al.*, 2008). Since functional traits are an essential link between the environment and organismal fitness (Violle *et al.*, 2007), these provide a powerful tool for ecological research (Anderegg, 2015). The analysis of climate impacts on tree traits is a common target in tree physiology, mostly carried out under controlled conditions (greenhouses) and usually focusing on seedlings. Although this kind of studies is useful to better understand the physiology of tree species, the potential of their outcome might be limited to understand and forecast responses of natural mature stands. Moreover, most of these studies are based on short-term frameworks (one-two years of observation), that hamper the study of climate-induced long-term acclimation. Recent studies also highlighted the differences of diverse tree functional types (conifers vs. broadleaf, anisohydric vs. isohydric), which can lead to a potentially better performance (Galiano *et al.*, 2010). However, a deep understanding of the physiological strategy of the different tree functional types under natural conditions in the current context of climate change is also still lacking.

Although the study of the relation between plant traits and climate has been mostly addressed at an inter-specific level, the intra-specific variability is less well understood despite the relevant implications, for instance, for vegetation modelling (Violle *et al.*,



2014). The degree of intra-specific trait variation has important consequences for the sensitivity of species to climatic shifts (Anderegg & Meinzer, 2015). For example, variations on hydraulic features within a single species could have a major effect on population demographics and responses to changes in climate extremes, such as a given population's ability to survive a drought (Anderegg & Meinzer, 2015). While some studies have examined tree traits at natural stands located at the southern limit of the species distribution, just a few have analysed spatial variation in tree traits (Martínez-Vilalta *et al.*, 2009; Anderegg & Hillerislambers, 2015). Moreover, the studies analysing spatial variation were mainly focused on edge-to-centre differences. Assessing the variability among rear-edge populations would give us some insights on trait variability and potential vulnerability to climate change at the dry and warm limits of species distributions.

## 2.2. Thesis aim

The main aim of this PhD thesis is to gain a better mechanistic understanding of short- and long-term responses to drought by characterizing adjustments in physiological and anatomical traits of temperate to boreal tree species from different functional types such as Scots pine (*Pinus sylvestris* L.) and sessile oak (*Quercus petraea* Matt. Liebl). The spatial variability of these adjustments was assessed across the rear-edge distributions limits of the studied species at the Mediterranean Basin. In order to achieve this main aim, a set of more specific objectives were defined for each research chapter. The obtained results will improve our ability to predict future changes in forest composition and coverage in the region.

A multidisciplinary approach was chosen in order to evaluate short- and long-term hydraulic performance, physiological and wood anatomical adjustments, indirect carbon allocation and photosynthetic activity estimators (i.e., tree rings and stable carbon isotopes, respectively). Moreover, a greenhouse experiment was set up to better recognize the species' strategies, particularly for those variables not possible to measure under field conditions. This investigation also sought to assess the spatial variability of these impacts along a longitudinal gradient in the Mediterranean Basin, to test if the west-east spatial differences described in tree growth are also observed in anatomical and physiological traits.



understand processes not possible to measure under the field/natural conditions due to the large study area.

The research chapter 4.2 focused on the differences in branch-level hydraulic traits of four Scots pine and four sessile oak natural stands located at the rear-edge in the western and central Mediterranean Basin (Spain, France and Italy) during a long and exceptionally dry spell in summer 2015. This publication is one of the first studies assessing the hydraulic traits of natural stands across a large area at the limit of the species' distributions. In combination with chapter 4.1, a full overview of the hydraulics of the studied tree species is given.

Long-term responses using dendrochronological techniques were assessed in chapters 4.3 and 4.4. The research chapter 4.3 aimed at a better understanding the sessile oak long-term xylem adjustment to drought by analysing and developing tree-ring width and vessel-related features chronologies of sessile oak rear-edge natural stands located across the Mediterranean Basin (Spain, Italy, Slovenia, Bulgaria and Romania). In the research chapter 4.4, tree-ring width and stable carbon isotope chronologies are used as surrogates of long-term carbon allocation and photosynthetic activity of five Scots pine and five sessile oak rear-edge natural stands located along the Mediterranean Basin (Spain, France, Italy, Slovenia, Bulgaria and Romania).

Chapter 5 highlights the significance of the results in relation to the emerging research questions and places these findings in the context of other studies and chapter 6 provides an outlook. References are listed in chapter 7.

### 3. Overview of methods

A brief overview of the applied methodology is given in this section. For further details, a complete description of the methods is explained in each research chapter.

#### 3.1. Study sites

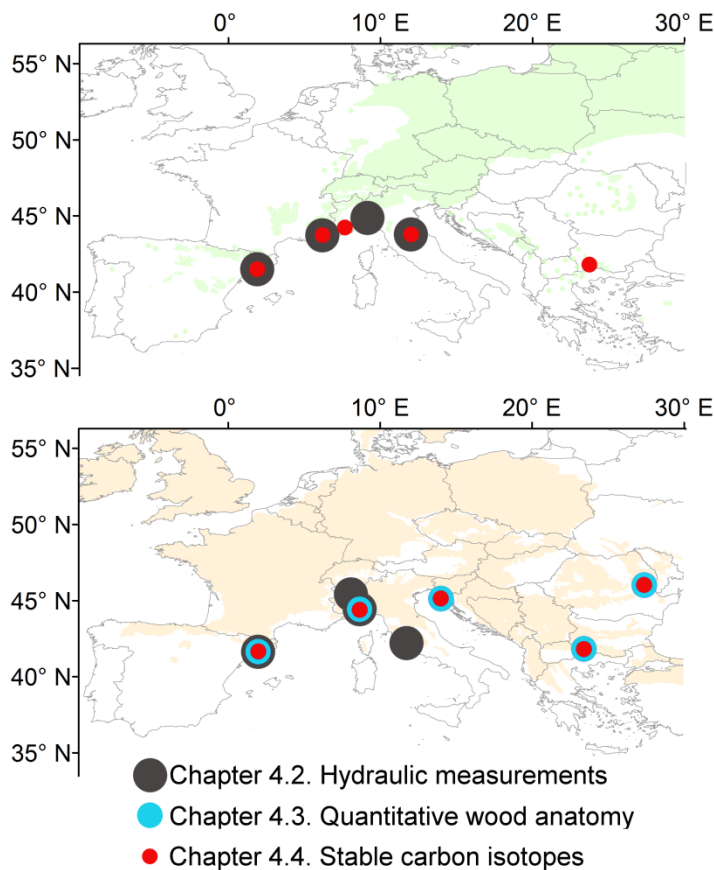


Figure 4. Distribution maps of the studied species (upper pannel, Scots pine; lower pannel, sessile oak) and location of the selected populations regarding the different measured parameters.

An accurate selection of populations across the studied species' distribution limits in southern Europe was performed (Figure 4 and 5). In total, 13 different populations were assessed in this PhD thesis (see exact locations in each research chapter). These populations were located in six different countries: Spain, France, Italy, Slovenia, Bulgaria and Romania. The population selection was optimized to cover a large climatic (Mediterranean and continental) and longitudinal gradient ( $41.7^{\circ}$  N –  $45.8^{\circ}$  N and  $2.0^{\circ}$  E –  $24.0^{\circ}$  E) along the Mediterranean Basin.

The study of the branch-level hydraulic traits (chapter 4.2) was focused on eight populations (four per species) in the western and central Mediterranean Basin located in Spain, France and Italy. In order to obtain comparable results, the sample collection took place during two weeks (July 2015) and more than 4300 km were driven during this short period to obtain all the samples.

The collection of dendrochronological samples (chapters 4.3 and 4.4) took place during the summers of 2013 and 2014. Additionally to the populations in the western and central Mediterranean Basin studied in chapter 4.1, a larger area including populations at the

eastern Mediterranean Basin (Romania and Bulgaria) were considered in these research chapters. Thus, high investments in economic and personal resources were required during these long and intense field campaigns across six different countries, as well as a continuous communication with local researchers and institutions for site localization and sampling permissions, respectively.



Figure 5. Scots pine (upper) and sessile oak (lower) study sites in chapters 4.3 and 4.4. Above from left to right: Sant Llorenç del Munt (Spain), Cuebris (France), Baiardo (Italy), Montesi (Italy) and Velingrad (Bulgaria). Below from left to right: Can Figueroles (Spain), Asti (Italy), Dolenja(Slovenia), Sushinta (Bulgaria) and, Focsani (Romania).

### 3.2. Temperature and drought manipulations

Seedling experiments offer some advantages to analyse drought responses since saplings are easier to manipulate. A two-year experiment was set up in order to better understand the physiological response of different functional tree types to warming and drought (chapter 4.1). This experiment was particularly important in the context of this PhD thesis to better characterize the species' responses to drought assessing processes not possible to measure in natural conditions such as stem water potential and stomatal conductance. Besides the two studied species, a third species (*Castanea sativa* Mill.) was also included in this experiment.



Figure 6. Field experiment of chapter 4.1. Heating system and rainout shelter are visible.

An extensive infrastructure was built up in an open space field located in the Gewächshauslaborzentrum Dürnast of the TUM School of Life Sciences Weihenstephan

in order to manipulate the climatic conditions (Figure 6). A heating system was set up, which aimed at heating the air up surrounding the seedlings by approximately 1-2 °C. A computer-based regulation ensured that the temperature did not exceed this limit. Water was heated up in the boiler and transported to the treatment plots through a corrugated network of black-plastic pipes. Additionally, drought conditions were achieved with a rainout shelter connected to a rain sensor which automatically prevented rain events. In comparison to permanent shelters, the temporary shelter avoided possible radiation artefacts or passive warming, resulting in more homogenous conditions among the seedlings. Moreover, soil composition of the plot was carefully selected to reduce water availability for the plants. The effect of seedling density was also assessed and two different seedling densities (3.69 and 7.57 seedling m<sup>-2</sup>) were arranged within each climatic treatment.

A total of 384 seedlings were planted on November 21<sup>st</sup> 2013 and had an acclimation period of seven months before the treatments were applied. The first treatment period started June 23<sup>rd</sup> 2014 and lasted until October 14<sup>th</sup> 2014 and the second treatment period ran from June 30<sup>th</sup> 2015 until September 15<sup>th</sup> 2015. The seedlings experienced normal site conditions between treatment periods.

In order to characterize the growing conditions and the efficiency of the climatic treatments during the treatment periods, air temperatures were continuously monitored using temperature sensors (FF-10V-INT-TE, B+B Thermo-Technik GmbH, Donaueschingen, Germany) installed in each treatment subplot. Soil moisture was measured twice a week at different depths using a profile soil moisture sensor (PR2 Profile Probe and HH2 Moisture Meer, Delta-T Devices Ltd., Cambridge, UK). Growth (height and diameter) and physiological parameters (stomatal conductance and stem water potentials) of the selected individuals were recorded once a month and weekly or biweekly, respectively. At the end of the whole experiment (September-October 2015), the hydraulic conductivities (maximum and native) as well as the percentage of loss of conductivity were measured using the methodology explained in the chapter 3.3 of this thesis.



### 3.3. Hydraulic conductivity measurements

The maximum and native hydraulic conductivity measurements included in chapter 4.1 and 4.2 were determined using the Sperry hydraulic apparatus (Sperry *et al.*, 1988) (Figure 7). The stem segments of length  $L$  were attached to a tubing system. This system consists of an upper and lower water reservoir, connected through tubes. The upper water reservoir was set at 0.3-0.5 m to create a pressure drop ( $\Delta P$ ) of 3-5 kPa across the stem segment. The lower reservoir was placed in an electronic balance (Mettler-Toledo XS204DR, Mettler-Toledo AG, Greifensee, Switzerland) connected to a computer to measure water flow rate every ten seconds ( $F$ ,  $\text{kg s}^{-1}$ ). Moreover, the background rate, which represents any osmotic and capillary uptake of water by the segment in the absence of an applied pressure difference, was also measured and equilibrated with the system. The resulting hydraulic conductivity ( $K_h$ ,  $\text{kg s}^{-1} \text{m MPa}^{-1}$ ) was obtained as:

$$K_h = (F L) / \Delta P \quad (\text{Eq. 1})$$

which was normalized by the xylem section (xylem-specific hydraulic conductivity,  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) and by the leaf area (leaf-specific hydraulic conductivity,  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ).

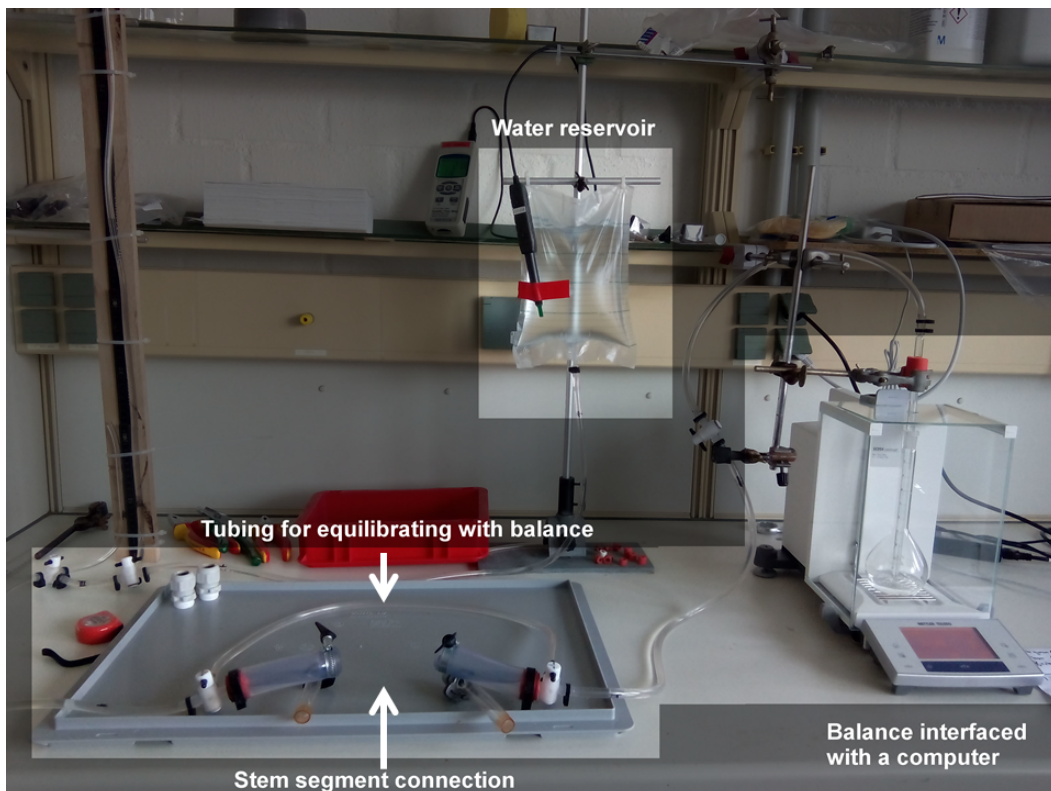


Figure 7. Sperry apparatus at TU München

### 3.4. Functional hydraulic traits

Several tree traits are involved in the regulation of water transport within the trees. In order to cope with adverse climatic conditions, trees can partly adjust some of these traits. Functional traits are defined as any trait which impacts fitness indirectly via its effects on growth, reproduction and survival (Violle *et al.*, 2007, 2014). These have become a fundamental tool of many ecological subdisciplines as in the case of plant hydraulics. Functional hydraulic traits in the regulation of the water transport were investigated in chapter 4.2 (Table 1) for better understanding of the strategy that the selected tree species followed to cope with stress conditions. Although several studies focused on the inter-specific differences of these hydraulic traits, only a few have investigated intra-specific variability of them under natural conditions (Mencuccini & Grace, 1995; Martínez-Vilalta *et al.*, 2009).

Table 1. Hydraulic traits analysed in Chapter 4.2. Modified from Anderegg & Hillerislambers (2015).

Hydraulic traits	Physiological implication
Xylem area-specific native hydraulic conductivity	Overall capacity to carry water in natural conditions (Venturas <i>et al.</i> , 2013)
Native percentage of loss hydraulic conductivity	Xylem vulnerability to cavitation under natural conditions (Maherali <i>et al.</i> , 2004)
Xylem area-specific maximum hydraulic conductivity	Increased $K_{S-MAX}$ indicates greater potential xylem hydraulic efficiency, which reduces hydraulic resistance and decreases the xylem tensions (Maherali <i>et al.</i> , 2004)
Leaf-specific maximum hydraulic conductivity	Increased $K_{L-MAX}$ indicates greater hydraulic support of each unit leaf area (Anderegg & Hillerislambers, 2015)
Leaf-to-xylem area ratio	Increased $A_L:A_X$ sapwood area-to-leaf area ratio increases hydraulic efficiency and reduces xylem tensions (Mencuccini & Grace, 1995; Martínez-Vilalta <i>et al.</i> , 2009)
Functional xylem fraction	Increased functional xylem section supposes higher sapwood area increasing the overall efficiency (Poyatos <i>et al.</i> , 2007)

In chapter 4.2, four natural stands per species were selected in the western and central Mediterranean Basin (Spain, France and Italy) and branches from six different trees were collected and analysed at each site. In order to homogenise the results, the five-year old internode of each branch was considered for the analyses. The samples were collected during July 2015, which was concurrent with one of the driest spells in the area over the



last years in a regional and global scale (NOAA National Centers for Environmental Information, 2016). The analysed traits covered different branch-level compartments including stems and leaves (Figure 8). The hydraulic conductivity measurements followed the methodology explained in the chapter 3.3 of this thesis.

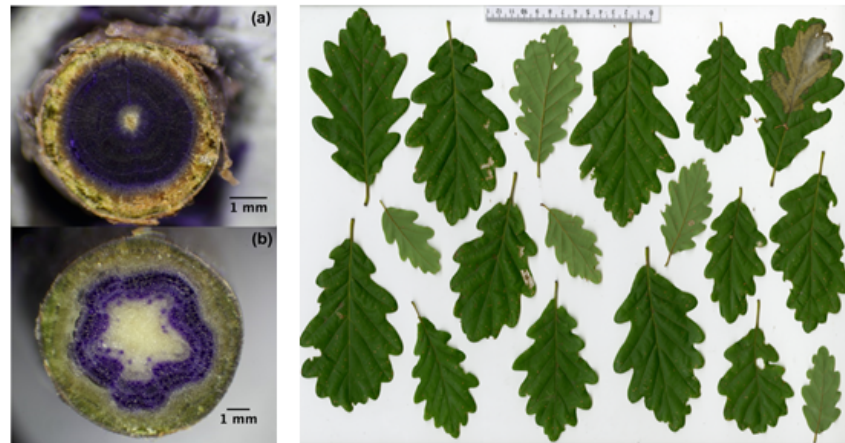


Figure 8. Example of hydraulic traits measured in chapter 4.2. Left images are examples of sapwood area and the right image is an example of leaf area.

### 3.5. Dendrochronology: a retrospective analysis

Trees record any environmental factor that directly or indirectly limits any process affecting radial growth or any of the tree-ring structures (Fritts, 1976). In seasonal temperate climates, the annual periodicity of cambial activity consisting of a winter dormancy and an active growing period leads to distinguishable growth rings in the wood (Schweingruber, 1993). Dendrochronology is the science that allows the identification of the exact year in which each tree ring was formed and the assignment of specific calendar dates to the tree rings with an absolute precision. This involves comparing patterns of wide and narrow rings or other anatomical characteristics among several tree-ring series, the so-called cross-dating. Although tree growth is affected by external and endogenous parameters (e.g. age, competition), climate is the principal driver of the inter-annual variability in the pattern of wide and narrow rings. Therefore, since trees accurately register events to which trees have been exposed during their lifetime, this dendrochronological approach offers the advantage of providing a long-time record of physiological processes of the tree, otherwise not achievable with any other method. Indeed, tree rings have been widely used to study long-term tree growth climate relationships (dendroecology) (Eilmann *et al.*, 2006; Gutiérrez *et al.*, 2011), to reconstruct climatic variables such as temperature and precipitation (dendroclimatology) (Büntgen *et*

*al.*, 2011; Dorado Liñán *et al.*, 2014), and to identify the dendro-provenance of archaeological and historical wood objects (dendroarcheology) (Grissino-Mayer & van de Gevel, 2007; Henderson *et al.*, 2009), among other applications.

Since the first works of Douglass in 1914 (Fritts, 1976), ring width has been, by far, the most explored tree-ring variable in dendrochronology for different purposes. However, in the recent decades the new technological advances supported novel subdisciplines such as dendrochemistry or quantitative wood anatomy that benefit of the annual resolution of the tree-ring series.

### 3.5.1. Quantitative wood anatomy

Wood anatomical features were formerly analysed in microsections of small blocks of tissue that required a laborious process. However, recent technological advances make tree-ring anatomy very attractive because time cost has considerably been reduced while larger samples can now be covered. The core-microtome was designed by Gärtner & Nievergelt (2010) to cut plane surfaces on entire cores and was the first step to obtain long-term series of anatomical features. New protocols were also developed taking advantage of this new tool and initiating a new research discipline (Gärtner & Schweingruber, 2013). Furthermore, the development of novel automated image-analysis systems such as WinCell or Roxas (von Arx & Carrer, 2014), together with the improvements with computer performance and power, significantly reduce the time investment per sample. Quantitative wood anatomy is the resulting discipline of all the above-mentioned improvements, which analyses the variability of xylem anatomical features in trees, shrubs, and herbaceous species. During the last decade, several studies applied these techniques for an increasing number of research topics such as climate-growth (Fonti & Garcia-Gonzalez, 2004; Fonti *et al.*, 2010; González-González *et al.*, 2015), stress responses (Fonti *et al.*, 2013), tree functioning (Petit *et al.*, 2011; Anfodillo *et al.*, 2012) or wood production (Pacheco *et al.*, 2015).

Quantitative wood anatomical techniques were applied in chapter 4.3 to understand the long-term climatic drivers of different ring structures of sessile oak populations. To obtain vessel-related information with an annual resolution, the procedure detailed in Figure 9 was applied.

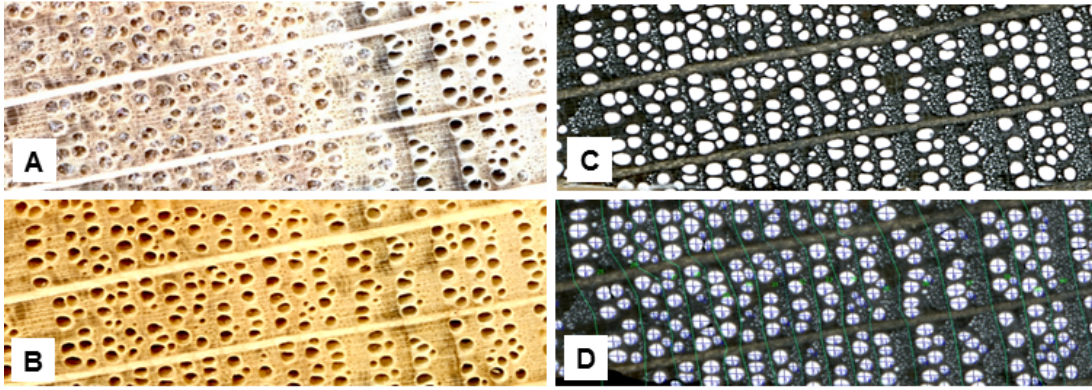


Figure 9. Procedure for sample preparation. A) Cores were surfaced with a sliding microtome. B) Air-water blast for wood dust and tyloses removal. C) Cores were stained with black ink and vessels were filled with white wax. D) Only vessels >10,000  $\mu\text{m}^2$  were measured.

Sessile oak wood cores from twelve trees were analysed at each of the five studied sites located in Spain, Italy, Slovenia, Romania and Bulgaria to build site chronologies of mean vessel area, number of vessels and tree-ring width, as well as the theoretical hydraulic conductivity calculated using the Hagen-Poiseuille law.

### 3.5.2. Stable carbon isotopes

Carbon, hydrogen and oxygen are the three components of cellulose, and all have more than one stable isotope (McCarroll & Loader, 2004). Stable isotopes of the same element have the same number of protons but differ in the number of neutrons. In the specific case of carbon, it has two stable ( $^{12}\text{C}$  and  $^{13}\text{C}$ ) and several radioactive isotopes.

The ratio between the two carbon stable isotopes,  $^{13}\text{C}$  to  $^{12}\text{C}$ , is determined by mass spectrometry, and by convention, is usually expressed in differential notation ( $\delta^{13}\text{C}$ ), in parts per thousand (‰), with reference to a standard material for which the isotopic ratio is known:

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad (\text{Eq. 2})$$

where  $R$  denotes the ratio between the heavy and the light isotope ( $^{13}\text{C}/^{12}\text{C}$ ) and  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the ratios in the sample and standard, respectively.

Plants with  $\text{C}_3$  photosynthetic pathway prefer  $^{12}\text{C}$ , their leaves and wood being depleted in  $^{13}\text{C}$  relative to air,  $\delta^{13}\text{C}$  ranging from -20‰ to -30‰ (McCarroll & Loader, 2004). This

change in ratios from a source to a product is known as fractionation, and the degree of fractionation is controlled to some extent by the response of the tree to its environment.

In plants, there are two processes in which carbon isotope fractionation occurs: stomatal conductance and assimilation rate (Francey & Farquhar, 1982; Farquhar *et al.*, 1989). The first one is related to the fractionation due to diffusion: when stomata are open, CO<sub>2</sub> diffuses easily into the cellular space, especially those including the lighter carbon isotope. The second fractionation occurs during carbon fixation by RuBisCO, which tends to use <sup>12</sup>C in preference over <sup>13</sup>C. When the rate of stomatal conductance is low, CO<sub>2</sub> is reduced resulting in less carboxylation discrimination against <sup>13</sup>C, and leading to higher  $\delta^{13}\text{C}$ . The fractionations that occur due to diffusion and carboxylation are constant processes (see chapter 4.4 for exact values), and the  $\delta^{13}\text{C}$  assimilated relies on the ratio of intercellular and atmospheric CO<sub>2</sub> concentrations. Consequently, under drought conditions, lower stomatal conductance would reduce the amount of intercellular carbon, forcing plants to assimilate CO<sub>2</sub> containing the heavier isotope (<sup>13</sup>C), and leading to high  $\delta^{13}\text{C}$  values. Several publications used this environmental signal of  $\delta^{13}\text{C}$  for dendroclimatological studies to reconstruct climatic variables such as temperature and precipitation (Treydte *et al.*, 2006; Loader *et al.*, 2010; Konter *et al.*, 2014) and also in ecophysiological studies to assess drought-induced responses and water-use efficiency of C3 plants (Saurer *et al.*, 2004; Andreu-Hayles *et al.*, 2011; Lévesque *et al.*, 2014; Frank *et al.*, 2015).

Stable carbon isotopes were used in chapter 4.4 of this PhD thesis to analyse the long-term physiological adjustments of the studied species over the last 50 years in a longitudinal transect across their southern distribution limits (Figure 4). The annual  $\delta^{13}\text{C}$  signal of wood cores from five different trees of each of the ten study sites was analysed for the period 1960-2012. Cellulose extraction followed the procedures explained by Schollaen *et al.* (2015) (Figure 10). This new technique consists in extracting cellulose directly from wood cross-sections, allowing the simultaneous extraction of several tree rings, and thereby considerably reducing the time costs of the extraction process.

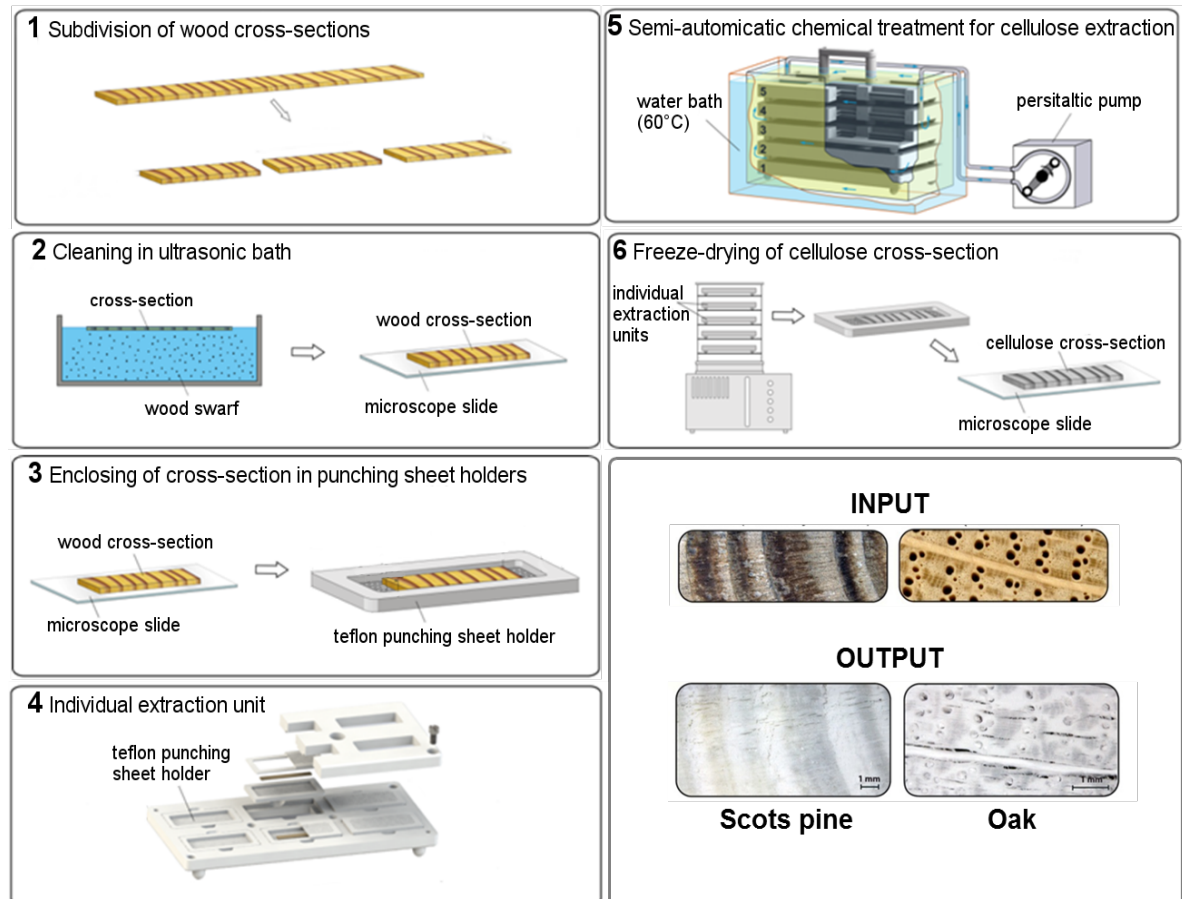


Figure 10. Cellulose extraction applying the new technique described by Schollaen *et al.* (2015). 1-6 all the steps included in the procedure. Last panel shows the initial cross-section and the final cross-section including only cellulose.

## 4. Abstracts of individual publications

### 4.1. Responses of contrasting tree functional types to air warming and drought

Elisabet Martínez-Sancho, Lizeth K. Vásconez Navas, Hannes Seidel, Isabel Dorado-Liñán and Annette Menzel (submitted), *Forests*

Climate change-induced rise of air temperatures and the increase of extreme climatic events, such as droughts, will largely affect plant growth and hydraulics, leading to mortality events all over the globe. In this study, we investigated the growth and hydraulic responses of seedlings of contrasting functional types. *Pinus sylvestris*, *Quercus* spp. and *Castanea sativa* seedlings were grown in a common garden experiment under four treatments: control, air warming, drought and their combination during two consecutive growing periods. Height and diameter increments, stomatal conductance and stem water potentials were measured during both growing seasons. Additionally, hydraulic parameters such as xylem-specific native and maximum hydraulic conductivities, and native percentage of loss of conductivity were measured at the end of the entire experiment. Our results clearly pointed to different adaptive strategies of the studied species. Scots pine displayed a relatively isohydric behavior with a strict stomata control prohibiting native embolism whereas sweet chestnut and oak as relatively anisohydric species displayed an increased loss of native conductivity as a results of low water potentials. Seasonal timing of shoot and diameter growth also differed among functional types influencing drought impacts. Additionally, the possibility of embolism reversal seemed to be limited under the study conditions.

#### Contributions:

EM-S, HS, ID-L and AM conceived and designed the experiments; LVN, HS and EM-S performed the measurements; EM-S, LVN and HS analyzed the data and EM-S led the writing with many comments from LVN, HS, ID-L and AM. About 50% of the work was done by myself.

## 4.2. Contrasting hydraulic architectures of Scots pine and sessile oak at their southernmost distribution limits

Elisabet Martínez-Sancho, Isabel Dorado-Liñán, Uwe G. Hacke, Hannes Seidel and Annette Menzel (2017) *Frontiers in Plant Science*, **8**, 598. DOI: [10.3389/fpls.2017.00598](https://doi.org/10.3389/fpls.2017.00598).

Many temperate European tree species have their southernmost distribution limits in the Mediterranean Basin. The projected climatic conditions, particularly an increase in dryness, might induce an altitudinal and latitudinal retreat at their southernmost distribution limit. Therefore, characterizing the morphological and physiological variability of temperate tree species under dry conditions is essential to understand species' responses to expected climate change. In this study, we compared branch-level hydraulic traits of four Scots pine and four sessile oak natural stands located at the western and central Mediterranean Basin to assess their adjustment to water limiting conditions. Hydraulic traits such as xylem- and leaf-specific maximum hydraulic conductivity ( $K_{S-MAX}$  and  $K_{L-MAX}$ ), leaf-to-xylem area ratio ( $A_L:A_X$ ) and functional xylem fraction (FX) were measured in July 2015 during a long and exceptionally dry summer. Additionally, xylem-specific native hydraulic conductivity ( $K_{S-N}$ ) and native percentage of loss of hydraulic conductivity (PLC) were measured for Scots pine. Inter-specific differences in these hydraulic traits as well as intra-specific variability between sites were assessed. The influence of annual, summer and growing season site climatic aridity (P/PET) on intra-specific variability was investigated. Sessile oak displayed higher values of  $K_{S-MAX}$ ,  $K_{L-MAX}$ ,  $A_L:A_X$  but a smaller percentage of FX than Scots pines. Scots pine did not vary in any of the measured hydraulic traits across the sites, and PLC values were low for all sites, even during one of the warmest summers in the region. In contrast, sessile oak showed significant differences in  $K_{S-MAX}$ ,  $K_{L-MAX}$ , and FX across sites, which were significantly related to site aridity. The striking similarity in the hydraulic traits across Scots pine sites suggests that no adjustment in hydraulic architecture was needed, likely as a consequence of a drought-avoidance strategy. In contrast, sessile oak displayed adjustments in the hydraulic architecture along an aridity gradient, pointing to a drought-tolerance strategy.

### Contributions:

AM, ID-L, UH, HS, and EM-S conceived the ideas. ID-L and EM-S collected the samples. EM-S and HS carried out the analyses with help from UH. The writing of the article was led by EM-S and many contributions and comments were made by ID-L, UH, HS and AM. About 65% of the work was done by myself.



### 4.3. Xylem adjustment of sessile oak at its southern distribution limits

Elisabet Martínez-Sancho, Isabel Dorado-Liñán, Ingo Heinrich, Gerhard Helle and Annette Menzel (2017) *Tree Physiology*, **37**, 903-914. DOI: [10.1093/treephys/tpx036](https://doi.org/10.1093/treephys/tpx036).

Drought is a key limiting factor for tree growth in the Mediterranean Basin. However, the variability in acclimation via xylem traits is largely unknown. We studied tree growth and vessel features of *Quercus petraea* (Matt.) Lieb. in five marginal stands across southern Europe. Tree-ring width (TRW), mean earlywood vessel area (MVA) and number of earlywood vessels (NV) as well as theoretical hydraulic conductivity ( $K_h$ ) chronologies were developed for the period 1963–2012. Summer drought signals were consistent among TRW chronologies; however, climatic responses of vessel features differed considerably among sites. At the three xeric sites, previous year's summer drought had a negative effect on MVA and a positive effect on NV. In contrast, at the two mesic sites, current year's spring drought negatively affected NV, while exerting a positive influence on MVA. In both cases,  $K_h$  was not altered by this xylem adjustment. All variables revealed identical east–west geographical patterns in growth and anatomical features. Sessile oak copes with drought in different ways: at xeric sites and after unfavourable previous summer conditions more but smaller vessels are built, lowering vulnerability to cavitation, whereas at mesic sites, dry springs partly lead to tree-rings with wider but fewer vessels. The variability of vessel-related features displays a similar geographical dipole in the Mediterranean Basin previously described for tree growth by other studies.

#### Contributions:

AM, ID-L and EM-S conceived the ideas. ID-L and EM-S collected the samples. EM-S carried out the analyses with help from ID-L, IH and GH. The writing of the article was led by EM-S and many contributions and comments were made by ID-L, IH, GH and AM. About 80% of the work was done by myself.



#### 4.4. Increased water-use efficiency translates into contrasting growth patterns of Scots pine and sessile oak at their southern distribution limits

Elisabet Martínez-Sancho, Isabel Dorado-Liñán, Emilia Gutiérrez-Merino, Michael Matiu, Ingo Heinrich, Gerhard Helle and Annette Menzel (accepted pending minor revisions), *Global Change Biology*

In forests, the increase of atmospheric CO<sub>2</sub> concentrations (Ca) has been related to enhanced tree growth and intrinsic water-use efficiency (iWUE). However, in drought-prone areas such as the Mediterranean Basin it is not yet clear to what extent this ‘fertilizing’ effect may compensate for drought-induced growth reduction. We investigated tree growth and physiological responses of Scots pine (*Pinus sylvestris* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) populations located at their southernmost distribution limits in Europe using annually resolved tree-ring width and  $\delta^{13}\text{C}$  data to track ecophysiological processes for the period 1960-2012. Results indicate that all the studied stands significantly increased their leaf intercellular CO<sub>2</sub> concentration (Ci), and consequently iWUE. Different trends in the theoretical gas exchange scenarios as a response to increasing Ca were found: generally, Ci tended to increase proportionally to Ca, except for trees from the driest sites in which Ci remained constant. Ci from the oak populations displaying higher water availability tended to increase at a comparable rate than Ca. Multiple linear models fitted at site level to predict basal area increment (BAI) using iWUE and climatic variables, better explained tree growth in pines (31.9-71.4 %) than in oak stands (15.8-46.8%). iWUE was negatively linked to pine growth whereas the effect on oak populations differed across sites. Tree growth at the western and central oak stands was negatively related to iWUE, whereas BAI from the easternmost population was positively associated to iWUE. Thus, some *Q. petraea* stands might have benefited from the ‘fertilizing’ effect of rising Ca, whereas none of the *P. sylvestris* stands seemed to compensate for the enhancement of the evaporative demand and trees showed in general growth reductions across sites. Additionally, the interannual variability of BAI and iWUE displayed a geographical polarity in the Mediterranean Basin similar to other studies.

##### Contributions:

AM, ID-L and EM-S conceived the ideas. ID-L and EM-S collected the samples. EM-S carried out the analyses with help from ID-L, EG-M, MM, IH and GH. The writing of the article was led by EM-S and many contributions and comments were made by ID-L, EG-M, MM, IH, GH and AM. About 80% of the work was done by myself.

## 5. Discussion

This PhD thesis and the publications linked to it aimed at better understanding the responses to drought of different functional tree types such as Scots pine and sessile oak under the effects of the on-going climate change at their rear-edge distribution limits in the Mediterranean Basin. In addition to the discussion presented in the individual publications, this chapter constitutes a general and unifying discussion of the results.

### 5.1. Species-specific responses

#### 5.1.1. Scots pine

The principal drought responses of Scots pine are indicated in Figure 11. The results obtained in this thesis agreed with the relatively isohydric behaviour (McDowell *et al.*, 2008) that has been typically associated with Scots pine (Irvine *et al.*, 1998; Poyatos *et al.*, 2008).

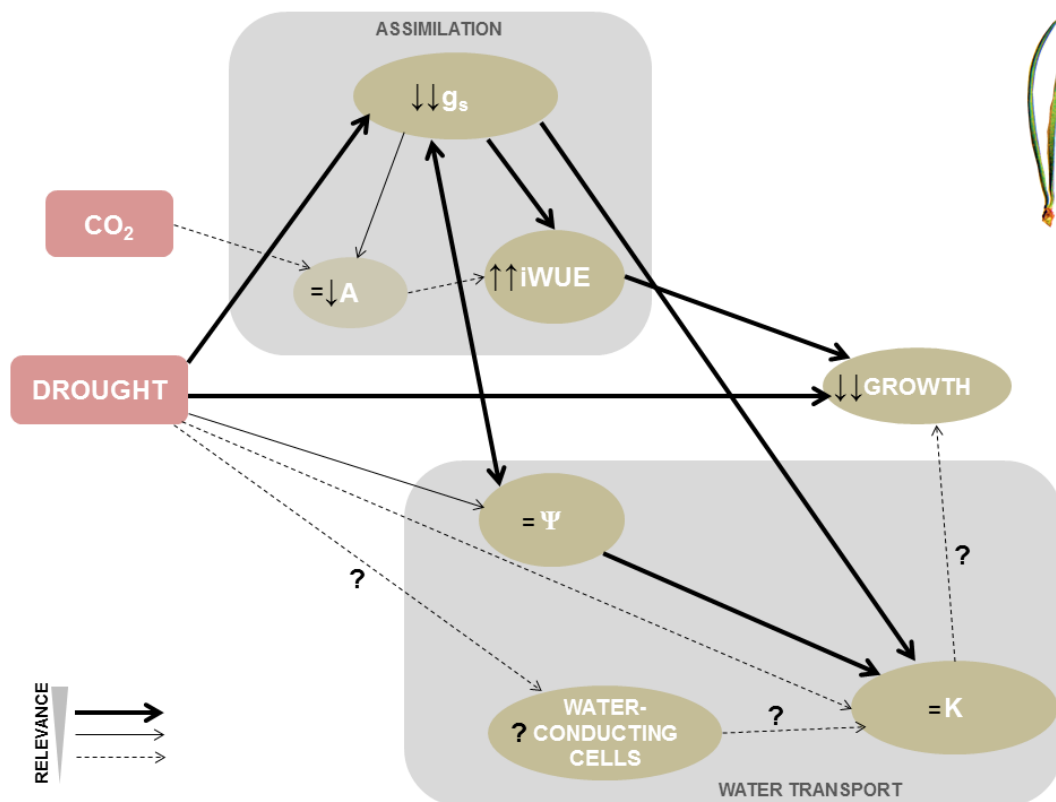


Figure 11. Theoretical framework summarizing the main results of this PhD thesis regarding Scots pine adjustments. g<sub>s</sub>, stomatal conductance; A, photosynthesis; iWUE, intrinsic water-use efficiency; K, hydraulic conductivity; Ψ, stem water potential; ↓, slightly reduced; ↓↓, strongly reduced; =, minor effect; ↑, slightly increased, ↑↑, strongly increased; ?, nonstudied.

The main physiological response of Scots pine to drought was the reduction of stomatal conductance. This was not only observed in seedlings under controlled conditions (chapter 4.1), but was also indirectly observed through the analyses of the stable carbon isotopes (chapter 4.4) and at the branch-level hydraulics in the studied natural populations (chapter 4.2). This strict mechanism had different consequences in the overall tree functioning. In the long-term, most of the studied populations did not benefit from the increase in atmospheric CO<sub>2</sub>; even showing a reduced capacity of intercellular carbon to follow atmospheric CO<sub>2</sub> rates (chapter 4.4). In turn, such avoidance strategy also compromises carbon uptake, forcing trees to rely on their carbon reserves, which may ultimately in a depletion of non-structural carbohydrates (McDowell, 2011). The combination of reduced stomatal conductance and the indirectly reduced photosynthesis resulted in a generalized increase in water-use efficiency, which has also been observed in other conifers (Granda *et al.*, 2014; Lévesque *et al.*, 2014) and in global studies considering several tree species (Peñuelas *et al.*, 2011; Silva & Anand, 2013). Indeed, one of the major findings of chapter 4.4 was that the increase in water-use efficiency was negatively associated with tree growth, pointing to a reduced carbon uptake, and consequently, negative growth trends. However, the strict stomatal control also helped to tightly maintain the stem water potential within the safety thresholds. These results were only observed in the seedling experiment (chapter 4.1), due to the logistical problems to measure field water potentials in our large study area. However, other studies carried out at the limit of the species distribution support this result in adult trees and under natural conditions (Fernández-De-Uña *et al.*, 2017).

As a result of the relatively high water potentials, reduced percentages of loss of conductivity (~10%) were measured at the stem level in seedlings under controlled conditions (chapter 4.1), and at branch level in natural conditions during one of the harshest dry spells in the region (chapter 4.2). These findings are in disagreement with other studies carried out in the NE Iberian Peninsula, where ~60% of native loss of conductivity was found (Aguadé *et al.*, 2015). However, it is worth mentioning that any of the studied populations included in this thesis did not suffer evident tree mortality in comparison to Aguadé *et al.* (2015), which might be related to the amount of embolisms that was found in their study. However, taking in consideration that the minimum xylem pressure measured in the field in relation to the xylem pressure causing 50% loss of hydraulic conductivity for Scots pine has been set up around -3.7 MPa (Martínez-Vilalta

*et al.*, 2002), our small percentages of loss of conductivity (10%), in relation to the measured water potentials ( $>-1$  MPa), are within the realistic values discarding possible measuring artefacts. These are in line with the global trends observed by Choat *et al.* (2012), who found that conifers operate with wider hydraulic safety margins, avoiding potential hydraulic impairments. Such protection of the hydraulic system was consistent with the functionality and duration of the xylem, since several tree rings were functional and contributed to the overall hydraulic conductivity (chapter 4.2). In fact, the next year's xylem only accounts for 15-20% of the tree-water transport in conifers (Urli *et al.*, 2013). Although the hydraulic conductivity is directly related to the xylem-conduit anatomy, in the case of Scots pine, the quantitative wood analyses are still pending and could not be included in this PhD thesis. However, no differences in maximum hydraulic conductivity nor in functional xylem area were found in chapter 4.2, indicating that, at least in the short-term, there is a limited impact of the potential anatomical adjustments on the overall conductivity.

Radial growth was strongly affected by drought at the rear-edge populations, as indicated by the amount of significant negative and positive correlations with temperature and precipitation, respectively (chapter 4.4). These, in combination with the negative impact of the increased water-use efficiency, resulted in negative long-term trends in all the studied populations. However, it is also worth highlighting the differences between seedlings' (chapter 4.1) and adult trees' growth responses to drought (chapter 4.4). In contrast to natural stands, young individuals growing under controlled conditions (chapter 4.1) showed reduced radial-growth sensitivity to drought. This could be explained by the fact that stress sensitivities vary from seedlings to adults, possibly reflecting greater inhibition of growth rather than of photosynthesis with increasing size (Niinemets, 2010). However, as recently highlighted by Cailleret *et al.* (2017), negative radial growth trends have been related to most of the cases of tree mortality, due to a gradual decline in hydraulic performance, coupled with depletion in carbon reserves, particularly in gymnosperms. Moreover, drought-stressed trees maybe more vulnerable to biotic attacks such as fungal pathogens (Desprez-Loustau *et al.*, 2006) and insects (Gaylord *et al.*, 2013), exacerbating and acceleration potential decline processes.

### 5.1.2. Sessile oak

The principal drought responses of sessile oak found in this PhD thesis are summarized in Figure 12. The obtained results confirmed the relatively anisohydric strategy displayed by this species, previously reported by other authors (Aranda *et al.*, 2000). The principal characteristic of such a strategy was a moderately relaxed stomata control, which allowed water potentials to fluctuate according to the climatic conditions (McDowell *et al.*, 2008). This relation between stomata control and water potentials was observed among the sessile oak seedlings in chapter 4.1. Due to the same reasons as for Scots pine, field water potentials were not measured but other authors have reported such behaviour under natural conditions (Bréda *et al.*, 1993a). Indirectly, the lack of correlations of the intercellular CO<sub>2</sub> concentrations with climatic conditions (chapter 4.4) also pointed to this behaviour.

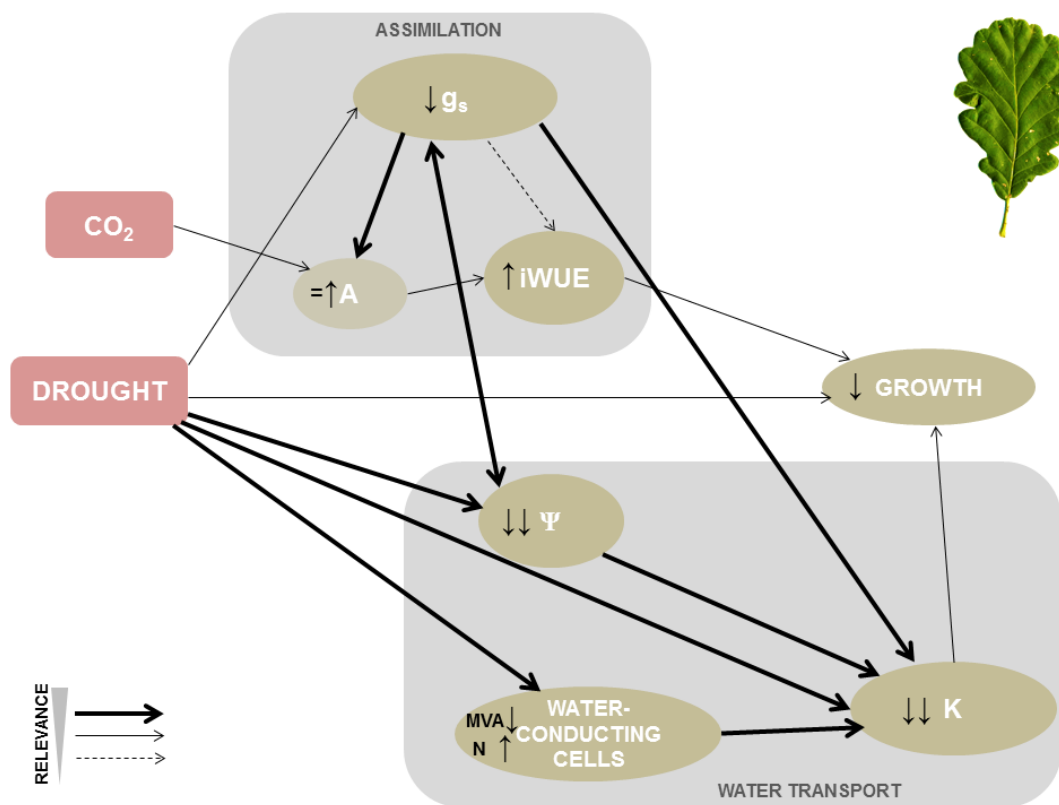


Figure 12. Theoretical framework summarizing the main results of this PhD thesis regarding sessile oak adjustments.  $g_s$ , stomatal conductance;  $A$ , photosynthesis;  $iWUE$ , intrinsic water-use efficiency;  $K$ , hydraulic conductivity;  $\Psi$ , stem water potential;  $MVA$ , mean vessel area;  $N$ , number of vessels; ↓, slightly reduced; ↓↓, strongly reduced; =, minor effect; ↑, slightly increased; ↑↑, strongly increased.

Furthermore, the intercellular CO<sub>2</sub> from all the oak populations could follow the changes in atmospheric CO<sub>2</sub> concentrations at the same rate or proportionally, indirectly pointing

to reduced photosynthetic limitations or even benefit from the increased atmospheric CO<sub>2</sub>. Therefore, the increase in water-use efficiency observed in sessile oak was lower compared to Scots pine, similar to the results reported at a continental level by Frank *et al.* (2015). The link between water-use efficiency and sessile oak growth seemed to be site-specific and displayed a geographical pattern. Similarly to Scots pine, the relation between water-use efficiency and oak growth was negative in populations located in the western of the Mediterranean Basin (Spain), but at a lesser degree. However, sessile oaks from the most eastern population located in Romania could have benefited from the increase of water-use efficiency. This situation is rarely reported in literature and is usually associated with an increment of photosynthetic rates rather than stomatal conductance reductions (Urrutia-Jalabert *et al.*, 2015).

Besides this, low water potentials had also major implications for the water-transport system. The large negative tensions within the xylem conduits led to several embolisms that impaired the native hydraulic conductivity and resulted in high percentages of conductivity loss (chapter 4.1). Unfortunately, this effect could not be tested under natural conditions due to the impossibility to measure samples with intact vessels, which could be longer than one metre in adult trees. However, large percentages of loss of conductivity (>60%) were observed in the seedling experiment (chapter 4.1) and reported in natural conditions by other authors (Cochard *et al.*, 1992; Bréda *et al.*, 1993b).

Drought also played a key role in modifying the wood anatomy of sessile oak (chapter 4.3). The xylem anatomy of sessile oak did not only adjust to environmental changes in order to avoid possible drought impacts and assure water transport, but also to efficiently transport water at sites without water limitations. This plastic response aimed to optimize maximum hydraulic conductivity towards aridity to avoid potential drought damages, but was significantly reduced since wood anatomy and maximum hydraulic conductivity are interrelated (Sperry *et al.*, 2008). Such adjustments were found in the long-term in the study of vessel anatomy (chapter 4.3) and in the short-term in the study of maximum hydraulic conductivity (chapter 4.2). Nevertheless, an increase in theoretical hydraulic conductivity was positively related to higher growth rates in all studied natural populations (chapter 4.2) as well as in seedlings (chapter 4.3).

The aforementioned xylem strategy needs to be discussed in the context with the functionality and duration of the species xylem. Sessile oak earlywood vessels are formed

in early spring before budburst and, therefore, entirely rely on stored carbohydrates (Bréda & Granier, 1996; Michelot *et al.*, 2012a). Since photosynthetic activity seems to be not disrupted under drought conditions in any of the studied populations included in this thesis, a certain pool of non-structural carbohydrates would be available for the next-year earlywood vessels formation, as also reported by other authors (Michelot *et al.*, 2012a). Although the characteristics (size and number) of the xylem conduits are adjusted after experiencing dry conditions in the previous year (chapter 4.2), the final size of the earlywood vessels is defined before stressful conditions occur, i.e. in summer, and cannot prevent potential damages due to extreme events (Anderegg & Hillerislambers, 2015). In this case, a large share of the earlywood vessels would be gas-filled due to cavitation events, impairing water transport (chapter 4.1). Differently to Scots pine, sessile oak has different strategies to tolerate this loss of conductivity. For instance, even if the earlywood vessels do not cavitate during summer, they most probably will during winter since they are prone to freeze-thaw cycles (Sperry *et al.*, 1994). Therefore, the duration and the functionality of the earlywood vessels are restricted to one or two growing seasons before being filled with tyloses, the so-called “throw away strategy” (Hacke & Sperry, 2001). Such a low share of functional xylem was also observed in chapter 4.2. Consequently, the next year’s earlywood vessels will again restore the major part of the hydraulic conductivity (Urli *et al.*, 2013). Furthermore, the latewood vessels are less sensitive to drought and frost damages, since cavitation vulnerability is also related to vessel size (Sperry *et al.*, 2006). Although latewood vessels remain functional during longer periods, contributing to the water transport during unfavourable conditions, they are less efficient in water transport than the earlywood ones. However, latewood vessels were not considered in this PhD thesis.

The inter-annual variability of sessile oak growth was negatively correlated to the lack of precipitation and drought in most of the studied natural populations (chapter 4.2), and in seedlings growing under controlled conditions (chapter 4.1). Indeed, several studies highlighted the sensitivity of oak growth to limiting-water conditions, despite performing an anisohydric strategy (Michelot *et al.*, 2012b; Delpierre *et al.*, 2016). This reinforced the view that sink activities (growth) are more sensitive and not necessarily dependent on source activities (photosynthesis) (Körner, 2015). Such a special sensibility was also observed during the seedling experiment (chapter 4.1), where stomatal conductance was kept under low water potentials, and presumably photosynthesis, whereas growth was

limited or stopped. Tree-ring width reductions in sessile oak are usually associated with variations in latewood width (Tardif & Conciatori, 2006), which could compromise the water-transport capability of the latewood area. Nevertheless, the combination of drought impacts, increase in water-use efficiency and the anatomical adjustment of sessile oak, resulted in site-specific long-term growth trends in the studied populations.

## 5.2. Trait variability across the species' southern limits

Assessing the spatial variability of hydraulic, anatomical and growth traits across the southern limits of the studied species' distributions, constituted an important aim of this PhD thesis. Generally, most publications have assessed trait changes from the edge of the species' distribution area to the centre, since the range of the climatic conditions leads to contrasting results. However, little is known about trait variability among rear-edge populations, in particular looking beyond radial growth. This document constitutes a first attempt to assess this.

In general, the results indicated a reduced plasticity of Scots pine traits at its southern distribution limits. None of the hydraulic traits measured at branch-level in chapter 4.2 differed across populations located in the western and central Mediterranean Basin (Spain, France and Italy). Similarly, studies analysing Scots pine plasticity at the continental scale found reduced variability in branch-level traits. Among these traits, the leaf-to-sapwood ratio was the only variable showing large differences across their studied sites (Mencuccini & Bonosi, 2001; Martínez-Vilalta *et al.*, 2009). Lower leaf-to-sapwood ratios at dry sites maintain constant water potentials within the stem (Mencuccini & Grace, 1995). Although there were differences in climatic variables such as site aridity among the studied sites included in this thesis (chapter 4.2), this pattern in leaf-to-sapwood ratios was not observed, suggesting that similar levels of stress occurred across the studied rear-edge populations.

Geographical patterns in Scots pine traits were found when studying more populations in a larger area, showing differences between western and eastern populations in the Mediterranean Basin. For instance, although the ratio of intercellular to atmospheric CO<sub>2</sub> from all the studied populations displayed significant sensitivity to water deficits, this



ratio only showed a negative relationship to increasing temperatures in the western and central populations located in Spain, France and Italy (chapter 4.4). This higher sensitivity to drought from the western and central stands translated into negative long-term trends of the  $^{13}\text{C}$  discrimination in these populations. Regarding radial growth, all the Scots pine populations displayed negative long-term trends, but these were only significant in the western and central populations (Spain, France and Italy). These results agree with other studies assessing Scots pine tree growth in the Mediterranean regions of Spain, France and Italy (Vacchiano *et al.*, 2012; Thabeet *et al.*, 2009; Hereş *et al.*, 2011; but see Martínez-Vilalta *et al.*, 2008). Indeed, a recent publication predicted an overall 16.4% growth reduction after 2050 for Scots pine populations located in the western Mediterranean Basin under the most severe climate change scenario (Sánchez-Salguero *et al.*, 2016). This points to a high vulnerability of this species to drought in the region.

In contrast to Scots pine, a few studies have assessed sessile oak trait variability under Mediterranean conditions. In fact, sessile oak populations have been largely managed and exploited in the area. However, during the second half of the 20<sup>th</sup> century, rural abandonment, land-use change and creation of protected areas, such as natural parks, have reduced the human pressure on oak stands. In this PhD thesis, sessile oak displayed a relatively high plasticity, with drought being one of the main drivers of these adjustments. In the case of the hydraulic traits, xylem- and leaf-specific maximum conductivities were directly linked to site aridity (chapter 4.1). Despite the non-direct relation between geographical longitude and aridity, the most western stand, located in the NE Spain, and the most southern central stand, located in central Italy, displayed the lowest values of xylem- and leaf-specific maximum conductivities, whereas the other two central stands located in northern Italy showed the highest. Such a relation with aridity rather than a geographical gradient was also confirmed in chapter 4.2, where sessile oak adjusted its anatomy in order to be more efficient in water transport in central populations with less water restrictions located in Italy and Slovenia, while western and eastern populations with reduced water availability located in Spain, Romania and Bulgaria adjusted to avoid drought damages. Similarly, the ratio of intercellular to atmospheric  $\text{CO}_2$  of all sessile oak populations was not limited by drought, and was even stimulated by higher temperatures in the case of the central populations with no water restrictions located in Italy and Slovenia. Thus, the discrimination against  $^{13}\text{C}$  of those populations increased during the last 50 years.

However, the growth trends of the sessile oak populations resembled a west-east pattern, which gradually shifted from significantly negative in the most western population located in Spain to significantly positive in the most eastern population located in Romania. This also suggests that wood formation is more sensitive to environmental conditions than any other measured variable, such as photosynthetic activity or earlywood formation (Delpierre *et al.*, 2016). The sensitivity of radial growth to climatic conditions also showed slight differences between western and eastern populations. Radial growth from eastern populations located in Bulgaria and Romania displayed significant positive relations to spring and summer temperatures (chapter 4.4), which could be associated with the resumption of tree growth after cold winters and the lengthening of the growing season (Gordo & Sanz, 2010).

Similar west-east patterns were found when assessing the inter-annual variability of all the studied parameters. Chapter 4.3 assessed the inter-annual variability of the characteristics related to earlywood vessels of sessile oak and chapter 4.4 analysed the inter-annual variability water-use efficiency and growth of sessile oak and Scots pine. All these series presented a clear west-east geographical pattern. Furthermore, in the case of water-use efficiency and growth, the geographical position overwrote species-specific particularities.

Seim *et al.* (2014) was the first publication to show such a geographical pattern in the Mediterranean region for pine growth. The authors concluded that different climatic drivers governed tree growth in the west and east of the Mediterranean Basin. Later, Chen *et al.* (2015) provided supportive evidence of a distinct west-east pattern of European beech sensitivity to temperature and precipitation effects; as well as insights of the effect of the North Atlantic Oscillation on tree growth. Recently, a study assessing the growth pattern of sessile oak and European beech rear-edge populations across the Mediterranean Basin related the west-east differences in tree growth to a prominent role of the intensification of the Summer North Atlantic Oscillation during the last decades (Dorado-Liñán *et al.*, 2017). This led to different climatic conditions in the region, and consequently, described the west-east tree-growth dipole. Thus, the west-east geographical polarity, displayed by the inter-annual series of all the studied parameters in this thesis, linked to the Summer North Atlantic Oscillation illustrates that not only forced-climate variability will determine survival, but also internal variability (e.g.

synoptic circulation patterns). However, more studies are needed to confirm this hypothesis and to find potential relationships to other circulation patterns such as the Hadley circulation which is expected to expand towards the poles under future climate change scenarios (Lu *et al.*, 2009; Lau & Kim, 2015).

### 5.3. Future perspectives

The contrasting strategies displayed by the studied species imply different benefits and disadvantages. The main difference relies on avoiding or tolerating drought. One could think that the drought-avoiding strategy displayed by most of the conifers might imply larger consequences. However, the most long-lived organisms in the world are conifers. The simplicity of their xylem prevailed during millennia. On the contrary, evolution developed to the efficiency of angiosperms. In general, the specialised xylem, deciduous foliage and better pollen dispersion allowed them to successfully establish across the world. However, the question that remains unanswered is: are the displayed strategies enough to resist this new climatic challenge in the Mediterranean region and compete with the better-adapted Mediterranean species?

The reduced variability found among the studied traits and the negative growth trends displayed by Scots pine during the last half of the 20<sup>th</sup> century across the rear-edge populations point to a potentially limited capability of the species to withstand the projected climate scenarios for the region. However, the drought-avoidance strategy reported for Scots pine in this thesis supports the species' resistance to extreme climatic events. At the same time, this conservative strategy could constrain the carbon uptake under long-lasting dry spells, as previously observed (Poyatos *et al.*, 2013). This, in combination with declining processes such as defoliation may force Scots pine to rely on carbon reserves to fulfil physiological demands. Despite the controversy around reserve depletion (Adams *et al.*, 2013; Sevanto *et al.*, 2014) and the source-sink limitations associated with it, the carbon reserve consumption could also weaken Scots pine ability to withstand biotic attacks, increasing its mortality risk. Although mortality events were not observed yet on the studied populations, the drying effect projected for the Mediterranean region during the next century (Giorgi & Lionello, 2008) will further threaten the Scots pine rear-edge populations (Sánchez-Salguero *et al.*, 2016). This is particularly important

for the western and central populations (Spain, France and Italy), which already showed significant negative growing trends and climatic limitations in their carbon uptake as presented in this thesis. Additionally, harsher summer conditions are expected in the western Mediterranean, also based on projected persistence of high Summer North Atlantic Oscillation phases during the next decades (Boé *et al.*, 2009). In fact, in the global study assessing tree mortality by Allen *et al.* (2010), most of the mortality events reported for Scots pine in the Mediterranean belonged to western and central populations, supporting this hypothesis. Moreover, recent publications found on-going replacements of Scots pine for holm oak (*Quercus ilex* L.) in some regions of the western Mediterranean Basin (Galiano *et al.*, 2010; Vayreda *et al.*, 2016) and in inner alpine valleys (Rigling *et al.*, 2013).

The large plasticity observed in sessile oak traits might guide to the assumption of a better fitness of the species in front of the forthcoming climate change. However, what is the limit of this plasticity? On one hand, the photosynthetic activity seemed not to be disrupted by climate in any of the studied populations, and it is even exacerbated by climate change in populations without water limitations located in Italy and Slovenia as reported in this thesis. This supports the hypothesis that anisohydric species may be less prone to suffer carbon starvation (Anderegg *et al.*, 2012). Moreover, the earlywood vessels, which are responsible for most of the tree-water transport, seem to also adjust to optimize the safety-efficiency equilibrium. On the other hand, these anatomical adjustments might not be enough to counterbalance the impairment in hydraulic conductivity due to extreme events, as high percentages of loss of conductivity were reported. In fact, the few mortality events for *Quercus* spp. reported by Allen *et al.* (2010) were usually associated with short-term extreme droughts. Xylem deterioration due to multiyear drought was the underlying reason of the widespread aspen forest die-off in North America (Anderegg *et al.*, 2013b), as aspen is also an anisohydric angiosperm, similar to sessile oak. The accumulated damage on the aspen xylem, due to consecutive droughts increased its vulnerability significantly to cavitation, the so-called cavitation fatigue (Hacke *et al.*, 2001). Thus, under the projection of having more recurrent extreme droughts in the forthcoming century, a higher risk of sessile oak mortality, triggered by hydraulic failure, could be expected. Moreover, the fact that wood formation is more sensitive to drought than photosynthetic activity, previously reported for the species (Delpierre *et al.*, 2016), was also observed in this thesis. Indeed, the most western

population (Spain) that displayed the lowest maximum hydraulic conductivities and a xylem-anatomy optimized towards aridity, also showed a negative growth trend during the last 50 years. This suggests that this population might be achieving the limits of the species' plasticity. Therefore, western populations could be more threatened under the forthcoming climatic scenarios, similar to Scots pine.

A similar study that analysed the dry-limit of distributions of two of the most widely distributed species in North America, ponderosa pine and trembling aspen, also agrees with the above conclusions (Anderegg & Hillerislambers, 2015). The drought-avoidance ponderosa pine would be more prone to suffer carbon limitations, whereas the drought-tolerance trembling aspen may be more vulnerable to extreme events. These different sensitivities between gymnosperms and angiosperms to carbon limitations and hydraulic failure were also observed in a global study, pointing that it might be a universal pattern across species (Adams *et al.*, 2017). Therefore, the intensity and duration of the forthcoming dry spells under the projected climate change scenarios seem to be key factors reshaping the distribution area of the studied species in the Mediterranean region.

## 6. Outlook

The presented thesis delivered some novel insights into the mechanistic understanding of contrasting functional types, such as Scots pine and sessile oak at their southern distribution limits. Although there was a tremendous effort invested into the sample collection and analyses, many research questions still remain due to lack of time, and not to lack of interest or ideas.

The most obvious unresolved question is the long-term response of Scots pine wood anatomy to climatic conditions. The results of this thesis indirectly indicate that little differences among sites could be found, but it is still unknown if this is due to common climatic drivers. Some studies that performed quantitative wood anatomical analyses on different Scots pine populations actually found some evidence of drought as a potential climatic driver of cell formation (Eilmann *et al.*, 2011; Martin-Benito *et al.*, 2013). However, analysing the complete Scots pine network included in this thesis will potentially help us to understand large-scale processes in the Mediterranean region. Further sample collection and preparation are in process (Figure 13) and the analyses will be carried out in future studies.

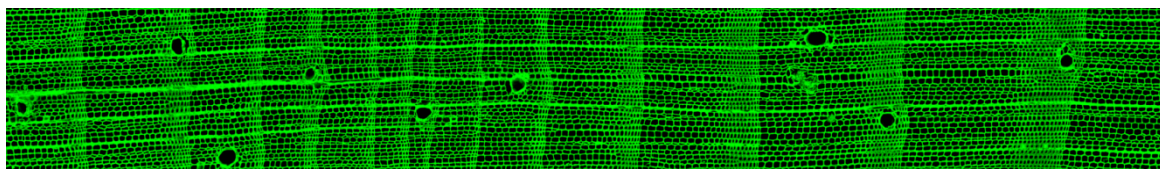


Figure 13. Scots pine core image using a confocal laser scan microscope.

The response to drought of latewood vessels from sessile oak was not investigated in this PhD thesis. Although they account for a lower share of the total hydraulic conductivity compared to the earlywood vessels, their contribution to the overall tree functioning is still unresolved and might be crucial for tree survival (Colangelo *et al.*, 2017).

Indeed, this PhD thesis and the publications linked to have mainly focused on responses or processes that occur within the tree xylem. However, assessing the response of other tree compartments might give us a complete picture of the species' acclimation strategies to climate change. For instance, below-ground processes such as higher carbon allocation in the root system (Taeger *et al.*, 2015; Aaltonen *et al.*, 2016) and different vulnerability of root-to-stem hydraulic conductivity under drought (Froux *et al.*, 2005), might be equally interesting to take into consideration when assessing the species' strategies.

Furthermore, the role of non-structural carbohydrates, including starch and soluble sugars, during drought is still unclear (Sala *et al.*, 2012). Some authors claim that carbon starvation may occur during dry conditions, due to impairments between carbon demand and uptake, resulting in a depletion of the reserves (Adams *et al.*, 2013; Sevanto *et al.*, 2014). Others state that these reserves cannot be allocated from source to sink organs, most likely due to phloem transport failure (Granda *et al.*, 2017). Moreover, differences in carbon dynamics between gymnosperms and angiosperms are also expected (Anderegg *et al.*, 2012), and would complement our understanding of the species' strategies.

Finally, disentangling the synergies between the climatic drivers, such as atmospheric circulation patterns that led the geographical gradients in the series from different tree variables (radial growth, wood anatomy and water-use efficiency), will be crucial to understand tree performance along the Mediterranean Basin. In particular, future predictions of a persistent stronger Summer North Atlantic Oscillation would enhance the drying conditions for western populations.

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Zhao M, Running SW (2010) Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. *Science*, **329**, 940–943. DOI: [10.1126/science.1192666](https://doi.org/10.1126/science.1192666).

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## Appendix

### A. Curriculum vitae

#### Personal information

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Name: Elisabet Martínez Sancho  
Date of birth: 26.05.1986  
Place of birth: Granollers (Spain)  
Address: Weizengasse 4  
85354 Freising (Germany)  
Nationality: Spanish

#### Education

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09/2013-09/2017 PhD student. “Physiological and anatomical acclimation of contrasting tree functional types under climate change along their southern range limits in Europe”. Ecoclimatology, Department of Ecology and Ecosystem Management. Technische Universität München. Freising, Germany.  
09/2010-09/2011 M.Sc. in Biodiversity. Thesis title: “Climate effects on the silver fir stand of Montseny Mountains: a dendroecological analysis”. Department of Ecology, University of Barcelona. Barcelona, Spain.  
09/2004-06/2009 Bachelor degree in Biology. Emphasis in Ecology and Plant physiology. Autonomous University of Barcelona. Barcelona, Spain.

#### Working experience

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12/2012-08/2013 Research assistant  
Project: “Ecophysiological characterization of the responses of several representative species of the subalpine stage to climate

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	change conditions”. Department of Ecology. University of Barcelona. Barcelona, Spain.
08/2012-11/2012	Research assistant Project: “Individual health and dynamics of natural populations”. Finnish Forest Research Institute Metsäntutkimuslaitos (METLA). Suonenjoki, Finland.
06/2011-09/2011	Field assistant Project: “Aerial surveillance of the quality of coastal waters”. Catalan Water Agency (ACA). Barcelona, Spain.
03/2011-06/2011	Research assistant Project: “Woody species identification of the museum art pieces”. National Art Museum of Catalonia (MNAC). Barcelona, Spain.
2010/2013	Field assistant Project: “Catalan Butterflies Monitoring Scheme (CBMS)”. Museum of Natural Sciences. Granollers, Spain.
06/2009-09/2010	Research and field assistant Project: “Conservation and monitoring of the olive ridley sea turtle ( <i>Lepidochelys olivacea</i> ) in playa Hermosa, Costa Rica”. Ministerio de Medio Ambiente y Energía. Playa Hermosa, Costa Rica.
02/2007-12/2007	Field assistant Project: “Analysis of socio-environmental conditions of the agricultural production in the region of Kukra (Nicaragua) and proposals for intervention”. Polytechnic University of Catalonia (UPC) and Desos, Opció solidària NGO. Bluefields, Nicaragua.

### **Mobility**

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11/2016-12/2016	Visiting scholar (PhD). University of Barcelona. Barcelona, Spain.
09/2016-10/2016	Visiting scholar (PhD). Helmholtz-Zentrum Potsdam. Potsdam, Germany.
02/2016-03/2016	Visiting scholar (PhD). University of Alberta. Edmonton, Canada.
10/2015-11/2015	Visiting scholar (PhD). Helmholtz-Zentrum Potsdam. Potsdam, Germany.

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03/2015-05/2015	Visiting scholar (PhD). Helmholtz-Zentrum Potsdam. Postdam, Germany.
09/2014-10/2014	Visiting scholar (PhD). University of Alberta. Edmonton, Canada.
02/2014-04/2014	Visiting scholar (PhD). Helmholtz- Zentrum Potsdam. Postdam, Germany.

### **Fellowships and Awards**

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09/2016-06/2017	Laura Bassi Award TU München (Germany). This award offers research stipends to support outstanding women in science.
08/2012-12/2012	ARGO scholarship from the Spanish government to work as a research assistant at the Finnish Forest Research Institute Metsäntutkimuslaitos (METLA). Suonenjoki, Finland.



## B. List of publications, conference contributions, and teaching

### List of publications

Publications marked with an asterisk (\*) are part of this thesis.

#### *Peer-reviewed*

##### *Published*

**Martínez-Sancho E**, Dorado-Liñán I, Hacke UG, Seidel H, Menzel A (2017) Contrasting hydraulic architectures of Scots pine and sessile oak at their southernmost distribution limits. *Frontiers in Plant Science*, 8, 598. DOI: [10.3389/fpls.2017.00598](https://doi.org/10.3389/fpls.2017.00598). \*

**Martínez-Sancho E**, Dorado-Liñán I, Heinrich I, Helle G, Menzel A (2017) Xylem adjustment of sessile oak at its southern distribution limits. *Tree Physiology*, 37, 903–914. DOI: [10.1093/treephys/tpx036](https://doi.org/10.1093/treephys/tpx036). \*

Dorado Liñán I, Zorita E, **Martínez-Sancho E**, Gea-Izquierdo G, Di Filippo A, Gutierrez E, Levanic T, Piovesan G, Vacchiano G, Zang C, Zlatanov T, Menzel A (2017) Large-scale atmospheric circulation enhances the Mediterranean East-West tree growth contrast at rear-edge deciduous forests. *Agricultural and Forest Meteorology*, 239, 86-95. DOI: [10.1016/j.agrformet.2017.02.029](https://doi.org/10.1016/j.agrformet.2017.02.029).

##### *Submitted/Under review/In revision*

**Martínez-Sancho E**, Dorado-Liñán I, Gutiérrez E, Matiu M, Helle G, Heinrich I, Menzel A. Increased water-use efficiency translates into growth patterns of Scots pine and sessile oak at their southern distribution limits. *Global Change Biology*. (Accepted pending minor revisions)\*

**Martínez-Sancho E**, Váscquez Navas LK, Seidel H, Dorado-Liñán I, Menzel A. Response of contrasting functioning tree types to air warming and drought. *Forests*. (Submitted)\*

Sánchez-Salguero R, Camarero JJ, Carrer M, Gutiérrez E, Alla AQ, Andreu-Hayles L, Hevia A, Koutavas A, **Martínez-Sancho E**, Nola P, Papadopoulos A, Pasho E, Toromani

E, Carreira JA, Linares JC. Climate extremes and predicted warming threaten Mediterranean fir refugia. *Proceedings of National Academy of Sciences*. (In revision)

Garcés-Pastor S, Gutiérrez E, **Martínez-Sancho E**, Dorado-Liñán I, Camarero JJ, Cañellas-Boltà N, Vegas-Vilarrúbia T. Subalpine forest dynamics reconstructed throughout the last 700 years in the Central Pyrenees by means of pollen and tree rings. *The Holocene*. (Under review)

*In preparation*

**Martínez-Sancho E**, Dorado-Liñán I, Heinrich I, Helle G, Menzel A. Climate influence on the tracheid features of the Scots pine at the southern distribution limits. (*New Phytologist*).

Hillabrand R, Lieffers V, Hogg E, **Martínez-Sancho E**, Menzel A, Hacke U. Impact of drought and insect defoliation events on wood anatomy in trembling aspen.

*Other publications*

**Martínez-Sancho E**, Gutiérrez E. Efectos del clima en el abetal de Passavets (Parque Natural del Montseny, Barcelona) (2014) IV International Yew Workshop.

Conference contributions

*Oral Presentations*

**Martínez-Sancho E**, Dorado-Liñán I, Heinrich I, Helle G and Menzel A (2015) Climate signals encoded in the earlywood vessels of sessile oak at its southern distribution limits. TRACE conference, 20.-23.05.2015, Seville, Spain.

*Poster Presentations*

**Martínez-Sancho E**, Dorado-Liñán I, Gutiérrez-Merino E, Matiu M, Helle G, Heinrich I and Menzel A (2017) Distinct effects of water-use efficiency on Scots pine and sessile oak growth at the rear-edges. EGU General Assembly, 23.-28.04.2017, Vienna, Austria.

**Martínez-Sancho E**, Dorado-Liñán I, Heinrich I, Helle G and Menzel A (2016) Preliminary results of the tree-ring and tracheid feature responses of Scots pine at its south-western distribution limits. TRACE conference, 12.-14.05.2016, Białowieża, Poland.

**Martínez-Sancho E** and Gutiérrez-Merino E (2015) Past and present of the southernmost silver fir stand in the Iberian Peninsula. TRACE conference, 20.-23.05.2015, Seville, Spain.

**Martínez-Sancho E**, Dorado-Liñán I, Heinrich I, Helle G and Menzel A (2014) Preliminary results of xylem hydraulic adjustment of sessile oak at its southern distribution limits. TRACE conference, 06.-10.05.2014, Aviemore, United Kingdom.

Dorado Liñán I, Zang C, Akhmetzyanov L, Chen K, **Martínez Sancho E**, Gea-Izquierdo G, Gutiérrez E, Castagneri D, Levanic T, Zlatanov T, Di Filippo A and Menzel A (2014) Growth trends and variability at European beech marginal populations in the Mediterranean Basin. TRACE conference, 06.-10.05.2014, Aviemore, United Kingdom.

## Teaching

### *Supervision*

Xylem hydraulic adjustment of *Castanea sativa*, *Pinus sylvestris* and *Quercus spp.* seedlings to air warming and drought. Lizeth K. Váscquez Navas, Master thesis in “Sustainable Resource Management”, Technische Universität München, 05/2017.

### *Lectures*

“Dendro in R” for Master and PhD students (16.12.2016). Departament of Biological Evolution, Ecology and Environmental Sciences, Universitat de Barcelona (Spain).