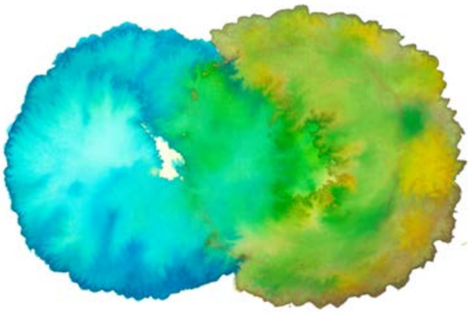
Universitat Autònoma de Barcelona

# Integrating plant hydraulics into functional traits framework to understand plant adjustments along a water availability gradient

**Teresa Rosas Torrent**



# Integrating plant hydraulics into functional traits framework to understand plant adjustments along a water availability gradient

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Bellaterra, March 2019

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*Als meus pares*
*i a la meva germana*

# Abstract

ONE of the main contributions of forest ecologists, in the 21st century, is to provide ecological theory and tools to describe and predict forests ecosystem changes caused by the ongoing global change. Over the last decade, ‘functional trait-based ecology’ has emerged as a refreshed discipline with the promise to turn ecology from a primarily descriptive science into a more mechanistic and predictive discipline. However, several foundational assumptions of trait-based ecology have not been rigorously tested. It is presumed that organ-level traits can be easily scaled-up to whole-plant traits, that intraspecific trait variability (ITV) can be largely overlooked, and that traits affect individual demographic outcomes and thus, are functional. Additionally, most trait-based approaches study ‘soft’ traits which are relatively easy and quick to measure for a large number of samples although they are not directly linked to specific physiological mechanisms. We argue that plant hydraulic traits can provide useful insights to the understanding of plant ecological strategies. Water transport throughout the plant affects both photosynthetic rate and growth. Plant hydraulics allow linking water to the carbon/nutrient economics, determine plants’ drought resistance and thus, are key factors when assessing forest vulnerability to climate change.

The main aim of this thesis is to integrate plant hydraulics into a functional trait-based framework, to assess trait variability, relationships and trade-offs at different ecological scales and to use this information to define strategies to cope with drought stress. To achieve this objective, two different study approaches were followed: one based on compiling a global dataset for 1149 species worldwide (Chapter 2), and another based on field data collection of a set of leaf, stem and hydraulic traits along a water availability gradient for six of the dominant tree species in Catalonia (NE Spain)

(Chapter 3 and Chapter 4). Specifically, in Chapter 2 we test a new framework relating hydraulic and more ‘standard’ traits across species at the global scale. In Chapter 3 we investigate the adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient at the interspecific and intraspecific levels. Finally, in Chapter 4 we test the functional importance of traits studied in the previous chapter, exploring the strength of the association between traits and tree growth also at the interspecific and intraspecific levels.

A significant finding to emerge from this thesis is that we do not find support for a world-wide ‘fast-slow’ plant economics spectrum that integrates across organs and resources (carbon, nutrients and water). Thus, scaling-up from organ level traits to whole-plant traits and resource use strategies may be more challenging than commonly anticipated because of compensatory responses within individuals. We also show that the ITV is especially relevant for integrative traits that involve more than one organ and that accounting for ITV is a necessary step forward towards improving our understanding of plant adjustments to environmental changes. Finally, we also show that our understanding of trait-growth (and by extension trait-performance) relationships can be greatly improved by selecting traits closely related to physiological functions and context-specific environmental drivers, integrating them along common axes of variation, and re-assessing the variables that are used to reflect whole-tree performance.

# Resum

UNA de les principals contribucions com ecòlegs forestals al segle XXI és proporcionar la teoria i aproximacions ecològiques per descriure i predir canvis dels ecosistemes forestals causats pel canvi global. En l'última dècada, l'ecologia basada en els trets funcionals ha sorgit com a una nova disciplina capaç de transformar l'ecologia merament descriptiva en una disciplina més mecanicista i predictiva. Tanmateix, algunes de les seves assumpcions fundacionals no s'han testat rigorosament. S'assumeix que els trets mesurats a nivell d'òrgan es poden escalar fàcilment a nivell de tota la planta, que la variabilitat intraespecífica dels trets (ITV) es pot ignorar en gran mesura i que els trets afecten les taxes demogràfiques dels individus i, per tant, són funcionals. A més, la majoria d'aproximacions estudien trets relativament fàcils i ràpids de mesurar per a un gran nombre de mostres, tot i que no estan directament relacionats amb mecanismes fisiològics específics. En aquesta tesis, mostrem que els trets hidràulics de les plantes poden ser de gran utilitat a l'hora d'entendre les principals estratègies ecològiques de les plantes. El transport d'aigua de les plantes afecta tant la seva taxa fotosintètica com el seu creixement. La hidràulica de les plantes permet incorporar l'aigua en l'economia del carboni i els nutrients, determina la resistència de les plantes a la sequera i per tant, és un factor clau a l'hora d'avaluar la vulnerabilitat dels boscos al canvi climàtic.

L'objectiu principal d'aquesta tesi és integrar els trets hidràulics en el marc dels trets funcionals clàssics, i determinar-ne la variabilitat, relacions i compromisos a diferents escales, així com usar aquesta informació per definir estratègies de les plantes per fer front a la sequera. Per tal d'aconseguir aquest objectiu, es van seguir dues aproximacions diferents: una basada en la compilació d'un conjunt de dades global de 1149 espècies d'arreu del món (Capítol 2); i una altra basada en dades de trets foliars, del tronc i

hidràulics mesurats al llarg d'un gradient de disponibilitat d'aigua, en sis de les espècies arbòries dominants a Catalunya (NE Espanya) (Capítol 3 i Capítol 4). Concretament, al Capítol 2 es testa un nou marc conceptual que relaciona els trets hidràulics amb els trets més clàssics a nivell global. En el Capítol 3 s'investiguen els ajustos i la coordinació dels trets hidràulics, foliars i del tronc al llarg d'un gradient de disponibilitat d'aigua a nivell interespecífic i intraespecífic. Finalment, al Capítol 4 s'avalua la importància funcional dels trets estudiats en el capítol anterior, explorant-ne les relacions amb el creixement dels arbres a nivell interespecífic i intraespecífic.

Un resultat rellevant d'aquesta tesi és que no hem trobat evidències que donin suport a l'existència d'un espectre econòmic global de tota la planta que n'integri els diferents òrgans i recursos (carboni, nutrients i aigua). D'aquesta manera, escalar els trets mesurats a nivell d'òrgan a trets de tota la planta i estratègies en l'ús de recursos, pot ser més difícil del que es sol preveure degut a les respostes compensatòries que es donen dins d'un mateix individu. També mostrem que la ITV és especialment rellevant en trets integradors que involucren més d'un òrgan i que incorporar la ITV és un pas necessari per millorar la nostra comprensió dels ajustos de les plantes als canvis ambientals. Finalment, il·lustrem que la nostra comprensió de les relacions entre el creixement i els trets pot millorar considerablement mitjançant la selecció de trets estretament relacionats amb funcions fisiològiques i factors ambientals específics del context d'estudi, integrant els trets al llarg d'eixos comuns de variació, i reavaluant les variables que s'utilitzen per reflectir el funcionament de la planta.

*"Y, de repente, vi el bosque."*
Brigitte Vasallo (2018)

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# Part I

# PRELIMINARIES

*“Just like a traditional painter or sculptor explores the properties of paint and stone, I work with the possibilities of plants and explore their properties.”*

SJOERD BUISMAN (1992)

*“We must consider the distinctive characters and the general nature of plants from the point of view of their morphology, their behaviour under external conditions, their mode of generation, and the whole course of their life.”*

THEOPHRASTUS (370-285 BC)

# 1

# Introduction

ALL of us depend on forests ecosystems and the services they provide. Forests play an important role in the regulation of climate and global biogeochemical cycles and contribute substantially to reducing the greenhouse effect (Bonan, 2008). It has been estimated a global net forest sink of ~1.1 Pg of carbon every year, i.e., a quantity equivalent in magnitude to 16% of anthropogenic carbon dioxide emissions (Pan *et al.*, 2011). Regarding water, plant transpiration returns to the atmosphere more than half of the rain that falls on the continents (Good *et al.*, 2015); which is to say that as much or more water circulates through the stems of the plants than through the Earth’s rivers. In addition, forests provide refuge for biodiversity, food, medicinal and other products, protection of soil resources, recreational uses, and fulfil spiritual needs and aesthetic values. Over the past 50 years, we have changed Earth’s ecosystems more rapidly and extensively than at any previous time of human history due to human population growth, increased resources consumption, and corresponding land use changes (Millennium Ecosystem Assessment, 2005; Ellis *et al.*, 2013). In the 21st century, humanity faces the huge challenge to adapt to this rapid global environmental change. As forest ecologists, our main contribution is to provide consistent ecological theory and tools that can better predict forests ecosystem changes across multiple ecological scales to guide governments, policymakers and the general public in efforts to mitigate and, especially, adapt to ongoing global change.

## Plant functional traits as an ecological tool

More than 2300 years ago in Ancient Greece, the philosopher Theophrastus was one of the first thinkers to classify plants according to their morphology, function and use. Nowadays, we are still using plant traits to develop general rules in pursuance of explanations and predictions of the fascinating and highly complex ecosystems that surround us. Over the last decade, the classic 'comparative ecology' renamed in the eighties as 'functional ecology' has emerged as a refreshed discipline under the name of 'trait-based ecology' (Shipley *et al.*, 2016), with the promise to turn ecology from a primarily descriptive science into a more mechanistic and predictive discipline (McGill *et al.*, 2006). A functional trait is defined as any morphological, physiological or phenological feature measurable at the individual level that impacts fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance (Violle *et al.*, 2007). There has been an exponential increase in the number of research studies that have successfully linked plant traits with plant ecological strategies (Reich *et al.*, 1997; Westoby *et al.*, 2002; Wright *et al.*, 2004), plant responses to climatic and other environmental factors (Lavorel & Garnier, 2002; Maherali *et al.*, 2004; Wright *et al.*, 2005; Ordoñez *et al.*, 2009) and community assembly processes (Shipley *et al.*, 2006; Sterck *et al.*, 2011; Laughlin, 2014). However, most of the potential of the functional traits approach remains to be realized and several foundational assumptions have not been rigorously tested (Escudero & Valladares, 2016; Shipley *et al.*, 2016; Funk *et al.*, 2017; Yang *et al.*, 2018). Resolving these issues is critical in order to define pathways towards a more robust and predictive discipline.

Research using trait-based approaches has mostly focused on understanding interspecific trait covariation to define general principles that constrain global phenotypic diversity. A recent global synthesis reports two roughly orthogonal axes summarizing variability in vascular plants and capturing three-quarters of the variation in six traits representing plant size and the leaf economics spectrum (LES) (Díaz *et al.*, 2016). The

LES summarizes the majority of interspecific variation in leaf morphology and function and it is probably the axis of variation that has received the most attention in recent years. It highlights the trade-off between the dry mass and nutrient investments in leaf construction and the time required for obtaining returns on those investments. Specifically, it runs from species with 'conservative' leaf traits (e.g. expensive leaf construction, slow physiological rates, long leaf lifespan) to species with 'acquisitive traits' (e.g, cheap leaf construction, fast returns on investments of carbon and nutrients, short leaf lifespan) (Reich *et al.*, 1997; Wright *et al.*, 2004). Leaf mass per area (LMA) has been recognized as a key trait capturing the core of the LES, as it reflects the expected return of the light intercepting area from the corresponding mass investment, resulting in acquisitive leaves with low LMA having a shorter depreciation time and, consequently, shorter leaf lifespan (Westoby, 1998). However, plants are more than leaves. For instance, Chave *et al.* (2009) proposed a second trait spectrum that relates mechanical aspects of wood with hydraulic stem properties. Similarly, recent studies have also started to explore the existence of a root economic spectrum analogous to the leaf economics spectrum, but results are not conclusive as data on below-ground traits are particularly hard to obtain (Mommer & Weemstra, 2012; Laliberté, 2017). To what extent these proposed axes are coordinated and define whole-plant economic strategies that integrate across organs (leaves, stems and roots) and resources (carbon, nutrients and water) remains a fundamental open question to be elucidated (Reich, 2014).

Plant traits have been traditionally averaged at the species level, without accounting for intraspecific variability. However, phenotypic variability within species can be large and can have significant implications for the structure and dynamics of ecological communities (Violle *et al.*, 2012). Indeed, recent studies have recognized the relevance of intraspecific trait variability (ITV), acknowledging its key role in shaping functional diversity, species coexistence and trait coordination at different ecological scales (Martínez-Vilalta *et al.*, 2009; Albert *et al.*, 2010; Messier *et al.*, 2010; Anderegg *et al.*, 2018). ITV has been shown to be particularly significant when moving from global to more regional scales and tends to be greater for whole-plant traits rather than traits involving a single organ (Marks, 2007; Siefert *et al.*, 2015). How to use continuous plant trait distributions accounting for ITV in new modelling approaches (e.g., Laughlin *et al.*, 2012; Van Bodegom *et al.*, 2014) is also an active research area that should enhance the low predictive power of Plant Functional Type (PFT) approaches in current dynamic vegetation models (Moran *et al.*, 2016). Consequently, incorporating ITV will be crucial for a good understanding of how the ecophysiological processes that determine plant function will respond to changes in environmental drivers (Jung *et al.*, 2014). It should be noted that not all trait plasticity is due to environmental adjustments as species can also change traits through other processes such as ontogeny (Mediavilla & Escudero, 2004).

Another foundational assumption that deserves more attention is the fact that, by definition, traits are considered functional to the degree they affect individual plant fitness (Violle *et al.*, 2007). However, studies relating traits with demographic rates are more of an exception than the rule in the literature and no consistent pattern has emerged on what are the traits with the greatest impact on demographic rates (Paine *et al.*, 2015). Most of these studies have been conducted in the tropics (Sterck *et al.*, 2006; Poorter *et al.*, 2008; Rüger *et al.*, 2012) and the considered traits often explained only a modest proportion of the total variation in growth and/or mortality (Wright *et al.*, 2010; Adler *et al.*, 2014; Poorter, 2018). This fact has raised the question of whether we are actually measuring the correct traits. Most studies include 'soft' traits that are relatively easy and quick to measure for a large number of samples, in contrast to more difficult to measure 'hard' traits that are more directly linked to specific physiological mechanisms and could potentially better capture essential plant axes of variation. In addition, in almost all these studies traits and demographic rates were aggregated at the species level by using mean values (but see Liu *et al.*, 2016; Poorter, 2018). How different environmental conditions modify the strength of correlations between traits and fitness it is often ignored. Thus, a more attentive focus on individual values rather than species-mean approaches, incorporating local environmental information and selecting traits with a stronger physiological basis could predict tree performance better.

Overall, great progress has been made in the last decades in understanding vegetation responses to environmental changes using plant traits. However, a number of remaining caveats remain in trait-based ecology approaches, which constitute a serious limitation and, at the same time, an opportunity for our current research agenda to respond to the imperious need to improve our predictions on vegetation responses to global drivers in a rapidly changing world. Thus, the challenges raised above justify the lens through which I[1] approach the following chapters of the present thesis.

## Forests in a drier world

Water is crucial to all life. Plants require water to survive and grow (e.g., Wullschleger *et al.*, 1998 determined that the maximal transpiration of 90% of trees varies between 10 L and 200 L per day). In plants, water and carbon economies are inextricably linked, because when plants open their stomata to assimilate one molecule of $CO_2$, hundreds of water molecules are inevitably lost by transpiration. In recent years, a global warming of the planet has occurred due to the increase in the concentration of greenhouse gases in the atmosphere, caused by human activity (IPCC, 2013). Drought-related tree mortality has been observed worldwide in all major forest biomes (Allen *et al.*, 2010) and it is expected to increase in most regions of the Earth, resulting from either decreased precipitation and/or an increase of the atmospheric evaporative demand (Dai, 2013; Allen *et al.*, 2015). The importance of drought as one of the major drivers shaping forest structure, composition and function is especially critical in the Mediterranean basin (Peñuelas *et al.*, 2001; Lloret *et al.*, 2004; Carnicer *et al.*, 2011), where two studies of the present dissertation are conducted, because many species have their southernmost distribution limit here and are hence particularly vulnerable to increases in aridity.

Understanding the physiological mechanisms underlying forest mortality is key to develop better predictive models of forest dynamics and function under drier conditions and identify the most important traits to assess mortality risk. A decade ago, McDowell *et al.* (2008) summarized prior knowledge and proposed a simple hydraulic framework with two main interdependent physiological mechanisms associated with tree mortality by drought: carbon starvation and hydraulic failure. These two processes could amplify or be amplified by the impact of biotic attacks. Hydraulic failure is hypothesized to cause tree death via dehydration, often associated with partial or complete loss of xylem function due to trapped gas emboli in the water transport system, which reduce

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[1] '*I*' is used for general discussion or information related to this thesis and '*we*' is used when referring to research chapters in which co-authors are involved.

the ability of plants to supply water to leaves and can result in tree death (McDowell *et al.*, 2008). The carbon starvation hypothesis poses that stomatal closure to minimize hydraulic failure during drought causes photosynthetic carbon uptake to decline to low levels, thereby promoting carbon starvation as carbohydrate demand continues for maintenance of metabolism and defense (McDowell *et al.*, 2008). Despite the intense research on this topic in the lasts years (Sala *et al.*, 2010; McDowell *et al.*, 2011; Sevanto *et al.*, 2014), how to model and predict drought-induced forest mortality still remains a great challenge, probably because plant responses to limited water availability include adjustments of complex traits networks at a variety of organizational and time scales (Hartmann *et al.*, 2018; Martinez-Vilalta *et al.*, 2018). However, hydraulic failure has emerged as the most common and consistent mechanism involved in drought-induced mortality (Anderegg, 2015; Rowland *et al.*, 2015; Adams *et al.*, 2017; Choat *et al.*, 2018), so that plant hydraulics offers us a promising tool to improve the predictive power of plant traits when assessing vegetation responses under global change.

## Plant hydraulics strategies and hydraulic traits

The xylem is the tissue involved in the plant transport system that moves water and dissolved nutrients from roots to leaves (Zimmerman 2002). Xylem conduits are dead and hollow cells in which the protoplasm has degenerated to minimize resistance against water flow. In gymnosperms, these conduits are called tracheids and consist of a single cell, which is generally less than a cm in length (Hacke *et al.*, 2015a). In angiosperms, multicellular conduits known as vessels (longer and wider than tracheids, from cm to m in length) are the ones primarily involved in water transport, although tracheids are also present (Jacobsen *et al.*, 2012). Water circulates between connected conduits of either type passing through pit membranes that connect conduit lumens. These membranes increase resistance to water flow and also play a crucial role in the safety of xylem (Sperry *et al.*, 2006). Interestingly, even if short tracheid lengths tend to cause a large resistance, because the torus-margo pit structure of conifers is more efficient per pit area in conducting water than the inter-vessel angiosperm pit, a comparable xylem resistivity is found between these two plant groups (Pittermann *et al.*, 2005). Extra-xylary paths also contribute to hydraulic resistance and vulnerability and should be included when describing plant hydraulic pathways although their exact role is still disputed (Scoffoni *et al.*, 2014).

The mechanism responsible for the ascent of water in plants from the soil to the leaves is reasonably well understood. In 1896 H. H. Dixon proposed the currently accepted cohesion-tension theory based on the pioneering work by Reverend Stephen Hales almost 300 years ago (Hales, 1727). According to this theory, the driving force of xylem water transport is generated by transpiration at the leaves' surface and water surface tension, and the continuity of the water column is maintained by cohesion between the water molecules because of hydrogen bonding and adhesion between the water column and xylem conduit walls (Tyree, 1997). This continuous long-distance transport mechanism is passive as does not require any energy besides solar radiation. However, it implies that the column of liquid water has to be below atmospheric pressure in a metastable state ('under tension'). As a result, water transport under dry conditions is prone to be disrupted by the formation of gas bubbles leading to cavitation and embolism, although interconduit pits operate as valves limiting the spread of embolisms through xylem conduits (Hacke & Sperry, 2001).

Xylem hydraulic properties have often been summarized by two main traits: hydraulic efficiency (maximum water transport capacity of the fully hydrated xylem) and hydraulic safety (ability to avoid the formation of gas emboli under stress). Hydraulic efficiency is usually expressed as the rate of water transport through a given area and length of sapwood, across a given pressure gradient (stem-specific hydraulic conductivity, $K_S$). Conversely, the water potential at which 50% of hydraulic conductivity is lost due to embolism ($P_{50}$) is the most commonly used trait to characterize xylem vulnerability to embolism and thus, hydraulic safety. Increasing the resistance to cavitation (more negative $P_{50}$ values) has been shown to be a mechanism of drought tolerance of species occupying drier habitats (Maherali *et al.*, 2004). It has long been proposed that there should be a trade-off between traits that provide safety from cavitation and the ones that confer higher transport efficiency. However, a recent global study has shown that this relationship is relatively weak because many species presented low safety but also low efficiency, although no species had high values for both traits (Gleason *et al.*, 2015). Xylem vulnerability curves quantify how much of the stem hydraulic conductivity of a stem segment is lost (y-axis) when it is exposed to more negative xylem pressures (x-axis). There are multiple methodologies for measuring vulnerability curves that mainly differ in how embolism is induced and how the hydraulic conductivity is quantified, which may fit a corresponding variety of experimental aims (Venturas *et al.*, 2017). In the present thesis, we used the bench dehydration method, which basically consists in exposing excised branches to dehydration in free air and then regularly measure hydraulic conductivity and xylem pressure on shorter segments (Sperry *et al.*, 1988). The relative change in hydraulic conductivity of drier segments compared with the fully conductive samples (measured with a flow meter) is then used to calculate the percentage of embolism. We selected this relatively time-consuming method because it better reproduces what plants experience in the field and allowed us to control for potential artefacts in long-vesselled species, a controversial topic in plant hydraulics research (Cochard *et al.*, 2013; Martin-StPaul *et al.*, 2014; Hacke *et al.*, 2015b).

Although most studies thus far have focused on stem traits when studying plant hydraulic responses to water availability, there is an increasing awareness of the importance of including also other traits. While $P_{50}$ and $K_S$ represent tissue-level traits, the hydraulic safety margin (HSM) and leaf-specific hydraulic conductivity ($K_L$) link these xylem-only traits to whole-plant water status and allocation and, thus, they represent more integrative variables describing plant hydraulic strategies. HSM is defined as the difference between the minimum leaf water potential that plants can achieve ($\psi_{min}$, a measure that combines environmental conditions and plant attributes, including rooting depth and stomatal control) and that causing xylem dysfunction (e.g., $P_{50}$). It has been shown that woody species across a wide range of biomes operate with a narrow safety margin from hydraulic failure regardless of their current rainfall environment, indicating a global convergence in the vulnerability to drought (Choat *et al.*, 2012). $K_S$ and $K_L$ are related by the sapwood/leaf area ratio, a trait known as Huber value (Hv); i.e., $K_L$=$K_S$·Hv. The Hv is a measure of allocation reflecting the cross-sectional stem surface available for xylem water supply vs. demand by leaves and can be adjusted to cope with dry conditions (Mencuccini & Bonosi, 2001; Martínez-Vilalta *et al.*, 2009). Another trait describing leaf water relations is the leaf water potential at turgor loss, or wilting point ($P_{tlp}$), which has also been recognized as a useful indicator of species drought tolerance (Bartlett *et al.*, 2012a,b).

Taken together, the integration of these multiple hydraulic traits in meaningful, multidimensional plant strategies to cope with drought at different ecological scales, and at a more general level, the link between hydraulic characteristics and the more usually studied plant traits, remain substantial challenges that I hope this dissertation contributes to elucidate.

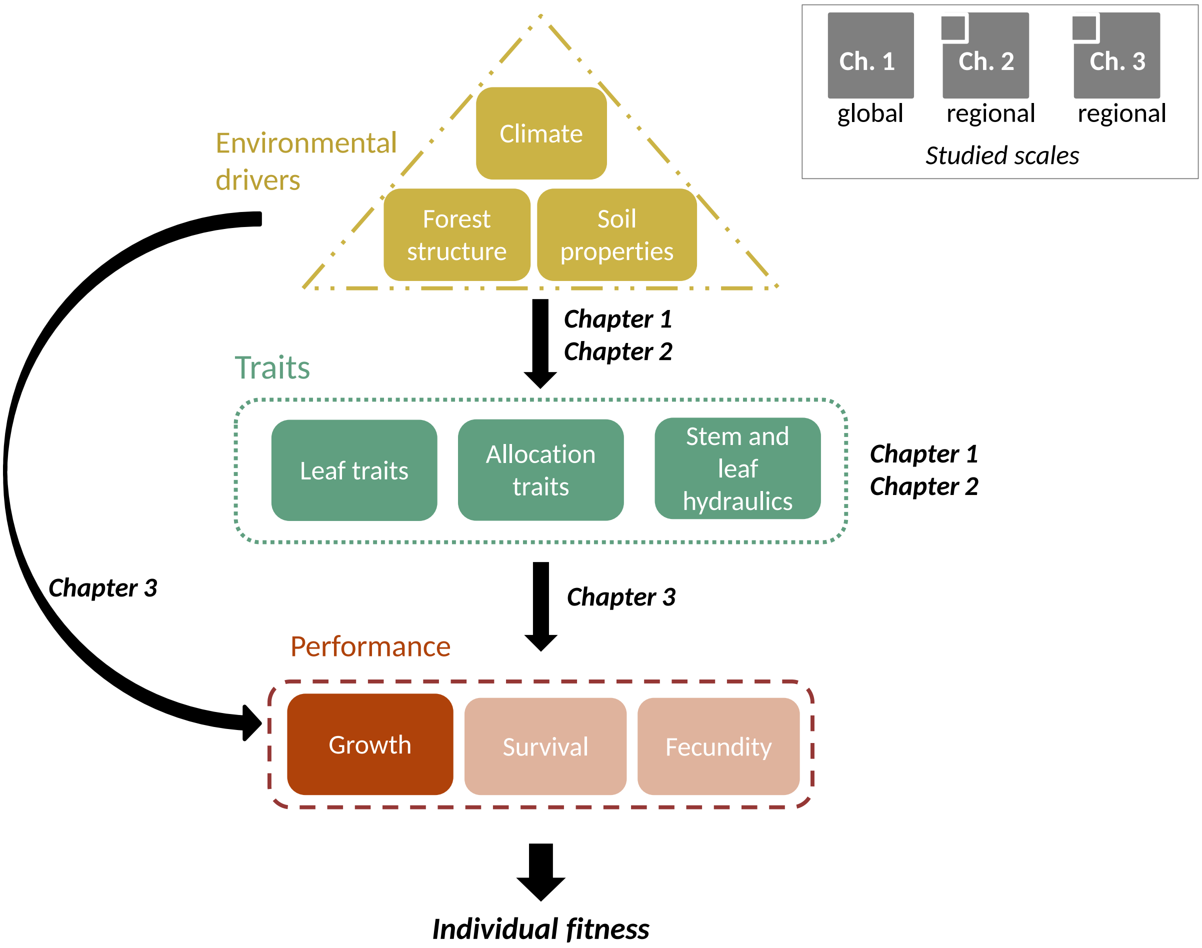

**Fig. 1.1:** Proposed framework to study the link between environmental drivers, traits and tree performance. Traits impact individual fitness indirectly via their effects on the three different components of individual performance, although only growth has been taken into consideration in this thesis. For clarity, interrelations among the different environmental drivers, traits and performance components are not represented.

## Research aims and thesis outline

In this thesis, I aim to integrate plant hydraulics into a functional trait-based framework, to assess trait variability, relationships and trade-offs at different ecological scales and to use this information to define plant strategies to cope with drought stress. To achieve this objective, two different study approaches were followed: one based on compiling a global dataset for 1149 species worldwide (**Chapter 2**) and another based on field data collection of a set of traits along a water availability gradient for six of the dominant tree species in Catalonia (NE Spain) (**Chapter 3** and **Chapter 4**). A conceptual framework of this dissertation is given in Fig. 1.1.

In **Chapter 2**, we aim to test a new framework relating hydraulic and more 'standard' traits across species at a global scale. Specifically, we examine (i) whether leaf economics spectrum (LES) traits co-vary with stem hydraulic traits and (ii) to what extent these relationships are maintained after accounting for climatic or phylogenetic effects reflecting basic functional coordination among traits. We also test (iii) whether the coordination between LES and hydraulic traits can be scaled-up to define overall plant strategies in resource use (*sensu* Reich, 2014).

In **Chapter 3**, we want to understand the adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient at the inter- and intraspecific levels. Specifically, we examine (i) how much trait variation is observed along the water availability gradient and how it is distributed among levels of organization, (ii) how are traits related to water availability within and between species and (iii) how are traits coordinated across and within species.

The main focus of **Chapter 4** is to test the functional importance of traits studied in the previous chapter, exploring the strength of the association between traits and tree growth at the inter- and intraspecific levels. We evaluate (i) what are the traits that better explain the variation of tree growth across and within species, (ii) how does trait coordination determine growth, and finally (iii) to what extent previous trait-growth associations are affected by the environment (climate and forest structure), tree size and trait coordination.

Finally, in **Chapter 5** I synthesize and integrate the results of the previous chapters addressing how this dissertation contributes to our understanding of plant ecological strategies, by drawing general conclusions to the main research questions and acknowledging the limitations of the approaches employed in the thesis. Finally, I also propose potential, and hopefully fruitful, paths to move this research topic forward.

# Part II

# CONTRIBUTIONS

# 2

# Supplying water to leaves: plant hydraulics and the leaf economics spectrum

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B. Choat, J. H. C. Cornelissen, J. M. Craine, A. G. Gutiérrez, S. Jansen, K. Kramer,
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## Summary

- A global leaf economics spectrum (LES) runs from species with ‘conservative’ leaf traits (i.e., expensive leaf construction, slow physiological rates, long leaf life-span) to species with ‘acquisitive traits’ (i.e., cheap leaf construction, fast returns on investments of carbon and nutrients, short leaf lifespan). The LES describes important aspects of plant ecological strategy and the underlying trade-offs among several key plant functional traits. But, are plant hydraulic and leaf-economic strategies coordinated? If so, can we discern a continuum of ‘slow’ to ‘fast’ whole-plant economic strategies that predicts plant responses to drought?

- Here, we compile trait data from 1149 species worldwide and show that plants with acquisitive leaves have sapwood with higher hydraulic conductivity (more efficient xylem) but are more vulnerable to embolism during drought (lower safety xylem). This coordination disappears, however, when more integrative hydraulic traits are considered. When hydraulic conductivity is normalized by leaf water demand (leaf-specific conductivity) and the vulnerability to embolism is normalized by the exposure to drought (hydraulic safety margin), they no longer scale with the LES, largely as a consequence of shifts in biomass allocation between leaves and sapwood. These patterns hold whether or not climatic or phylogenetic effects are accounted for in our analyses.

- These results indicate that LES traits are not sufficient to characterize whole-plant performance, and that models predicting vegetation responses to ongoing climate change can benefit from the incorporation of integrative plant hydraulics traits.

> *"In the biosphere, water cannot be separated from life, and life cannot be separated from water."*
>
> V.I. VERNADSKY (1926)

EVOLUTION has resulted in a wide diversity of plants varying in form and function, both within communities and among communities arrayed along environmental gradients. Underlying this variability in form and function are trade-offs among traits. A recent global synthesis reports two roughly orthogonal axes that summarize three-quarters of the variation in six key traits, representing plant size and the LES (Díaz *et al.*, 2016). Specific leaf area (SLA) is a core trait in the LES, describing the light-intercepting area constructed for a given dry mass investment in leaves. Higher SLA leaves tend to have lower construction costs per unit leaf area (Villar & Merino, 2001) but are less physically robust (Onoda *et al.*, 2011) and have shorter leaf lifespans (Wright *et al.*, 2004). High SLA species tend to have higher leaf N and P concentrations and show faster metabolic rates (Wright *et al.*, 2004). If these leaf economic properties translated into overall plant and resource-use strategies (Reich, 2014), this would reduce the number of traits needed to describe plant form and function in global models. However, synthesis studies have shown that SLA and growth are associated in seedlings, but not in adult plants (Gibert *et al.*, 2016), and this association tends to be weak even in seedlings (Paine *et al.*, 2015). This suggests that additional traits may improve predictions.

Hydraulics offer a promising avenue to understand how leaf-level trade-offs scale up to the whole plant, particularly under drought stress. Long-distance water transport connects supply (from roots) and demand (transpiration) and distributes resources to all plant tissues and organs, including leaves, meristems and reproductive structures (West *et al.*, 1999), allowing to incorporate water into the carbon/nutrient economics spectrum (Reich, 2014). Importantly, hydraulic traits have been directly related to plant performance (e.g., growth, mortality rates) even in moist tropical forests (Engelbrecht *et al.*, 2007; Rowland *et al.*, 2015; Anderegg *et al.*, 2016), highlighting their relevance for predicting demographic responses under changing climatic conditions (Skelton *et al.*, 2015).

The xylem represents a significant proportion of the entire water transport pathway, both in its contribution to plant hydraulic resistance, and in terms of carbon investment (Mencuccini, 2003). Xylem hydraulic efficiency, normally defined as the capacity to transport water per unit time and pressure gradient, is often expressed per unit of sapwood area (stem-specific hydraulic conductivity, $K_S$). $K_S$ increases with the size and density of xylem conduits (Zimmermann, 1983). When considering whole-plant performance however, a more relevant metric is leaf-specific xylem hydraulic conductivity ($K_L$). $K_L$ represents the balance between xylem supply and leaf demand for water or, in other words, the pressure gradient needed to sustain a given transpiration rate. $K_S$ and $K_L$ are related via the ratio of sapwood cross-sectional area to total leaf area (Hv, the Huber value).

Hydraulic conductivity, however, decreases as xylem water potential (MPa) becomes increasingly negative, because of gas emboli blocking xylem conduits. The water potential at which 50% conductivity is lost ($P_{50}$) commonly characterizes vulnerability to embolism. More negative $P_{50}$ values indicate xylem that is more resistant to embolism. Perennial species with lower $P_{50}$ in arid or semi-arid habitats also experience more negative water potentials, compared to species from mesic habitats. Therefore, hydraulic safety is normally characterized by relating $P_{50}$ to $\psi_{min}$, the pre-dawn shoot water potential measured during the driest time of year (Choat *et al.*, 2012). $\psi_{min}$ reflects both site water availability and plant water-use strategy. The hydraulic safety margin (HSM), i.e., HSM = $\psi_{min}$ - $P_{50}$, quantifies the degree of conservatism in hydraulic behaviour, with low (or even negative) values indicating higher risk of embolism. Woody species across a wide range of biomes operate with narrow HSM, converging globally in their vulnerability to drought, independent of climate (Choat *et al.*, 2012).

Our aim here is to build a global-scale understanding of the coordination between leaf economic strategies and plant hydraulic strategies. We formulate two paired hypotheses. On one hand, species with conservative leaf economic strategies (i.e. lower SLA) will tend to occupy drier habitats and be more exposed to drought stress (lower

$\psi_{\text{min}}$), and hence will need to have a safer xylem (lower $P_{50}$). On the other hand, because the Hv is simply the sapwood area per unit leaf mass divided by SLA, we expect lower SLA species to have more sapwood per unit leaf area (higher Hv), and hence require lower xylem transport efficiency (lower $K_S$). If these relationships reflect basic functional coordination among traits, they would be maintained after accounting for climatic effects despite the fact that SLA (Wright *et al.*, 2005), $P_{50}$ (Choat *et al.*, 2012), Hv (Tyree & Ewers, 1991; Mencuccini & Grace, 1995) and $\psi_{\text{min}}$ (Bhaskar & Ackerly, 2006; Martínez-Vilalta & Garcia-Forner, 2017) are all known to vary with climate. Finally, we asked whether the coordination between LES and hydraulic traits can be scaled-up to define overall plant strategies in resource use (*sensu* Reich, 2014). To test this we compared leaf economic strategies with xylem hydraulic conductivity normalized by leaf water demand (approximated here as the xylem conductivity per unit leaf area, $K_L$) and with the vulnerability to embolism normalized by exposure to drought (defined as the HSM).

## Materials and methods

### Dataset description

We compiled a global dataset with information on seven traits, two related to LES and five to hydraulics (Fig. A.1): specific leaf area (SLA, $m^2$ $Kg^{-1}$), nitrogen concentration per mass ($N_{\text{mass}}$, mg $g^{-1}$), stem-specific hydraulic conductivity ($K_S$, Kg $m^{-1}$ $MPa^{-1}$ $s^{-1}$), leaf-specific hydraulic conductivity ($K_L$, Kg $m^{-1}$ $MPa^{-1}$ $s^{-1}$ ), water potential at 50% loss of conductivity ($P_{50}$, MPa), twig-based Huber value (sapwood area/ leaf area, Hv; $cm^2$ $m^{-2}$) and minimum water potential ($\psi_{\text{min}}$, MPa). We also calculated hydraulic safety margins (HSM, MPa), i.e., $\psi_{\text{min}}$ - $P_{50}$. For all trait records, we calculated the mean at the species level obtaining a final dataset (HydraTRY) with 1149 species although sample size was smaller for bivariate relationships (414 species for SLA-$P_{50}$, 468 for SLA-$K_S$, 387 for SLA- $\psi_{\text{min}}$, 820 for SLA-Hv, 199 for SLA-HSM, 460 for SLA-$K_L$). The majority of traits related to LES were obtained from TRY (Plant Trait Database, *www.try-db.org*) (Kattge *et al.*, 2011) while hydraulic traits were compiled from an updated version of Choat *et al.* (2012) and additional datasets (Patiño *et al.*, 2012; Manzoni *et al.*, 2013, 2014; Martínez-Vilalta *et al.*, 2014; Nardini & Luglio, 2014; Togashi *et al.*, 2015), primarily covering China, the Amazon and Australia. Our $\psi_{\mathrm{min}}$ data combines measurements taken on covered leaves, to equilibrate xylem and leaf water potentials, with measurements taken on uncovered leaves. In this latter case, measured water potentials may be lower that xylem water potential due to the pressure drop in leaves associated with transpiration. However, this effect is likely to be minimized in our dataset because $\psi_{\mathrm{min}}$ is usually recorded under extreme conditions when stomata are completely closed, as confirmed also by the analyses conducted by Choat *et al.* (2012) using a dataset largely overlapping with ours.

To bring species binomials to a common taxonomy across data sets, names were matched against accepted names in The Plant List using *taxonstand* R package (Cayuela *et al.*, 2012). Any binomials not found in this list were matched against the International Plant Names Index (IPNI; *www.ipni.org*) and Tropicos (*www.tropicos.org*). The final list with unresolved species nomenclature was carefully checked manually. We then used *taxonlookup* R package (Pennell *et al.*, 2016) to complete species information at genus, family, order and group level. To standardize hydraulic trait data, we removed trait records measured on juvenile plants and on plants grown under controlled conditions (i.e., greenhouse, manipulative experiments), as well as duplicates and clearly erroneous entries. Hydraulic records measured on roots and exponentially-shaped vulnerability curves were excluded from the dataset (Cochard *et al.*, 2013; Bartlett *et al.*, 2016). We identified potential outliers following the approach described in TRY, so that trait records with a distance of >4 standard deviations from the mean of species, genus, family or higher-rank taxonomic group were excluded from the dataset. We also paid special attention to avoid identifying as outliers truly extreme values. The remaining dataset was used to calculate species trait means.

We calculated species climatic envelopes using *speciesmap*, an R package that obtains species occurrences from GBIF (Global Biodiversity Information Facility, *www.gbif.org*), downloading WorldClim climate layers (*www.worldclim.org*) and Global Potential Evapo-Transpiration dataset from CGIAR-CSI (Consortium for Spatial Information data, *www.cgiar-csi.org*) (Zomer *et al.*, 2008), rasterizing species occurrences to the same resolution as WorldClim (10 min = 18.6 x 18.6 km at the equator) and, finally, extracting means and

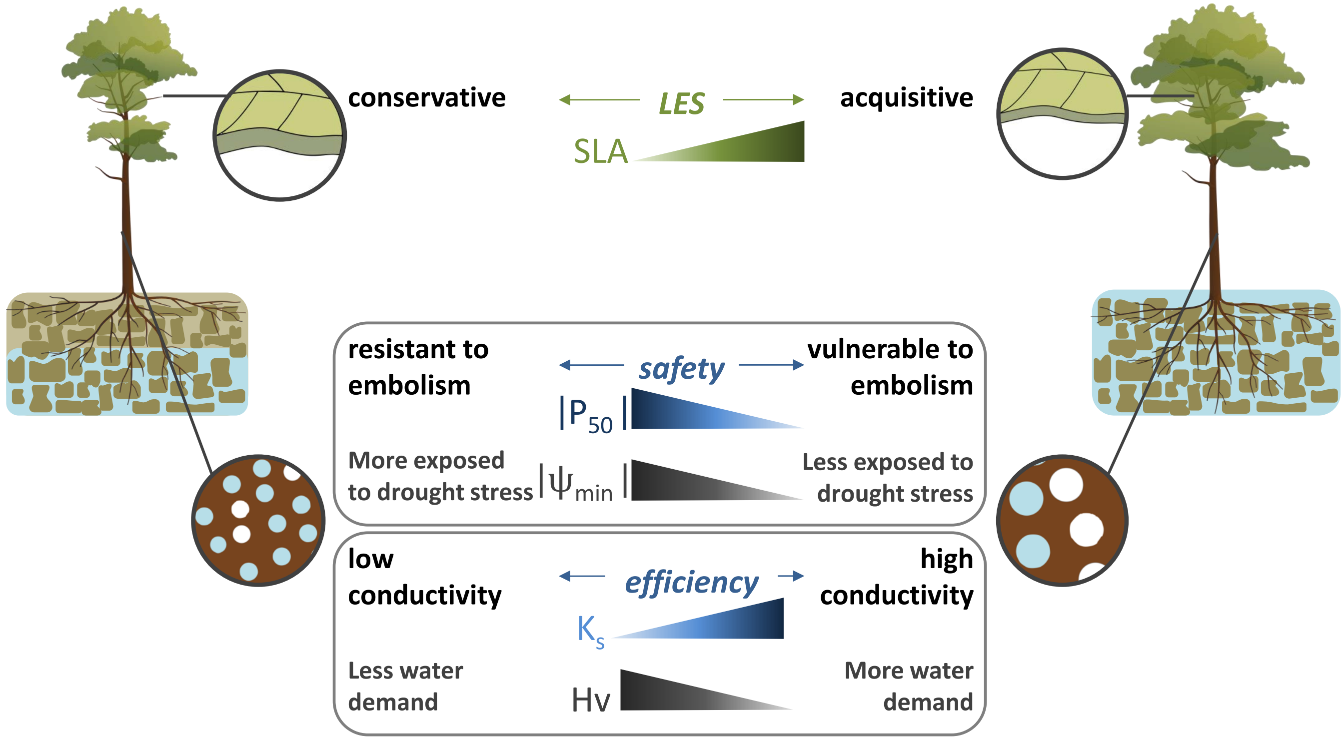

**Fig. 2.1:** Conceptual diagram relating leaf strategies and hydraulic traits and the two paired hypothesis between the LES and plant hydraulics in terms of safety and efficiency. Plants with conservative leaves (low-SLA) are hypothesized to occupy drier sites (more negative $\psi_{min}$) and have a more resistant xylem (more negative $P_{50}$). At the same time, plants with conservative leaves are hypothesized to have more sapwood per unit leaf area (higher Hv), and hence require lower xylem transport efficiency (lower $K_S$).

0.05 and 0.95 quantiles for mean annual precipitation (MAP), mean annual temperature (MAT) and mean annual potential evapotranspiration (PET) across all grid cells of the species occurrence region. We then calculated a Moisture Index as the ratio of MAP to PET as defined by the United Nations Environment Programme (Middleton & Thomas, 1992). Converting the occurrence data into presence/absence grid cells allowed us to reduce sampling bias ensuring that poorly sampled areas were equally weighted in the climate envelope estimates. Species classification into biomes was obtained from a Whittaker diagram of MAT and MAP.

We built a phylogeny $\sim$60% of the total number of species with trait information, starting from the largest time-scaled plant phylogenetic tree available (Zanne *et al.*, 2014). To improve the overlap between phylogeny information and trait data we used *phyndr* (Pennell *et al.*, 2016) to swap species with no available information in our tree with phylogenetically equivalent species.

## Data analysis

All analyses were carried out in R (version 3.3.2). All trait variables with the exception of HSM were natural-log transformed to achieve normality. Negative variables ($\psi_{min}$, $P_{50}$) were converted to positive prior to transformation.

To maximize sample size in some analyses, we used SLA as a proxy for the LES and we used standardized major axis (SMA) (*smart* package, (Warton *et al.*, 2012)) to relate it to hydraulics traits ($P_{50}$, $K_S$, $\psi_{min}$, Hv, HSM, $K_L$). We repeated these analyses on all species and on gymnosperms and angiosperms separately (Supplementary material Tables A.1, and Fig. 2.2). Phylogenetic effects were taken into account (Supplementary material Table A.4) using phylogenetic generalized least squares (PGLS) (Paradis *et al.*, 2004) using *ape* (Paradis *et al.*, 2004) and *caper* (Orme *et al.*, 2013) R packages. When using PGLS, model selection was based on Akaike's information criterion (AIC). We started from the saturated model and progressively removed non-significant explanatory variables until the minimal adequate model with lowest AIC was found. Models within 2 AIC units were considered equivalent and the simplest one was selected. To confirm the regression results based on SLA, principal components analysis (PCA) was also used to partition bivariate variation in SLA and $N_{mass}$ onto two orthogonal axes enabling the use of species' scores along the first axis as a proxy for the LES (Supplementary Tables A.2 and A.3). This axis explained 81% of the variance in SLA and $N_{mass}$. To account for trait plasticity (within species) in our analyses, we performed a random resampling (n=1000) adjusting all variables to a normal distribution and assuming a coefficient of variation of 10% for SLA, 18% for $P_{50}$, 18% $\psi_{min}$, 51% for $K_S$, 29% for Hv, 39% for $K_L$, based on preliminary analyses of our own database before aggregating at the species level (Supplementary Table A.5).

Finally, we performed two path analyses with *laavan* R package (Oberski, 2014) (one for safety and another for efficiency) to explore conceptual models of how hydraulic traits and SLA (used as a proxy for the LES) are related with each other and with climate in terms of mean annual precipitation (MAP) and mean annual temperature (MAT). We repeated both path analyses using Moisture Index (Moisture index = MAP/PET) as exogenous variables instead of MAT and MAP (Supplementary Fig. A.4). All the variables were standardized prior to fitting the path models. We started with a model including direct climate effects on SLA, $\psi_{min}$ and $P_{50}$ (safety model) and on SLA, Hv and $K_S$ (efficiency model), plus all possible covariations among those traits, and direct effects of those traits on the integrative variable characterizing xylem hydraulic conductivity normalized by leaf water demand ($K_L$) and the vulnerability to embolism normalized by exposure to drought (HSM). We then simplified the model by removing non-significant paths until the minimal adequate model with the lowest BIC (Bayesian Information Criterion) was found. Models within 2 BIC units were considered equivalent and the simplest one was selected. We considered one index from each fit class (absolute, parsimony and comparative) to assess overall goodness of the fit of path models (Brown, 2014). Full-information Maximum Likelihood method was used to deal with missing data (Arbuckle, 1996; Enders & Bandalos, 2001).

## Results and discussion

We used SLA as the chief proxy for a species' position along the LES. As predicted, species with more conservative leaf economic strategies (lower SLA) had more embolism-resistant xylem (more negative $P_{50}$) ($R^2 = 0.17$, $P < 0.001$, Fig. 2.2a, Supplementary Table A.1).

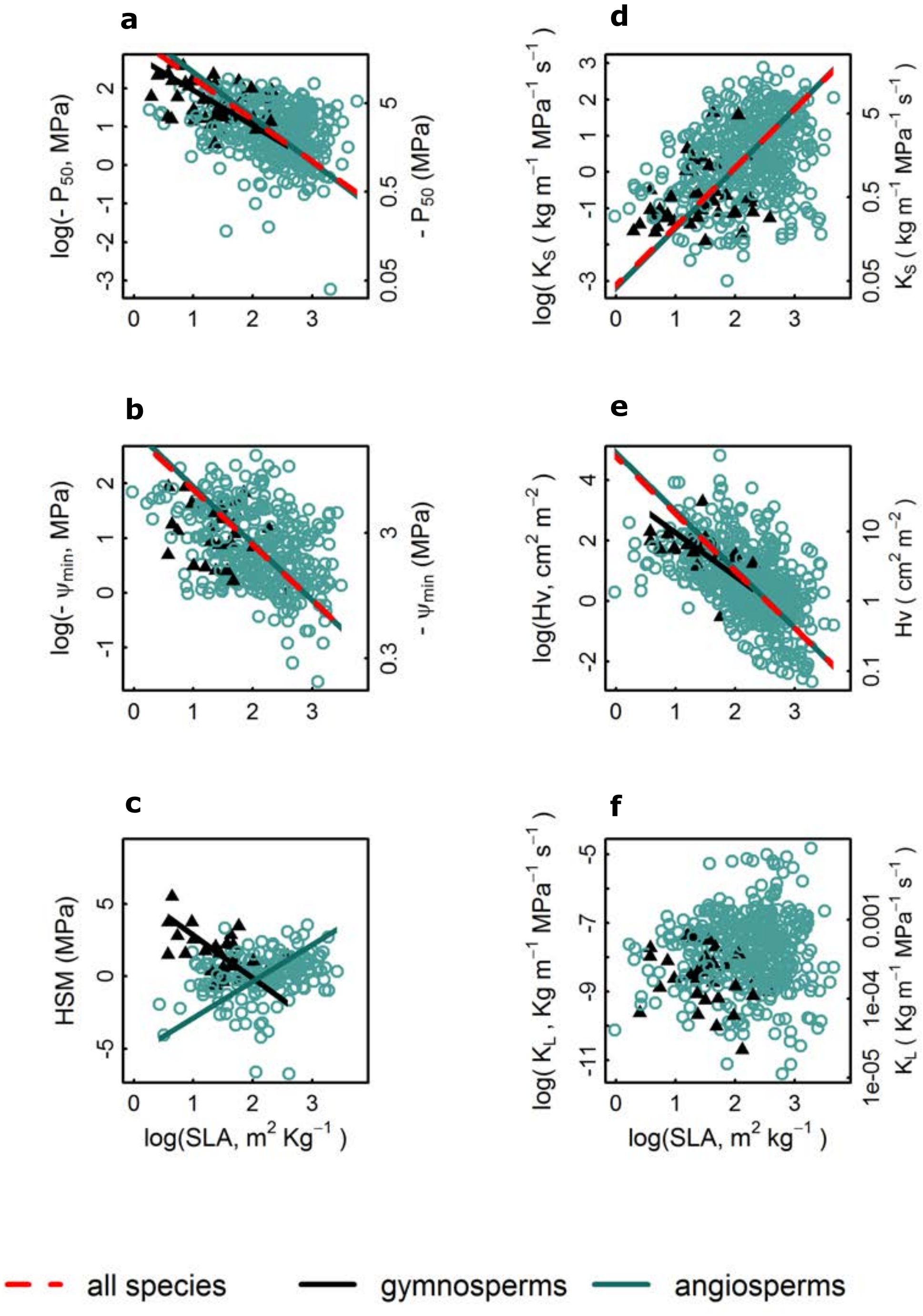

**Fig. 2.2:** Standardised Major Axis (SMA) regressions between specific leaf area (SLA, X axis) and hydraulic traits: (a) water potential at 50% loss of hydraulic conductivity ($P_{50}$), (b) xylem minimum water potential ($\psi_{min}$), (c) hydraulic safety margin (HSM), (d) stem-specific hydraulic conductivity ($K_S$), (e) Huber value (sapwood area / leaf area, Hv), and (f) leaf-specific hydraulic conductivity ($K_L$) for all species (red regression line), gymnosperms (black line and triangle symbols) and angiosperms (green line and round symbols). Only statistically significant relationships are shown ($P<0.05$). All data are natural-log transformed with the exception of HSM.

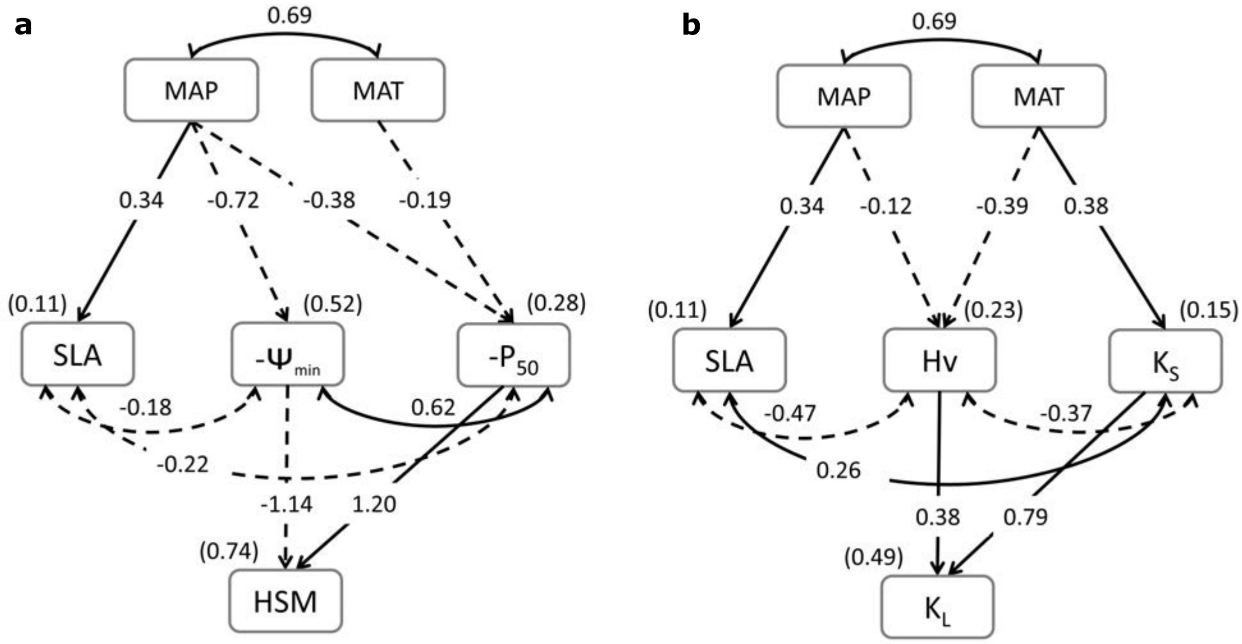

**Fig. 2.3:** Path analysis relating specific leaf area (SLA), mean annual precipitation (MAP) and mean annual temperature (MAT) and hydraulic traits characterizing (a) plant hydraulic safety and (b) plant hydraulic efficiency. Only the values of the path coefficients that were significant in the models are shown (standardized values). Positive effects are indicated by solid lines and negative effects by broken lines. The number in brackets over a given endogenous variable in the path diagram corresponds to the $R^2$ value, indicating the percentage of the variance in that variable that is explained by the model. The models were tested considering one index from each goodness of fit class: SRMR<0.08 (absolute fit), CFI>0.95 (fit adjusting for model parsimony) and RMSEA<0.06 (comparative fit) where SRMR=0.01, CFI=0.98, RMSEA=0.03 for both models. All variables are natural-log transformed except HSM. Abbreviations: $P_{50}$, water potential at 50% loss of hydraulic conductivity; $K_S$, stem-specific hydraulic conductivity; $\psi_{min}$, minimum leaf water potential; Hv, Huber value (sapwood area/leaf area); HSM, hydraulic safety margin; $K_L$, leaf-specific hydraulic conductivity.

Lower SLA was also associated with lower $\psi_{\text{min}}$ ($R^2$ = 0.16, P < 0.001, Fig. 2.2b, Supplementary Table A.1). In both cases the fitted log-log scaling slope was ca. – 1, meaning that a 5-fold increase in SLA was accompanied by 5-fold decrease in both $P_{50}$ and $\psi_{\text{min}}$. As a result, variation in HSM was unrelated to that in leaf economics, considered across all species ($R^2 \approx 0$, P = 0.55, Fig. 2.2c, Supplementary Table A.1). Thus, species with conservative leaf economic strategies did not require wider HSM to support longer leaf lifespans. That said, SLA and HSM were negatively correlated in gymnosperms and positively correlated in angiosperms when analysed separately (Fig. 2.2c, Supplementary Table A.1). This is consistent with a different leaf-plant coordination strategy for gymnosperms (Zimmermann, 1983), but these results have to be taken with caution due to the low sample size for this clade and the difficulty in obtaining accurate estimates of $\psi_{\text{min}}$ at the species level.

Our second prediction-pair was also clearly supported. Lower SLA species had less conductive xylem ($R^2$= 0.13, P<0.001, Fig. 2.2d, Supplementary Table A.1), but higher xylem cross-sectional area for a given leaf area ($R^2$ = 0.29, P < 0.001, Fig. 2.2e, Supplementary Table A.1). Thus, high values of Hv in plants with conservative leaves imply a considerable higher xylem construction cost, but presumably the cost or risk of lower conductance must be equally large, given their lower $K_S$. In sum, the two scaling relationships of SLA with $K_S$ and Hv showed similar magnitude slopes, but were opposite in sign (Fig. 2.2d, 2.2e and Table A.1), meaning that SLA and $K_L$ were unrelated ($R^2$=0.01, P=0.10, Fig. 2.2f, Supplementary Table A.1). This last result is consistent with the fact that acquisitive leaves have been shown to have higher light-saturated photosynthetic rates per unit of leaf mass, but not leaf area (Wright *et al.*, 2004), the relevant comparison here. This was also true when considering gymnosperms and angiosperms separately (Supplementary Table A.1).

In all cases, similar results were obtained when species' positions along the LES were indexed with SLA and leaf N concentration (Supplementary Fig. A.3 and Table A.3), when incorporating phylogenetic information (Supplementary Table A.4) and when accounting for intraspecific variation due to trait plasticity (Supplementary Table A.5).

Finally, we used path analysis to explore the correlation network among SLA, hydraulic traits and climate (mean annual precipitation, MAP, and mean annual temperature, MAT) at the recording sites of each species (Fig. 2.3). Two key conclusions can be drawn: firstly, SLA varied independently from both HSM and $K_L$ (Fig. 2.3a-b); that is, leaf economic variation was largely orthogonal to variation in normalized hydraulic traits characterizing safety (relative to exposure) and efficiency (per unit leaf water demand). Secondly, relationships among SLA and all six hydraulic variables were consistent with the bivariate analyses, indicating that they represent direct coordination among traits, rather than climate-driven correlations. Similar results were obtained when repeating path analyses with annual moisture index (mean annual precipitation divided by potential evapotranspiration) instead of MAP and MAT as climatic variables (Supplementary Fig. A.4).

Overall, our results show that leaf traits are coordinated with xylem safety ($P_{50}$) and efficiency ($K_S$) at the global scale, suggesting whole-plant syndromes linking water and carbon/nutrient economies consistent with well-known physiology. However, independently of these relationships, plants also regulate the balance between water supply and demand and between vulnerability and exposure to drought stress, via control of Hv and $\psi_{min}$, both of which depend on allocation and organ physiology. As a result, the fast/slow continuum in leaf properties does not map directly onto an axis of plant hydraulic performance in terms of more integrative traits such as HSM and $K_L$.

Our findings that LES and xylem traits are coordinated at the global scale but that LES is largely decoupled from more integrative plant hydraulic traits may be interpreted in two ways. On the one hand, it may imply that these more integrative measures that involve more than one organ do not reflect fundamental ecological trade-offs, but are rather universal functional set-points driven by biophysical constraints. If HSM and $K_L$ represented universal set-points, we would expect them to be much less variable than corresponding xylem traits. Alternatively, large coefficients of variation (CV) in HSM and $K_L$ would reflect meaningful differences across species-specific strategies that are independent from the LES. Our results suggest that the latter is the case, e.g., CV of $K_S$, Hv and $K_L$ are 116%, 200% and 145% respectively, whereas CV for $P_{50}$, $\psi_{min}$ and HSM are 71%, 68% and 541%, respectively. This suggests that whole-plant biomass allocation strategies (represented here by Hv, but also likely to affect $\psi_{\mathrm{min}}$) drive compensatory responses that decouple overall plant hydraulic strategies from the LES. Other traits may also be involved in these compensatory responses (e.g., specific rooting length, root tissue density, leaf shedding or stomatal control during drought) but this does not alter our main conclusion. Extra-xylary tissue also contributes to hydraulic resistance and vulnerability and therefore, should be considered when describing plant hydraulic strategies (Cruiziat *et al.*, 2002). Clearly, a broader evaluation of the key trait dimensions describing plant function is needed. This is particularly important for land surface models and shows that incorporating allocation and hydraulics is required to scale up from leaf to plant in forecasting vegetation changes under new climatic conditions.

* *

# 3

# Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient

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## Summary

- Trait variability in space and time allows plants to adjust to changing environmental conditions. However, we know little about how this variability is distributed and coordinated at different levels of organization.

- For six dominant tree species in NE Spain (three Fagaceae, three Pinaceae) we quantified the inter- and intraspecific variability of a set of traits along a water availability gradient. We measured leaf mass per area (LMA), leaf nitrogen concentration (N), carbon isotope composition in leaves ($\delta^{13}$C), stem wood density (WD), the Huber value (Hv, the ratio of cross-sectional sapwood area to leaf area), sapwood-specific and leaf-specific stem hydraulic conductivity ($K_S$ and $K_L$ , respectively), vulnerability to xylem embolism ($P_{50}$ ) and the turgor loss point ($P_{tlp}$).

- Differences between families explained the largest amount of variability for most traits, although intraspecific variability was also relevant. Species occupying wetter sites showed higher N, $P_{50}$ and $P_{tlp}$, and lower LMA, $\delta^{13}$C and Hv. However, when trait relationships with water availability were assessed within species they held only for Hv and $P_{tlp}$.

- Overall, our results indicate that intraspecific adjustments along the water availability gradient relied primarily on changes in resource allocation between sapwood and leaf area and in leaf water relations.

> *“It is not the strongest of species that survive or the most intelligent but the ones most responsive to change.”*
>
> CHARLES R. DARWIN (1859)

UNDERSTANDING the patterns underlying the huge diversity in plant form and function across different organizational levels is a central goal for ecologists. This diversity arises from a combination of genetic variation and phenotypic plasticity and results in adaptations to a range of environmental conditions across space and time (Bradshaw, 1965, 2006). In the last decades, trait-based ecology has emerged as a renewed discipline with the potential to be applied to dynamic global vegetation models (Van Bodegom *et al.*, 2012; Harper *et al.*, 2016) and improve predictions of vegetation responses to environmental changes (Lavorel & Garnier, 2002; McGill *et al.*, 2006). The use of traits emphasizes species phenotypic values over taxonomic characteristics, facilitating the comparison among species and environments (Westoby & Wright, 2006). Identifying trade-offs that appear repeatedly because of evolutionary constraints has become a major research topic because they have the potential to reflect ecological strategies (Westoby *et al.*, 2002; Laughlin, 2014; Adler *et al.*, 2014). One of the dimensions that has received more attention is the leaf economics spectrum (LES), which highlights the trade-off between the dry mass and nutrient investments in leaf construction and the time required for obtaining returns on those investments (Reich *et al.*, 1997; Wright *et al.*, 2004). However, how exactly to describe and integrate complex community dynamics and predict ecosystem-level responses to environmental changes from individual-level trait measurements remains a challenge (Shipley *et al.*, 2016; Funk *et al.*, 2017).

Functional variability of plants has been frequently collapsed at the species level by using mean values, thus ignoring intraspecific trait variability (ITV). However, much work has shown that ITV is relevant when making predictions about plant community assembly and ecosystem functioning (Violle *et al.*, 2012). This is particularly the case when we move from global to more regional scales (Messier *et al.*, 2010; Albert *et al.*, 2012; Violle *et al.*, 2012; Siefert *et al.*, 2015) and from organ-level traits to integrative traits involving several organs, as the latter tend to be more sensitive to the environment and show higher ITV as a result of local genetic adaptation and phenotypic plasticity (Marks, 2007; Siefert *et al.*, 2015). Thus, incorporating the variability of traits along environmental gradients among different levels of organization (family, species, population and individual) may help elucidate how traits respond to environmental variation and thus, improve trait-based models. For example, (Reich *et al.*, 2014) showed that accounting for ITV in gymnosperm needle longevity with latitude across boreal forests impacted significantly on carbon cycling projections.

A related challenge is to understand how trait covariation changes at different ecological levels (organizational levels and spatial scales) (Levin, 1992; Chave, 2013). Previous work has shown that correlation patterns are not always conserved across scales. For example, several studies have failed to find some of the central LES trade- offs, defined across species means at the global scale, when working at smaller spatial or organizational scales (Wright & Sutton-Grier, 2012; Laforest-Lapointe *et al.*, 2014; Niinemets, 2015; Messier *et al.*, 2016; Anderegg *et al.*, 2018). This is because traits that appear closely coordinated at certain scales may have different sensitivities to scale- dependant drivers of variation, which can effectively decouple them at finer scales (Messier *et al.*, 2016). These results have important implications for trait-based ecology: if we want to predict species responses to changing environmental conditions, we need to elucidate intraspecific trait covariance structures to understand the adaptive value of trait combinations in different environments. At the same time, we should be cautious when interpreting trait relationships across species as fundamental trade-offs among functions and strategy dimensions. The study of trait correlation networks is a step forward in formalizing multiple factors shaping an integrated plant phenotype (Poorter *et al.*, 2014; Messier *et al.*, 2017) and allowing comparisons across scales.

The complexity of trait variation has usually been condensed in a few easily measured ('soft') traits that are not necessarily good predictors of demographic rates (Poorter *et al.*, 2008; Paine *et al.*, 2015; Yang *et al.*, 2018). For example, leaf mass per area (LMA), one of the most commonly measured traits, is usually weakly associated with growth rate, especially in adult plants (Wright *et al.*, 2010; Gibert *et al.*, 2016). Moving from ‘soft’ traits to more mechanistic (‘hard’) traits that have a clearer physiological basis and are likely to be stronger determinants of fitness should improve our capacity to elucidate vegetation dynamics under changing environmental conditions. This is particularly the case for drought-related impacts on forest function and dynamics (Skelton *et al.*, 2015; Sperry & Love, 2015; Brodribb, 2017), which are expected to increase in most regions of the Earth under climate change (Allen *et al.*, 2015).

Several studies have related hydraulic traits to plant performance under drought in terms of growth and mortality rates (Rowland *et al.*, 2015; Anderegg *et al.*, 2016; Choat *et al.*, 2018). Hydraulic traits define the efficiency of the plant water transport system, usually defined in terms of stem-specific hydraulic conductivity ($K_S$ ) and its safety against failure under drought stress, typically characterized as the water potential at which 50% stem conductivity is lost due to xylem embolism ($P_{50}$ ). In addition, allocation to sapwood cross-sectional area relative to leaf area (the Huber value, Hv) regulates supply capacity per unit of water demand, and it is thus a key component of plant hydraulic architecture (Mencuccini & Bonosi, 2001). It has been shown that plants can respond to drier conditions by increasing the resistance to xylem embolism (e.g., Blackman *et al.*, 2014), decreasing the leaf water potential at turgor loss in leaves (Bartlett *et al.*, 2012b) and/or increasing their sapwood-to-leaf area ratio (Martínez-Vilalta *et al.*, 2009). Thus , these hydraulic traits can be used to describe the range of plant hydraulic strategies in diverse communities (Skelton *et al.*, 2015) and may provide stronger insights into the drivers of forest dynamics than the more commonly measured ‘soft’ traits (Brodribb, 2017).

If trait variation across scales in commonly measured ‘soft’ traits remains poorly understood, knowledge is even more limited regarding hydraulic traits. A recent meta-analysis found that 33% of the variation in $P_{50}$ was contributed by differences within species (Anderegg, 2015). However, part of this variability could be due to methodological aspects (Cochard *et al.*, 2013) and several individual studies have shown low plasticity in embolism resistance across climatically contrasted populations (Maherali & DeLucia, 2000; Martínez-Vilalta *et al.*, 2009; Lamy *et al.*, 2011, 2014; López *et al.*, 2016). The degree of coordination between leaf economics traits and hydraulic traits is also a leading research subject. A universal ‘fast-slow’ whole-plant economics spectrum that integrates resource use strategies (for water, carbon and nutrients) across organs has been proposed (Reich, 2014), but the evidence remains mixed (Brodribb *et al.*, 2007; Blonder *et al.*, 2011; Markesteijn *et al.*, 2011; Méndez-Alonzo et al., 2012; Sack *et al.*, 2013; Li *et al.*, 2015).

To address these critical issues, we studied the variability of a set of hydraulic, leaf and stem traits along a water availability gradient in six dominant tree species in Catalonia (NE Spain), focusing on the following questions.

(1) How much trait variation is observed and how is it distributed among levels of organization? We hypothesize that differences between families (Pinaceae vs. Fagaceae) will explain the largest part of trait variability in this temperate system, although ITV will be substantial, especially for more integrative traits such as $K_L$ and Hv.

(2) How do traits vary along the water availability gradient within and between species? We hypothesize that hydraulic traits will be more closely linked to water availability than other stem and leaf traits. Most of the trait changes along the water availability gradient will entail species substitutions and, thus, the strength of trait-environment relationships will be weaker within than across species, reflecting lower capacity for functional adjustment within species.

(3) How are traits coordinated across and within species? Across species, we hypothesize the existence of a general 'fast-slow' strategy at the whole-plant level that combines LES and hydraulic traits (e.g., low LMA will be associated with high $K_S$ and high vulnerability to embolism). At the same time, we expect that intraspecific correlation networks may differ from those across species because relatively weak evolutionary or physiological trade-offs can be reversed due to plasticity within-species.

## Materials and methods

### Study site and sampling design

The study area included all the forested territory of Catalonia (NE Spain) that encompasses 1.2 million ha, around 38% of its total land area. Catalonia is very diverse both topographically and climatically: mean annual temperature ranges from 18 °C (at the southern coast) to 3 °C (in the Pyrenees) and annual rainfall varies from 400 mm to >1,500 mm (CDAC, *www.opengis.uab.cat/acdc*). We selected six of the most dominant tree species in Catalonia (3 Pinaceae and 3 Fagaceae), accounting for $\sim$75% of the total forest area (Gracia *et al.*, 2004, see also Table B.1): *Pinus sylvestris* L, Pinus nigra J.F.Arnold., *Pinus halepensis* Mill., *Fagus sylvatica* L., *Quercus pubescens* Willd . and *Quercus ilex* L. For each species, 15 plots from the Spanish forest inventory (IFN) were resampled in which the target species was dominant (minimum 50% of the total basal area), maximizing the water availability gradient occupied by each species in the study region. Water availability was quantified as the precipitation to potential evapotranspiration ratio, P/PET, for the spring-summer period (see below). Five plots per species were sampled for each of three species-specific P/PET ranges following a stratified random design (dry, corresponding to P/PET < $33^{rd}$ percentile; wet for P/PET > $66^{th}$ percentile; and mild for the rest) (Fig. B.1, B.2). Plots with the two highest stoniness levels and those that had been managed during the last 14 years according to previous IFN surveys were discarded.

Within each plot, five non-suppressed canopy trees of the target species with diameter at breast height (DBH) > 12.5 cm were randomly selected, all within 25 m of the centre of the plot. All samples and data were collected from May to December 2015. To minimise phenological variation in traits within species, species were sampled sequentially (*P. halepensis*, mid-May to end June; *Q. pubescens*, end June and July; F. sylvatica, August; *P. sylvestris*; September to mid-October; *Q. ilex*, mid-October to mid- November; *P. nigra*, mid-November to mid-December). From each tree, two branches (one for leaf measurements and the other for hydraulic measurements) were sampled from the exposed part of the canopy in the top half of the crown. Sampled branches were at least 70 cm long for Pinus spp., 150 cm for Quercus spp. and 80 cm for Fagus, to account for differences in the maximum length of xylem conduits (see below). Branches were transported to the laboratory inside plastic bags under cool and dark conditions and measurements were taken within 24h.

## Environmental variables

At each plot, four soil samples (20 cm deep) were taken using a soil core at the four cardinal points at 5 m distance from the centre of the plot. The topsoil (O horizon) was removed to exclude the organic deposit and litterfall, and the four samples were merged. The following variables were measured on each pooled sample: N-$NO_3$ concentration (colorimetric method; Keeney & Nelson, 1982), phosphorus content (available phosphorous-Olsen phosphorous; Olsen & Sommers, 1982), soil humidity (gravimetric soil water content; Gardner, 1986), organic matter fraction (organic carbon content estimated with acid dichromate oxidation method; Nelson & Sommers, 1982) and soil texture classes defined by the USDA system (sedimentation-Robinson pipette; Gee & Or, 2002). To integrate the different components of soil texture into one single variable, the exponent of the Saxton equation (Saxton *et al.*, 1986) was calculated as follows:

$$b = -3.140 - 0.00222\,(\%\ clay)^2 - 3.484.10^{-5}(\%\ sand)^2(\%\ clay) \tag{3.1}$$

where less negative values of $b$ indicate sandy soils with lower soil water retention capacity.

Forest structure data for each plot were also available from the last Spanish forest inventory (IFN4) that was conducted over the same time period as our sampling. Forest structural data included total plot basal area, stand density, mean diameter at breast height and the 90th percentile for height of all trees in the plot. Climate data were obtained from the Climatic Digital Atlas of Catalonia (Ninyerola *et al.*, 2005), a collection of digital maps at 200 x 200 m resolution including average annual radiation, mean annual temperature, minimum annual temperature, and annual precipitation for the period 1951-2010. PET values were calculated according to the Hargreaves-Samani method (Hargreaves & Samani, 1982) and used to estimate P/PET for the spring- summer period and P/PET for the summer period.

## Leaf traits and wood density

Standard protocols (Pérez-Harguindeguy *et al.*, 2013) were followed for all trait measurements (Table 3.1) . Previous-year needles (conifers) and current-year leaves (broadleaves) were selected to measure fully expanded leaves. Twigs with leaves were cut under water and placed into flasks with the cut end submerged in deionized water in the dark overnight before measurements.

Leaf mass per area (LMA) is a measure of biomass investment in leaves per unit light interception and gas exchange (Poorter *et al.*, 2009). For LMA determinations,

| **Trait** | **Symbol** | **Units** |
|---|---|---|
| Leaf mass per area | LMA | g $cm^{-2}$ |
| Leaf nitrogen concentration | N | mg $g^{-1}$ |
| Leaf carbon isotope composition | $\delta^{13}C$ | ‰ |
| Wood density (stem) | WD | g $cm^{-3}$ |
| Huber value, sapwood to leaf area ratio (branch) | Hv | $cm^2$ $m^{-2}$ |
| Leaf-specific xylem hydraulic conductivity (branch) | $K_L$ | kg $m^{-1}$ $s^{-1}$ $MPa^{-1}$ |
| Stem-specific xylem hydraulic conductivity (branch) | $K_S$ | kg $m^{-1}$ $s^{-1}$ $MPa^{-1}$ |
| Pressure causing 50% xylem embolism (branch) | $P_{50}$ | MPa |
| Leaf water potential at turgor loss point | $P_{tlp}$ | MPa |

**Table 3.1:** Traits measured in this study.

twenty leaves were randomly selected, scanned and their areas were measured with ImageJ software (Wayne Rasband-National Institute of Health, Bethesda, MD, USA). Afterwards, samples were oven-dried at 60 °C and weighed, and LMA was calculated as leaf dry mass/ fresh area.

The Huber value is the ratio of cross-sectional sapwood area to subtended leaf area, and can be viewed therefore as the ratio of hydraulic and mechanical investment costs over the expected gains obtained by leaf display. Leaves from terminal branches (65 cm long from the tip) were oven dried and weighted, and LMA was used to convert the total dry weight of the distal leaves of each branch into total branch leaf area. In order to calculate branch level Hv, and to make values comparable across species, maximum leaf area was estimated taking into account species phenology and the time of sampling. Sapwood area was obtained through measuring total xylem area on digital images of stained (safranin-astra blue) 15-20 micrometer thin sections in ImageJ (v 1.440 - Wayne Rasband, USA).

We used leaf carbon isotope composition ($\delta^{13}$C) and leaf nitrogen concentrations (N) to further characterize leaf functioning. Less negative $\delta^{13}$C values suggesting lower discrimination against the heavier $^{13}$C are indicative of greater stomatal control and water-use efficiency (Farquhar *et al.*, 1989), whereas higher leaf N concentrations are usually associated to higher photosynthetic capacity because of the high N content of photosynthetic machinery (Evans, 1989). Leaf $\delta^{13}$C and N were determined using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC- Davis Stable Isotopes Facility (California, USA). Samples were previously oven-dried at 60 °C for four days, grounded with a Retsch MM400 ball mill (Verder Group, Haan, Germany) and placed in tin capsules for analysis. Carbon stable isotope concentrations were expressed in relation to the Pee-Dee Belemnite (PDB) standard.

Leaf osmotic potential ($\psi_{\mathrm{O}}$) was measured with a VAPRO 5500 vapor pressure osmometer (Wescor, Logan, UT, USA). Leaves were wrapped in foil to limit condensation and evaporation, were submerged in liquid nitrogen for two minutes and were sealed in a plastic zip bag at ambient conditions. After letting them defrost, they were put inside a syringe and squeezed until 10 $\mu$l of sap were obtained. Finally, $\psi_{\mathrm{O}}$ was used to predict the leaf water potential at which leaf cells lose turgor closing their stomata and ceasing gas exchange and growth ($P_{\mathrm{tlp}}$) (Brodribb *et al.*, 2003) following the equation described in Bartlett *et al.* (2012a):

$$P_{\mathrm{tlp}} = 0.832\psi_{\mathrm{O}} - 0.631 \tag{3.2}$$

Wood density (WD) is considered a central trait shaping the wood economics spectrum (Chave *et al.*, 2009). We measured WD on one stem core per individual extracted using a hand increment borer (5 mm diameter; Suunto, Vantaa, Finland). The core was sealed in plastic tubes upon collection and taken to the laboratory under cold conditions. Fresh core volume of all wood was calculated after removing the bark by the dimensional method, measuring its total length and its diameter using a caliper. Cores were then oven dried at 100 °C for 48 h and weighed. Wood density was calculated as the oven-dry mass divided by fresh volume.

## Hydraulic traits

Before hydraulic measurements, maximum vessel length was estimated using the air infiltration technique (Ewers & Fisher, 1989) on eight 2 m branches per species. We flowed compressed air ($\sim$0.15 MPa) through the branches with their basal end immersed in water and successively shortened the stem until bubbling was observed. Because compressed air at low pressures cannot pass through vessel end walls, the bubbling indicated the presence of open xylem conduits. The resulting estimates of maximum conduit length were used to decide the minimum length of the sampled branches (see *Study site and sampling design* section above).

Vulnerability curves were established by measuring the hydraulic conductivity of stem segments at different water potentials, using a variation of the bench dehydration method (Sperry & Tyree, 1988; Cochard *et al.*, 2013; Choat *et al.*, 2015). Hydraulic conductivity was measured using a commercial XYL'EM apparatus (Bronkhorst, Montigny-les-Cormeilles, France) as the ratio between the flow through the stem segment and the pressure gradient (5 kPa). The initial hydraulic conductivity ($K_i$) was measured in three subsamples (segments) per branch that were excised underwater at the terminal part of the shoots (Martin-StPaul *et al.*, 2014). An initial cut was applied to allow xylem tension in the branch segment to relax before measurements, avoiding artefacts associated with the cutting under tension (Wheeler *et al.*, 2013). After the segments were cut again to their final size ($\sim$2 cm in length), their proximal ends were connected to the tubing system of the XYL'EM, which was filled with deionized filtered water with 10 mM KCl and 1 mM $CaCl_2$ that had been previously degassed using a membrane contactor (Liqui-Cell Mini-Module membrane 1.7x5.5, Charlotte, USA). After measuring the initial conductivity, the segments were flushed once at 0.15 MPa for 10 minutes (for *Quercus* spp. and *F. sylvatica*) or held in the solution under partial vacuum during 48h (for *Pinus* spp., as flushing conifer segments often results in the pit membranes being permanently pushed against tracheid cells walls) in order to measure their maximal conductivity ($K_{max}$) as above. The values of $K_i$ and $K_{max}$ were used to compute the percent loss of hydraulic conductivity (PLC). The previous measurements were repeated a second time on a different set of stem segments after branches had been dehydrated on the bench to obtain PLC estimates at lower water potentials. The timing of this second measurement was adjusted for different species and branches (between two and eight days) to cover a wide range of PLC values. The tubing system was regularly cleaned using 10% bleach solution for at least 20 min to prevent microorganism's growth and, afterwards, flushed with a degassed solution. Additionally, we used the apical part of each measured twig segment to measure water potential ($\psi$) with a Scholander pressure chamber (Solfranc Tecnologias, Tarragona, Spain).

To fit vulnerability curves to each set of PLC and water potential measurements, the following sigmoid function was used (Pammenter & Willigen, 1998):

$$PLC = 100/(1 + exp(a(\psi - P_{50}))) \tag{3.3}$$

where $\psi$ is the water potential, $a$ is the slope of the curve and thus determines the rate at which conductivity is lost as water potential declines, and $P_{50}$ determines the position of the curve on the abscissa and gives the pressure causing 50% loss of conductivity. Parameters were estimated by fitting a separate nonlinear mixed model for each species, using the *nlme* R package (Pinheiro *et al.*, 2018). The model accounted for individual nested in plot as a random effect on coefficient $P_{50}$ . Preliminary analyses confirmed that this model structure provided the best fit to the data.

In addition, all distal leaves of each segment were removed to determinate their area as explained above. Leaf-specific hydraulic conductivity ($K_L$ ) was calculated as $K_{max}$ divided by the distal leaf area supported. Similarly, stem-specific hydraulic conductivity ($K_S$ ) was calculated as $K_{max}$ divided by cross-sectional sapwood area.

## Statistical analyses

To assess trait variability, the quartile coefficient of dispersion (QCD) was calculated for each trait as the ratio between half the interquartile range ((Q3-Q1)/2) and the average of the quartiles ((Q1+Q3)/2). QCD was used as a more robust measure of dispersion than the coefficient of variation (CV), as the latter is not appropriate for datasets including isotopic measurements (Brendel, 2014) or log-transformed data (Canchola *et* *al.*, 2017) (see also the Supporting Information Table B.2). To understand the distribution of variability for each trait, we used different sets of linear mixed models, always fitting separate models for each trait. In the first ones, family, species and population were introduced as nested random factors to assess how trait variability was distributed among these different levels of organization. In the second ones, models were fit separately for each family, and included only species and population (nested) to assess trait variability among- and within-species (within each family). All variables were checked for normality and natural-log transformed whenever required to ensure normality.

Before exploring the effect of environmental factors on trait variation, three separate principal components analyses (PCAs) were performed to summarize soil, forest structure and climate data (Supporting Information Fig. B.3). As before, all variables were checked for normality and natural-log transformed if required. For further analyses, the two most orthogonal variables showing the highest axes loading in each PCA were selected as integrated measures of environmental predictors. Coefficient b from Saxton equation (Eqn. 3.1) and soil P were selected to describe soil characteristics, the mean tree diameter at breast height and total plot basal area to describe forest structure, and spring-summer P/PET and annual radiation to describe the climate. A first mixed model for each trait was fit starting with the 'saturated' model including all six environmental variables as fixed explanatory variables (without interactions). We included plot nested in species as random effects on the intercept of the model. Preliminary analyses showed that including a random species effect on the slopes did not improve model fit. This model was simplified stepwise removing the least significant term until a minimal adequate model with the lowest AIC (Akaike information criterion) was obtained. Models within 2 AIC units were considered equivalent in terms of fit and the simplest one was selected (Zuur *et al.*, 2009).

To explore specifically the variability of each trait along the P/PET gradient imposed by our sampling design, a second mixed model was fit for each trait. To separate the intraspecific from the interspecific component of trait responses to P/PET, we split P/PET into two additive variables which were included as separate fixed factors in the model: mean P/PET at the species level and centred P/PET. The latter variable was calculated as the difference between plot P/PET and the average P/PET for the corresponding species. We also included plot nested within species as a random effect on the intercept. As before, preliminary analyses showed that including a random species effect on the slope did not improve model fit. Model selection was carried out as described above. In all cases, the residuals of the selected models showed no obvious pattern and were approximately normally distributed. Linear mixed effects models were fit using the *lme4* R package (Bates *et al.*, 2015).

Finally, to characterize trait coordination within- and between-species, statistically significant correlations among traits were graphically represented using trait covariation networks with the *igraph* R package (Csardi & Nepusz, 2006). Traits were represented as nodes and their correlation as the edges linking them. Two indicators of network centrality were calculated for each trait: the degree (D), defined as the number of edges of a node and the weighted degree (Dw), defined as the sum of all significant coefficients of correlation of a node (Supporting Information Table B.5). In these latter analyses, all traits were natural-log transformed to improve the linearity of relationships. All analyses were carried out with R Statistical Software version 3.3.2 (R Core Team, 2017).

## Results

### Magnitude and distribution of trait variability

Most trait variation occurred between families (Pinaceae vs Fagaceae), with the exception of $K_L$ and $P_{tlp}$ for which the contribution of family was close to zero (Fig. 3.1b). Pinaceae tended to have higher LMA, Hv and $\delta^{13}$C than Fagaceae, whereas the opposite was true for leaf N, WD, $K_S$ and $P_{50}$ (Fig. B.4). Overall, the proportion of variance explained at the intraspecific level (among and within populations) was on average 23.11% (Fig. 3.1b). Within Pinaceae, $K_S$ , $K_L$ , Hv, WD and $\delta^{13}$C showed a higher variability within than among species, while in Fagaceae this was only the case for Hv (Fig. B.5). Other traits, such as $P_{50}$ , showed substantial variability within families (4.51 MPa range within Pinaceae and 3.84 MPa range within Fagaceae) but most of this variance occurred across species (Table B.2 and Fig. B.5). $K_S$ , $K_L$ , LMA and Hv were the most variable traits, while $\delta^{13}$C, $P_{tlp}$ and WD showed the least variation (Fig. 3.1a).

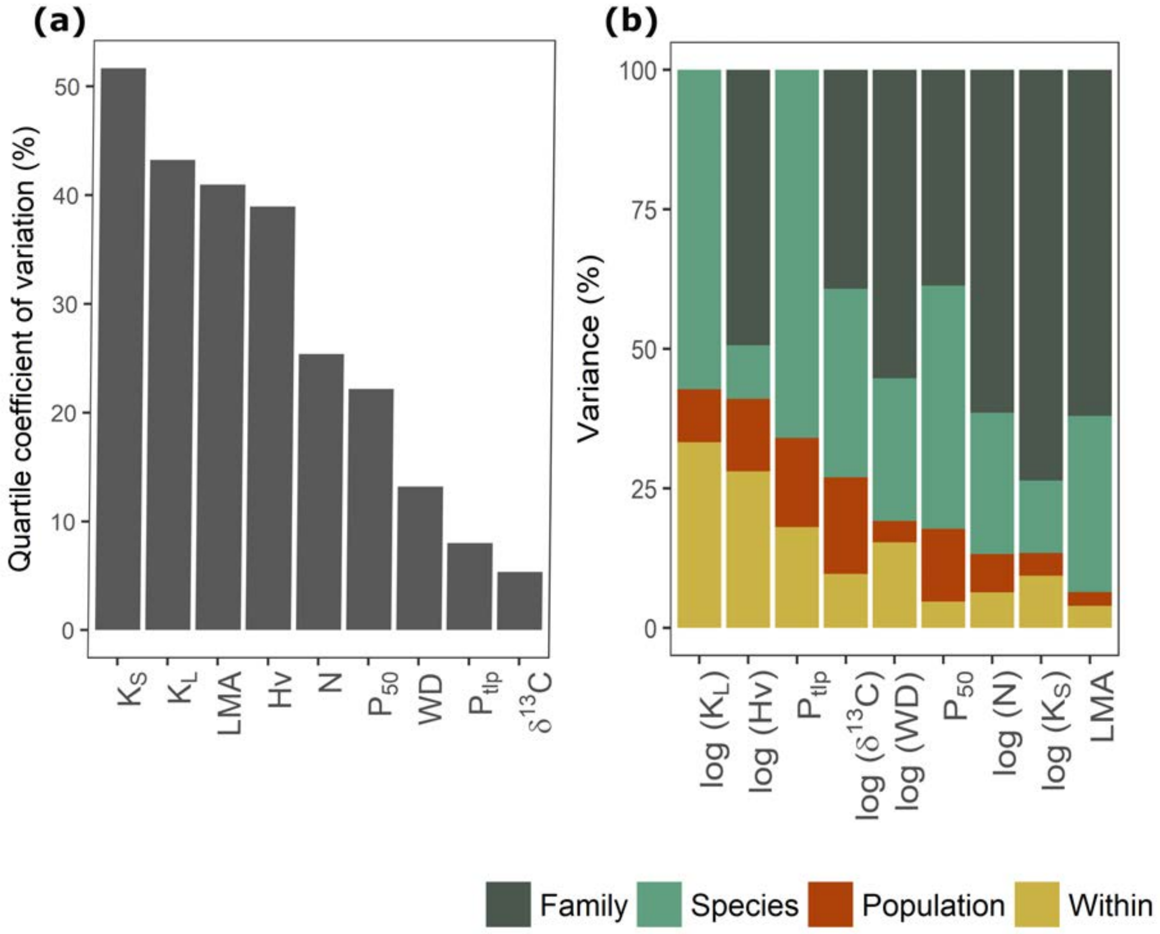

**Fig. 3.1:** (a) Quartile coefficient of dispersion of the studied traits and (b) variance partitioning across different ecological levels of organization. '*Within*' denotes variance between individuals of the same population. Traits are ordered (left to right) from higher to lower total variation in panel (a), and from higher to lower % variation within species in panel (b). See Table 3.1 for definition of symbols.

|  | LMA | log (N) | log( -$\delta^{13}$C) | log (WD) | Hv | $K_L$ | $K_S$ | -$P_{50}$ | -$P_{tlp}$ |
|---|---|---|---|---|---|---|---|---|---|
| **Fixed Parts** |  |  |  |  |  |  |  |  |  |
| soil P | **0.08**<br>**(0.02 – 0.13)*** | -0.08<br>(-0.17 – 0.01) | n.i. | 0.08<br>(-0.00 – 0.17) | n.i. | n.i. | n.i. | n.i. | n.i. |
| log (b.Saxton) | n.i. | n.i. | n.i. | 0.05<br>(-0.01 – 0.12) | n.i. | n.i. | n.i. | n.i. | -0.03<br>(-0.13 – 0.07) |
| log (DBH mean) | **-0.07**<br>**(-0.12 – -0.01)*** | 0.09<br>(-0.00 – 0.17) | 0.12<br>(0.00 – 0.24) | **-0.11**<br>**(-0.19 – -0.03)**** | **-0.14**<br>**(-0.26 – -0.01)*** | **-0.14**<br>**(-0.24 – -0.03)*** | -0.08<br>(-0.16 – -0.00) | n.i. | n.i. |
| Basal Area | -0.05<br>(-0.10 – 0.01) | n.i. | n.i. | 0.01<br>(-0.06 – 0.09) | n.i. | n.i. | n.i. | 0.08<br>(-0.03 – 0.20) | n.i. |
| Annual radiation | **0.06**<br>**(0.01 – 0.10)*** | n.i. | **-0.23**<br>**(-0.33 – -0.13)***** | n.i. | 0.09<br>(-0.01 – 0.20) | 0.07<br>(-0.02 – 0.17) | n.i. | n.i. | n.i. |
| log (P/PET) | n.i. | n.i. | **0.25**<br>**(0.09 – 0.41)**** | n.i. | **-0.27**<br>**(-0.44 – -0.10)**** | n.i. | n.i. | -0.18<br>(-0.35 – -0.02) | **-0.36**<br>**(-0.53 – -0.20)***** |
| **Random Part** |  |  |  |  |  |  |  |  |  |
| $\sigma^2$ | 0 | 0.009 | 0.001 | 0.009 | 0.178 | 0.153 | 0.087 | 0.071 | 0.024 |
| τ00, PLOT:SP | 0 | 0.009 | 0.001 | 0.002 | 0.065 | 0.039 | 0.036 | 0.193 | 0.017 |
| τ00, SP | 0.005 | 0.084 | 0.002 | 0.035 | 0.134 | 0.216 | 0.521 | 0.775 | 0.053 |
| $R^2$ marginal | 0.02 | 0.01 | 0.22 | 0.01 | 0.14 | 0.03 | 0 | 0.02 | 0.14 |
| $R^2$ conditional | 0.94 | 0.91 | 0.84 | 0.8 | 0.59 | 0.64 | 0.86 | 0.93 | 0.78 |
| Observations | 444 | 444 | 444 | 444 | 439 | 444 | 444 | 444 | 425 |

**Table 3.2:** Results of the linear mixed models examining the relationships between traits and environmental variables characterizing the soil, the climate and the stand structure. A different mixed effects model including all environmental variables in the fixed part and plot nested within species in the random part was fit for each trait. The model's standardized coefficients including confidence intervals (in brackets) are shown. Significant correlations (* $P<0.05$, ** $P<0.01$, *** $P<0.001$) are shown in bold. Information on the random effect variances ($\sigma^2$, total; $\tau$00, PLOT:SP, within-species; and $\tau$00, SP, cross-species), the proportions of explained variance by fixed effects ($R^2$ marginal) and explained variance by fixed and random effects ($R^2$ conditional) are also provided. DBH mean, plot mean diameter at the breast height; b.Saxton, b Saxton coefficient; soil P, soil phosphorus content; P/PET, precipitation to potential evapotranspiration ratio; n.i., not included in the best model. See Table 3.1 for definition of symbols.

## Trait responses along a water availability gradient

Traits responded differently to environmental factors (Table 3.2). Regarding soil properties, only soil phosphorus concentration showed a significant effect (positive) on LMA. As for stand structure, mean DBH had the strongest predictive effect across all models. Plots with larger trees on average were associated with lower LMA, lower WD, lower Hv and lower $K_L$ . Stand basal area did not have significant effects on any trait. Finally, regarding climatic variables, high annual radiation was associated with leaves with high LMA and high (less negative) $\delta^{13}$C. Plots with higher P/PET values had trees with more negative $\delta^{13}$C, lower Hv and less negative $P_{tlp}$. Overall, environmental variables at the plot level were not strong predictors of trait variation, as showed by relatively low values of the marginal $R^2$ (variation explained by the fixed effects) (Table 3.2). The fact that conditional $R^2$ values (Table 3.2) were normally much higher indicates that a large proportion of the variance in all traits is explained by differences among species and plots not captured by the environmental variables included in our analysis. Similar results were obtained if we used PCA axes as fixed factors describing environmental variation in models instead of individual variables (Table B.3).

When we specifically explored the variability of each trait along the water availability (P/PET) gradient, considering both species means and plot-scale deviations from the means (centred values), higher marginal $R^2$ values and generally stronger effects were obtained (*cf.*, Supporting Information Table B.4). Significant relationships between P/PET and traits across species were consistent with the results reported in the previous paragraph, but we also found a positive relationship between P/PET and $P_{50}$ (which was only marginally significant in the previous analysis) and a positive relationship with leaf N concentrations (Fig. 3.2). Importantly, trait-environment relationships were scale-dependent and when these patterns were analysed within species, we only found significant relationships between centred P/PET and Hv and $P_{tlp}$. In these two cases, the relationships had the same (negative) sign but shallower slopes than the corresponding relationships across species (Fig. 3.2 and Supporting Information Table B.4). Similar results were obtained when the mean DBH, the strongest explanatory variable in the initial mixed model (*cf.* previous paragraph), was included as a fixed factor in this latter model (not shown).

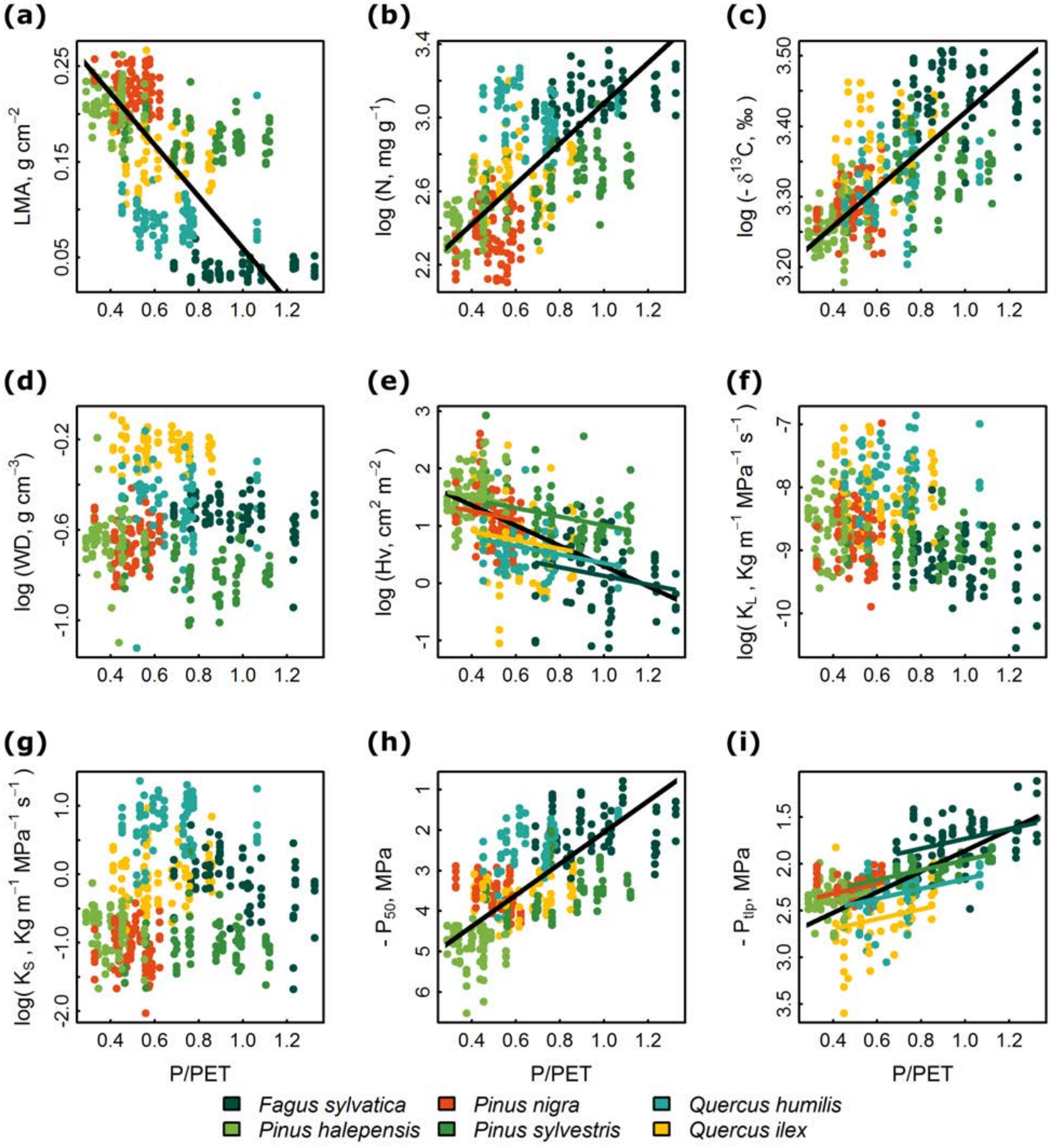

**Fig. 3.2:** Relationship between water availability (in terms of the precipitation to potential evapotranspiration ratio, P/PET) and studied traits. The black regression lines give the overall cross-species relationships, and the coloured lines the corresponding within-species relationships, when significant (P<0.05). Variables were natural-log transformed whenever required to satisfy normality assumptions. See Table 3.1 for definition of symbols.

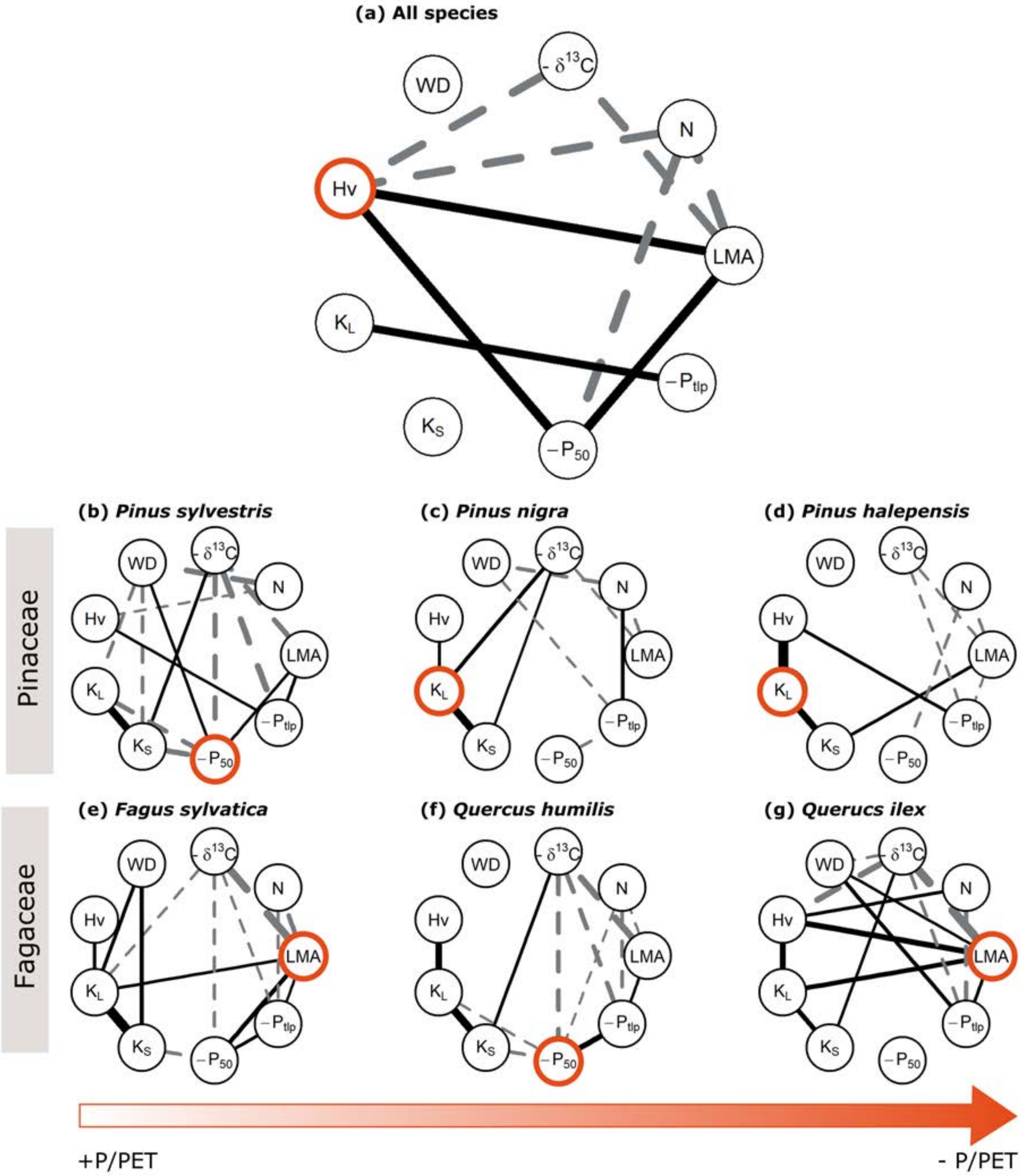

**Fig. 3.3:** (a) Trait correlation networks across species (n=6) and for each studied species separately (b-g). Solid black and grey dashed edges show positive and negative correlations, respectively. Correlation strength is represented by edge thickness. Only significant correlations are shown ($P<0.05$). Traits identified by red circles show the highest centrality value in terms of weighted degree (the sum of all the significant coefficients of correlation of a node). All traits were natural-log transformed before analysis. See Table 3.1 for definition of symbols.

### Trait correlation networks

Trait coordination differed within- and among- species (Fig. 3.3). When species means were considered, LMA and Hv were the traits showing highest values of centrality across species (Supporting Information Table B.5). These two traits were positively related to each other and tightly linked to leaf N, $\delta^{13}$C and $P_{50}$ , so that higher allocation to sapwood area relative to leaf area was correlated with a greater construction cost per unit leaf area, lower N, higher water use efficiency (less negative $\delta^{13}$C values) and higher cavitation resistance (more negative $P_{50}$ ). $P_{tlp}$ and $K_L$ also showed a positive relationship. Surprisingly, $K_L$ and $K_S$ were unrelated across species, although a consistent, positive relationship appeared when species were analysed separately (Fig. 3.3).

When analysing trait coordination within species, the strong LMA-Hv relationship observed across species was only significant in one species (*Q. ilex*). At the intraspecific level, the negative correlation between LMA and -$\delta^{13}$C and the positive correlation between $K_S$ and $K_L$ were the only relationships present in all cases (Fig. 3.3). $K_L$ showed the highest centrality in two out of the three measured gymnosperms, while it was never central in angiosperms. On the other hand, LMA was the trait with the highest centrality in two out of three studied angiosperms species. However, caution is needed when considering these results due to the limited number of species sampled within each family. When centrality was expressed as simple count of number of significant correlations (degree), $\delta^{13}$C and $P_{tlp}$ appeared also particularly important, especially in Fagaceae (Table B.5). Finally, taking into account the overall network, *P. sylvestris*, *F. sylvatica*, and *Q. ilex* were the species showing more correlations among traits and the highest weighted degree (Table B.5).

## Discussion

We found that traits varied primarily between tree families but that ITV also accounted for a relevant amount of total variation, especially in more integrative traits ($K_L$ , Hv). Most study traits responded to water availability, with increasing N, $P_{50}$ and $P_{tlp}$ and decreasing LMA, $\delta^{13}$C and Hv with P/PET across species. However, at the intraspecific level we only found trait variation along the water availability gradient for Hv and $P_{tlp}$.

Finally, trait coordination was scale-dependent and we did not find clear evidence of a single, dominant axis of variation reflecting a fast-slow, whole-plant economics spectrum.

## Magnitude and distribution of trait variability

Our results show that traits differ substantially in their variability along the same environmental gradient, with an order of magnitude difference in the quartile coefficient of dispersion between the most variable ($K_S$ and $K_L$ ) and the least variable traits ($\delta^{13}C$ and $P_{tlp}$). The high variability of $K_S$ and $K_L$ agrees with previous studies across species (Maherali *et al.*, 2004; Martínez-Vilalta *et al.*, 2004; Gleason *et al.*, 2015), and may be caused by their high sensitivity to small differences in wood anatomy (particularly conduit diameter), which varies substantially across and within species (Tyree *et al.*, 1994; Sperry *et al.*, 2008). The higher variability of $K_S$ relative to $K_L$ likely reflects that the latter is normalized by water demand in terms of leaf area. More generally, however, the ecological implications of this high variability in xylem transport capacity, both within and among species, remains to be elucidated, particularly considering that in our study $K_S$ and $K_L$ did not respond consistently to water availability. On the other hand, $P_{tlp}$ showed very low variability in comparison with other hydraulics traits, also in agreement with previous findings (Mencuccini et al., 2015; Bartlett *et al.*, 2016).

Not surprisingly, trait variability was mostly distributed across families, reflecting the contrasting trait syndromes between angiosperm and gymnosperm clades (Wright *et al.*, 2004; Chave *et al.*, 2009; Carnicer *et al.*, 2013). Our results also confirm previous findings for hydraulic traits, with higher Hv, lower $K_S$ and higher resistance to embolism in conifers relative to angiosperm trees (Becker *et al.*, 1999; Choat *et al.*, 2012; Gleason *et al.*, 2015). The high proportion of variation attributed to the family level for $K_S$ is explained by xylem conduit properties, as unicellular conifer tracheids are substantially narrower and more than an order of magnitude shorter than angiosperm vessels (Sperry *et al.*, 2006). Besides the direct effect of these different dimensions on $K_S$ , the fact that we measured relatively short length segments implies that our $K_S$ estimates corresponded mostly to lumen conductivity for the Fagaceae and to total conductivity (lumen and end-wall) for Pinaceae species. Interestingly, the family effect disappeared when xylem conductivity was normalized by leaf area ($K_L$ ) because conifers also tend to have more sapwood per unit of leaf area (higher Hv, Fig. B.4) (see also Becker *et al.*, 1999).

Intraspecific trait variability contributed to a substantial amount of the total variance (from 6 to 42% depending on the trait). This is consistent with a growing body of evidence showing that ITV is relevant (Albert *et al.*, 2012; Laforest-Lapointe *et al.*, 2014), especially when we move from organ-specific traits (leaves, stems or roots) to more integrative traits involving several organs (e.g. $K_L$ , Hv) (Siefert *et al.*, 2015). Studies addressing ITV in hydraulic traits are less frequent (but see Martínez-Vilalta *et al.*, 2009; Wortemann et al., 2011; Lamy *et al.*, 2014; Hajek *et al.*, 2016). In line with our results, Hv and $K_L$ have been reported to be among the most plastic hydraulic properties in pines (DeLucia et al., 2000; Martínez-Vilalta *et al.*, 2009) while other traits such as $P_{50}$ usually show low plasticity (Maherali & DeLucia, 2000; Martínez-Vilalta *et al.*, 2009; Lamy *et al.*, 2014; López *et al.*, 2016). Further studies are needed to investigate whether these patterns are generalizable across other plant families. It should also be noted that we probably underestimated the magnitude of ITV because we did not cover the whole species distribution range, species were sampled sequentially to minimise phenological variation within species, and we always selected healthy-looking mature trees with sun-exposed branches according to standard trait sampling protocols (Pérez- Harguindeguy *et al.*, 2013). These factors, however, would also affect total trait variation and it remains unclear what their impact would be on the percentage contribution of ITV.

## Trait responses along a water availability gradient

In agreement with findings reported in others studies (Vilà-Cabrera *et al.*, 2015; Anderegg *et al.*, 2018), trait-environment relationships were not very tight, suggesting that unaccounted species-specific differences and/or other plot variables not included in our study were stronger drivers of trait variability. Mean DBH was the strongest determinant of trait variation. Specifically, plots with larger trees on average tended to have lower LMA, WD, Hv and $K_L$ , in line with previous findings (Laforest-Lapointe *et al.*, 2014; Gleason *et al.*, 2018). The effect of P/PET, our target environmental factor, was significant or marginally significant for $\delta^{13}$C, Hv, $P_{tlp}$ and $P_{50}$, but not for LMA, N or WD when controlling for the effect of other environmental factors. This indicates that hydraulic and water related traits responded more strongly to water availability than LES or other stem traits, as hypothesized.

When we assessed the overall response of each trait to P/PET, without accounting for the effect of other environmental variables that co-varied along the environmental gradient studied, a higher proportion of trait variance was explained, because species means were explicitly included in the model (Table B.4). In this broader assessment, LMA and N were also related to water availability, besides the hydraulic/water relations variables identified in the previous analysis. Wetter sites were associated with species with leaf traits related to acquisitive resource strategies (low LMA and high N). Several studies have shown that LMA tends to be higher at drier sites as a result of water stress adaptation through increasing wilting resistance (Schulze *et al.*, 1998; Cunningham *et al.*, 1999). Regarding the relationship between N and water availability, contrasting results have been reported. While some studies have reported that species from drier sites present higher N leaf concentration to enhance water conservation during photosynthesis (Wright & Westoby, 2002), others have found no general relationship (Killingbeck & Whitford, 1996; Vilà-Cabrera *et al.*, 2015).

Vulnerability to xylem embolism was lower (more negative $P_{50}$) in species occupying drier sites, consistent with the notion that cavitation resistance is a key determinant of species distributions (Maherali *et al.*, 2004; Jacobsen *et al.*, 2007; Martínez-Vilalta *et al.*, 2012; Choat *et al.*, 2012; Blackman *et al.*, 2014; Trueba *et al.*, 2017; Li *et al.*, 2018; Skelton *et al.*, 2018). Similarly, another key drought tolerance trait, $P_{tlp}$, also showed a significant relationship with P/PET across species, with lower (more negative) $P_{tlp}$ associated with drier habitats, allowing the maintenance of leaf turgor and gas exchange under drier conditions (Brodribb *et al.*, 2003; Lenz *et al.*, 2006; Bartlett *et al.*, 2012b). This did not prevent, however, an increase in water use efficiency (less negative $\delta^{13}$C values) and increased allocation to sapwood area relative to leaf area (Hv) at drier sites, consistent with previous reports (Warren *et al.*, 2001; Martínez-Vilalta *et al.*, 2004, 2009; Gebrekirstos *et al.*, 2011). Interestingly, species hydraulic efficiency ($K_S$ , $K_L$ ) did not vary consistently along the water availability gradient. Overall, our results across species suggest that increasing tolerance to hydraulic dysfunction in drier sites implies increasing carbon costs per unit leaf area in terms of leaf and sapwood construction.

Importantly, trait-environment relationships were scale dependent (Anderegg *et al.*, 2018) and, as hypothesized, relationships within species were generally less strong than across species. Hv and $P_{tlp}$, two of the three traits with higher %ITV, were the only traits that responded to P/PET within species. These two intraspecific relationships had the same sign but shallower slopes than the corresponding relationships with P/PET among species, which likely reflects lower capacity for hydraulic adjustment within than among species due to relatively fixed drought response strategies at the species level. This result highlights the importance of Hv and $P_{tlp}$ in shaping plastic responses along water availability gradients. Lower leaf area per unit of sapwood (which reduced water demand) and osmotic adjustment may be needed to balance water and carbon costs under reduced water availability in the context of relatively constant hydraulic safety thresholds within species, measured here as stem $P_{50}$. This is consistent with the view that $P_{50}$ is an (evolutionarily) canalized trait buffered against genetic and environmental variation (Lamy *et al.*, 2014). Overall, adjustments along the water availability gradient in the six species studied rely more on changes in stomata closure and resource allocation between sapwood and leaf area than changes in hydraulic safety and efficiency, consistent with previous results comparing pine populations (Mencuccini & Grace, 1995; Mencuccini & Bonosi, 2001; Poyatos *et al.*, 2007; Martínez-Vilalta *et al.*, 2009).

## Trait correlation networks

To our knowledge, our study is the first attempt to test simultaneously the covariation between traits related to leaf economics (LMA, N), xylem hydraulics in terms of safety and efficiency ($P_{50}$, $K_S$), allocation ($K_L$, HV) and traits related to leaf gas exchange ($\delta^{13}C$, $P_{tlp}$), both at the interspecific and intraspecific levels. We found weak evidence for the existence of a unique coordination between hydraulics and more standard leaf and stem traits, which would be required for the existence of universal, resource use strategies at the whole plant-level (our last hypothesis, *cf.* Reich, 2014). In our study, species with conservative leaf economic strategies (i.e., higher LMA) presented a safer xylem (lower

$P_{50}$), possibly to support longer leaf lifespans (Wright *et al.*, 2004). However, this interpretation should also consider that species occupying drier sites are also likely to be exposed to lower water potentials, which would affect their hydraulic safety margins and possibly result in higher hydraulic risk in drier locations. On the other hand, although higher LMA species showed also higher Hv, this pattern did not result in any relationship with xylem transport efficiency (either $K_S$ or $K_L$ ). This lack of a universal 'fast-slow' whole-plant economics spectrum is reinforced when we assessed trait covariation at the intraspecific level. We provide evidence that rather than a single dominant axis of 'fast-slow' plant economics spectrum, multiple combinations of traits are possible depending on the species and the environment. Caution is thus needed when interpreting the comparatively simple trait covariation structures revealed in global studies using relatively few traits (Díaz *et al.*, 2016), and comprehensive assessments including wider sets of traits may improve our ability to represent the patterns underlying the huge diversity in plant form and function.

The increase in water use efficiency (estimated from $\delta^{13}$C) with increasing LMA was the only correlation present in all studied trait networks. This relationship is commonly reported (Körner *et al.*, 1991; Hultine & Marshall, 2000) and it is probably due to an increase in length in the internal diffusion pathway from the stomata to the chloroplasts reducing carbon dioxide supply at the site of carboxylation (Evans *et al.*, 1986). We did not find support for a trade-off between hydraulic safety and efficiency across species and only in two cases within species, consistent with a recent global synthesis that found that many species presented low safety and low efficiency (Gleason *et al.*, 2015). At the intraspecific level, of the two traits that responded to water availability at the intraspecific level, Hv was typically loosely linked to the rest of the trait network (except in *Q. ilex*), whereas $P_{tlp}$ retained a more central role. Higher leaf tolerance to low water potentials (more negative $P_{tlp}$) was associated to higher water use efficiency (less negative $\delta^{13}$C) and to higher leaf construction costs (higher LMA) in most species, suggesting an adaptation to drier and hotter conditions (Wright *et al.*, 2005). It should be noted, however, that our results on trait coordination across species should be considered with caution, as only six species were measured. In addition, our experimental design does not allow disentangling associations resulting from fundamental constraints from those arising from indirect relationships through third variables (in our case driven by changing water availability), which should constitute a priority for future research.

## Conclusion

Our study shows that plant adjustment along a water availability gradient involve many different suites of traits, and highlight the importance of ITV for understanding the capacity of plants to buffer against environmental changes. Availability of individual/plot level trait data coupled with environmental and site information will allow more accurate model parameterization and, therefore, better predictions of species responses to global change (Moran *et al.*, 2016). We show that, within species, plant adjustments along a water availability gradient rely more on changes in allocation (Hv) and leaf tolerance to low water potentials ($P_{tlp}$) than on changes in xylem safety or efficiency. Finally, we show that the use of trait networks could accommodate the intricate, multivariate relationships shaping plant strategies to a much greater degree than approaches based on bivariate relationships (Poorter et al., 2014; Messier *et al.*, 2017). Scale-dependent trait covariation networks can provide powerful insights when assessing the architecture of plant plasticity and its limits under changing environmental conditions.

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# 4

# Are leaf, stem and hydraulic traits good predictors of individual tree growth?

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Frank Sterck and Jordi Martínez-Vilalta

## Summary

- A major foundation of trait-based ecology is that traits have an impact on individual fitness. However, trait-growth relationships have been poorly tested in plants, especially outside tropical ecosystems. In addition, measuring traits directly related to physiological processes ('hard traits') remains difficult and the differences between inter- and intraspecific relationships are seldom explored.

- Here, we use individual-level data on a set of hydraulic, leaf and stem traits to explore which traits are the best predictors of basal area increment (BAI) and growth efficiency (BAI per unit of tree leaf area, GE) among and within species for six dominant tree species along a water availability gradient in Catalonia (NE Spain).

- Traits were better predictors of GE than BAI and significant relationships were largely driven by differences among species means. BAI was negatively associated with wood density and hydraulic efficiency while 'conservative' leaf and stem traits enhanced GE. Climate effects on BAI and GE were indirectly mediated by changes in traits, stand structure and tree size.

- Our study suggests that trait integration along common axes of variation together with a revaluation of the variables that better reflect whole-tree performance can greatly improve our understanding of trait-fitness relationships.

> *"Prediction is very difficult, especially about the future."*
>
> Niels Bohr (1971)

A major challenge in ecology is to understand the link between plant demographic rates and key functional traits to better understand life-history strategies and improve our ability to predict vegetation dynamics and the impacts of climate change on ecosystem structure and functionality. In the last two decades, trait-based approaches have concentrated on investigating the rules that constrain global phenotypic diversity across species, focusing on organ-level spectra such as the leaf economics spectrum (Reich *et al.*, 1997; Wright *et al.*, 2004), the wood economics spectrum (Chave *et al.*, 2009) or belowground traits economics spectrum (Freschet *et al.*, 2010; Mommer & Weemstra, 2012). The assumption that traits have an impact on plant performance and, thus, can provide a basis to scale up from organisms to ecosystems function (Lavorel & Garnier, 2002) has been an important foundation of this research area. However, our understanding of the relationships between traits and demographic rates such as growth is still limited (Yang *et al.*, 2018). This is particularly true for Mediterranean and temperate biomes (but see Klooster *et al.*, 2007; Martínez-Vilalta *et al.*, 2010; Gleason *et al.*, 2018), since most evidence thus far has been gathered in highly diverse tropical ecosystems (Sterck *et al.*, 2006; Poorter *et al.*, 2008; Kraft *et al.*, 2010; Wright *et al.*, 2010; Rüger *et al.*, 2012; Iida *et al.*, 2014). Among the most commonly measured traits, wood density has emerged as the most consistent predictor of tree growth and mortality rates, with lower wood densities generally associated with faster growth rates and lower survival (Poorter *et al.*, 2008; Martínez-Vilalta *et al.*, 2010; Rüger *et al.*, 2012; Iida *et al.*, 2014). However, commonly measured traits frequently explain only a modest proportion of the observed variability in demographic rates (Adler *et al.*, 2014; Paine *et al.*, 2015).

This is especially true when we focus on growth rates, and particularly for adult trees instead of saplings or juvenile trees (Wright *et al.*, 2010; Iida *et al.*, 2014; Gibert *et al.*, 2016). Most studies relating functional traits with growth use absolute growth metrics (such as the basal area increment, BAI) or relative metrics that account for overall size (such as the relative growth rate, RGR) (Gibert *et al.*, 2016). Although it has received little attention in trait-based studies, growth efficiency (GE), defined as the ratio of stemwood production to crown leaf area (Waring, 1983), could be an informative and complementary metric to characterize growth. GE is a physiologically meaningful metric that normalizes overall (radial) growth by the leaf area exchanging carbon and water with the atmosphere. As such, it may be a more precise indicator of physiological performance than other measures of growth, which are strongly affected by allometric scaling (Hérault *et al.*, 2011).

Almost all studies relating traits with demographic rates have been conducted using a species mean approach. However, an increasing number of studies show the importance of taking into account intraspecific trait variation (Albert *et al.*, 2010; Violle *et al.*, 2012; Siefert *et al.*, 2015). The capacity of populations to adjust their traits along environmental gradients could have the potential to maintain or even enhance performance under environmental changes (Laforest-Lapointe *et al.*, 2014). Interestingly, the few studies exploring trait-demography relationships at the individual level have yielded contrasting conclusions. Li *et al.* (2016) showed that individual-level traits better predict individual tree growth than using species mean values provided that context information is added to analyses (e.g., stand structure or soil nutrients), while the opposite was concluded in an extensive study on 25 traits measured on more than 383 species also in the tropics (Poorter, 2018). However, none of these studies was able to distinguish the extent by which the observed trait-growth relationships at the individual level were due to variation across species and/or within species.

Another caveat is that most commonly investigated traits are relatively easy to measure but they are only indirectly related to physiological processes (‘soft’ traits, *sensu*

Hodgson *et al.*, 1999; Weiher *et al.*, 1999). This fact questions whether we are underestimating other plant dimensions that could provide more insights into community structure, functioning and dynamics, although they may be more time-consuming and technically challenging to characterize. This is particularly the case of traits related to drought vulnerability and plant water use (Brodribb, 2017). Drought and heat stress have been recognized as major drivers of forest mortality worldwide in the last decades (Allen *et al.*, 2010). Drought severity and frequency are expected to increase in most regions of the Earth resulting from either decreased precipitation and/or increased evaporative demand (Dai, 2013). Although there has been an intense debate in the last decades regarding the physiological mechanisms underlying drought-induced mortality in trees (McDowell *et al.*, 2008; Sala *et al.*, 2010; Hartmann *et al.*, 2018), all recent reports agree on the key role of plant hydraulics in the process (Anderegg, 2015; Rowland *et al.*, 2015; Adams *et al.*, 2017; Choat *et al.*, 2018).

Plant hydraulic strategies have often been summarized by two main traits that characterize the xylem safety and efficiency of plants. The maximum water transport capacity (conductivity) of the fully hydrated xylem (usually normalized per unit of sapwood area, $K_S$) is commonly used as a measure of efficiency, while xylem safety is often expressed as the xylem water potential at which 50% of hydraulic conductivity is lost ($P_{50}$). The leaf water potential at turgor loss ($P_{tlp}$) has also been used to assess physiological drought tolerance across species (Brodribb *et al.*, 2003; Lenz *et al.*, 2006; Bartlett *et al.*, 2012b). Hydraulic safety margin, i.e., the difference between the minimum xylem water potential experienced by a plant and that causing xylem dysfunction, has emerged as a good predictor of both growth and mortality rates across species (Anderegg *et al.*, 2016; Eller *et al.*, 2018). Similarly, traits related to plant allocation and hydraulic architecture, such as the sapwood-to-leaf area ratio (the Huber value, Hv) have also received attention, and have been recognized as key components of plant strategies to adjust to changes in water availability (Mencuccini & Grace, 1995; DeLucia *et al.*, 2000; Mencuccini & Bonosi, 2001; Poyatos *et al.*, 2007; Martínez-Vilalta *et al.*, 2009; Rosas *et al.*, 2019). However, the few studies that have investigated the association between hydraulic traits and plant performance (Poorter *et al.*, 2010; Russo *et al.*, 2010; Anderegg *et al.*, 2016; Iida *et al.*, 2016; Hietz *et al.*, 2017; Eller *et al.*, 2018), have used values averaged at the species level (see Liu *et al.*, 2016 for one exception). Integrating different plant traits to consider the intrinsic multidimensionality of plant phenotypes and linking common axes of variation to plant performance remains an essential question to be addressed (Kraft *et al.*, 2015; Laughlin & Messier, 2015).

In this study, we use individual-level data on a set of hydraulic, leaf and stem traits to explore which traits are the best predictors of tree growth for six dominant tree species along a water availability gradient in Catalonia (NE Spain). Specifically, we address the following questions:

(1) What traits are more closely associated with tree growth? And are they more tightly related to BAI or to GE? We hypothesized that traits will better predict GE than BAI because absolute (radial) growth standardised by leaf-area is more closely related to physiological performance and less dependent on plant size. We also predict that trees with acquisitive leaf traits and efficient water transport will have greater growth rates.

(2) To what extent are the trait-growth associations explained by differences across versus within species? We hypothesized that trait-growth relationships will be significant within species, but that differences across species means will dominate these relationships, because of higher variation in both variables across species than within species.

(3) How does trait coordination determine growth? We hypothesized that relationships between growth and composite trait metrics will be stronger than when only single traits are considered, because trait integration better reflects axes of variation important for whole-plant performance.

(4) To what extent are trait-growth associations affected by the environment (climate and forest structure) and tree size? We hypothesize that climate will have an indirect effect on growth through its effects of traits. High plot density will increase competition among individuals, which will be negatively associated with growth. Finally, tree size will be negatively associated with growth rates due to size-related declines in net assimilation rates (Ryan *et al.*, 2004; Mencuccini *et al.*, 2005).

# Materials and Methods

## Study site and sampling design

The study area included all the forested territory of Catalonia (NE Spain), which encompasses 1.2 million ha, around 38% of its total land area. Catalonia is very diverse both topographically and climatically: mean annual temperature ranges from 18 °C (at the southern coast) to 3 °C (in the Pyrenees) and annual rainfall varies from 400 mm to >1,500 mm (CDAC, *www.opengis.uab.cat/acdc*). We selected six of the most dominant tree species in Catalonia (3 Pinaceae and 3 Fagaceae), accounting for ∼75% of the total forest area (Gracia *et al.*, 2004): *Pinus sylvestris* L, Pinus nigra J.F.Arnold., *Pinus halepensis* Mill., *Fagus sylvatica* L., *Quercus pubescens* Willd . and *Quercus ilex* L. For each species, 15 plots from the Spanish forest inventory (IFN) were resampled in which the target species was dominant (minimum 50% of the total basal area), maximizing the water availability gradient occupied by each species in the study region. Water availability was quantified as the precipitation to potential evapotranspiration ratio (P/PET) for the spring-summer period (see below). Five plots per species were sampled for each of three species-specific P/PET ranges, following a stratified random design (dry, corresponding to P/PET < $33^{th}$ percentile; wet for P/PET > $66^{th}$ percentile; and mild for the rest) (Fig. C.1). Plots with the two highest stoniness levels and those that had been managed during the last 14 years according to the third IFN survey were discarded.

Within each plot, five non-suppressed canopy trees of the target species with a diameter at breast height (DBH) > 12.5 cm were randomly selected, all within 25 m of the centre of the plot. All samples and data were collected from May to December 2015. To minimise phenological variation in traits within species, species were sampled sequentially (*P. halepensis*, mid-May to end June; *Q. pubescens*, end June and July; *F. sylvatica*, August; *P. sylvestris*; September to mid-October; *Q. ilex*, mid-October to mid-November; *P. nigra*, mid-November to mid-December). From each tree, two branches (one for leaf measurements and the other for hydraulic measurements) were sampled from the exposed part of the canopy in the top half of the crown. Branches were transported to the laboratory inside plastic bags under cool and dark conditions and measurements were taken within 24h.

## Individual trait data

For each of the target trees, 9 traits were measured (Table 4.1): leaf mass per area (LMA) as a measure of biomass investment in leaves per unit light interception and gas exchange (Poorter *et al.*, 2009); leaf nitrogen concentration (N); leaf water use efficiency ($\delta^{13}$C) where less negative values are indicative of greater water-use efficiency (Farquhar *et al.*, 1989); wood density (WD); the Huber value at the branch level (Hv), defined as the ratio of cross-sectional sapwood area to subtended leaf area; the sapwood-specific hydraulic conductivity ($K_S$) as a measure of xylem efficiency; the water potential at 50% loss of hydraulic conductivity ($P_{50}$) as a measure of xylem safety; and the leaf water potential at which leaf cells lose turgor, which is normally accompanied by stomatal closure and cessation of gas exchange ($P_{tlp}$) (Brodribb *et al.*, 2003). Standard protocols (Pérez-Harguindeguy *et al.*, 2013) were followed for all trait measurements. Hydraulic vulnerability curves were established by measuring the hydraulic conductivity of stem segments at different water potentials, using a variation of the bench dehydration method (Sperry & Tyree, 1988; Cochard *et al.*, 2013; Choat *et al.*, 2015). See Chapter 3 for a complete description of the methods used.

| **Trait** | **Symbol** | **Units** |
|---|---|---|
| Leaf mass per area | LMA | g cm$^{-2}$ |
| Leaf nitrogen concentration | N | mg g$^{-1}$ |
| Leaf carbon isotope composition | $\delta^{13}$C | ‰ |
| Wood density (stem) | WD | g cm$^{-3}$ |
| Huber value, sapwood to leaf area ratio (branch) | Hv | cm$^{2}$ m$^{-2}$ |
| Leaf-specific xylem hydraulic conductivity (branch) | $K_L$ | kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$ |
| Stem-specific xylem hydraulic conductivity (branch) | $K_S$ | kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$ |
| Pressure causing 50% xylem embolism (branch) | $P_{50}$ | MPa |
| Leaf water potential at turgor loss point | $P_{tlp}$ | MPa |

**Table 4.1:** Traits measured in this study.

## Growth data

The data on individual tree growth were obtained from the third and fourth Spanish National Forest Inventories (IFN3 and IFN4), the latter conducted over the same time period as our sampling. The interval between inventories per plot varied between 13.89 and 15.35 yr. We calculated individual basal area increment (BAI) as the difference between final and initial over-bark basal area, divided by the census interval.

The diameter of all primary branches was measured for each tree to estimate the total tree leaf area. Branch-level ratios between leaf biomass and branch diameter were measured for 2-8 branches per tree, spanning a range of branch size. In order to make values comparable across species, seasonal maximum leaf area was estimated, taking into account species phenology and the time of sampling. The relationship between leaf biomass (natural-log transformed) and branch diameter (natural-log transformed) was fitted separately for each species using a linear mixed effects model including individual nested within plot as a random effect on the intercept ($R^2 > 0.90$ in all models). Preliminary analyses showed that including a random plot effect on the slope did not improve model fit. Then, we estimated the leaf biomass of each sampled tree by summing the leaf biomass of all its branches. LMA measured on the same tree was used to convert total tree leaf biomass in total tree leaf area. Finally, we calculated individual growth efficiency (GE) as the ratio between individual basal area increment and total tree leaf area.

Spanish National Forest Inventory plots are circular with variable radius, so that the size of the inventory plots depends on the diameter of the measured trees. Specifically, within 5 m from the centre of the plots all trees with diameter at breast height (DBH) $\geq$ 7.5 cm are measured, between 5 and 10 m from the centre of the plots only trees with DBH $\geq$ 12.5 cm are considered, whereas at 10-15 m from the centre of the plots only trees with DBH $\geq$ 22.5 cm are included, and at 15-25 m from the centre only large trees (DBH $\geq$ 42.5 cm) are measured. For this reason, tree growth data was not available for all trees where traits were measured (97 missing data points out of 450 measured trees) and thus, were not included in the analyses. Total plot basal area was also available for both inventories.

### Climatic data

To estimate P/PET as a measure of water availability for each study plot, climate data were obtained from the Climatic Digital Atlas of Catalonia (Ninyerola *et al.*, 2005), a collection of digital maps at 200 x 200 m resolution including average annual radiation, mean annual temperature, maximum annual temperature, minimum annual temperature and annual precipitation for the period 1951-2010. Then, PET values were calculated according to the Hargreaves-Samani method (Hargreaves & Samani, 1982) and used to estimate P/PET for the spring-summer period for each sampled plot.

## Statistical analyses

All variables were checked for normality and natural-log transformed whenever required. First, Pearson correlation coefficients were used to quantify the association between studied traits and growth rates (BAI and GE). Secondly, to separate the intraspecific from the interspecific component of trait-growth relationships, we fitted two linear mixed effects models for each trait, one with BAI as the response variable and the other with GE. Two variables were included as non-interacting explanatory factors in each model: the mean trait value at the species level and the species-centred trait value. The latter was calculated as the difference between the trait value for a given tree and the average value of the corresponding species. Including both variables allows isolating the relative significance of across versus within-species effects on growth. We included plot nested in species as random effects on the intercept of each model. Preliminary analyses showed that including a random species effect on the slope did not improve model fit. The residuals of all models showed no obvious pattern and were approximately normally distributed. Linear mixed effects models were fit using the *lme4* R package (Bates et al., 2015).

To summarize studied traits into overall plant axes of variation, a principal components analysis (PCA) was performed on the 9 traits considered in the study. The first and the second PCA axes explained 49% and 24% of the variability, respectively. Then, two linear mixed effects models were used to evaluate how trait coordination determined BAI and GE using the first two axes of the PCA as explanatory factors. To separate the interspecific from the intraspecific component, we used the mean of each PCA score at the species level and the centred score value, which resulted in a total of four explanatory variables. As before, centred values were calculated as the difference between individual PCA score values and the average value of the corresponding species. A second PCA performed using centred trait values at the species level confirmed that the previous PCA axes, which were driven by trait variability both within- and among-species, also reflected the main axes of variation at the intraspecific level (Fig. C.3). This second, intraspecific PCA, however, explained a lower proportion of the total variance (22% the first and 19.9% the second) (Fig. C.3).

Finally, to evaluate the direct and indirect effects of climate (P/PET), forest structure (initial plot basal area), tree size (initial tree basal area) and trait covariation on growth rates, two different piecewise structural equation models (SEM) were performed, one for BAI and the other for GE. We started with the ‘saturated’ model including all possible directional effects of the first two previous PCA axes, climate, forest structure and tree size on growth rate, as well as directional effects of climate on forest structure, tree size and traits (PCA axes), plus all possible covariations among them. An important advantage of the piecewise SEM approach over classical covariance-based structural equations models (e.g. Oberski, 2014) is that it allows piecing multiple individual linear mixed models together into a single causal network, taking into account the hierarchical structure of the data (Lefcheck, 2016). Thus, we included plot nested in species as random effects on the intercept in all SEM sub-models. The overall SEM fit was evaluated using Shipley’s test of d-separation (Shipley, 2013): Fisher’s C statistic ($P>0.05$ indicates that no significant paths are missing and a good model fit) and Akaike information criterion (AIC). Models were simplified stepwise by removing the least significant path until a minimal adequate model with the lowest AIC was obtained. Models within 2 AIC units were considered equivalent in terms of fit and the simplest one was selected (Zuur *et al.*, 2009). We reported the standardized coefficient for each path from each component models, as well as the marginal (variance explained by fixed factors) and conditional (variance explained by fixed and random factors) $R^2$ values (Nakagawa &

Schielzeth, 2013). SEM models were fitted with the R package *piecewiseSEM* (Lefcheck, 2016). All analyses were carried out using R statistical software v.3.3.2 (R Core Team, 2017).

## Results

### Individual trait-growth associations

Pinaceae species tended to have higher values of BAI than Fagaceae and differences between families increased when growth was normalized by total tree leaf area (Fig. 4.1a and 4.1b). Individual-level correlations between trait values and GE were stronger than trait-BAI associations, although correlation coefficients were always r<0.65 (Fig. C.2). We subsequently examined the extent by which significant associations were caused by across-species versus within-species variability. A substantial percentage of the variance was explained by differences among species and plots (high difference between conditional and marginal $R^2$), especially for BAI (Tables C.1-C.2). $K_L$ and WD were the only traits that showed a significant relationship with BAI across species, whereby higher growth rates were associated with lower WD and lower $K_L$ values (Fig. 4.2 and Table C.1). Within species, only $\delta^{13}$C showed a significant (positive) effect with BAI (Fig. 4.2 and Table C.1). Regarding GE, species with higher BAI per unit of total tree leaf area were associated with conservative leaf resource use strategies (high LMA and low N), a higher water use efficiency (less negative $\delta^{13}$C) and a lower vulnerability to xylem embolism (more negative $P_{50}$) (Fig. 4.3 and Table C.2). Species with higher GE also showed a positive relationship with Hv and a negative effect of $K_S$ that resulted in a non-significant relationship with $K_L$ (Fig. 4.3 and Table C.2). However, when trait-GE relationships were assessed within species, significant (positive) relationships were found for $K_L$, Hv and LMA (Fig. 4.3 and Table C.2). Interestingly, for these last two variables, the intraspecific slopes were shallower than the corresponding interspecific slopes.

## Associations between growth and composite trait metrics

Trait data showed two orthogonal axes of variation that explained 72.8% of the total variance (48.8% and 24%, respectively). The first axis was interpreted in terms of conservative leaf resource use and drought resistance strategies, since high LMA, low N, resistance to embolism (more negative $P_{50}$) and water use efficiency (less negative $\delta^{13}C$) all had highly positive factor loadings (Fig. 4.4). The second axis was associated with higher values of $K_L$ and $K_S$ (as well as wood density and $P_{tlp}$) and, thus, it was interpreted as a proxy for hydraulic efficiency (Fig. 4.4). Associations between these trait axes and growth metrics were generally in line with the individual trait-growth associations reported in the previous section. When we explored the effect of trait covariation on BAI, we found a negative relationship with the hydraulic efficiency axis both within and among species (Table C.2). Regarding the first PCA component, related to conservative leaf resource use and drought resistant strategies, a negative effect on BAI was found only at the intraspecific level (Table C.2). For GE, more conservative leaf resource use and drought resistance strategies (PCA 1), as well as lower hydraulic efficiency (PCA 2) were positively associated with GE, but only across species (Table C.2). Noteworthy, when trait coordination was taken into account, a higher proportion of variance was explained: mean and centred PCA axes explained 14% and 52% of BAI and GE, respectively (Table C.2).

## Effects of trait axes, environment and tree size on plant growth

P/PET was positively associated with initial tree basal area and initial plot basal area. P/PET had a negative effect on both PCA components, whereby higher values were related with traits associated with more acquisitive leaf resource use strategies, lower drought tolerance and lower hydraulic efficiency (Fig. 4.5). Tree basal area had a strong positive effect on BAI, while a weaker and opposite effect was found for GE (Fig. 4.5). Plot basal area only showed a significant negative relationship with growth. Relationships between trait PCA axes and growth were weaker when accounting for climate, forest structure and tree size. BAI was negatively affected by traits related to higher hydraulic efficiency (PCA 2) (Fig. 4.5a) while a positive effect on GE was found with traits related to conservative leaf resource use and drought resistance strategies (PCA

1) (Fig. 4.5b). Overall, the model accounted for 58% of the total variability on BAI and 64% on GE, but the variance explained by the fixed factors was relatively low (40% for BAI and 10% for GE), in part reflecting that we could not explicitly disentangle inter- and intraspecific trait effects in SEM models. In the GE SEM model the variance explained by the fixed factor was particularly low because tree size had a lower effect than in BAI SEM model (Fig. 4.5b).

## Discussion

The results of this investigation showed that traits were better predictors of GE than BAI and that trait-growth relationships were largely driven by differences among species. Higher values of WD and leaf-related hydraulic efficiency were negatively associated with BAI across species. On the other hand, species with conservative leaf and stem traits (e.g. high leaf mass area, low N, high leaf water-use efficiency, more negative $P_{50}$, low $K_S$) showed higher values of GE. The composite trait metrics defined by taking into account trait coordination better predicted growth rates and depicted similar relationships to those identified using individual traits. Finally, we showed that climate effects on BAI and GE were indirectly mediated by changes in traits, stand structure and tree size.

### Stem traits were the best predictors of BAI

WD and $K_L$ were the best predictors of BAI whereby tree volumetric growth decreased with increasing WD and $K_L$ across species (Fig. 4.2). A decline in growth rates with denser wood has been previously reported in both tropical and Mediterranean ecosystems (Poorter *et al.*, 2008, 2010; Wright *et al.*, 2010; Martínez-Vilalta *et al.*, 2010; Rüger *et al.*, 2012) but the rationale behind it is still controversial. Denser wood is more expensive to construct, as a smaller volume is produced by the same carbon investment, but it has also been argued that respiration maintenance costs due to lower trunk surface area are lower (Larjavaara & Muller-Landau, 2010). Additionally, some studies have posed that individuals with denser wood show lower growth rates because they have lower hydraulic and photosynthetic capacity that result in a lower carbon gain and thus, lower growth (Santiago *et al.*, 2004; Chave *et al.*, 2009; Poorter *et al.*, 2010; but see Eller *et al.*, 2018). At the same time, denser wood has been proposed to confer high cavitation resistance due to an increase in the mechanical strength of xylem conduits (Hacke *et al.*, 2001). This relationship may allow trees with denser wood and more resistant xylem to keep on functioning for longer periods and, thus, eventually recover the higher initial carbon investments on wood construction (Eller *et al.*, 2018). However, we did not find support for these arguments as no relationship was found between BAI and traits such as $P_{50}$ or $K_S$.

We did find a negative relationship between BAI and $K_L$ across species, probably mediated by WD, as higher values of WD, $K_S$ and $K_L$ all positively contributed to the second PCA axis interpreted as a proxy of hydraulic efficiency (Fig.4. 4). This fact could be explained because we are studying gymnosperms and angiosperms species with contrasted and highly coupled wood and hydraulic properties. This is particularly true for conifers that have lighter wood and the main volume of wood is occupied by tracheids with low $K_S$ (Fig.4.4) (Sperry *et al.*, 2006). In contrast, angiosperm hydraulic properties can vary more independently from wood structural attributes, because of the large wood volume proportions of fibres and parenchyma (Venturas *et al.*, 2017). Using a global dataset, it has been reported that no relationship exists between hydraulic conductivity and mechanical wood strength in angiosperms (Zanne *et al.*, 2010). Indeed, in our study system, the relevance of the WD in the second PCA axis decreased significantly when the PCA was performed using centred trait values at the species level (Fig. C.3). However, we could not distinguish whether trait associations resulted from indirect correlations with traits not considered in our study or imply a direct coordination.

## Conservative leaf and stem traits enhanced GE

Interestingly, traits better predicted GE than BAI, as we hypothesized, probably because GE is a performance variable more closely linked to physiological performance in terms of carbon and water economies. Thus, further studies may benefit from comparing different growth performance metrics and perhaps alternative variables that could potentially better describe whole-plant growth. In contrast to our hypothesis, higher

GE rates were associated with leaf traits related to conservative resource use strategies across species (high LMA and low N) (Fig. 4.3). Photosynthetic capacity typically decreases with LMA when expressed on a mass basis, but when photosynthetic capacity is expressed per unit leaf area it is almost unrelated with LMA at the global scale (Wright *et al.*, 2004) and typically increase with LMA within species (Reich *et al.*, 1994; Poorter *et al.*, 2009). It has been proposed that higher photosynthetic rates per unit leaf area related with higher values of LMA could be explained by a greater leaf thickness associated with thicker photosynthetic mesophyll layers instead of structural mass components (Niinemets, 1999; Osnas *et al.*, 2018). In fact, some studies have found that high LMA had a positive effect on growth even without normalizing it by leaf area under water- or nutrient-limited conditions in the tropics (Prado-Junior *et al.*, 2016; Poorter, 2018; Van der Sande *et al.*, 2018).

Xylem traits related to drought tolerance, particularly more negative $P_{50}$ values, were associated with conservative leaf traits and likewise enhanced GE across species (Fig. 4.3), possibly because these traits allowed species to persist functionally for longer time during periods of drought stress, which are common in Mediterranean ecosystems. It should be noted, however, that we did not find any significant effect of $P_{\mathrm{tlp}}$ on GE, despite the fact that $P_{\mathrm{tlp}}$ has been also associated with drought tolerance (Bartlett *et al.*, 2012b). Regarding traits related to hydraulic efficiency, we found a compensation between Hv and $K_S$, such that species showing higher GE rates had lower $K_S$ but also higher Hv, resulting in a non-significant interspecific relationship with $K_L$ (Fig. 4.3). However, when we take trait coordination into account, the marginal, individual effect of WD on GE ($p<0.07$, Table C.2) probably contributes to a significant overall negative effect of the second PCA axis related to hydraulic efficiency across species (Table C.2). Overall, and as hypothesized, the predictive power of traits increased when trait coordination was considered (Table C.2). Taken together, these results suggest that in our study system, interspecific differences in GE rely more on conservative leaf traits and drought tolerance strategies rather than on more efficient water transport to leaves.

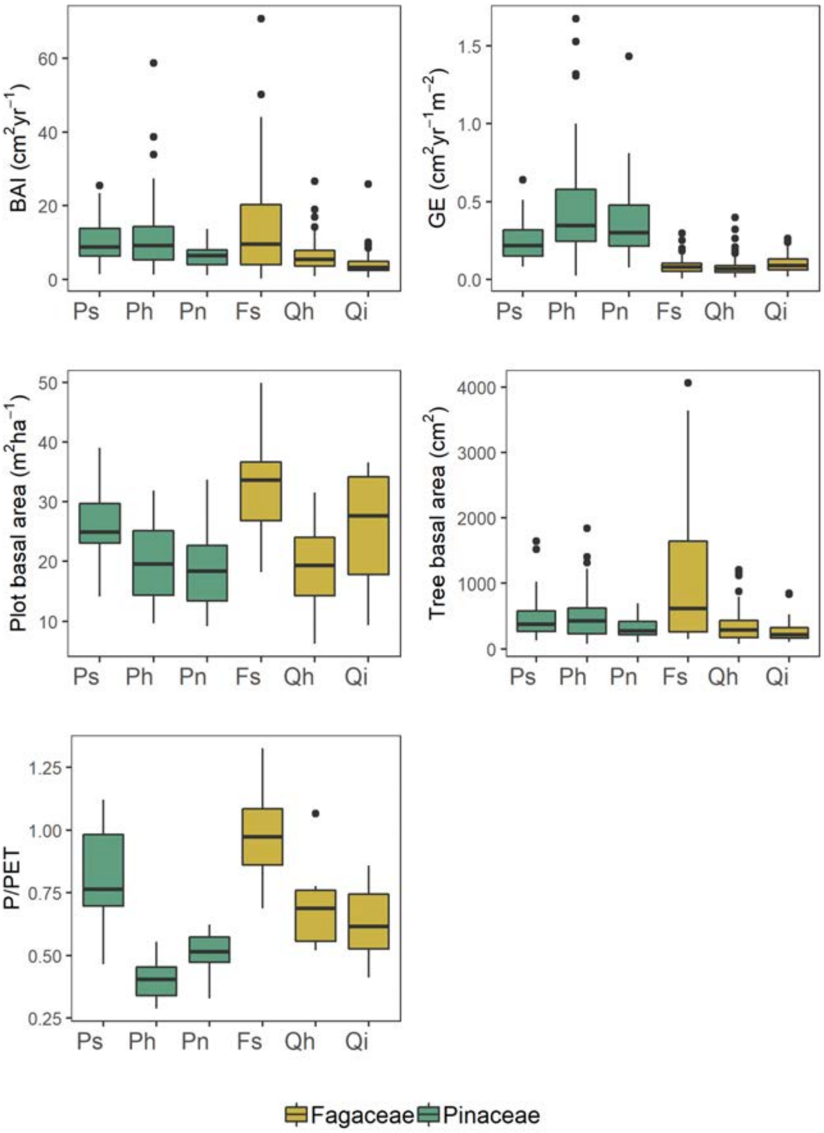

**Fig. 4.1:** Boxplot of (a) basal area increment (BAI), (b) growth efficiency (basal area increment per unit of total tree leaf area, GE), (c) plot basal area, (d) tree basal area, and (e) precipitation to potential evapotranspiration ratio (P/PET) as a function of species and family (Pinaceae vs Fagaceae). The limits of boxes indicate the first and third quartiles, and the horizontal line within each box corresponds to the median. The upper whisker extends from the third quartile to the highest value within 1.5 x IQR (interquartile range) of the third quartile. The lower whisker extends from the first quartile to the lowest value within 1.5 × IQR of the first quartile. Abbreviations: Fs, *Fagus sylvatica*; Qi, *Quercus ilex*; Qh, *Quercus humilis*; Ps, *Pinus sylvestris*; Ph, *Pinus halepensis*; Pn, *Pinus nigra*.

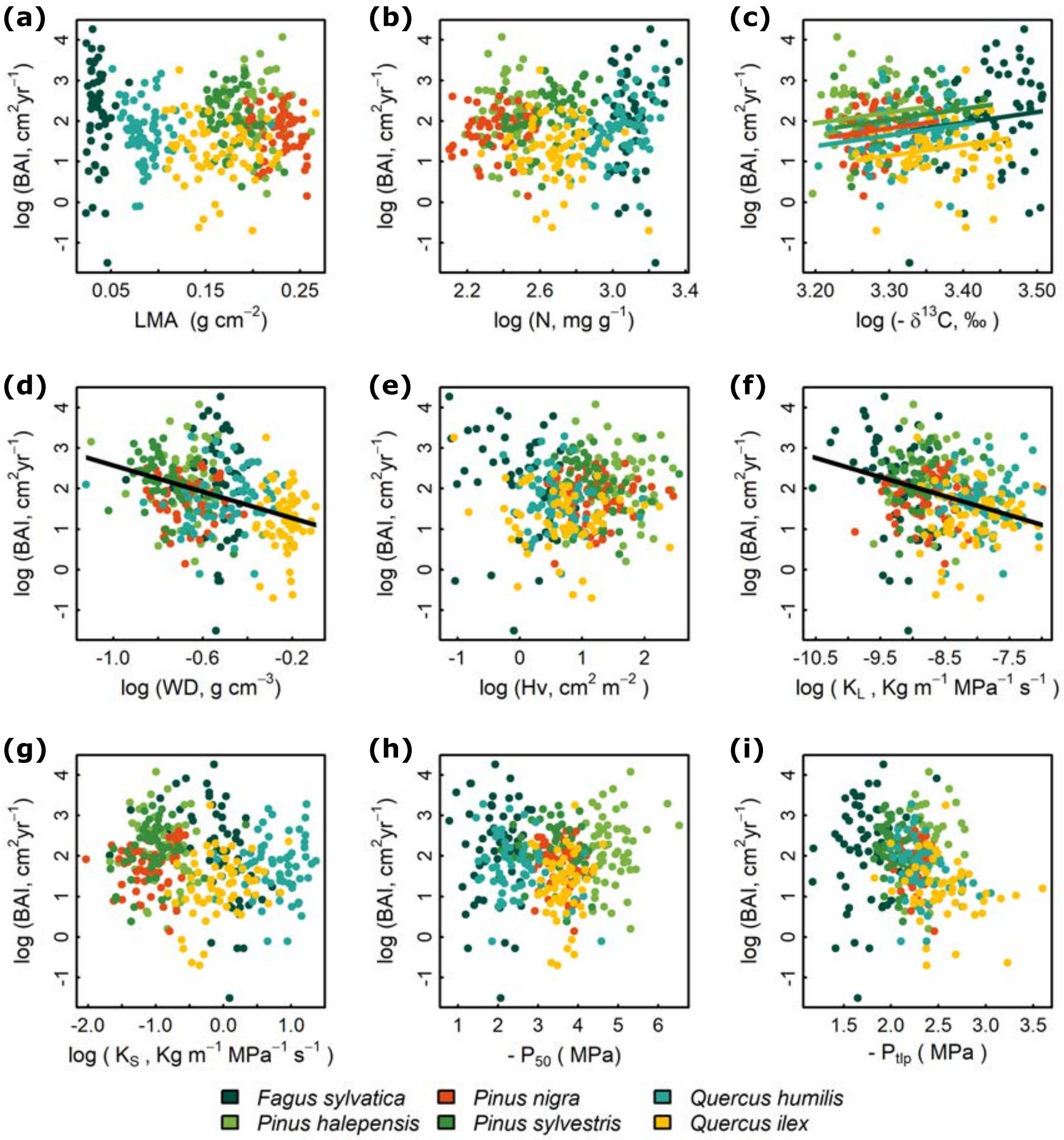

**Fig. 4.2:** Relationship between basal area increment (BAI) and studied traits. The black regression lines give the overall cross-species relationships, and the coloured lines the corresponding within-species relationships, when significant ($P < 0.05$). Variables were natural-log transformed whenever required to satisfy normality assumptions. See Table 4.1 for definition of symbols.

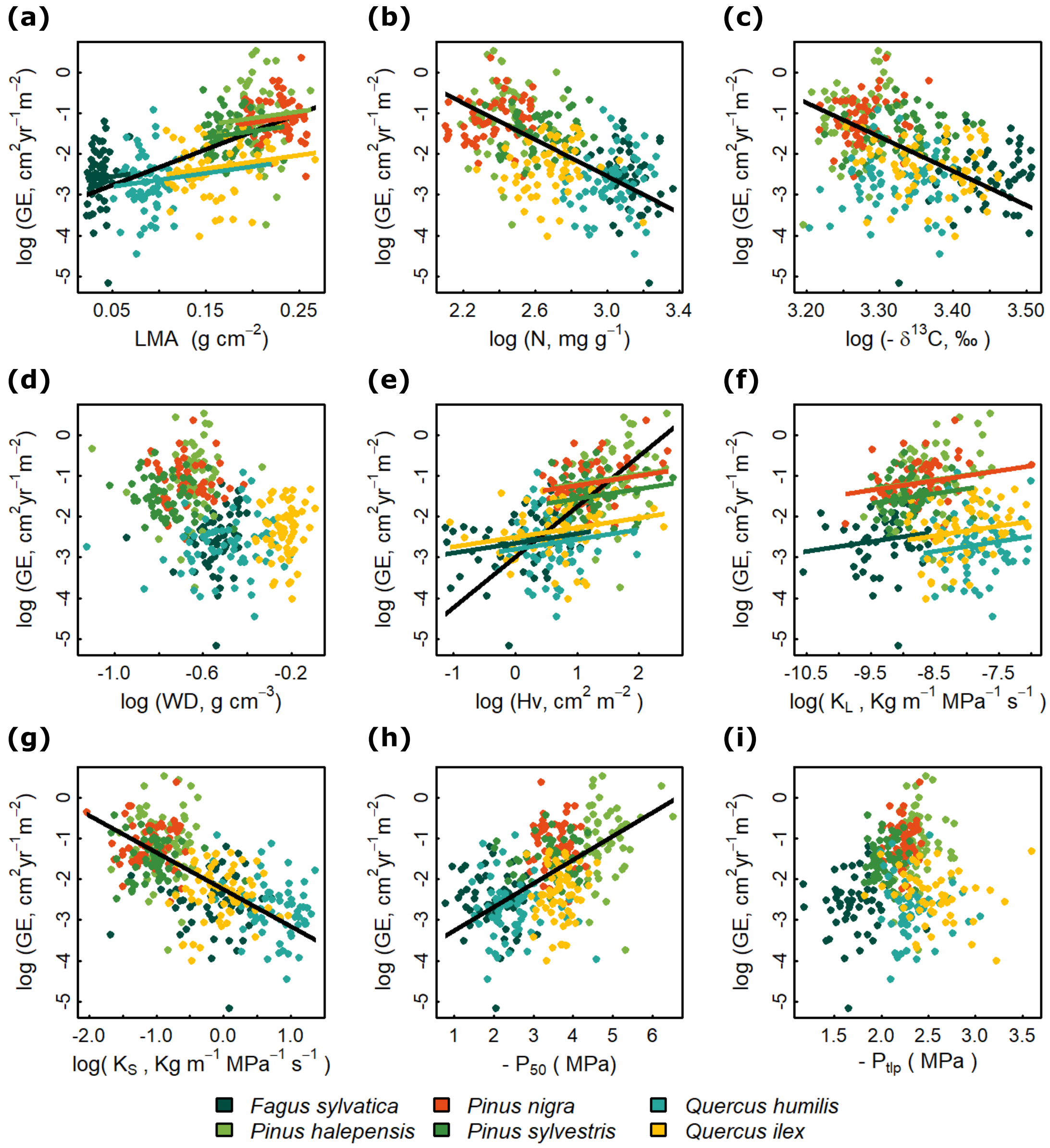

**Fig. 4.3:** Relationship between growth efficiency (basal area increment per unit of total tree leaf area, GE) and studied traits. The black regression lines give the overall cross-species relationships, and the coloured lines the corresponding within-species relationships, when significant ($P < 0.05$). Variables were natural-log transformed whenever required to satisfy normality assumptions. See Table 4.1 for definition of symbols.

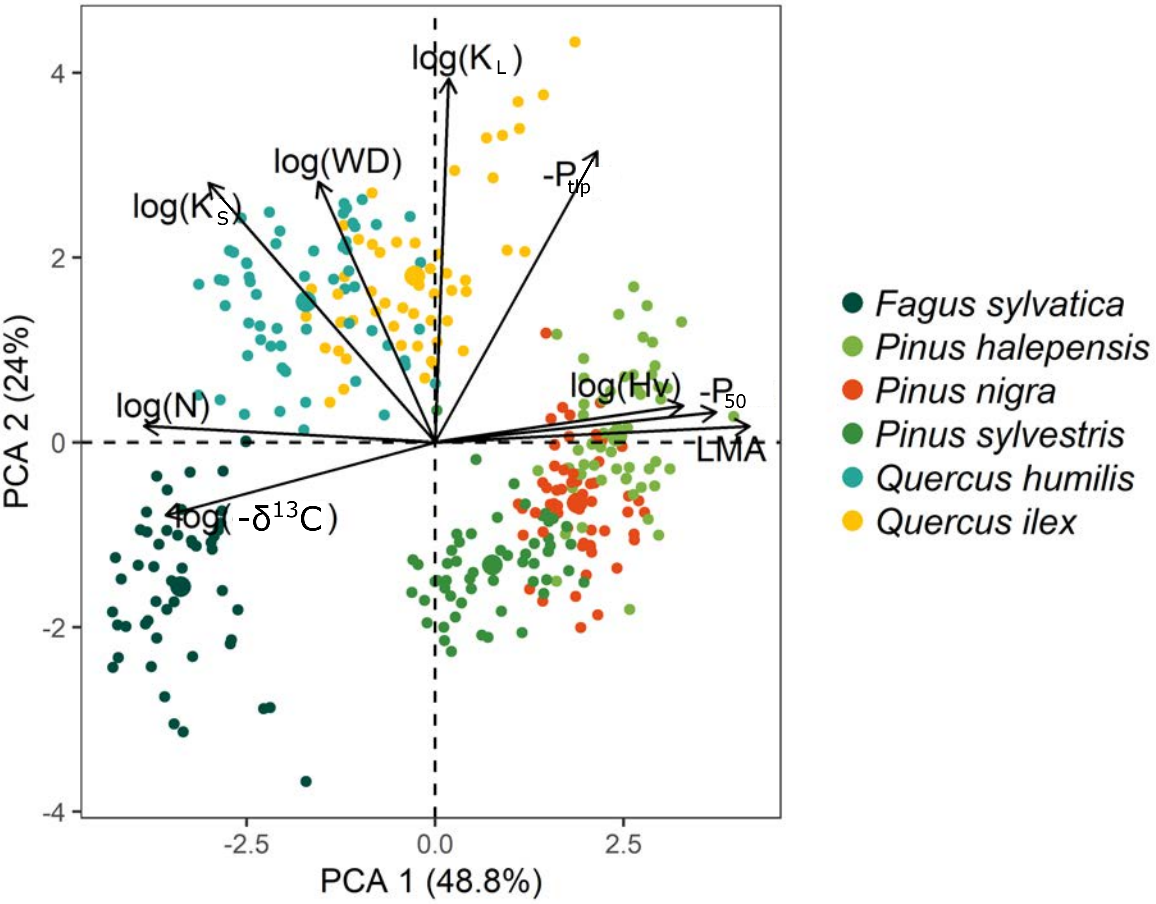

**Fig. 4.4:** Principal component analysis (PCA) summarizing trait variability across individual sampled trees. The first two PCA axes with the percentage of explained variance (in brackets) are shown. Variables were natural-log transformed whenever required to satisfy the normality assumptions. See Table 4.1 for definition of symbols.

| | Log (BAI) | | | Log (GE) | | |
|---|---|---|---|---|---|---|
| **Fixed Factors** | Estimates | CI | P | Estimates | CI | P |
| (Intercept) | **1.83** | **1.61 – 2.06** | **<0.001** | **-1.9** | **-1.99 – -1.80** | **<0.001** |
| PCA axis 1 mean | 0.02 | -0.09 – 0.13 | 0.741 | **0.31** | **0.27 – 0.36** | **<0.001** |
| PCA axis 1 centred | **-0.17** | **-0.32 – -0.02** | **0.027** | 0.03 | -0.10 – 0.16 | 0.646 |
| PCA axis 2 mean | **-0.22** | **-0.39 – -0.04** | **0.015** | **-0.2** | **-0.27 – -0.12** | **<0.001** |
| PCA axis 2 centred | **-0.13** | **-0.26 – -0.00** | **0.045** | 0.07 | -0.03 – 0.18 | 0.180 |
| **Random Effects** | | | | | | |
| $\sigma^2$ | 0.47 | | | 0.31 | | |
| τ00 PLOT:SP | 0.14 | | | 0.12 | | |
| τ00 SP | 0.06 | | | 0 | | |
| Observations | 331 | | | 331 | | |
| Marginal $R^2$ | 0.14 | | | 0.52 | | |
| Conditional $R^2$ | 0.39 | | | 0.65 | | |

**Table 4.2:** Results of the linear mixed models examining the relationships between absolute radial growth (BAI) and growth efficiency (basal area increment per unit of total tree leaf area, GE), and the first two PCA axes (*cf.* Fig. 4.4) within (centred) and among species (mean). The model's fixed effects coefficients including confidence intervals (CI) and p-values (P) are shown. Information on the random effect variances ($\sigma^2$, total, $\tau00$ PLOT:SP, within-species and $\tau00$,SP, cross-species) as well as the proportions of explained variance by fixed effects ($R^2$ marginal) and by fixed and random effects ($R^2$ conditional) is also provided. Abbreviations: PCA axis 1, first PCA component; PCA axis 2, second PCA component.

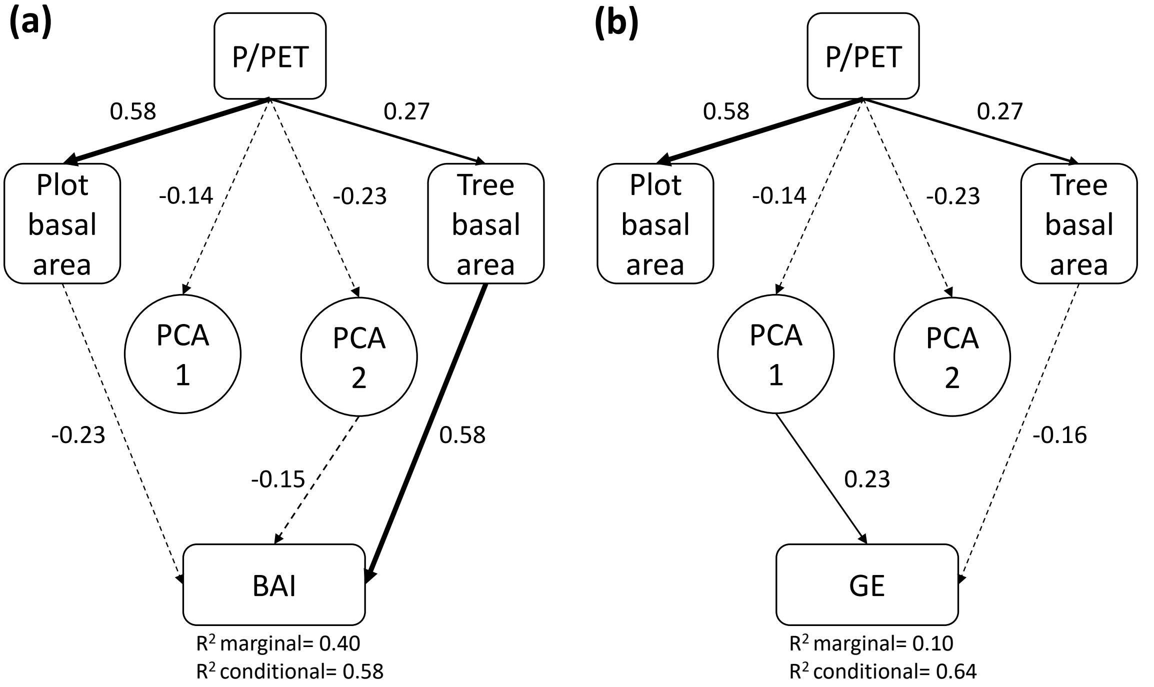

**Fig. 4.5:** Piecewise structural equation models relating climate (in terms of precipitation over potential evapotranspiration ratio, P/PET), forest structure (in terms of initial plot basal area), tree size (in terms of initial tree basal area) and traits (using the first PCA component, PCA 1; and the second PCA component, PCA 2; *cf.* Fig. 4.4). Panel (a) shows the results for basal area increment (BAI) and panel (b) for growth efficiency (basal area increment per unit of total tree leaf area, GE). Arrows indicate significant links between variables. Solid and dashed lines indicate positive and negative relationships, respectively. Standardized path coefficients, as well as the marginal and conditional $R^2$ values, are provided.

## Trait-growth association were more predictable across species than within species

This is, as far as we know, the first time that a study aims to disentangle interspecific from intraspecific effects in trait-growth associations. As hypothesized, growth rates were largely driven by differences across species means (Fig. 4.2 and Fig. 4.3), reflecting a higher trait variability among species that changed along the water availability gradient (Fig. 4.1e) compared to the relatively low trait plasticity at the intraspecific level (*cf.* Poorter, 2018). Specifically, within species, we only found a positive relationship between BAI and lower WUE values (more negative $\delta^{13}$C). This relationship is probably indirect, and reflects the fact that xeric populations tend to show both higher WUE and lower BAI. In addition, it has been seen that, over time, increasing WUE does not necessarily stimulate tree growth, especially in water-limited environments were higher WUE values can result from a decrease in stomatal conductance rather than an increase in photosynthetic capacity (Peñuelas *et al.*, 2011; Lévesque *et al.*, 2014). Regarding GE, we only found significant positive trends at the intraspecific level with LMA, Hv and $K_L$. Because $K_L$ is the product of Hv and $K_S$ and intraspecific relationships with GE were found only for Hv (not for $K_S$) (Fig. 4.3), the positive relationship between $K_L$ and GE within species appears to be driven by the intraspecific plasticity in allocation between sapwood and leaves. Therefore, while we found a compensation between Hv and $K_S$ across species, the dominant driver within species appears to be the climatic sensitivity of allocation to the water availability gradient (P/PET) (Chapter 3; Rosas *et al.*, 2019) with its carry-over effects on GE differences across plots. It should be noted that selecting healthy individuals with sun-exposed branches and sampling species sequentially to minimize phenological variation, together with the fact that we did not cover the whole distribution ranges of study species, could have resulted in an underestimation of intraspecific trait variability. More studies are needed to confirm whether the observed patterns can be generalized to other study systems and wider sets of species.

### Climate effects on BAI and GE were indirectly mediated by changes in traits, stand structure and tree size

In Chapter 3 (Rosas *et al.*, 2019), we showed that tree size and P/PET affect trait variability along the sampled gradient. Here we examine how these same variables affect growth responses. In Chapter 3, we showed that the strong response of traits to P/PET was partly caused by the parallel variation of tree size (but not basal area) along the gradient. Here we do not find a direct effect of P/PET on growth metrics but we only find indirect effects via traits and tree size and stand structure. As expected, plots with higher water availability (higher P/PET values) were denser and trees presented a higher basal area. Wetter sites were also associated with acquisitive leaf and stem resource use strategies (low LMA, less negative $P_{50}$) as previously seen in other studies (e.g. Maherali *et al.*, 2004; Wright *et al.*, 2005). We also found a negative relationship between P/PET and hydraulic efficiency axis (PCA 2), although we did not detect any significant climatic effect on $K_S$ , $K_L$ or WD in a previous study in the same study system (Chapter 3; Rosas *et al.*, 2019). Higher plot basal area, presumably associated to competition intensity, only showed a negative effect on BAI, while no significant relationship was found with GE, probably because this latter variable already captures compensatory changes in tree leaf area as a function of competitive environment (Jump *et al.*, 2017). Not surprisingly, trees with higher basal area showed higher BAI but the opposite was true when we normalized BAI by total tree leaf area. These results are in line with the age-related declines in net assimilation rates reported in other studies, which have been explained by size effects rather than a function of age per se related to cellular senescence (Mencuccini *et al.*, 2005; Martínez-Vilalta *et al.*, 2007). Importantly, when environmental drivers and tree size were simultaneously considered, results were consistent with trait-growth associations obtained with mixed models of individual traits: high hydraulic efficiency and dense wood (PCA 2) were associated with low BAI, while GE was enhanced by conservative leaf resource use and drought tolerance strategies (PCA 1) (Fig. 4.5). Our finding that climate effects on growth were largely indirect, mediated by changes in stand structure, tree size and plant traits is consistent with earlier reports across species (Martínez-Vilalta *et al.*, 2010) and helps highlight the importance of the ecological context in interpreting trait-growth relationships.

## Conclusions

In summary, our investigation showed that the studied traits better predict GE than BAI and thus, highlights that studying performance variables more closely related to whole-plant performance can provide complementary insights in trait-based studies. Trait-growth relationships were more predictable across than within species, reflecting a relatively restricted plastic capacity at the intraspecific level. Stem traits (WD, $K_L$) were the best predictors of BAI and traits related to conservative leaves and drought tolerance strategies (high LMA, low N, more negative $P_{50}$, low $K_S$) were positively associated with GE. Thus, caution is needed when interpreting conservative-acquisitive continuum traits at the organ level as indicators of plant performance. Our results revealed that integrating a wide set of traits defining main plant ecological strategies can improve our ability to predict demographic rates, thus increasing the functional relevance of trait-based approaches. Finally, we also showed that climate effects on BAI and GE were indirectly mediated by changes in traits, stand structure and tree size. Future studies will benefit from further characterization of the importance of traits and their covariation on the different components of performance (growth, survival and fecundity) and under different ecological contexts, in order to improve our understanding of overall plant resource use strategies and their overall relationship to fitness.

# Part III

# DISCUSSION AND CONCLUSIONS

*"Caminante son tus huellas*
*El camino nada más;*
*caminante no hay camino*
*se hace camino al andar."*
ANTONIO MACHADO, 1912

*"There is much to be done. There is also a real hope*
*that we may be getting somewhere."*
MARK WESTOBY (2002)

# 5

# Discussion and Conclusions

THE imperious need to forecast changes in vegetation responses to global environmental drivers has soared the search for simplification of the huge diversity of plants in a few measurable traits relevant to plant functioning and ecosystem processes. This thesis integrates plant hydraulic traits into a functional trait-based framework and examines some of the foundational assumptions of trait-based plant ecology. Specifically, this dissertation improves our understanding of trait coordination and trait adjustments to the environment at different ecological scales as well as it empirically tests to what extent studied traits can determine individual growth rates. In this respect, it attempts to contribute to strengthen the theory of trait-based ecology and provide tools that guide model development opening up an avenue for predicting changes in forest ecosystem function under ongoing climate change better.

## Is there a whole-plant economics spectrum?

A central aim in ecology is to understand trade-offs between critical traits explaining the main plant ecological strategies. A global leaf economics spectrum (LES) has been described, spanning from conservative leaves with long life-span and slow returns on carbon and nutrients to acquisitive leaves with short life-span and fast returns on investment (Reich *et al.*, 1997; Wright *et al.*, 2004). The functional significance of this spectrum for whole plants remains to be elucidated. Reich (2014) proposed the existence of a world-wide 'fast-slow' plant economics spectrum that integrates and couples all organs (leaves, stems and roots) and resources (carbon, nutrient, water) regardless of environmental conditions. Thus, the proposed spectrum goes from 'slow' plants with high tissue density, long tissue life span, low rates of resource acquisition and flux (including water), to 'fast species' with the opposite features. This hypothesis assumes that being fast at any organ or resource will require being fast in all others and, even more importantly, that traits can be easily scaled-up from the organ to the whole individual. We[1] evaluated this hypothesis at the global scale throughout data compilation from 1149 species worldwide (Chapter 2), and at the regional scale for the six most dominant tree species in Catalonia (NE Spain) along a water availability gradient and assessing patterns at the inter- and intraspecific levels (Chapter 3).

Specifically, in Chapter 2 we found that leaf economics and xylem hydraulics were coordinated at the global scale supporting the idea that similar strategies can be identified based on the traits of individual organs. Plants with acquisitive leaves had more efficient xylem (higher hydraulic conductivity, $K_S$) and were more vulnerable to embolism during drought (more negative $P_{50}$, that is, the water potential at which 50% of hydraulic conductivity is lost due to embolism). However, we also showed that plants regulate the balance between water supply and demand and between vulnerability and exposure to drought stress, via control of the Huber value (the sapwood to leaf area ratio, Hv) and minimum water potential ($\psi_{\text{min}}$), both of which depend on allocation and organ physiology. Plants with low specific leaf area (SLA) leaves had a xylem more resistant to

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[1] '*I*' is used for general discussion or information related to this thesis and '*we*' is used when referring to research chapters in which co-authors are involved.

drought but, at the same time, those plants operated at more negative plant water potentials. Similarly, although high SLA was associated with high $K_S$, the correspondingly lower Hv more than counterbalanced the hydraulic advantage at the organ scale. As a result, when hydraulic conductivity was normalized by leaf water demand (leaf-specific conductivity, $K_L$) and the vulnerability to embolism was normalized by the exposure to drought (hydraulic safety margin, HSM), they no longer scale with the leaf economics spectrum. Thus, we showed a discrepancy between organ-level and whole-plant scales, whereby the 'fast/slow' continuum in leaf properties does not map directly onto an axis of plant hydraulic performance in terms of more integrative traits such as HSM and $K_L$. Interestingly, the previous patterns hold whether or not climatic or phylogenetic effects were accounted for in our analyses. Similarly, this lack of integration of functional strategies across resources, but also organs, was also confirmed in Chapter 3 where we studied how leaf economics and hydraulic traits were related at both interspecific and intraspecific levels. We showed that trait coordination was scale-dependent and that, rather than a single dominant axis of 'fast-slow' plant economics spectrum, the relationships among traits were likely to occupy a wide space in which multiple combinations were possible depending on the species and the environment.

Taken together, these results suggest that scaling-up traits from the organ to the whole-plant level, which is an essential step to predict how communities assemble and shape ecosystem processes, may be not as easy and straightforward as frequently assumed, because of the existence of compensatory responses within (and probably across) individuals. This fact has important implications for global vegetation models and suggests that a re-evaluation of the key trait dimensions to describe whole-plant function is needed. Belowground traits, although they have not been investigated during this thesis, also merit further research efforts to incorporate them successfully in the functional trait syndrome conceptual framework (Mommer & Weemstra, 2012; Laliberté, 2017). Thus, we proved that a shift from 'soft' to more mechanistic traits (*sensu* Brodribb, 2017) whose function can be clearly defined physiologically (e.g., $P_{50}$) can provide useful insights when trying to understand the dominant axes of plant functional variation. In particular, in this thesis, I advocate the importance of the inclusion of plant hydraulic traits within a functional traits framework. We showed that plant hydraulics allowed us linking water in the carbon/nutrient economics described in LES as a key resource closely coupled to carbon assimilation because of the role of stomata in gas exchange (Cowan, 1978). Thus, we argue that understanding the coordination between LES and hydraulics is essential to select the best minimum set of traits that summarize functionally important aspects of plant diversity. Additionally, because hydraulic traits have been related to plant performance under stress, their value can be critical when predicting vegetation responses under future changing climatic conditions (Choat *et al.*, 2012; Anderegg *et al.*, 2012; Rowland *et al.*, 2015; Anderegg *et al.*, 2016; Choat *et al.*, 2018).

❋ **In sum, a significant finding to emerge from this thesis is that we did not find support for a world-wide 'fast-slow' plant economics spectrum. Scaling-up from organ level traits to whole-plant traits and resource use strategies may be more challenging than commonly anticipated because of compensatory responses within individuals.**

## What is the extent of ITV? What role does it play in terms of acclimation to the environment?

Traits can vary between and within species but most trait-based approaches have used species mean trait values without taking into account variability within species (ITV). Although recent studies have started acknowledging the importance of ITV when making predictions about plant community assembly and ecosystem functioning in commonly measured 'soft' traits (Albert *et al.*, 2010; Violle *et al.*, 2012; Jung *et al.*, 2014; Anderegg *et al.*, 2018), knowledge regarding hydraulic traits is more limited. To bring light to this gap of knowledge, in Chapter 3 we studied how much variation was observed in a set of hydraulic, leaf and stem traits and how it was distributed among levels of organization (family, species, population) for the six dominant species of Catalonia (NE Spain) along a water availability gradient.

Specifically, we showed that trait variability was mostly distributed across the two studied families (Pinaceae and Fagaceae), which correspond to the dominant tree families in temperate forests. However, intraspecific variability contributed to a substantial amount of the total variance (from 6% to 42% depending on the trait), especially for integrative traits involving more than one organ (e.g. $K_L$ or Hv). Acclimation is a more restrictive concept than plasticity (defined as the capacity of a species to vary its trait values), as it implies an adjustment of the trait values to the environment, so that performance is maintained (or improved) under different environmental conditions. Thus, trait plasticity does not necessarily imply acclimation because within species traits can also vary due to other factors, such as ontogeny (Spasojevic *et al.*, 2014). In Chapter 3 we also tested how stem and leaf traits varied along a water availability gradient within and across species. We found that trait-environment relationships were scale-dependent and that relationships across species were generally stronger than within species. Across species, wetter sites were associated with species with leaf traits related to acquisitive resource use strategies and showing a higher vulnerability to drought. However, intraspecific adjustments along a water availability gradient relied more on changes in allocation (Hv) and leaf tolerance to low water potentials ($P_{tlp}$) than on changes in xylem safety or efficiency.

These results highlight the importance to determine the relative extent of ITV for a wide set of traits in a given study system and geographical context, to enhance our understanding of the plants' capacity to buffer against environmental changes. Thus, many more studies are needed to identify and describe patterns in plasticity and acclimation of traits, especially for 'hard' traits such as the ones related to plant hydraulics, for which no global compilation exists. This will allow us to better understand how traits acclimate in response to different environmental factors (temperature, $CO_2$, nutrients, light, etc.) and which traits are more plastic and which are less labile. Besides a better quantification of ITV, further research efforts should also test how this variation impacts on community assembly and ecosystem processes (e.g. Jung *et al.*, 2010), to determine in what cases ITV can ultimately be safely ignored and when should be included.

✻ **In sum, according to the data gathered during this research, we showed the ITV was especially relevant for integrative traits that involve more than one organ. Accounting for ITV is a necessary step forward towards improving our understanding of plant adjustments to environmental changes.**

## Can we predict individual growth rates from traits?

Despite the fact that the understanding of the relationships between traits and individual performance (growth, fecundity and survival) is still very limited, the term 'functional traits', which implies such an association, is widely used. Thus, another trait-based foundational assumption that I examined during this thesis is the expectation that traits determine growth rates. In Chapter 4, we found that traits were better predictors of growth efficiency (basal area increment per unit of total tree leaf area, GE) than absolute growth (basal area increment, BAI), highlighting that a re-evaluation of the variables that better reflect whole-tree performance can greatly complement our understanding of trait-growth relationships. Similar to my earlier findings when analyzing trait changes along the same water availability gradient (Chapter 3), also here significant relationships were largely driven by differences among species means. Specifically, BAI was negatively associated with wood density and hydraulic efficiency ($K_L$) across species, while traits related to conservative leaf resource use and to drought tolerance enhanced species GE. Interestingly, composite trait metrics defined by taking into account trait coordination better predicted growth rates and generally confirmed previous individual trait-growth relationships.

On the other hand, in Chapter 3 we showed that tree size and climate (in terms of precipitation over potential evapotranspiration, P/PET) affected trait variability along the sampled water availability gradient. In Chapter 4, we examined how these same variables affected trait-growth associations. We did not find a direct effect of P/PET on growth metrics, but only indirect effects of climatic water availability via their effect on traits, stand structure and tree size. These results highlight the importance of the ecological context when interpreting trait-growth relationships. Interestingly, Hv and $P_{tlp}$ (i.e., the traits that adjusted within species along the water availability gradient, Chapter 3), showed contrasted responses when evaluating their effect on growth rates: while a positive relationship was found between Hv and GE across and within species, $P_{tlp}$ did not show any significant relationship with growth (Chapter 4). It has been argued that acclimation in traits allows plants from the same species to maintain or improve their performance under contrasting environmental conditions (Sultan, 2000; Valladares *et al.*, 2007). Although we did not explicitly test this hypothesis, we speculate that in our study system plants adjusted $P_{tlp}$ and Hv to mitigate or even over-compensate the negative effects of water scarcity on growth. This fact could partially explain why $P_{tlp}$ variation within species along the water availability gradient did not contribute to explain intraspecific growth variation, and also that higher allocation to sapwood area relative to leaf area (high Hv) within species, associated with drier sites (Chapter 3), enhanced individual growth efficiency (Chapter 4). It is also possible that trait adjustments along the environmental gradient studied had an effect on performance components not considered in this thesis (survival or fecundity).

We argue that expecting that one single trait will predict individual performance across temporal, spatial and taxonomic scales is pretentious. Firstly, our results stressed that understanding the role of traits on growth will benefit by moving from single-trait approaches to a whole-tree approach, in which a wider set of traits are integrated. However, it should be noted that the fact that the main axes of plant variation do not necessarily translate into main plant 'functional' axes, together with the evidence that different trait combinations can provide similar individual fitness in a given environment (Marks & Lechowicz, 2006), may limit our interpretations. Secondly, traits and their associated functions are invariably context-specific. Thus, adding environmental data into analyses can be an essential step towards a more predictive discipline (Yang *et al.*, 2018). Therefore, we should improve our efforts by selecting traits that are closely tied with the main environmental drivers in the study system of interest, although they can be more technically difficult to obtain. Last but not least, a very recent paper discusses the term 'function' in ecology from a coral reef perspective. They suggest that a better definition of the word 'function' would be simply anything that relates to 'the movement or storage of energy or material', because it makes all aspects of functioning as part of a functional continuum, but consistently coupled to the process-based unifier of material fluxes (Bellwood *et al.*, 2019). Thus, perhaps it is time to step back a bit in order to have a closer look to the conceptual definition of 'function' in trait-based studies and limit its use to cases where the link between function and traits has really been tested.

❋ **In sum, we showed that our understanding of trait-growth (and by extension trait-performance) relationships can greatly improve by: (i) selecting traits closely related to physiological function and context-specific environmental drivers, (ii)**

**integrating them along common axes of variation, and (iii) re-evaluating the variables that are used to reflect whole-tree performance.**

## Final remarks

In the questions addressed throughout this dissertation, as with much of ecology, complexity abounds. We are living in times of changing conditions, where human activity hampers ecosystem predictability and continuously poses new challenges on the research agenda. Further progress will depend on combining empirical, theoretical and modelling approaches and will involve smooth collaboration across disciplines. Diversity (that is, not just scientific background, but also including aspects such as gender, ethnicity, age and economic status) will be essential for more creative solutions to tackle complex questions in ecology (Intemann, 2009; Nielsen *et al.*, 2017).

In 1896, Francis Darwin, when commenting on Dixon's cohesion-tension theory (the basis of our current knowledge of plant water transport), noted: 'To believe that columns of water should hang in the tracheals like solid bodies, and should, like them, transmit downwards the pull exerted on them at their upper ends by the transpiring leaves, is to some of us equivalent to believing in ropes of sand.' I like the idea to finish this dissertation with an example that highlights the very non-definitive nature of science. Science cannot offer completely conclusive solutions to our questions; it must always be open to the possibility that some new data defy what is established. I must confess that the notion that what you have read along these pages can be easily collapsed, has given me an appearance of fragility not always easy to manage when trying to build my first scientific principles. However, during these four years I have learned that this apparent fragility is the strength and the beauty of knowledge, which should always encourage one to acknowledge one's own ignorance and be challenged by curiosity.

**

# Part IV

# APPENDICES

# A

# Supplementary material for Chapter 2

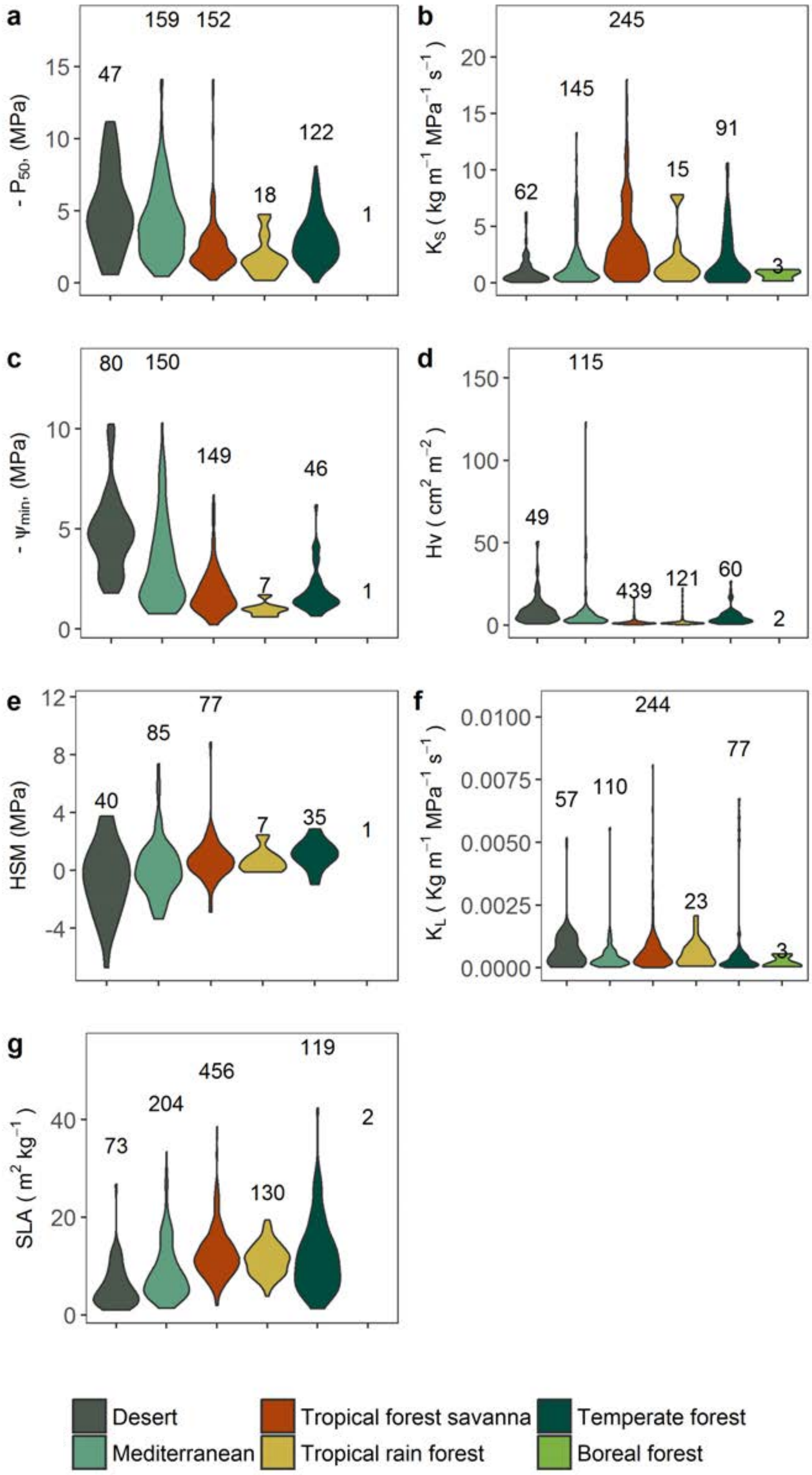

**Fig. A.1:** Frequency distribution of each trait as a function of species biome: (a) water potential at 50% loss of hydraulic conductivity ($P_{50}$), (b) xylem minimum water potential ($\psi_{min}$), (c) hydraulic safety margin (HSM), (d) stem-specific hydraulic conductivity ($K_S$), (e) Huber value (sapwood area / leaf area, Hv), and (f) leaf-specific hydraulic conductivity ($K_L$). Frequency distributions were estimated by a non-parametric kernel method.

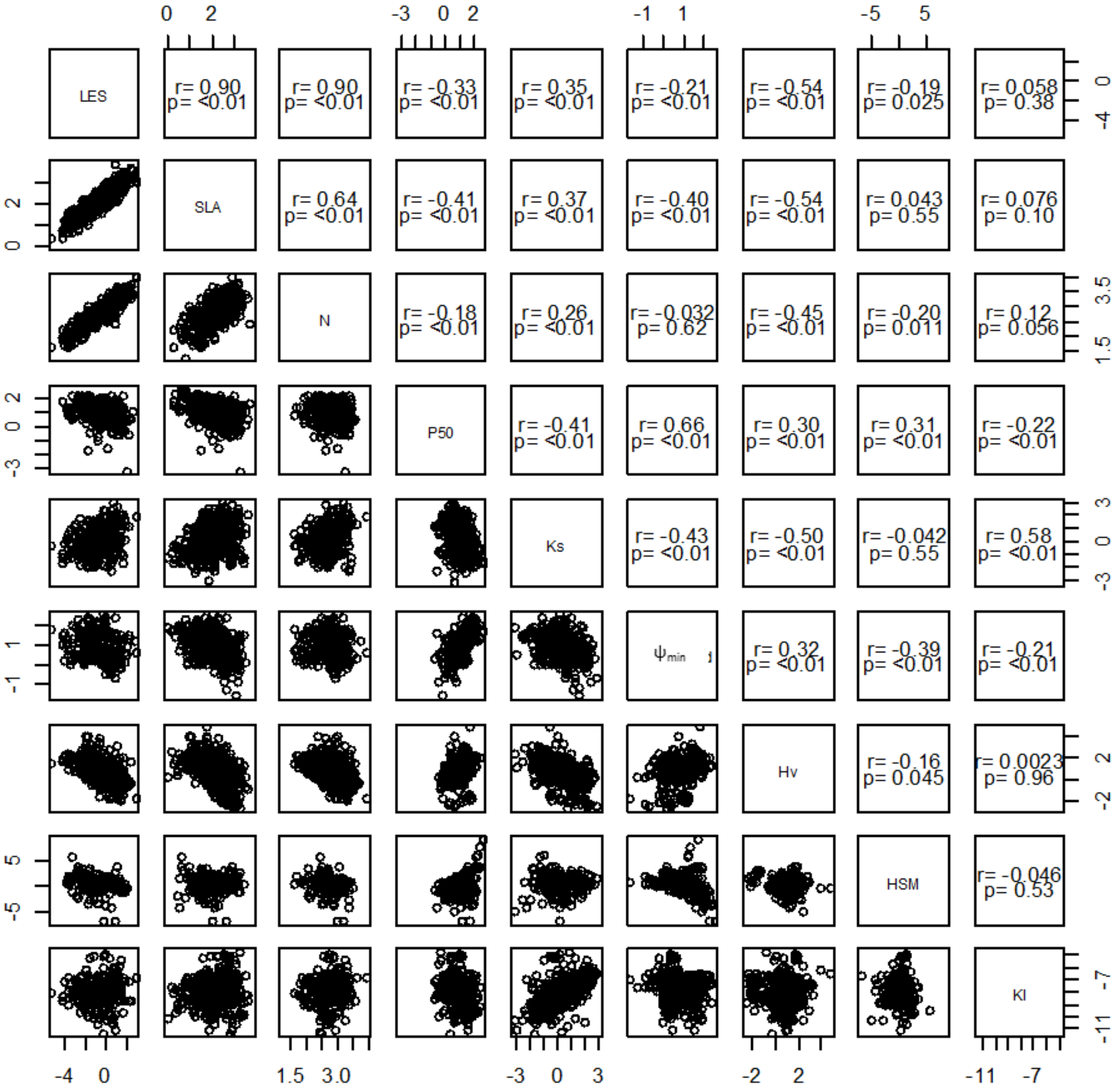

**Fig. A.2:** Bivariate relationships among all traits of interest, showing scatterplots, correlation coefficients (r) and the corresponding p-values (p). All data are natural-log transformed with the exception of HSM. $\psi_{min}$ and $P_{50}$ were previously converted to positive. Abbreviations: $P_{50}$, water potential at 50% loss of hydraulic conductivity; $K_S$, stem-specific hydraulic conductivity; $\psi_{min}$, minimum leaf water potential; Hv, Huber value (sapwood area/leaf area); HSM, hydraulic safety margin; $K_L$, leaf-specific hydraulic conductivity; SLA, specific leaf area; N, nitrogen concentration per mass; LES, new variable combining SLA and $N_{mass}$ and used as proxy for the leaf economics spectrum (see methods Chapter 2).

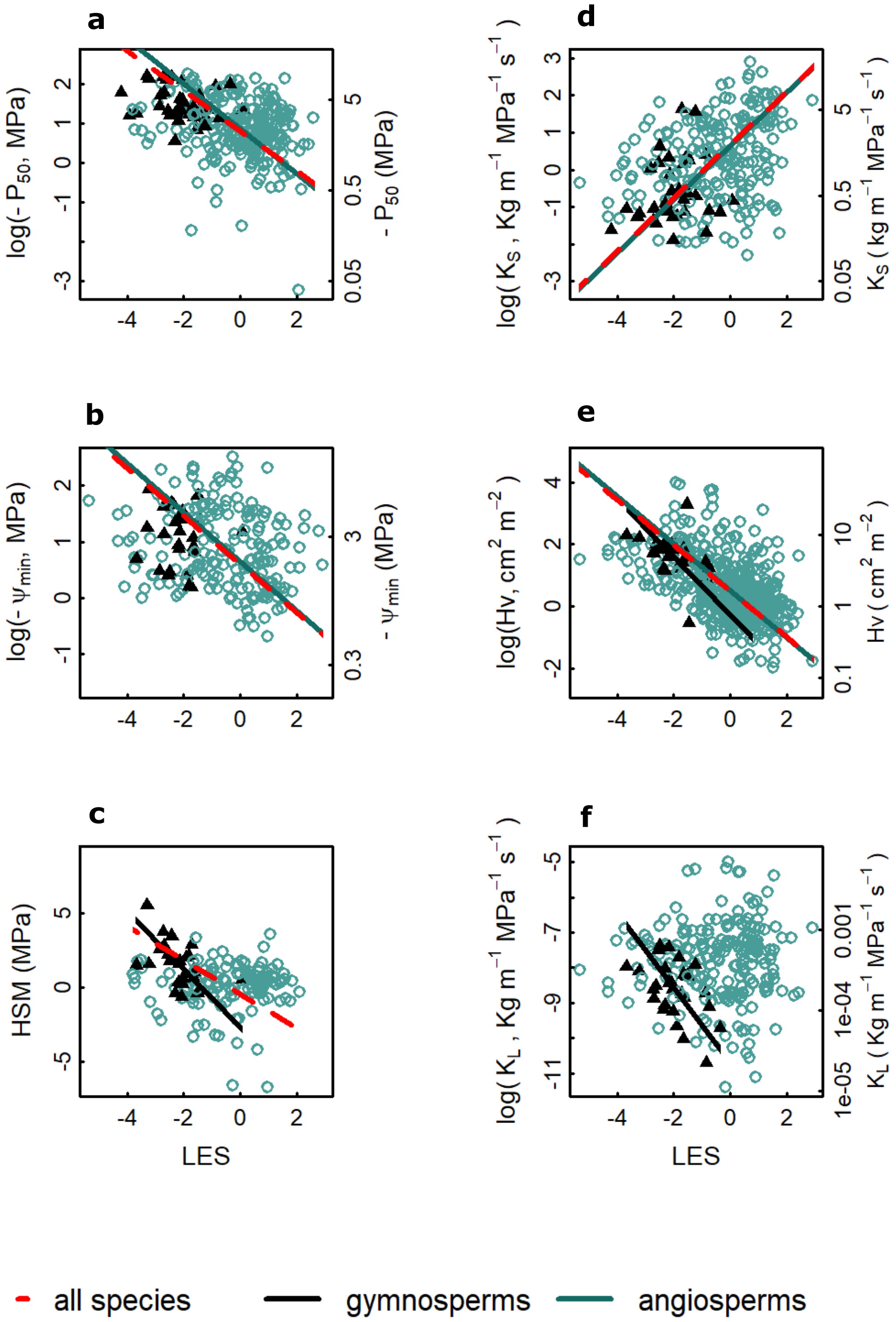

**Fig. A.3:** Relationships between the first PCA axis combining SLA and $N_{mass}$ data (LES variable, X axis) and hydraulic traits: (a) water potential at 50% loss of hydraulic conductivity ($P_{50}$), (b) xylem minimum water potential ($\psi_{min}$), (c) hydraulic safety margin (HSM), (d) stem-specific hydraulic conductivity ($K_S$), (e) Huber value (sapwood area / leaf area, Hv), and (f) leaf-specific hydraulic conductivity ($K_L$) for all species (red regression line), gymnosperms (black line and triangle symbols) and angiosperms (green line and round symbols). Only statistically significant relationships are shown ($p<0.05$). All data are natural-log transformed with the exception of HSM.

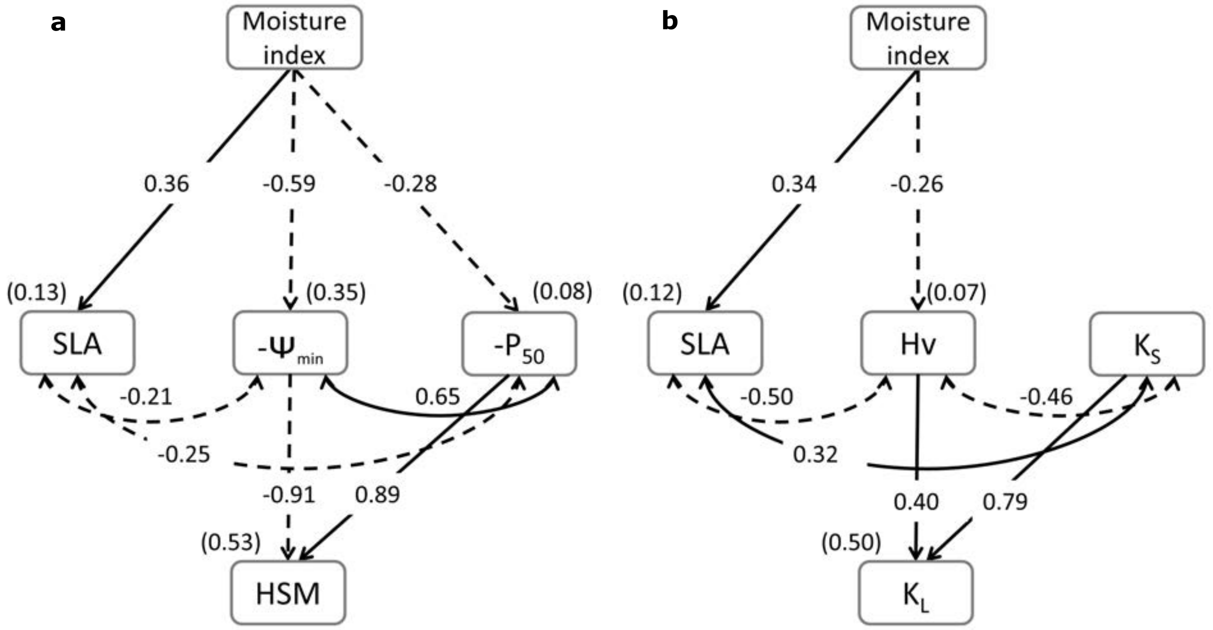

**Fig. A.4:** Path analysis relating specific leaf area (SLA), moisture index (mean annual precipitation/ mean annual potential evapotranspiration) and hydraulic traits characterizing (a) plant hydraulic safety and (b) plant hydraulic efficiency. Only the values of the path coefficients that were significant in the models are shown (standardized values). Positive effects are indicated by solid lines and negative effects by broken lines. The number in brackets over a given endogenous variable in the path diagram corresponds to the $R^2$ value, indicating the percentage of the variance in that variable that is explained by the model. The models were tested considering one index from each goodness of fit class: SRMR<0.08 (absolute fit), CFI>0.95 (fit adjusting for model parsimony) and RMSEA<0.06 (comparative fit) where SRMR=0.01, CFI=0.98, RMSEA=0.03 for both models. All variables are natural-log transformed except HSM. Abbreviations: $P_{50}$, water potential at 50% loss of hydraulic conductivity; $K_S$, stem-specific hydraulic conductivity; $\psi_{min}$, minimum leaf water potential; Hv, Huber value (sapwood area/leaf area); HSM, hydraulic safety margin; $K_L$, leaf-specific hydraulic conductivity.

| **SLA~** | | **All species** | **Gymnosperms** | **Angiosperms** |
|---|---|---|---|---|
| **$P_{50}$** | elevation | 3.35 | 2.87 | 3.59 |
| | slope | -1.08 | -0.92 | -1.17 |
| | p-value | 7.00E-18 | 6.20E-04 | 1.60E-08 |
| | $R^2$ | 0.17 | 0.22 | 0.08 |
| | n | 414 | 50 | 364 |
| **$K_S$** | elevation | -3.11 | -2.72 | -3.19 |
| | slope | 1.62 | 1.41 | 1.65 |
| | p-value | 2.63E-16 | 0.08 | 3.94E-10 |
| | $R^2$ | 0.13 | 0.07 | 0.09 |
| | n | 468 | 44 | 424 |
| **$\Psi_{min}$** | elevation | 2.9 | 2.7 | 2.98 |
| | slope | -1.02 | -1.14 | -1.04 |
| | p-value | 2.14E-16 | 0.19 | 7.01E-15 |
| | $R^2$ | 0.16 | 0.06 | 0.16 |
| | n | 387 | 28 | 359 |
| **Hv** | elevation | 4.77 | 3.68 | 4.89 |
| | slope | -1.89 | -1.44 | -1.93 |
| | p-value | 1.52E-62 | 0.02 | 8.10E-55 |
| | $R^2$ | 0.29 | 0.19 | 0.26 |
| | n | 820 | 26 | 794 |
| **HSM** | elevation | -4.85 | 5.82 | -5.39 |
| | slope | 2.49 | -2.97 | 2.53 |
| | p-value | 0.55 | 0.01 | 4.14E-04 |
| | $R^2$ | 0 | 0.23 | 0.07 |
| | n | 199 | 27 | 172 |
| **$K_L$** | elevation | -11.31 | -6.38 | -11.41 |
| | slope | 1.58 | -1.51 | 1.62 |
| | p-value | 0.1 | 0.14 | 0.45 |
| | $R^2$ | 0.01 | 0.07 | 0 |
| | n | 460 | 31 | 429 |

**Table A.1:** Standardised Major Axis regression (SMA) slopes and intercepts of specific leaf area (SLA) against water potential at 50% loss of hydraulic conductivity ($P_{50}$), stem-specific hydraulic conductivity ($K_S$), minimum xylem water potential ($\psi_{min}$), Huber value (sapwood area / leaf area, Hv), hydraulic safety margin (HSM) and leaf-specific hydraulic conductivity ($K_L$) for all species, gymnosperms and angiosperms, respectively. Statistical significance, sample size (n) and adjusted-$R^2$ of each model are also shown. All data were natural-log transformed before analysis, with the exception of HSM.

| | | **Comp.1 (LES)** | **Comp.2** |
|---|---|---|---|
| Standard deviation | | 1.28 | 0.61 |
| Proportion of Variance | | 0.81 | 0.19 |
| Loadings | | | |
| | *SLA* | 0.7 | -0.7 |
| | *N* | 0.7 | 0.7 |

**Table A.2:** Summary of the Principal Components Analysis (PCA) used to reduce the bivariate variation in SLA and $N_{mass}$ onto two orthogonal axes enabling the use of species' scores along the first axis to create a new variable (LES) as a representative proxy for the LES.

| **LES~** | | **All species** | **Gymnosperms** | **Angiosperms** |
|---|---|---|---|---|
| **$P_{50}$** | elevation | 0.8 | 0.53 | 0.87 |
| | slope | -0.51 | -0.45 | -0.57 |
| | p-value | 1.94E-08 | 0.17 | 6.12E-04 |
| | $R^2$ | 0.11 | 0.05 | 0.05 |
| | n | 277 | 39 | 238 |
| **$K_S$** | elevation | 0.68 | 1.32 | 0.65 |
| | slope | 0.72 | 0.96 | 0.72 |
| | p-value | 1.32E-05 | 0.22 | 2.53E-05 |
| | $R^2$ | 0.13 | 0.04 | 0.08 |
| | n | 244 | 35 | 209 |
| **$\psi_{min}$** | elevation | 0.6 | -0.44 | 0.65 |
| | slope | -0.43 | -0.7 | -0.44 |
| | p-value | 2.39E-03 | 0.87 | 7.21E-03 |
| | $R^2$ | 0.04 | 0 | 0.04 |
| | n | 210 | 26 | 184 |
| **Hv** | elevation | 0.48 | -0.29 | 0.5 |
| | slope | -0.74 | -0.94 | -0.76 |
| | p-value | 6.29E-47 | 0.05 | 2.72E-38 |
| | $R^2$ | 0.29 | 0.16 | 0.25 |
| | n | 606 | 24 | 582 |
| **HSM** | elevation | -0.45 | -2.73 | 0.5 |
| | slope | -1.15 | -1.97 | 1.18 |
| | p-value | 0.02 | 0.012 | 0.93 |
| | $R^2$ | 0.04 | 0.24 | 0 |
| | n | 144 | 25 | 119 |
| **$K_L$** | elevation | -7.49 | -10.72 | -8.13 |
| | slope | 0.75 | -1.07 | -0.77 |
| | p-value | 0.38 | 0.01 | 0.97 |
| | $R^2$ | 0 | 0.21 | 0 |
| | n | 238 | 28 | 210 |

**Table A.3:** Standardised Major Axis regression (SMA) slopes and intercepts of the LES axis against water potential at 50% loss of hydraulic conductivity ($P_{50}$), stem-specific hydraulic conductivity ($K_S$), minimum xylem water potential ($\psi_{min}$), Huber value (sapwood area / leaf area, Hv), hydraulic safety margin (HSM) and leaf-specific hydraulic conductivity ($K_L$) for all species, gymnosperms and angiosperms, respectively. Statistical significance, sample size (n) and adjusted-$R^2$ of each model are also shown. All data were natural-log transformed before analysis, with the exception of HSM.

| | **$P_{50}$** | | | **$K_S$** | |
|---|---|---|---|---|---|
| term | Estimate | p.value | term | Estimate | p.value |
| (Intercept) | 2.28 | 3.97E-13 | (Intercept) | 2.07 | 6.18E-07 |
| P50 | -0.24 | 1.38E-08 | Ks | 0.13 | 9.20E-07 |
| lambda | 0.73 ( 0.57 , 0.84 ) | | lambda | 0.87 ( 0.78 , 0.92 ) | |
| n obs./ $R^2$ | 330 / 0.09 | | n obs./ $R^2$ | 321 / 0.07 | |
| | **$\psi_{min}$** | | | **Hv** | |
| term | Estimate | p.value | term | Estimate | p.value |
| (Intercept) | 2.2 | 9.38E-10 | (Intercept) | 2.11 | 4.23E-10 |
| $\psi_{min}$ | -0.32 | 7.08E-10 | Hv | -0.21 | 0 |
| lambda | 0.77 ( 0.62 , 0.87 ) | | lambda | 0.85 ( 0.77 , 0.91 ) | |
| n obs./ $R^2$ | 256 / 0.14 | | n obs./ $R^2$ | 464 / 0.2 | |
| | **HSM** | | | **$K_L$** | |
| term | Estimate | p.value | term | Estimate | p.value |
| (Intercept) | 1.77 | 1.78E-09 | (Intercept) | 1.83 | 2.09E-09 |
| HSM | 0.07 | 0.02 | Kl | -0.02 | 0.56 |
| lambda | 0.6 ( 0.35 , 0.8 ) | | lambda | 0.76 (0.62, 0.85) | |
| n obs./ $R^2$ | 150 / 0.04 | | n obs./ $R^2$ | 460/ 0 | |

**Table A.4:** Summary of the phylogenetic generalized linear models (PGLS) of specific leaf area (SLA) as a function of water potential at 50% loss of hydraulic conductivity ($P_{50}$), stem-specific hydraulic conductivity ($K_S$), minimum xylem water potential ($\psi_{min}$), Huber value (sapwood area / leaf area, Hv), hydraulic safety margin (HSM) and leaf-specific hydraulic conductivity ($K_L$). Sample size (n obs.), adjusted-$R^2$ and the value of lambda (a measure of the phylogenetic effect on trait evolution) are also provided for each model. All data were natural-log transformed before analysis, with the exception of HSM.

|  | quantiles | elevation | slope | $R^2$ | p-value | %sig.p-value |
|---|---|---|---|---|---|---|
| **$P_{50}$** | 2.50% | 3.23 | -1.11 | 0.12 | 1.85E-18 |  |
|  | 50% | 3.33 | -1.07 | 0.15 | 9.79E-16 |  |
|  | 97.50% | 3.42 | -1.03 | 0.17 | 5.20E-13 | 100 |
| **$\psi_{min}$** | 2.50% | 2.8 | -1.05 | 0.12 | 6.73E-17 |  |
|  | 50% | 2.9 | -1.01 | 0.14 | 1.59E-14 |  |
|  | 97.50% | 2.98 | -0.97 | 0.17 | 2.16E-12 | 100 |
| **HSM** | 2.50% | -8.65 | -4.09 | 0 | 0.06 |  |
|  | 50% | -6.61 | 3.33 | 0 | 0.56 |  |
|  | 97.50% | 8.83 | 4.26 | 0.02 | 0.97 | 2.1 |
| **$K_S$** | 2.50% | -3.3 | 1.57 | 0.1 | 5.83E-17 |  |
|  | 50% | -3.15 | 1.64 | 0.12 | 3.99E-14 |  |
|  | 97.50% | -3 | 1.71 | 0.14 | 6.85E-12 | 100 |
| **Hv** | 2.50% | 4.51 | -1.92 | 0.22 | 1.09E-57 |  |
|  | 50% | 4.68 | -1.85 | 0.24 | 2.43E-51 |  |
|  | 97.50% | 4.85 | -1.78 | 0.27 | 6.06E-46 | 100 |
| **$K_L$** | 2.50% | -16.13 | -3.71 | 0 | 0.01 |  |
|  | 50% | -15.47 | 3.49 | 0 | 0.44 |  |
|  | 97.50% | 0.24 | 3.77 | 0.01 | 0.96 | 8.2 |

**Table A.5:** Summary of the resampling results (n=1000) assessing the impact of trait plasticity (within species) on bivariate trait relationships. The resulting distribution of Standardised Major Axis regression (SMA) slopes and intercepts are reported for the relationships between specific leaf area (SLA) and water potential at 50% loss of hydraulic conductivity ($P_{50}$), stem-specific hydraulic conductivity ($K_S$), minimum xylem water potential ($\psi_{min}$), Huber value (sapwood area / leaf area, Hv), hydraulic safety margin (HSM) and leaf-specific hydraulic conductivity ($K_L$). Statistical significance, adjusted-$R^2$ and the percentage of significant resampling events (%sig.pval) of each model are also shown. All data were natural-log transformed before analysis, with the exception of HSM.

**

# B

# Supplementary material for Chapter 3

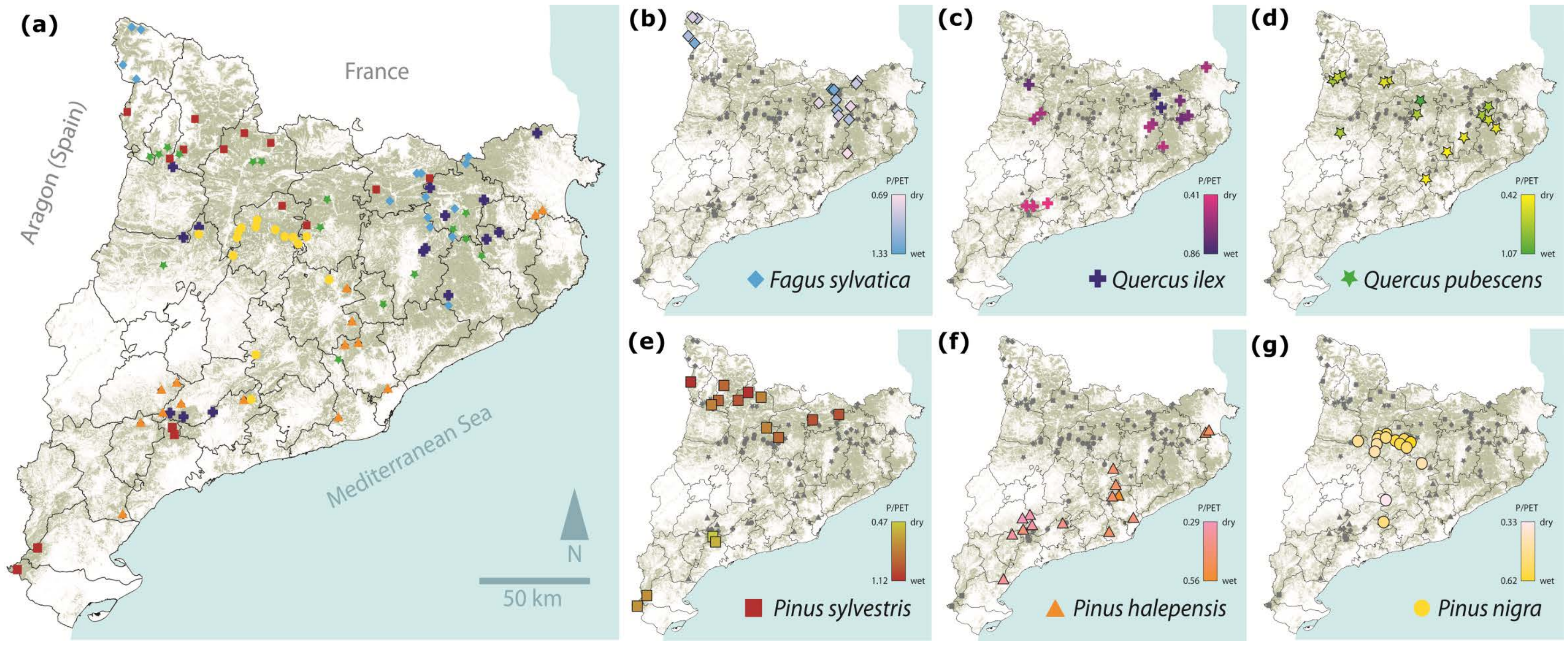

**Fig. B.1:** Map of Catalonia showing the distribution of (a) all the 90 sampling plots along the water availability gradient (precipitation to potential evapotranspiration ratio, P/PET) where different symbols indicate dominant species at each plot and (b-g) separately for each dominant species in the plot. *Fagus sylvatica*: blue diamond; *Quercus ilex*: purple cross; *Quercus pubescens*: green star; *Pinus sylvestris*: red square; *Pinus halepensis*: orange triangle and *Pinus nigra*: yellow circle.

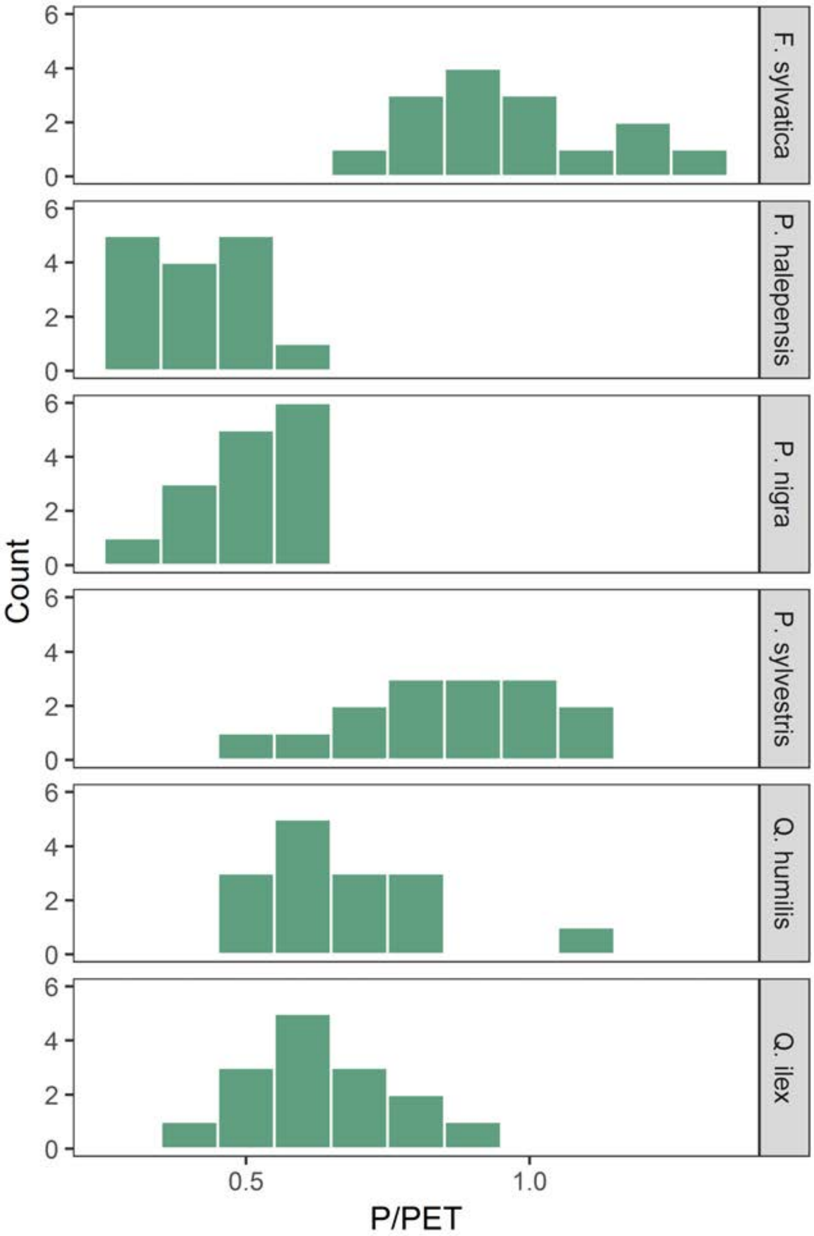

**Fig. B.2:** Distributions of the precipitation to potential evapotranspiration ratio (P/PET) plot values for each sampled species.

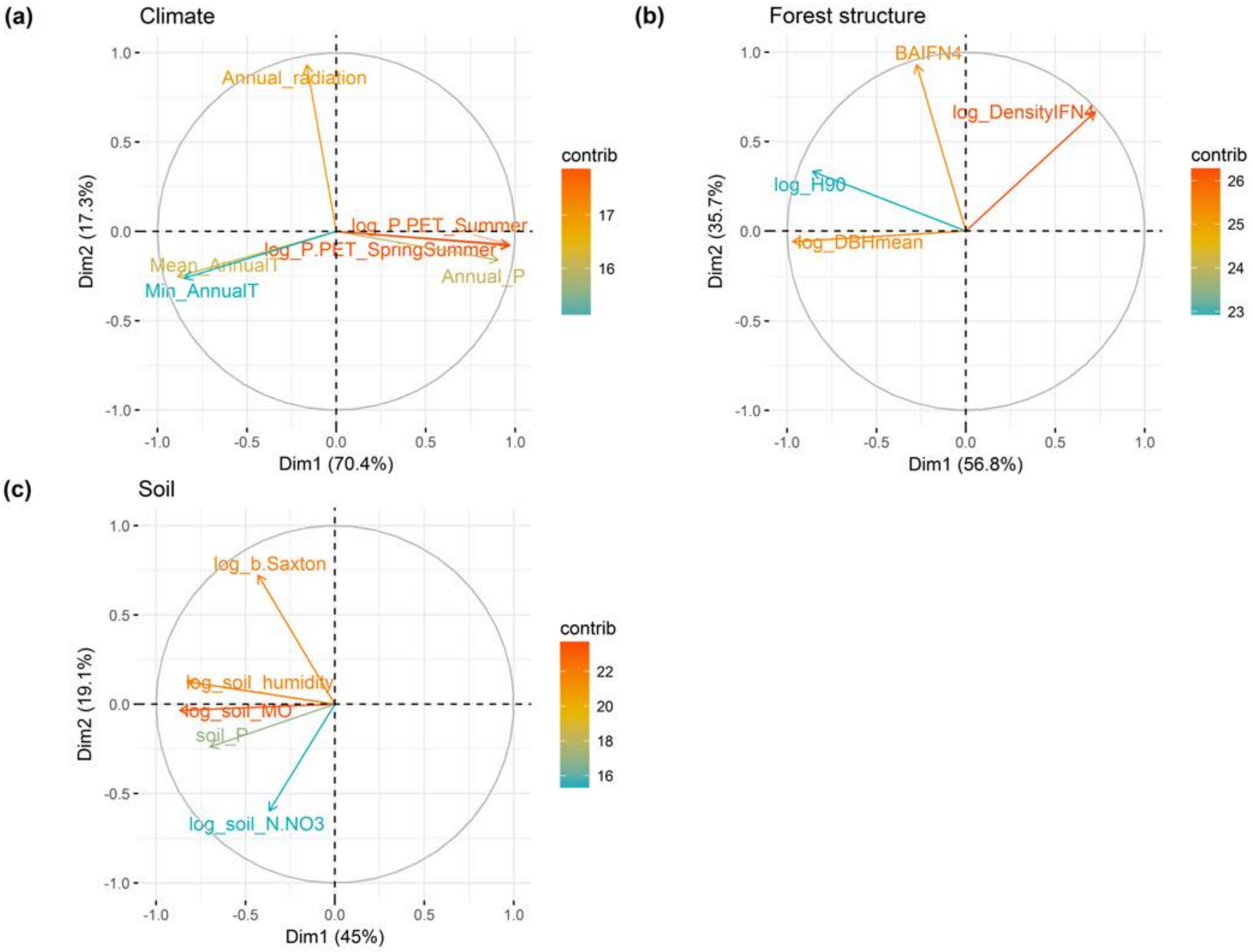

**Fig. B.3:** Principal component analysis (PCA) summarizing environmental variables at the plot level related with (a) climate, (b) forest structure and (c) soil characteristics. The percentages in brackets in the axis labels indicate the variance explained by each axis. Redder colors denote a higher loading on the axes. Environmental variables: Annual radiation; Mean_AnnualT, mean annual temperature; Min_AnnualT, minimum annual temperature; Annual_P, annual precipitation; P/PET from spring-summer, spring-summer precipitation to potential evapotranspiration ratio; P/PET_summer, summer precipitation to potential evapotranspiration ratio; DBHmean, plot mean diameter at the breast height; ABIFN4, total plot basal area; H90, plot mean quantile 0.9 tree height; DensityIFN4, total plot number of stems per ha; b.Saxton, b Saxton coefficient; soil P, soil phosphorus content; soil_N.NO3 , soil N-$NO_3$ content; soil_P, soil phosphorus content; soil humidity; soil_MO, soil organic matter fraction. Variables were natural-log transformed whenever required to satisfy the normality assumptions.

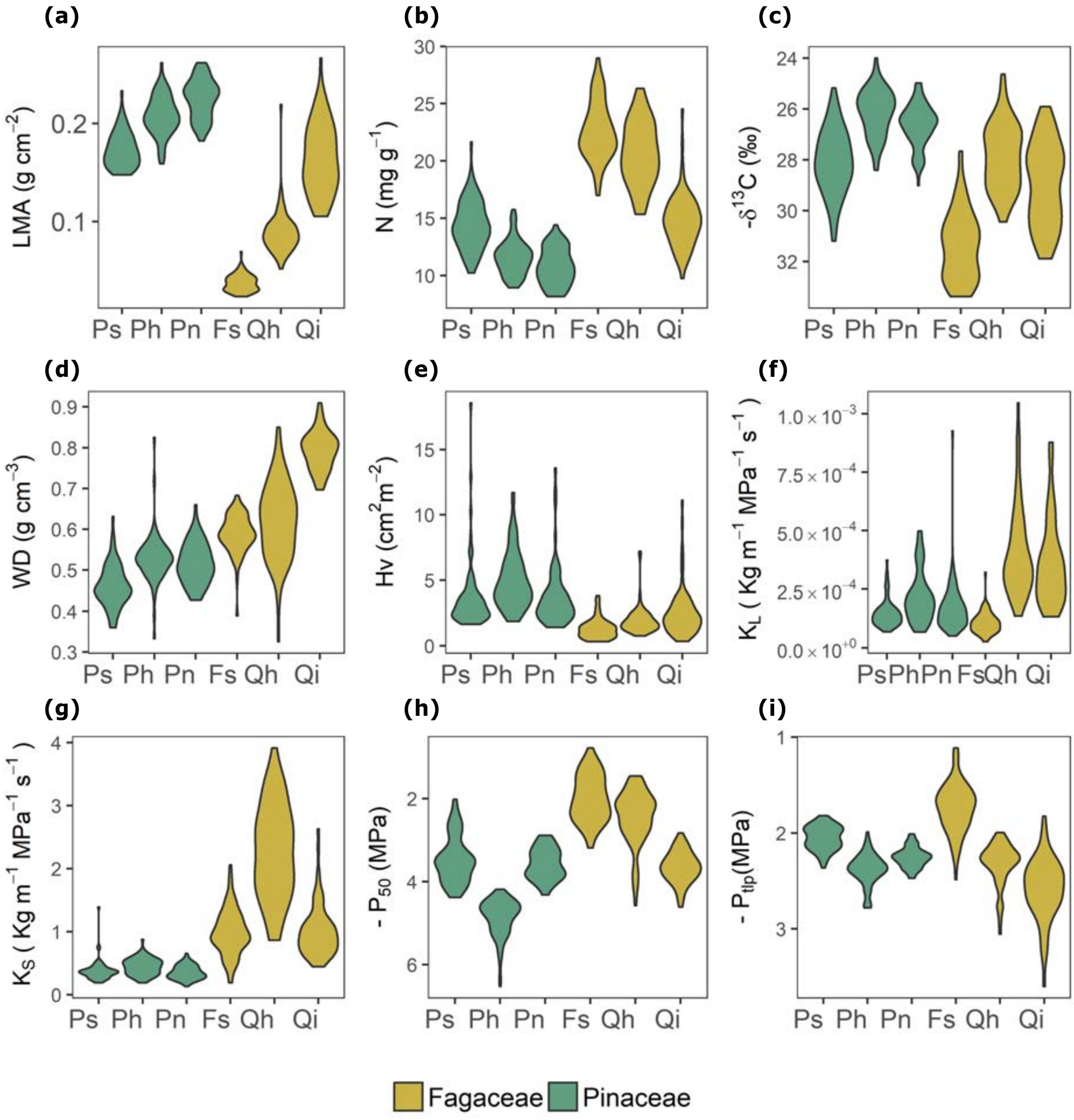

**Fig. B.4:** Frequency distribution of each trait estimated by a non-parametric kernel method as a function of species and family (Pinaceae vs Fagaceae): (a) leaf mass per area (LMA), (b) leaf nitrogen concentration (N), (c) leaf carbon isotope discrimination ($\delta^{13}$C), (d) wood density (WD), (e) Huber value (sapwood area / leaf area, Hv), (f) leaf-specific hydraulic conductivity ($K_L$), (g) stem-specific hydraulic conductivity ($K_S$), (h) the water potential at 50% loss of hydraulic conductivity ($P_{50}$) and (i) the water potential at turgor loss point ($P_{tlp}$). The shape represents all possible trait values; with thickness indicating how common they are (the thickest section represents the mode average). Abbreviations: Fs, *Fagus sylvatica*; Qi, *Quercus ilex*; Qh, *Quercus humilis*; Ps, *Pinus sylvestris*; Ph, *Pinus halepensis*; Pn, *Pinus nigra*.

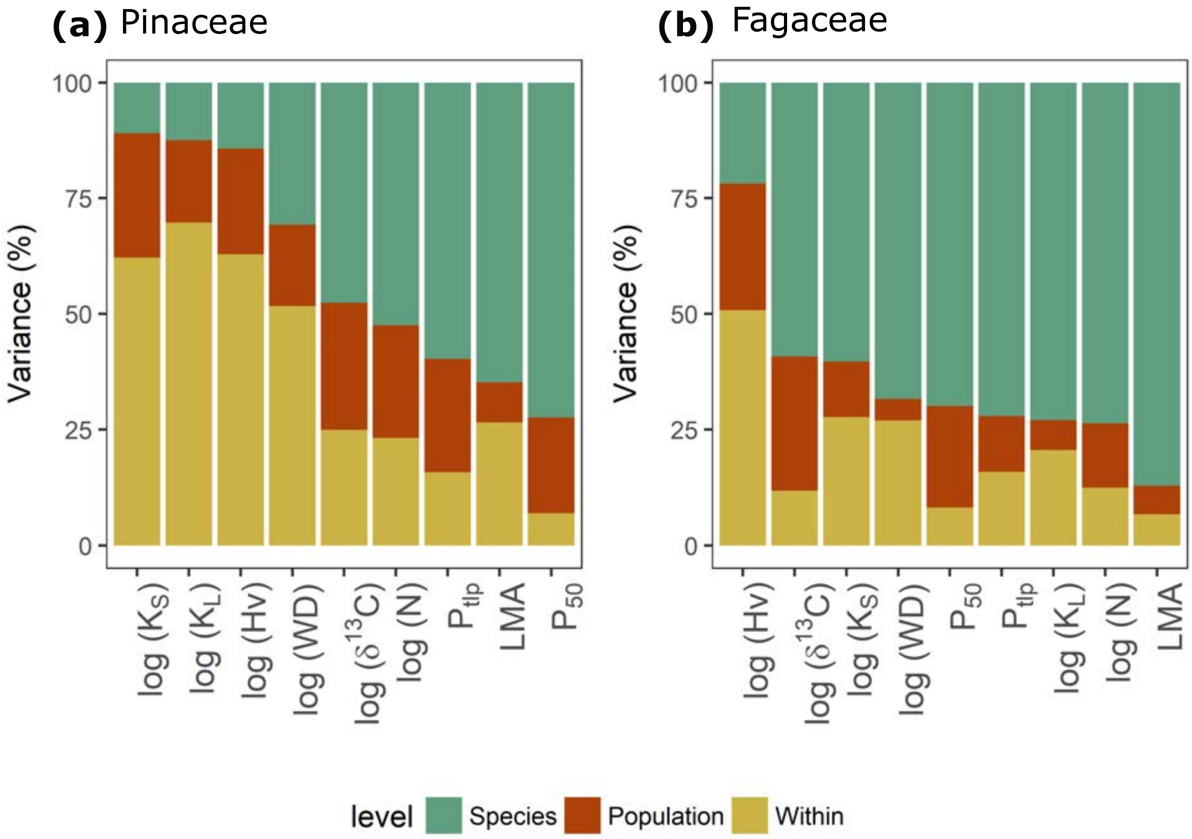

**Fig. B.5:** Variance partitioning within (a) Pinaceae and (b) Fagaceae families. 'Within' denotes variance between individuals of the same population. Abbreviations: LMA, leaf mass per area; N, leaf nitrogen concentration; $\delta^{13}$C , leaf carbon isotope discrimination; WD, wood density; Hv, Huber value (sapwood area / leaf area); $K_L$, leaf-specific hydraulic conductivity; $K_S$, stem-specific hydraulic conductivity; $P_{50}$, the water potential at 50% loss of hydraulic conductivity; $P_{tlp}$, the water potential at turgor loss point.

| | P/PET percentil | DBH (cm) | | Height (m) | | Total plot basal area ($m^2$ $ha^{-1}$) | | Total area in the study region | |
|---|---|---|---|---|---|---|---|---|---|
| | | *mean* | *SD* | *mean* | *SD* | *mean* | *SD* | (ha) | % |
| ***P. sylvestris*** | < 33 | 21.84 | 4.44 | 14.82 | 2.60 | 31.50 | 4.26 | 219754 | 18.4 |
| | 33-66 | 25.37 | 3.60 | 15.38 | 2.89 | 33.23 | 9.05 | | |
| | > 66 | 27.60 | 9.34 | 16.04 | 3.58 | 36.70 | 8.30 | | |
| ***P. nigra*** | < 33 | 19.37 | 1.70 | 13.60 | 2.11 | 19.94 | 5.32 | 140627 | 11.8 |
| | 33-66 | 19.40 | 1.72 | 12.15 | 2.60 | 24.69 | 5.01 | | |
| | > 66 | 20.33 | 3.39 | 15.05 | 3.29 | 30.39 | 9.34 | | |
| ***P. halepensis*** | < 33 | 23.08 | 4.91 | 13.78 | 3.39 | 22.96 | 8.15 | 239092 | 20 |
| | 33-66 | 26.65 | 4.60 | 15.07 | 3.52 | 27.90 | 10.49 | | |
| | > 66 | 27.00 | 5.26 | 16.55 | 2.47 | 25.09 | 6.62 | | |
| ***F. sylvatica*** | < 33 | 23.69 | 3.26 | 25.58 | 3.79 | 33.71 | 5.04 | 28726 | 2.4 |
| | 33-66 | 32.50 | 12.74 | 26.94 | 8.31 | 34.87 | 7.36 | | |
| | > 66 | 37.38 | 12.33 | 28.04 | 8.69 | 43.73 | 12.13 | | |
| ***Q. humilis*** | < 33 | 18.00 | 8.61 | 13.58 | 3.05 | 21.00 | 10.49 | 75000 | 6.3 |
| | 33-66 | 22.46 | 4.90 | 16.02 | 3.85 | 21.81 | 8.39 | | |
| | > 66 | 23.34 | 4.17 | 16.78 | 4.17 | 24.74 | 9.64 | | |
| ***Q. ilex*** | < 33 | 17.76 | 3.86 | 11.49 | 3.58 | 28.78 | 9.96 | 184654 | 15.5 |
| | 33-66 | 14.35 | 1.22 | 9.33 | 2.20 | 25.26 | 7.55 | | |
| | > 66 | 15.39 | 1.24 | 11.26 | 1.85 | 37.18 | 6.78 | | |

**Table B.1:** Characterization of the dominant species in the study plots (n = 5 plots per species and P/PET percentile). The percentage of ha for each studied species is relative to the total forest area in Catalonia.

| | N | SD | mean | median | range | range Pinaceae | range Fagaceae | CV* | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **LMA** | 449 | 0.07 | 0.15 | 0.17 | 0.02-0.27 | 0.15-0.26 | 0.02-0.27 | 46.46 | 0.09 | 0.21 | 40.97 |
| **N** | 449 | 4.91 | 15.81 | 14.63 | 8.18-28.97 | 8.18-21.65 | 9.75-28.97 | 31.34 | 11.86 | 19.94 | 25.40 |
| **-$\delta^{13}$C** | 449 | 2.10 | 28.16 | 27.77 | 24.00-33.37 | 24.00-31.19 | 24.63-33.37 | 7.32 | 26.54 | 29.54 | 5.35 |
| **WD** | 449 | 0.12 | 0.58 | 0.56 | 0.33-0.91 | 0.33-0.83 | 0.33-0.91 | 20.49 | 0.50 | 0.65 | 13.20 |
| **Hv** | 449 | 2.25 | 3.05 | 2.45 | 0.32- 18.58 | 1.42-18.58 | 0.32-11.11 | 76.38 | 1.65 | 3.75 | 38.95 |
| **$K_L$** | 444 | 1.70E-04 | 2.37E-04 | 1.81E-04 | 2.62e-05- 1.05e-03 | 5.05e-05-9.27e-04 | 2.62e-05-1.05e-03 | 71.41 | 1.20E-04 | 3.03E-04 | 43.24 |
| **$K_S$** | 449 | 0.77 | 0.91 | 0.57 | 0.13-3.92 | 0.13-1.39 | 0.19-3.92 | 86.89 | 0.37 | 1.15 | 51.67 |
| **-$P_{50}$** | 449 | 1.05 | 3.34 | 3.45 | 0.78-6.53 | 2.02-6.53 | 0.78-4.62 | 31.41 | 2.55 | 4.00 | 22.19 |
| **-$P_{tlp}$** | 430 | 0.34 | 2.21 | 2.24 | 1.11-3.60 | 1.82-2.78 | 1.11-3.60 | 15.23 | 2.03 | 2.38 | 8.02 |

**Table B.2:** Statistical descriptors of studied traits. Leaf mass per area (LMA, g cm$^{-2}$), leaf nitrogen concentration (N, mg g$^{-1}$), leaf carbon isotope discrimination ($\delta^{13}$C, ‰), wood density (WD, g cm$^{-3}$), Huber value (sapwood area / leaf area, Hv; cm$^2$ m$^{-2}$), leaf-specific hydraulic conductivity ($K_L$, kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$), stem-specific hydraulic conductivity ($K_S$, kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$), the water potential at 50% loss of hydraulic conductivity ($P_{50}$, MPa) and the water potential at turgor loss point ($P_{tlp}$, MPa). Abbreviations: N, sample size; SD, standard deviation; CV, coefficient of variation; Q1, first quartile; Q3, third quartile; IQR, interquartile range, *CV for natural-log transformed data was calculated following Canchola *et al.* (2017).

|  | LMA | log (N) | log( -$\delta^{13}$C) | log (WD) | Hv | $K_L$ | $K_S$ | -$P_{50}$ | -$P_{tlp}$ |
|---|---|---|---|---|---|---|---|---|---|
| **Fixed Parts** |  |  |  |  |  |  |  |  |  |
| Soil.PCA1 | -0.04 | n.i. | n.i. | -0.08 | -0.12 | 0.06 | n.i. | 0.12 | n.i. |
|  | (-0.08 – 0.01) |  |  | (-0.15 – -0.01) | (-0.23 – -0.01) | (-0.04 – 0.16) |  | (0.00 – 0.23) |  |
| Soil.PCA2 |  |  |  |  |  | 0 |  |  |  |
|  |  |  |  |  |  | (-0.10 – 0.10) |  |  |  |
| Structure.PCA1 | **0.11** | **-0.14** | **-0.2** | 0.08 | 0.16 | 0.13 | 0.05 | n.i. | n.i. |
|  | **(0.06 – 0.17)*** | **(-0.24 – -0.05)*** | **(-0.33 – -0.08)*** | (-0.00 – 0.16) | (0.03 – 0.29) | (0.01 – 0.25) | (-0.03 – 0.14) |  |  |
| Structure.PCA2 | **-0.06** | 0.07 | 0.09 |  |  | -0.01 |  |  |  |
|  | **(-0.11 – -0.01)*** | (-0.02 – 0.15) | (-0.02 – 0.20) |  |  | (-0.12 – 0.10) |  |  |  |
| Climate.PCA1 | n.i. | n.i. | 0.18 | -0.12 | **-0.22** | -0.14 | -0.06 | -0.11 | **-0.39** |
|  |  |  | (0.02 – 0.35) | (-0.24 – -0.01) | **(-0.39 – -0.06)*** | (-0.30 – 0.03) | (-0.17 – 0.05) | (-0.28 – 0.06) | **(-0.56 – -0.23)**** |
| Climate.PCA2 | **0.07** | 0.06 | **-0.23** | -0.07 | n.i. | 0.05 | n.i. | n.i. | 0.09 |
|  | **(0.02 – 0.11)*** | (-0.02 – 0.14) | **(-0.34 – -0.13)**** | (-0.14 – 0.00) |  | (-0.06 – 0.16) |  |  | (-0.02 – 0.19) |
| **Random Parts** |  |  |  |  |  |  |  |  |  |
| $\sigma^2$ | 0 | 0.009 | 0.001 | 0.009 | 0.178 | 0.153 | 0.087 | 0.009 | 0.024 |
| τ00, PLOT:SP | 0 | 0.008 | 0.001 | 0.002 | 0.063 | 0.041 | 0.037 | 0.028 | 0.017 |
| τ00, SP | 0.004 | 0.083 | 0.002 | 0.034 | 0.148 | 0.192 | 0.547 | 0.092 | 0.048 |
| NPLOT:SP | 89 | 89 | 89 | 89 | 88 | 89 | 89 | 89 | 87 |
| NSP | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Observations | 444 | 444 | 444 | 444 | 439 | 444 | 444 | 444 | 425 |

**Table B.3:** Results of the linear mixed models examining the relationships between environmental variables using the PCA axis and traits. The model's standardized coefficients including confidence intervals (in brackets) are shown. Significant correlations (* $P<0.05$, ** $P<0.01$, *** $P<0.001$) are shown in bold. Information on the random effect variances ($\sigma^2$, total; $\tau 00$, PLOT:SP, within-species; and $\tau 00$, SP, cross-species) are also provided. Abbreviations: n.i., not included in the best model. See Table 3.1 for definition of symbols.

| | LMA | log (N) | log( -$\delta^{13}$C) | log (WD) | Hv | $K_L$ | $K_S$ | -$P_{50}$ | -$P_{tlp}$ |
|---|---|---|---|---|---|---|---|---|---|
| **Fixed Part** | | | | | | | | | |
| (Intercept) | **0.33**<br>**(0.21 – 0.45)** *** | 1.98<br>**(1.46 – 2.50)** *** | **3.15**<br>**(3.07 – 3.23)** *** | -0.54<br>(-1.04 – -0.04) | **2.07**<br>**(1.17 – 2.98)** ** | **-7.79**<br>**(-9.00 – -6.57)** *** | -1.02<br>(-2.89 – 0.84) | **5.93**<br>**(4.33 – 7.52)** *** | **2.97**<br>**(2.47 – 3.47)** *** |
| P/PET_centered | -0.02<br>(-0.04 – 0.01) | 0.15<br>(-0.00 – 0.30) | 0.05<br>(-0.01 – 0.10) | -0.08<br>(-0.18 – 0.01) | **-0.75**<br>**(-1.24 – -0.26)** * | -0.25<br>(-0.65 – 0.15) | -0.17<br>(-0.52 – 0.17) | -0.64<br>(-1.31 – 0.04) | **-0.53**<br>**(-0.74 – -0.31)** *** |
| P/PET_mean | **-0.27**<br>**(-0.44 – -0.10)** * | **1.1**<br>**(0.35 – 1.85)** * | **0.27**<br>**(0.16 – 0.39)** ** | -0.02<br>(-0.74 – 0.70) | **-1.77**<br>**(-3.08 – -0.47)** * | -1.15<br>(-2.90 – 0.59) | 0.94<br>(-1.74 – 3.62) | **-3.88**<br>**(-6.17 – -1.58)** ** | **-1.11**<br>**(-1.83 – -0.39)** * |
| **Random Part** | | | | | | | | | |
| $\sigma^2$ | 0 | 0.009 | 0.001 | 0.009 | 0.178 | 0.153 | 0.087 | 0.071 | 0.024 |
| τ00, PLOT:SP | 0 | 0.009 | 0.001 | 0.002 | 0.07 | 0.043 | 0.037 | 0.189 | 0.015 |
| τ00, SP | 0.002 | 0.031 | 0.001 | 0.029 | 0.09 | 0.168 | 0.404 | 0.285 | 0.028 |
| $R^2$ marginal | 0.55 | 0.47 | 0.52 | 0.003 | 0.27 | 0.12 | 0.06 | 0.5 | 0.44 |
| $R^2$ conditional | 0.93 | 0.9 | 0.87 | 0.77 | 0.61 | 0.63 | 0.85 | 0.93 | 0.8 |
| Observations | 444 | 444 | 444 | 444 | 439 | 444 | 444 | 444 | 425 |

**Table B.4:** Results of the linear mixed models examining the effect of precipitation to potential evapotranspiration ratio (P/PET) on each trait within (P/PET_centred) and among species (P/PET_mean). The model's fixed effects coefficients including confidence intervals (in brackets) are shown. Significant correlations are shown in bold (* $P<0.05$, ** $P<0.01$, *** $P<0.001$). Information on the random effect variances ($\sigma^2$, total; $\tau$00, PLOT:SP, within-species; and $\tau$00, SP, cross-species) as well as the proportions of explained variance by fixed effects ($R^2$ marginal) and by fixed and random effects ($R^2$ conditional) are also provided. See Table 3.1 for definition of symbols.

|  | **All species** |  | ***P. sylvestris*** |  | ***P. nigra*** |  | ***P. halepensis*** |  | ***F. sylvatica*** |  | ***Q. humilis*** |  | ***Q. ilex*** |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | *Dw* | *D* | *Dw* | *D* | *Dw* | *D* | *Dw* | *D* | *Dw* | *D* | *Dw* | *D* | *Dw* | *D* |
| **LMA** | 3.61 | 4 | 0.98 | 3 | 0.51 | 2 | 1.05 | 4 | 1.89 | 5 | 1.01 | 3 | 2.09 | 5 |
| **N** | 2.74 | 3 | 0.69 | 2 | 0.91 | 3 | 0.56 | 2 | 0.63 | 2 | 0.77 | 3 | 0.63 | 2 |
| **$\delta^{13}C$** | 1.76 | 2 | 1.64 | 4 | 0.77 | 3 | 0.49 | 2 | 1.41 | 4 | 1.58 | 4 | 2.03 | 5 |
| **WD** | 0.00 | 0 | 1.33 | 4 | 0.59 | 2 | 0.00 | 0 | 0.63 | 2 | 0.00 | 0 | 0.79 | 3 |
| **Hv** | 3.64 | 4 | 0.49 | 2 | 0.26 | 1 | 1.05 | 2 | 0.27 | 1 | 0.52 | 1 | 1.71 | 4 |
| **$K_L$** | 0.83 | 1 | 1.29 | 3 | 1.17 | 3 | 1.32 | 2 | 1.85 | 5 | 1.39 | 3 | 1.25 | 3 |
| **$K_S$** | 0.00 | 0 | 1.62 | 4 | 0.84 | 2 | 0.84 | 2 | 1.38 | 3 | 1.23 | 3 | 0.63 | 2 |
| **$P_{50}$** | 2.75 | 3 | 1.72 | 5 | 0.28 | 1 | 0.28 | 1 | 1.23 | 4 | 1.63 | 5 | 0.00 | 0 |
| **$P_{tlp}$** | 0.83 | 1 | 1.04 | 3 | 0.84 | 3 | 0.75 | 3 | 1.02 | 4 | 1.40 | 4 | 1.27 | 4 |
| ***Overall network*** | *16.16* | *18* | *10.80* | *30* | *6.15* | *20* | *6.34* | *18* | *10.30* | *30* | *9.53* | *26* | *10.42* | *28* |

**Table B.5:** Descriptors of trait networks (a) across species (n=6) and (b-g) for each studied species separately. Abbreviations: Dw, weighted degree defined as the sum of all significant coefficients of correlation of a node; D, degree defined as the number of edges of a node. See Table 3.1 for definition of symbols.

# C
# Supplementary material for Chapter 4

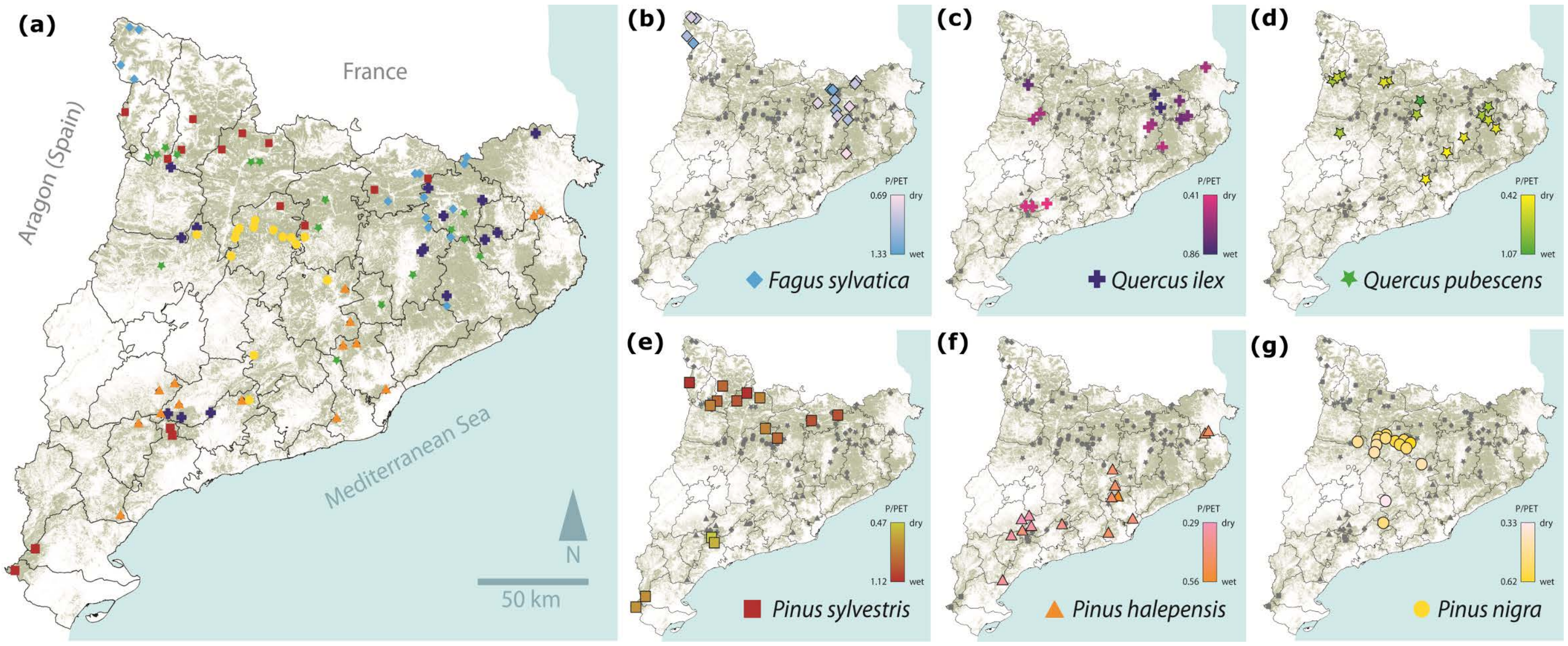

**Fig. C.1:** Map of Catalonia showing the distribution of (a) all the 90 sampling plots along the water availability gradient (precipitation to potential evapotranspiration ratio, P/PET) where different symbols indicate dominant species at each plot and (b-g) separately for each dominant species in the plot. *Fagus sylvatica*: blue diamond; *Quercus ilex*: purple cross; *Quercus pubescens*: green star; *Pinus sylvestris*: red square; *Pinus halepensis*: orange triangle and *Pinus nigra*: yellow circle.

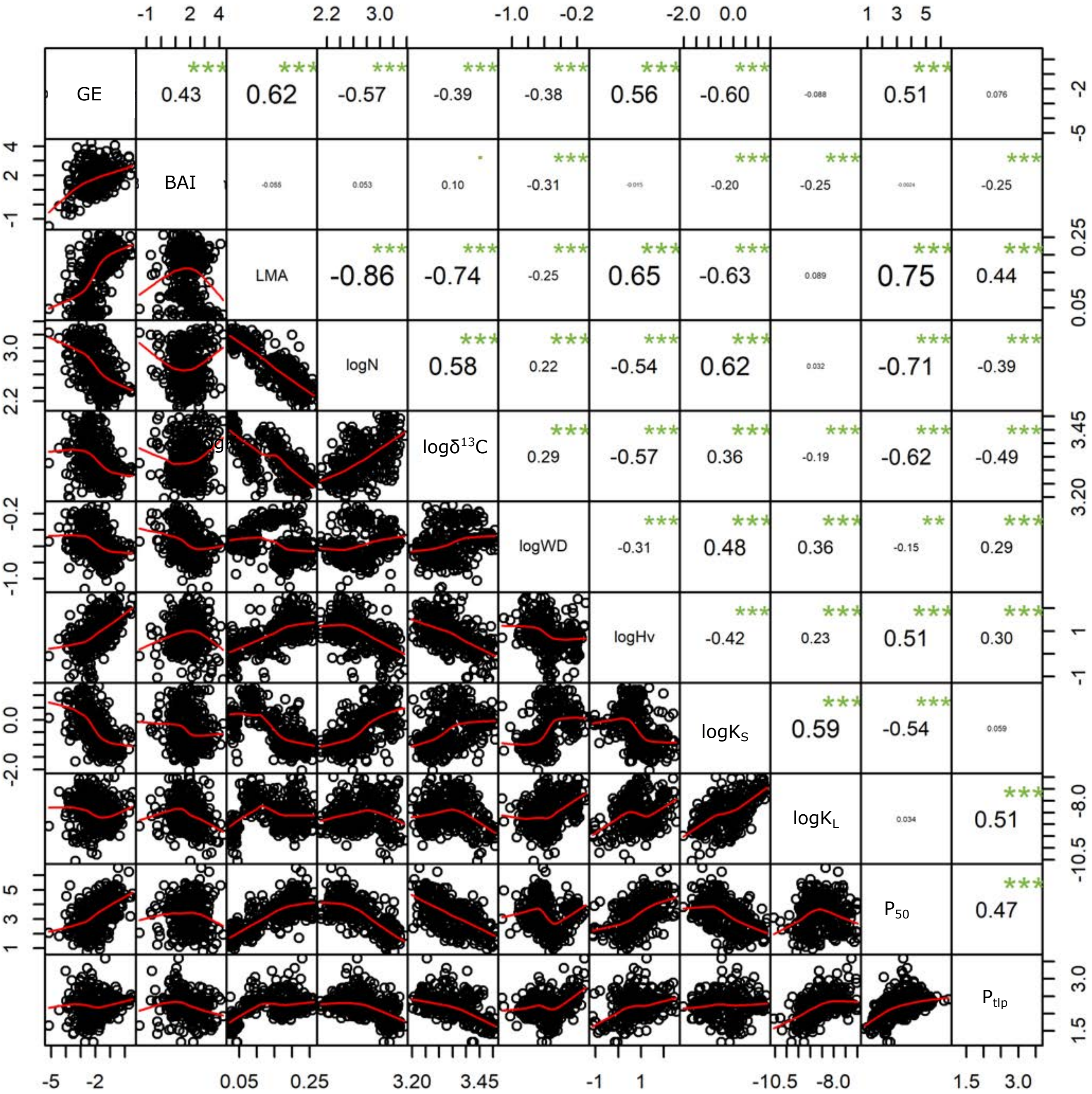

**Fig. C.2:** Pairwise correlations among traits, basal area increment (BAI) and growth efficiency (basal area increment per unit of total tree leaf area, GE). Pearson correlation coefficients are given for each relationship. Significance levels: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Variables were natural-log transformed whenever required to satisfy the normality assumptions. See Table 4.1 for definition of symbols.

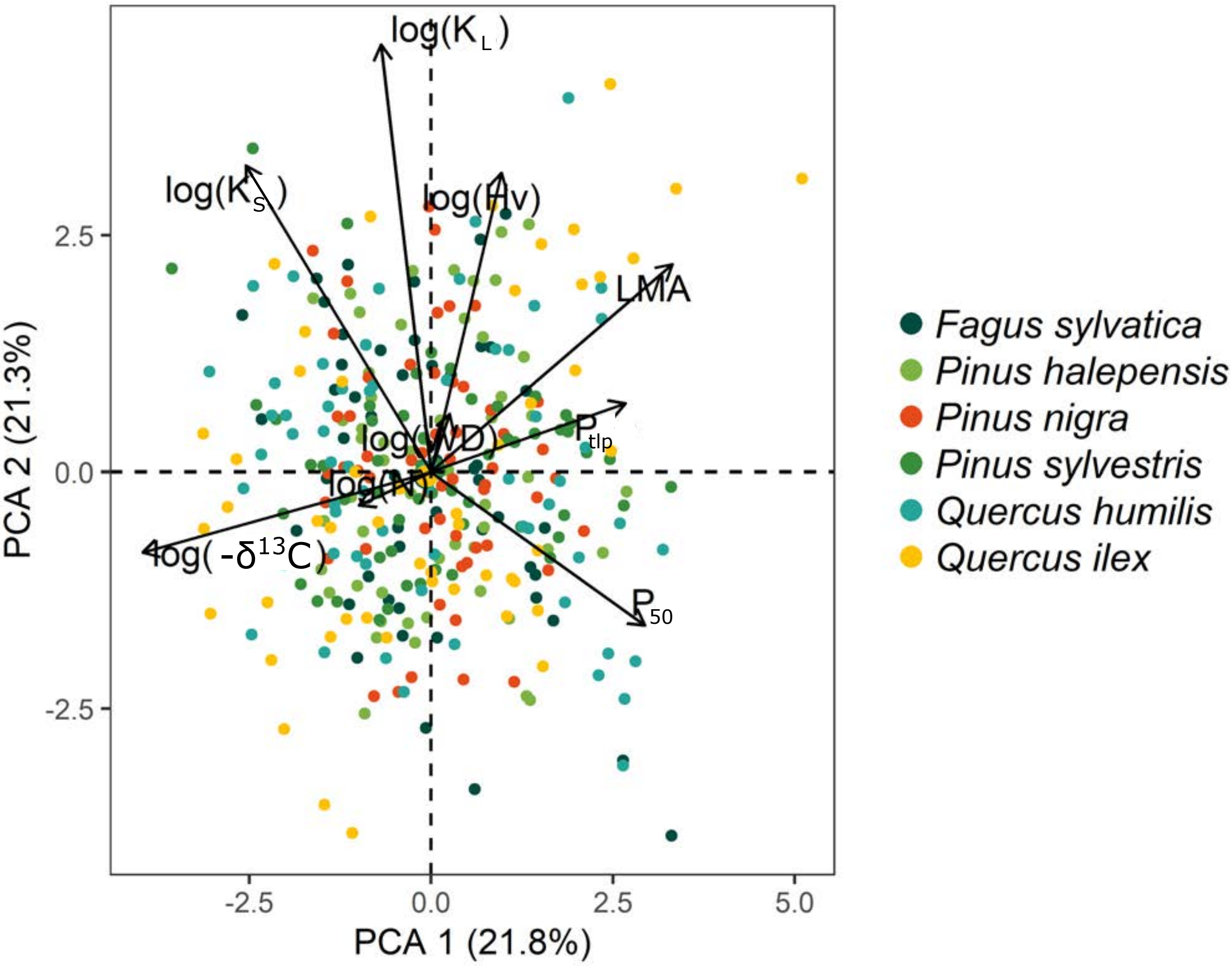

**Fig. C.3:** Principal component analysis (PCA) summarizing trait variability within species. The first two PCA axes and the percentage of the explained variance (in brackets) are shown. Variables were natural-log transformed whenever required to satisfy the normality assumptions. See Table 4.1 for definition of symbols.

| | **LMA** | | | **N** | | | **-$\delta^{13}$C** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Fixed factors** | Estimates | CI | P | Estimates | CI | P | Estimates | CI | P |
| (Intercept) | 1.92 | 1.13 – 2.71 | <0.001 | 1.85 | -1.35 – 5.05 | 0.258 | 2.45 | -15.26 – 20.15 | 0.787 |
| Mean trait | -0.4 | -5.23 – 4.42 | 0.870 | 0 | -1.17 – 1.18 | 0.994 | -0.18 | -5.48 – 5.13 | 0.948 |
| Centred trait | -1.78 | -5.62 – 2.05 | 0.362 | 0.44 | -0.23 – 1.12 | 0.197 | **2.67** | **0.51 – 4.82** | **0.015** |
| **Random Effects** | | | | | | | | | |
| $\sigma^2$ | 0.47 | | | 0.47 | | | 0.47 | | |
| $\tau$00 PLOT:SP | 0.17 | | | 0.16 | | | 0.15 | | |
| $\tau$00 SP | 0.14 | | | 0.14 | | | 0.14 | | |
| ICC PLOT:SP | 0.22 | | | 0.2 | | | 0.2 | | |
| ICC SP | 0.18 | | | 0.18 | | | 0.19 | | |
| Observations | 352 | | | 352 | | | 352 | | |
| Marginal R$^2$ | 0.003 | | | 0.005 | | | 0.018 | | |
| Conditional R$^2$ | 0.398 | | | 0.392 | | | 0.396 | | |
| | **WD** | | | **Hv** | | | **$K_L$** | | |
| **Fixed factors** | Estimates | CI | P | Estimates | CI | P | Estimates | CI | P |
| (Intercept) | 0.95 | 0.35 – 1.56 | 0.002 | 1.72 | 1.08 – 2.35 | <0.001 | -2.18 | -6.22 – 1.85 | 0.289 |
| Mean trait | **-1.62** | **-2.66 – -0.58** | **0.002** | 0.16 | -0.47 – 0.78 | 0.621 | **-0.47** | **-0.94 – -0.00** | **0.049** |
| Centred trait | -0.39 | -1.14 – 0.36 | 0.310 | -0.14 | -0.31 – 0.03 | 0.116 | -0.13 | -0.31 – 0.06 | 0.185 |
| **Random Effects** | | | | | | | | | |
| $\sigma^2$ | 0.47 | | | 0.47 | | | 0.46 | | |
| $\tau$00 PLOT:SP | 0.17 | | | 0.17 | | | 0.18 | | |
| $\tau$00 SP | 0.03 | | | 0.13 | | | 0.06 | | |
| ICC PLOT:SP | 0.26 | | | 0.22 | | | 0.25 | | |
| ICC SP | 0.04 | | | 0.17 | | | 0.09 | | |
| Observations | 352 | | | 349 | | | 352 | | |
| Marginal R$^2$ | 0.107 | | | 0.013 | | | 0.075 | | |
| Conditional R$^2$ | 0.377 | | | 0.395 | | | 0.388 | | |
| | **$K_S$** | | | **$-P_{50}$** | | | **$-P_{tlp}$** | | |
| **Fixed factors** | Estimates | CI | P | Estimates | CI | P | Estimates | CI | P |
| (Intercept) | 1.77 | 1.43 – 2.10 | <0.001 | 1.82 | 0.60 – 3.04 | 0.003 | 3.83 | 1.66 – 6.01 | 0.001 |
| Mean trait | -0.22 | -0.65 – 0.21 | 0.307 | 0.01 | -0.34 – 0.36 | 0.948 | -0.9 | -1.87 – 0.07 | 0.070 |
| Centred trait | -0.15 | -0.39 – 0.09 | 0.226 | -0.06 | -0.26 – 0.14 | 0.579 | -0.17 | -0.61 – 0.27 | 0.447 |
| **Random Effects** | | | | | | | | | |
| $\sigma^2$ | 0.47 | | | 0.47 | | | 0.47 | | |
| $\tau$00 PLOT:SP | 0.18 | | | 0.17 | | | 0.17 | | |
| $\tau$00 SP | 0.11 | | | 0.14 | | | 0.08 | | |
| ICC PLOT:SP | 0.24 | | | 0.22 | | | 0.23 | | |
| ICC SP | 0.14 | | | 0.18 | | | 0.11 | | |
| Observations | 352 | | | 352 | | | 334 | | |
| Marginal R$^2$ | 0.033 | | | 0.001 | | | 0.071 | | |
| Conditional R$^2$ | 0.4 | | | 0.403 | | | 0.392 | | |

**Table C.1:** Results of the linear mixed models examining the effect of each trait on basal area increment (BAI) among species (mean trait) and within species (centred trait). The model's fixed effects coefficients including confidence intervals (CI) and p-values (P) are shown. Information on the random effect variances ($\sigma^2$, total, $\tau 00$ PLOT:SP, within-species and $\tau 00$,SP, cross-species) as well as the proportions of explained variance by fixed effects (R$^2$ marginal) and by fixed and random effects (R$^2$ conditional) are also provided. See Table 4.1 for definition of symbols.

| | **LMA** | | | **N** | | | **-$\delta^{13}$C** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Fixed factors** | Estimates | CI | P | Estimates | CI | P | Estimates | CI | P |
| (Intercept) | **-3.21** | **-4.00 – -2.41** | **<0.001** | **4.18** | **1.57 – 6.80** | **0.002** | **25.94** | **2.21 – 49.67** | **0.032** |
| Mean trait | **8.82** | **3.96 – 13.67** | **<0.001** | **-2.24** | **-3.20 – -1.28** | **<0.001** | **-8.34** | **-15.45 – -1.23** | **0.022** |
| Centred trait | **3.31** | **0.16 – 6.45** | **0.039** | 0.18 | -0.38 – 0.74 | 0.530 | 1.16 | -0.64 – 2.97 | 0.208 |
| **Random Effects** | | | | | | | | | |
| $\sigma^2$ | 0.31 | | | 0.32 | | | 0.32 | | |
| $\tau$00 PLOT:SP | 0.13 | | | 0.12 | | | 0.12 | | |
| $\tau$00 SP | 0.15 | | | 0.09 | | | 0.28 | | |
| ICC PLOT:SP | 0.22 | | | 0.23 | | | 0.17 | | |
| ICC SP | 0.25 | | | 0.18 | | | 0.39 | | |
| Observations | 352 | | | 352 | | | 352 | | |
| Marginal $R^2$ | 0.364 | | | 0.411 | | | 0.255 | | |
| Conditional $R^2$ | 0.663 | | | 0.649 | | | 0.669 | | |

| | **WD** | | | **Hv** | | | **$K_L$** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Fixed factors** | Estimates | CI | P | Estimates | CI | P | Estimates | CI | P |
| (Intercept) | -3.35 | -5.06 – -1.65 | <0.001 | **-2.99** | **-3.57 – -2.40** | **<0.001** | -5.37 | -16.41 – 5.66 | 0.340 |
| Mean trait | -2.62 | -5.55 – 0.30 | 0.078 | **1.23** | **0.65 – 1.81** | **<0.001** | -0.41 | -1.69 – 0.88 | 0.535 |
| Centred trait | 0.51 | -0.10 – 1.13 | 0.102 | **0.24** | **0.10 – 0.38** | **0.001** | **0.24** | **0.09 – 0.39** | **0.002** |
| **Random Effects** | | | | | | | | | |
| $\sigma^2$ | 0.31 | | | 0.31 | | | 0.31 | | |
| $\tau$00 PLOT:SP | 0.12 | | | 0.11 | | | 0.1 | | |
| $\tau$00 SP | 0.37 | | | 0.12 | | | 0.6 | | |
| ICC PLOT:SP | 0.15 | | | 0.21 | | | 0.1 | | |
| ICC SP | 0.46 | | | 0.22 | | | 0.59 | | |
| Observations | 352 | | | 349 | | | 352 | | |
| Marginal $R^2$ | 0.207 | | | 0.405 | | | 0.048 | | |
| Conditional $R^2$ | 0.691 | | | 0.657 | | | 0.708 | | |

| | **$K_S$** | | | **-$P_{50}$** | | | **-$P_{tlp}$** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Fixed factors** | Estimates | CI | P | Estimates | CI | P | Estimates | CI | P |
| (Intercept) | **-2.25** | **-2.58 – -1.93** | **<0.001** | **-3.84** | **-5.34 – -2.33** | **<0.001** | -2.77 | -8.33 – 2.80 | 0.330 |
| Mean trait | **-0.91** | **-1.33 – -0.49** | **<0.001** | **0.58** | **0.15 – 1.02** | **0.008** | 0.39 | -2.10 – 2.88 | 0.758 |
| Centred trait | -0.17 | -0.36 – 0.03 | 0.100 | 0.02 | -0.14 – 0.18 | 0.810 | -0.11 | -0.47 – 0.26 | 0.559 |
| **Random Effects** | | | | | | | | | |
| $\sigma^2$ | 0.31 | | | 0.31 | | | 0.31 | | |
| $\tau$00 PLOT:SP | 0.13 | | | 0.12 | | | 0.13 | | |
| $\tau$00 SP | 0.11 | | | 0.23 | | | 0.66 | | |
| ICC PLOT:SP | 0.23 | | | 0.18 | | | 0.12 | | |
| ICC SP | 0.2 | | | 0.35 | | | 0.6 | | |
| Observations | 352 | | | 352 | | | 334 | | |
| Marginal $R^2$ | 0.414 | | | 0.292 | | | 0.01 | | |
| Conditional $R^2$ | 0.666 | | | 0.667 | | | 0.72 | | |

**Table C.2:** Results of the linear mixed models examining the effect of each trait on growth efficiency (basal area increment per unit of total tree leaf area, GE) among species (mean trait) and within species (centred trait). The model's fixed effects coefficients including confidence intervals (CI) and p-values (P) are shown. Information on the random effect variances ($\sigma^2$, total, $\tau$00 PLOT:SP, within-species and $\tau$00,SP, cross-species) as well as the proportions of explained variance by fixed effects ($R^2$ marginal) and by fixed and random effects ($R^2$ conditional) are also provided. See Table 4.1 for definition of symbols.

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# Part V

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