STOMATAL CONTROL, XYLEM HYDRAULICS AND LEAF MORPHOLOGY IN THE 40 BEF-CHINA TREE SPECIES: TRAIT INTERRELATIONSHIPS, FUNCTIONAL DIVERSITY AND TREE GROWTH PREDICTION

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“My precious”

John Ronald Reuel Tolkien (1937)

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Chapter 1

1.1 Summary

Research on biodiversity-ecosystem functioning (BEF) has undergone considerable progress especially due to lessons learned in BEF experiments lately. With 400.000 trees and shrubs planted in 566 plots and a tree species diversity gradient between 1 and 24 species per plot, BEF-China is one of the largest BEF experiments with woody plants. The present thesis consists of four papers all based on data from the BEF-China tree species.

The first three studies that form part of this thesis deal with the interrelationships of plant functional traits in the 40 subtropical broadleaved tree species planted in the BEF-China experiment. Investigating the interplay between different plant functional traits, this thesis addressed the following questions: How are traits related to each other and are there any ecophysiologically determined trade-offs in different trait complexes such as xylem hydraulics and stomatal regulation parameters? Are these ecophysiological characteristics related to leaf traits captured by the leaf economics spectrum (LES)?

Several new key parameters that describe stomata conductance (g_S) regulation were extracted from g_S-vapour pressure deficit curves. As hypothesized, traits characteristic for the LES, for example, leaf nitrogen concentration and leaf carbon-to-nitrogen ratio, were related to mean g_S. Interestingly, other leaf traits not correlated with the LES, such as stomatal traits, were found to be good predictors of stomatal regulation characteristics.

Water flow in vascular plants connects xylem hydraulics and leaf morphology and anatomy. The quantification of the specific xylem hydraulic conductivity and the xylem pressure at which 50% loss of the maximum specific xylem hydraulic conductivity of the study species occurs (Ψ_{50}) revealed that cavitation resistance was closely linked to the traits of the LES, but not to any parameter of g_S regulation though. Moreover, several leaf traits that typically are not considered in research, for instance, microscopy and morphologic traits such as leaf thickness, the log ratio of palisade to spongy mesophyll thickness and microstructure of the leaf surface, determined g_S regulation significantly. For xylem hydraulics, however, the leaf habit overruled any further signal from other traits.

Although analysis of plant functional traits represents a reliable approach in biodiversity ecology, BEF studies that would focus on the parallel analysis of the impact of biotic and abiotic effects on ecosystem functions such as productivity in forest ecosystems have been scarce so far. Thus, the dominant drivers of forest growth like the environment, the identity of

specific species or the magnitude of functional diversity in the respective communities have not been fully understood yet.

The fourth study focused on the impact that the following factors may have on the mean tree crown increment of the experimental plots: the environmental conditions, community weighted mean (CWM) traits or functional diversity. The trait values aggregated in CWM displayed the biggest effect on the variation in productivity of the forest plots; FD scored medium impact whereas the environment, contrary to expectation, had only a very limited impact. Thus, already at an early stage of the experiment, biotic influences overruled effects from the environmental setting.

Summing up, this thesis provides deeper insight into stomatal control and contributes to the knowledge about xylem vulnerability and morphological proxies for physiological leaf traits. In addition, a framework to predict ecosystem functioning was tested and environment was found to be the least important parameter. The results of this thesis demonstrate that the specific plant functional traits employed in BEF research should be chosen carefully in order to maximize methodological efficiency and to minimize unwanted data redundancy.

1.2 摘要

近年来关于生物多样性与生态系统功能关系的研究取得了长足的进步，特别是通过一系列的控制实验研究。中国亚热带森林生物多样性与生态系统功能实验（BEF-China）是目前世界上规模最大的木本植物BEF控制实验之一。该实验共设置有566个样方，包含40多万棵乔木和灌木，并按照单种到24种六种不同的多样性水平分别进行了设计配植。本文的四章内容均是基于来自BEF-China样地40种物种在不同多样性水平下的数据而完成。

论文的前三章主要探讨了这40种阔叶树种功能性状之间的相互关系，涉及的关键科学问题有：功能性状间之间是怎样相互关联的？不同的功能性状复合空间里是否存在一定的生理生态平衡关系，比如木质部水力导度与气孔调节参数是否相关？这些生理生态性性状是否和典型的叶片形态性状之间存在一定关系？

本文的研究结果表明，用于描述气孔导度调节机制的参数是从气孔导度-蒸汽压差曲线中提取的。正如假设所预测，属于叶片经济谱型（LES）的功能性状都和气孔平均导度显著相关，如叶氮含量和叶片碳氮比。而其它和叶片经济谱型无显著相关的气孔形态性状却能很好的预测气孔调节机制。

此外，对目标物种的木质部导水率和水势的量化结果发现，植物抗空穴化能力和属于叶片经济谱型的性状紧密相关，和气孔导度调节机制的参数却没有显著关系。而另外一些不常用的叶片功能性状却和气孔调节紧密相关，如一些微观和形态性状：叶片厚度、叶片栅栏和海绵组织的厚度比以及叶表皮的微观结构。对于木质部导度，叶片性状的影响明显胜过其它性状。

尽管已经有大量研究证明基于功能性状的方法是研究生物多样性的可靠途径，然后真正将其应用于生物多样性与生态系统功能研究中用来解释生物和非生物因子对生态系统功能影响的研究还为之甚少，比如究竟是环境、特定物种还是功能多样性驱动了森林树木生长还有待解决。本文的第四章即是探讨环境、群落水平的功能性状以及功能多样性对林冠生长的相对重要性。结果表明群落水平的功能性状能够最多解释样地生产力的变异，其次是功能多样性，而环境因子所起的作用很小。换句话说，即使在实验的早期阶段，已经发现生物因子对生态系统功能的影响超过了非生物因子。

综上所述，本论文从全新的角度分析了森林树木的气孔控制机制、木质部脆弱性以及叶片生理性状的形态学特征。并且通过验证多个能够预测生态系统功能的因子发

现环境在其中起着并不重要的作用。结果也提出在今后关于BEF的研究中应慎重选择特定植物功能性状的重要性，以最大化提高方法的利用率以及最小化数据的冗余性。

1.3 Zusammenfassung

Die Biodiversitätsforschung auf ökosystemarer Ebene hat vor allem durch Erkenntnisse aus Experimenten, die den Zusammenhang zwischen Diversität und Ökosystemfunktionen untersuchen, beträchtliche Fortschritte erzielt. Das Projekt BEF-China (*Biodiversity-Ecosystem Functioning*) ist mit 400.000 angepflanzten Bäumen und Sträuchern in 566 Untersuchungsflächen eines der größten Experimente dieser Art weltweit. Auf einem Areal in Südost-China wurde in den Jahren 2009 und 2010 eine Bepflanzung realisiert, die Untersuchungsflächen mit bis zu 24 Baumarten umfasste. Daten aus diesem Experiment bilden die Grundlage der vier Manuskripte auf denen die vorliegende Dissertation basiert.

Die ersten drei Manuskripte beschäftigen sich mit Zusammenhängen zwischen funktionalen Pflanzenmerkmalen der 40 subtropischen Laubbaumarten, die im BEF-China Experiment gepflanzt worden sind. Dabei wurden folgende Fragen gestellt: Über welche Funktionen sind die Merkmale untereinander verknüpft? Gibt es ökophysiologisch getriebene Kompromisse in der Ausprägung eines Merkmals, das unterschiedliche Funktionen hat, so zum Beispiel Merkmale des Xylems und Faktoren, die stomatäre Leitfähigkeit regulieren? Sind diese ökophysiologischen Kenngrößen mit Blattmerkmalen assoziiert, welche durch das *leaf economics spectrum (LES)* abgebildet werden?

Aus Funktionskurven zwischen stomatärer Leitfähigkeit und Dampfdruckgefälle wurden neue Schlüsselgrößen, die die Regulierung stomatärer Leitfähigkeit beschreiben, abgeleitet. Wie zuvor angenommen, standen Merkmale, welche typisch für das *LES* sind, wie die Stickstoff-Konzentration und das Kohlenstoff-Stickstoff-Verhältnis, im Zusammenhang mit mittlerer stomatärer Leitfähigkeit. Interessanterweise ließ sich von weiteren Merkmale, wie Dichte und Größe der Stomata, die als nicht korreliert mit dem *LES* bekannt sind, gut auf die Regulation von stomatärer Leitfähigkeit schließen.

Der Wasserfaden in Gefäßpflanzen verbindet in funktionaler Weise das Xylem mit der Blattmorphologie und -anatomie. Mittels der Quantifizierung von spezifischer hydraulischer Leitfähigkeit des Xylems und des Xylemdruckes, bei dem 50% Verlust der maximalen spezifischen hydraulischen Leitfähigkeit des Xylems auftraten, konnte gezeigt werden, dass die Kavitationsresistenz eng mit Merkmalen des *LES* verknüpft ist, jedoch mit keinem stomatären Regulationsparameter. Verschiedene Blattmerkmale, welche sonst nur sehr selten untersucht werden, so zum Beispiel mikroskopische und morphologische Merkmale wie Blattdicke, das logarithmierte Verhältnis der Stärke von Schwamm- und Palisadenparenchym und Mikrostrukturen der Blattoberfläche, beeinflussten in signifikanter Weise die Regulation von stomatärer Leitfähigkeit. Im Bereich der Xylemmerkmale war dagegen das Signal der Blattlebensdauer maßgeblich.

Funktionale Pflanzenmerkmale stellen einen bewährten Ansatz in der Biodiversitäts- und Ökosystemfunktionsforschung dar. Dennoch sind Biodiversitäts- und Ökosystemfunktionsstudien, welche zugleich die Einflüsse von sowohl biotischen als auch abiotischen Effekten auf Ökosystemfunktionen, wie zum Beispiel Produktivität in Waldökosystemen, untersuchen, immer noch selten. Daher sind die wichtigsten Einflussgrößen auf Waldwachstum wie Umwelt, Artidentitätseffekte oder der Einfluss von funktioneller Diversität noch nicht vollkommen erforscht.

Das vierte Manuskript zeigt, inwieweit das mittlere Kronenwachstum der Bäume einer Untersuchungsfläche durch die abiotische Umwelt, gemittelte Eigenschaften aller Bäume der Untersuchungsfläche (*community weighted means, CWM)* oder funktionelle Diversität erklärt werden kann. Die durch *CWM* zusammengefassten Merkmale erklärten den größten Anteil an Variation in der Produktivität der Walduntersuchungsflächen, funktionale Diversität erreichte einen mittleren Einfluss, wohingegen die unbelebte Umwelt nur sehr geringe Erklärungskraft bot. Trotz des frühen Sukzessionsstadium der Untersuchungsflächen überwogen biotische Effekte gegenüber Umwelteinflüssen.

Zusammengefasst führt diese Dissertation zu neuen Erkenntnissen über stomatäre Kontrolle, Kavitationsresistenz und morphologische Schätzwerte für physiologische Pflanzenmerkmale. Die Analysen wurden mit einem umfangreichen Datensatz durchgeführt, so dass die gewonnenen Erkenntnisse von hoher Generalisierbarkeit sind. Zusätzlich wurde ein Konzept zur Vorhersage von Ökosystemfunktionen getestet, wobei festgestellt wurde, dass Umwelteinflüsse den geringsten Effekt auf die Produktivität hatten. Die Ergebnisse dieser Arbeit belegen die große Bedeutung der Auswahl von spezifischen funktionalen Pflanzenmerkmalen für die Maximierung methodischer Effizienz und die Minimierung ungewollter Redundanz der Daten in der Biodiversitäts- und Ökosystemforschung.

1.4 Introduction

1.4.1 How is biodiversity linked to ecosystem functioning?

Humankind depends on stable and reliable ecosystem functioning (Díaz et al. 2006; Haines-Young and Potschin 2010). Ecosystem functioning is defined as all biological, geochemical and physical processes that control cycles of organic matter, nutrients and energy in an environment, for example, primary production, nutrient cycling or decomposition (Maynard et al. 2010; Cardinale et al. 2012). In turn, the complexity of these processes depends on biodiversity. Compelling evidence for many positive links between diversity of ecosystems and their functioning has been found in the growing body of experiments in recent research on biodiversity–ecosystem functioning (BEF) (Balvanera et al. 2014; Tobner et al. 2014). Productivity defined as the rate of biomass production in an ecosystem is one example of ecosystem functioning. A more specific term - net primary production - refers to productivity of autotrophs, here plants (Chapin et al. 2011).

However, the specific mechanisms behind the interplay of biodiversity and ecosystem key functions such as productivity have not been satisfactorily understood yet (Cardinale et al. 2006; Balvanera et al. 2014). One of the reasons is the complexity of biodiversity itself that includes all variability in living creatures and ecological complexes they are part of, expressed in genetic, species and ecosystem variability (Balvanera et al. 2006; Cardinale et al. 2006; Cardinale et al. 2012; CBD). To achieve efficient future land use strategies and conservation policy, further research is needed to extend the current knowledge about a wide range of systems and environments. Regardless the specific definition or level of biodiversity one refers to (Mace et al. 2012), if species decline in frequency or become extinct, they are no longer available for ecosystems (Hooper et al. 2012) such as those examined in this dissertation.

Drawing conclusions across different ecosystems, environments and species represents a great challenge. Systematic approaches under comparable settings are therefore indispensable and should be strived for. BEF experiments may be an answer to this challenge and represent the state of the art in the current ecological research.

The present thesis consists of four papers that are all based on the data and the design of the BEF-China experiment (Yang et al. 2013; Bruelheide et al. 2014). On two experimental sites 40 broadleaved subtropical tree species native to the surrounding forests have been planted in a design with diversity levels ranging from monocultures to 2, 4, 8, 16 and 24 species per plot. The sites are located in Jiangxi province, 400 km west of Shanghai.

In order to relate functioning to biodiversity in BEF experiments, different facets of biodiversity need to be quantified (Reiss et al. 2009). However, there is no simple measure as biodiversity comprises many different facets including diversity at the genetic, species or ecosystem levels (Davies and Cadotte 2011). The concept of diversity is based on differences that can be described by several indices, one of which is functional diversity (FD) (Schleuter et al. 2010). FD is considered to perform better than pure species richness index in prediction of ecosystem functioning, for example, in prediction of productivity of a community, since FD captures various facets of the communities. In contrast, species richness is only a compilation of species presence data for a community (Tilman 1997; Petchey et al. 2004). In the case of functional diversity FD, these differences can be partitioned into two main components: distances in qualities of objects, on the one hand, and relative abundances of these objects, on the other hand (Purvis and Hector 2000). In ecology these “qualities” are termed traits. A functional trait is a measurable attribute of an individual plant which affects its performance in terms of growth, reproduction or survival (Lavorel and Garnier 2002; Violle et al. 2007; Perez-Harguindeguy et al. 2013). Consequently, FD is defined as the range of trait dissimilarities and divergence and as the distribution of species in a community (Tilman 2001).

1.4.2 Plant functional traits

Functional traits represent a major area of interest within the field of plant ecology (Messier et al. 2010; Perez-Harguindeguy et al. 2013). Since plant functional traits carry a large part of relevant information about a species, they may help to predict the interactions between species as well as the characteristics of communities and even of the whole ecosystems (Naeem and Wright 2003; Grime 2006; Luck et al. 2009). However, this relationship is not a simple one because traits are often pleiotropic: one trait usually affects several functions, and, vice versa, one function is often driven by several traits. Elementary trade-offs between functional traits are one of the basic concepts in functional ecology (Sterck et al. 2011). These trade-offs may have different causes: they can either be allocation-based, such as specific leaf area (SLA) and leaf nitrogen-to-carbon ratio (Evans and Poorter 2001), or have an evolutionary basis, such as the different strategies in leaf defence mechanisms between polyphenols and leaf toughness (Schuldt et al. 2014). It has been shown that trade-offs can act in a general way across biomes and can cause limitations in possible trait combinations in plant species even on a global scale (Reich et al. 1999).

Among all traits of a plant, particularly leaf traits have been confirmed to be highly effective predictors of plant performance due to their central role in the plant's functionality and due to their key player function, for instance, in gas exchange or light acquisition (Reich et al. 1999; Poorter et al. 2004; Sterck et al. 2006; Kröber et al. 2012). Thus, leaf traits not only play a central role in primary productivity (Wilson et al. 1999; Marron et al. 2005; Pontes et al. 2007; Cadotte et al. 2009) but also capture many of the essential trade-offs over a large array of functions. An important framework that can explain a large amount of leaf trait interrelationships is the leaf economics spectrum (LES) that sorts species according to their nutrient investment strategies from fast-return to slow-return ones (Wright et al. 2004). However, it has not been fully understood so far how different trait complexes, for example, anatomical and physiological leaf traits, are connected to the LES. The LES runs from short-lived leaves, which are highly physiologically active and contain high nutrient concentrations, to long-lived leaves, which are less physiologically active and contain lower nutrient concentrations (Shipley et al. 2006; Osnas et al. 2013). The LES describes the intensity of photosynthetic assimilation in a leaf and its dark respiration rate and is thus reflected in leaf traits such as SLA, leaf nitrogen concentration and leaf phosphorous concentration (Maire et al. 2013). Nutrient contents discriminate plant species globally along a gradient from acquisitive to conservative investment strategies (Wright et al. 2004; Freschet et al. 2010). The LES, in addition, validly accounts for variation in leaf habit (Edwards et al. 2014). Besides explaining the nutrient use in leaves, the LES may also predict other physiologically relevant aspects such as water use (Reich 2014). Recent studies have revealed that the traits represented in the LES are related to plant water use and thus to traits that reflect stomatal conductance (g_S) (Aasamaa and Sõber 2011), xylem hydraulics (Hoeber et al. 2014) and anatomical features like leaf vein density (Blonder et al. 2014). However, the relationship between LES and plant water relations has been seen controversially as some studies do not confirm clear links between LES and physiological parameters related to stomatal control (Juhrbandt et al. 2004; Sales-Come and Hölscher 2010). Thus, this thesis aimed to test for such relationships in the set of trees in the BEF-China experiment.

Stomatal conductance traits, xylem hydraulic traits & leaf microscopy traits

The regulation of g_S is a key process for the water balance in vascular plants. The most frequently measured parameters of stomatal regulation are mean und maximum g_S. However, these parameters do not capture the regulatory abilities of tree species. In particular, they do not take into account the responsiveness to external environmental factors, of which the most important is vpd (Farquhar and Sharkey 1982). During periods of drought stress, sensitive and precise stomatal regulation is crucial to prevent cavitation (Hacke et al. 2006). Although some earlier studies modelled the relationships between g_S and vpd, they mostly employed linear relationships (Schulze et al. 1994; Oren et al. 1999). Only few attempts were made to quantify the point of down-regulation at high vpd, and most studies focused on the vpd range, in which the degree of stomata opening increases (Leuning 1995; Patanè 2011). So far, no consistent model has been proposed to predict the vpd at which plants down-regulate g_S. One of the aims of the first manuscript in this thesis (see Chapter 2) was to develop a model that would allow to extract these parameters. Subsequently, these new parameters could be tested for relationships with traits such as those described by the LES and traits that have not been often studied yet (Walker et al. 2014). For instance, g_S regulation is known to be affected by leaf habit. In general, deciduous species were found to have higher g_S values; under drought conditions, however, their g_S values were lower than those of evergreen species (Sobrado 1998; Liu et al. 2011; Zhang et al. 2013). Table 1 shows the list of particular traits that were included in this study to test for such relationships.

In contrast to the process of determining the parameters of g_S regulation, methods that assess sensitivity of xylem hydraulics are well established (Sperry et al. 1988; Tyree and Sperry 1989). Specific xylem hydraulic conductivity and sensitivity to cavitation are quantified via flow-through measurements and using a modified Scholander pressure chamber. Nevertheless, these methods require sophisticated lab equipment and are highly labour- and time-consuming. Thus, detecting proxies for xylem hydraulic traits would increase the efficiency in screening plant species in comparative physiology studies. Several studies on trees have shown that high xylem hydraulic conductivity and high sensitivity to cavitation are related to low wood density and thus to high SLA (Poorter et al. 2010; Markesteijn et al. 2011a; Choat et al. 2012). The parameters of g_S control and xylem hydraulics may be expected to be related because transpiration and xylem hydraulics are tightly linked through a specific water use strategy of a particular plant species. Consequently, tree species that under drought conditions down-regulate g_S at high vpd quickly and efficiently often have xylem vessels highly sensitive to cavitation (Martorell et al. 2014; Franks and Casson 2014; Dow and Bergmann 2014; Nardini and Luglio 2014). In contrast, tree species with cavitation-resistant vessels are able to sustain high g_S in phases of high vpd and therefore tolerate low water potentials in the xylem. Conversely, a high photosynthetic capacity of leaves, as expressed by a high SLA, might be associated with high xylem hydraulic conductivity to facilitate sufficient water supply required for high g_S. These expectations were tested in the second manuscript of this thesis: the specific xylem hydraulic conductivity and the xylem pressure that leads to 50% loss of the maximum specific xylem hydraulic conductivity (Ψ_{50}) were quantified and related to the LES traits (Table 1).

Various studies have aimed at finding mechanistic links between xylem hydraulics and plant functional traits assigned to other complexes in order to predict xylem hydraulics (Poorter et al. 2010; Markesteijn et al. 2011a; Choat et al. 2012). In such studies, these were mainly the LES traits that were tested for relationships to stomatal regulation and xylem hydraulics. However, a much bigger variety of traits is available for research, and these traits seem to be worth analysing since they may describe physiological processes better than the established LES traits (Somavilla et al. 2014; DosAnjos et al. 2014). Because other traits have been rarely studied, not much is known about their interrelationships (Wylie 1951; Jackson 1967). Particularly the traits describing aspects of the leaf surface may hold information relevant to plant water relations since the leaf surface is the main barrier for water loss and the occurrence of specific microscopy structures on the leaf surface may be related to specific physiological parameters describing the plant’s water use strategy (Eglinton and Hamilton 1967; Jeffree 1986; Barthlott et al. 1998). Since the internal leaf structure links xylem and stomata, it might hold causal information on how leaf functionality connects different physiological demands (Evans 1999; Mediavilla et al. 2001; Santiago et al. 2004).

Table 1

Overview of all functional traits examined in this thesis with references to the respective publications. The traits are arranged by trait complexes. The main functions each trait presumably has are listed. Traits that are associated with the same function may be related to each other.

Trait		Function				Manuscript no.			
Code sorted by trait complex	Meaning	Productivity	Longevity	Water transport efficiency	Drought resistance	1	2	3	4
Physiological leaf traits									
CONMEAN	average g_S [1]	+++		+	-	x	x		x
CONMAX	maximum g_S [2]	+++	-	++		x	x		x
VPDMAX	vpd at CONMAX	+++				x	x		x
CONMAXFIT	fitted max. g_S [2]	+++				x	x	x	x
VPDMAXFIT	vpd at CONMAXFIT	+	++		+	x	x	x	x
VPDPOI	vpd at the second point of inflection of the g_S-vpd model	+	++		++	x	x		x
Chemical leaf traits									
N	Leaf nitrogen content [3]	+++	---			x	x		x
C	Leaf carbon content [3]	---	+++		+	x	x		x
CN	Leaf carbon to nitrogen ratio [3]	---	+++			x	x		x
CA	Leaf calcium content [4]	+	-			x	x		x
K	Leaf potassium content [5]	+	-			x	x		x
MG	Leaf magnesium content [6]	+++	---			x	x		x
Macromorphological leaf traits									
LEAFHABIT	Leaf duration [7, 8]	+	-			x		x	
LA	Leaf area [7]	+	-			x	x		x
LDMC	Leaf dry matter content [7]	-	+			x	x		x
SLA	Specific leaf area [7]	-				x	x		x
LEAFTOUGH	Leaf toughness [9]	---	+++			x	x		x
EXTRAFLORAL	Extrafloral nectaries [10]	+				x	x	x	x
LEAFPIN	Leaf pinnation [11]	+				x	x		
LEAFMAR	Leaf margin type [11]		+			x	x		
Microscopy leaf traits									
STOMDENS	Stomata density [12]				+++	x	x		x
STOMSIZE	Stomata size [12]			+		x	x		x
STOIND	Stomata index [12]			+		x	x		x
DIAMVEIN1	Diameter veins 1st order [13]			+++		x	x	x	x
DIAMVEIN2	Diameter veins 2nd order [13]			+++		x	x	x	x
VEINLENGTH	Length of veins in 1 mm^2 [13, 14]			+++		x	x	x	x
TRICHOM	Type of trichomes [15]	-			++			x	
COVTRIUP	Cover of trichomes on adaxial side of leaf [15]				++			x	
COVTRILO	Cover of trichomes on abaxial side of leaf [15]				++			x	
CUTFOLDUP	Folding of upper cuticle [16]				+			x	
CUTFOLDLO	Folding of lower cuticle [17, 18]				+			x	

Table 1 continued

Trait		Function				Manuscript no.			
Code sorted by trait complex	Meaning	Productivity	Longevity	Water transport efficiency	Drought resistance	1	2	3	4
Microscopy leaf traits continued									
EPIWAXUP	Pattern of upper epicuticular wax layer [17, 18]		+		+			x	
EPIWAXLO	Pattern of lower epicuticular wax layer [17]		+		+			x	
UPPEREPI	Thickness of upper epidermis [19]		+					x	x
PALIS	Thickness of palisade parenchyma [19]	+++						x	x
SPONGY	Thickness of spongy parenchyma [19]	+		+				x	x
LOG10RATIO	$\log_{10}$ of the ratio of thickness of palisade to spongy parenchyma [20, 21]	+++						x	x
LEAFTHICK	Leaf thickness [22]	-	+					x	x
SUBEPID	Presence of a subepidermis [19]							x	x
EPICELLSIZ	Ratio of the cell size of upper and lower epidermis [19]							x	x
PALSTR	Layer structure of palisade parenchyma [21, 23]	++						x	x
EXCRET	Presence of excretory glands [22]	-						x	x
DENSINTCEL	Density of spongy parenchyma [24, 25]			-				x	x
COLSCLER	Presence of column of sclerenchyma cells through the leaf [19]	-	+					x	x
PAPILL	Presence of papillae [17]				+			x	x
Physiological wood traits									
WPOT	Water potential [26]						x		x
PSI_{50}	Loss of 50% initial conductivity [26]		---		---		x	x	x
HYDCOND	maximum specific xylem hydraulic conductivity [26]	+++		+++			x	x	x
B	Parameter b (sigmoid regression of PSI_{50})						x		x
Microscopy wood traits									
MEANAREA	mean area of conducting vessels [26]	+		+++	---		x		
MEANROUND	mean roundness of conducting vessels [27]			++	+		x		
WOODDENS	Wood density [26]	---	+++				x		x
DHYD	hydraulically weighted diameter of conducting vessels [13, 27]			+++	---		x		

[1] (Ewers et al. 2007), [2] (Schulze et al. 1994), [3] (Hiyama et al. 2005), [4] (White and Broadley 2003), [5] (Humble and Raschke 1971), [6] (Wasilewska et al. 2008), [7] (Pringle et al. 2011), [8] (Aerts 1995),[9] (Schuldt et al. 2014), [10] (Koptur 1992), [11] (Taylor 1975), [12] (Franks and Farquhar 2007), [13] (Sack et al. 2013), [14] (Lambers et al. 2008), [15] (Schuepp 1993), [16] (Arambarri et al. 2008), [17] (Wilkinson 1979), [18] (Riederer and Schreiber 2001), [19] (Slaton et al. 2001), [20] (Zhang et al. 2012a), [21] (Dineva 2004), [22] (Gerosa et al. 2003), [23] (Vogelmann et al. 1996), [24] (Van Wittenberghe et al. 2012), [25] (Bussoti 2008), [26] (Markesteijn et al. 2011b), [27] (Tyree and Zimmermann 2002)

Microscopy traits characterising anatomical features of the leaf such as spongy mesophyll thickness may be related to carbon assimilation strategies of a plant, which in turn should be inseparably related to the plant's water use strategy. As decreasing cuticle thickness, stomatal densities and the ratio of palisade to spongy mesophyll ratio have been associated with increased shading (Qin et al. 2012) and since increased shading often translates into lower g_S values, it may be assumed that morphological leaf traits related to shaded environment are also related to lower g_S values (Kitajima 1994; Poorter 1999; Aranda et al. 2005). The same comparison may be made for drought conditions. Bacelar *et al.* (2004) observed that several structural leaf traits connected with sclerophylly increase under more severe drought conditions, thus the same traits may be more pronounced in tree species with high xylem cavitation resistance. Hence, the aim of the third manuscript in this thesis (Chapter 4) was to assess leaf microscopy traits of the 40 BEF-China species and to test for their relationships to physiological parameters (see Table 1).

1.4.3 Are ecosystem functions better explained by the environment, by community weighted means or by functional diversity?

The final challenge of this thesis was to identify the factors that best describe ecosystem functioning of the experimental communities in BEF-China. As a key function in ecosystems the thesis focused on productivity. As the BEF-China experimental sites at Xingangshan cover an exceptionally wide environmental range of different slopes and aspects which are associated with a high variability in microclimatic conditions between the experimental plots, a strong environmental impact on productivity would be expected. It is well known that environmental effects at a local scale can alter valid patterns in trait variation globally (Wright and Sutton-Grier 2012). Besides the impact of the environment, ecosystem functioning may be affected by the community's functional composition. The functional composition of a community can be described by two different measures that highlight complementary aspects: the community weighted mean (CWM) and functional diversity (FD) (Ricotta and Moretti 2011). CWM is defined as the mean in trait values across all occurring species of a community weighted by their abundances (Garnier et al. 2004; Lavorel et al. 2008). Therefore, CWM allows for direct comparisons of communities in absolute trait values; FD, in contrast, captures information on the range of values and abundances of traits in a community (Chapin et al. 2000; Díaz and Cabido 2001; Loreau et al. 2001; Hooper et al. 2005).

It has also to be considered that ecosystem functioning is dependent neither on the environment nor on CWM and FD exclusively. Not only does the environment affect ecosystem functioning, for example, productivity, directly but also acts indirectly on the relative abundance and composition of species in a community over time. Thus, when trying to tear these different factors apart it is crucial to control for environmental influence in experimental settings.

Certain base parameters are used to estimate maximum productivity for a given community at a given place. The magnitude of biomass production is ultimately related to resource supply, such as nutrient availability, photosynthetically active photon flux density and water availability. Other essential parameters include the amount of these resources captured by the individuals of a community and, finally, the efficiency in resource use, in other words, how efficiently plants transform these necessary elements into biomass production. These terms were aggregated by Monteith et al. (1977) and then further refined by Binkley et al. (2004) and Richards et al. (2010) in the Production Ecology Equation:

$$p = r * f * e$$

where p is biomass production, r is resource supply, f is fraction of resources captured and e is the efficiency of resource use (p & r being area normalized). When transposed into the terms introduced above, the environment is assumed to have the strongest impact on r. In contrast, f & e both represent a summary of the relevant traits of the species in the community, as captured by CWM and FD. However, equating r with environment and f & e with traits in the Production Ecology Equation is certainly too simple since the environment does also affect f & e. In particular, f & e are also affected by stem density, canopy layer structure and successional stage of the community. One of the major advantages of the BEF-China experiment is that these last two factors have been controlled for as the trees were planted at the same time in equal density.

FD is positively linked to various ecosystem functions, for example, to biomass production, although the magnitude of its impact may be lower than environmental effects or specific species characteristics (Paquette and Messier 2011; Vilà et al. 2013; Isbell et al. 2013). Functional leaf and wood traits of a community averaged by CWM are considered specifically significant in predicting tree growth and thus in predicting productivity (Healy et al. 2008; Ricotta and Moretti 2011). Many authors provided evidence for a positive relationship between forest biodiversity and productivity (Kelty 1988; Caspersen and Pacala 2001; Liang et al. 2007; Lei et al. 2009; Young et al. 2011; Zhang et al. 2012b; Vilà et al. 2013; Gamfeldt et al. 2013).

In the Jena grassland experiment Roscher et al. (2012) observed that CWM had a stronger effect on productivity than FD; however, their study did not explicitly include environmental factors. Despite differing environmental conditions across the experimental plots, such as distance to the river, environment did not affect productivity predictions since in the experimental design employed random blocks, which accounted for differing environmental conditions statistically. Only few studies analysed the combined impact of the environment and FD on ecosystem properties (Díaz et al. 2007). In one of these studies, Vilà et al. (2013) established that tree species richness positively influenced the tree wood production at the local scale across a large range of environments. However, in BEF studies the biodiversity signal has to be separated from environmental impacts. The need to do so was demonstrated, for example, in the Sardinilla Experiment in Panama, where Healy et al. (2008) found the environment to create a greater impact on the plot-level productivity than biodiversity. Nevertheless, effects of biodiversity could account for 23-30% in variation of productivity.

It remains unclear which specific functional traits might be the best predictors for productivity. In general, traits that are functionally associated with productivity (see Table 1) should be good predictors in systematic approaches where a multitude of functional traits is tested for its impact on community biomass production.

1.5 Objectives of the thesis

The overall aim of this thesis is to contribute to the general understanding of how biodiversity loss entails loss in ecosystem functioning. Therefore, mechanisms were studied that potentially relate functionality to biodiversity in ecosystems. This can be achieved in BEF experiments where biodiversity has been experimentally manipulated and most of the confounding environmental factors are controlled for. Consequently, this thesis made use of the large–scale biodiversity–ecosystem function experiment BEF-China.

On the basis of the experimental setup, this dissertation addressed the following main questions: How are the LES, the parameters of stomatal control, xylem hydraulics and microscopy leaf traits, interrelated in the 40 broadleaved tree species planted in the

BEF-China experiment? Which traits, in addition to the LES, predict plant physiological key functions such as g_S regulation and xylem sensitivity to cavitation?

Taking productivity as an important ecosystem function, how can differences in trait composition of plots explain variation in productivity? More specifically, how can plot-level tree crown increment be best predicted from traits?

1.5.1 Outline

This thesis is composed of four papers and is structured as follows: the four papers are presented in chapters two to five, and the discussion is found in the final chapter six. The first paper determines the parameters of stomatal regulation extracted from g_S – vpd models of different tree species and tests how these parameters are linked to functional leaf traits, with a particular focus on traits related to the LES. The second paper deals with xylem hydraulics and the relationships between xylem hydraulics and stomatal control. The third paper focuses on leaf microscopy traits, and asks how these rarely studied traits can explain aspects of leaf physiology that are not covered by the LES. The first three papers form the basis for the fourth paper. This last paper asks how tree growth of the experimental plots in BEF-China can be best explained, by environment, CWM or FD.

Figure 1 shows how the four papers build upon each other. Whereas paper one and two take a physiological perspective, paper three puts the emphasis on leaf morphology. Finally, paper four focuses on ecosystem functioning exemplified by biomass production. The studies have all been conducted at the same geographical site and with the same species under identical conditions. The advantage of such systematic approach is that the results presented in all four papers are highly comparable.

Table 2 gives a synopsis of the species examined and data used for each study. The aim was to include all species of the two sites A and B in all papers. However, the number of species included in the studies differed slightly (see Table 2) because species had not been sufficiently established at the sites and tree growth data had not been available for both plots at the time of writing this thesis.

The final chapter draws upon the entire thesis tying up various theoretical and empirical strands. It also discusses implications of the findings for the future research into this area. Finally, the conclusion gives a brief summary and critical assessment of the findings.

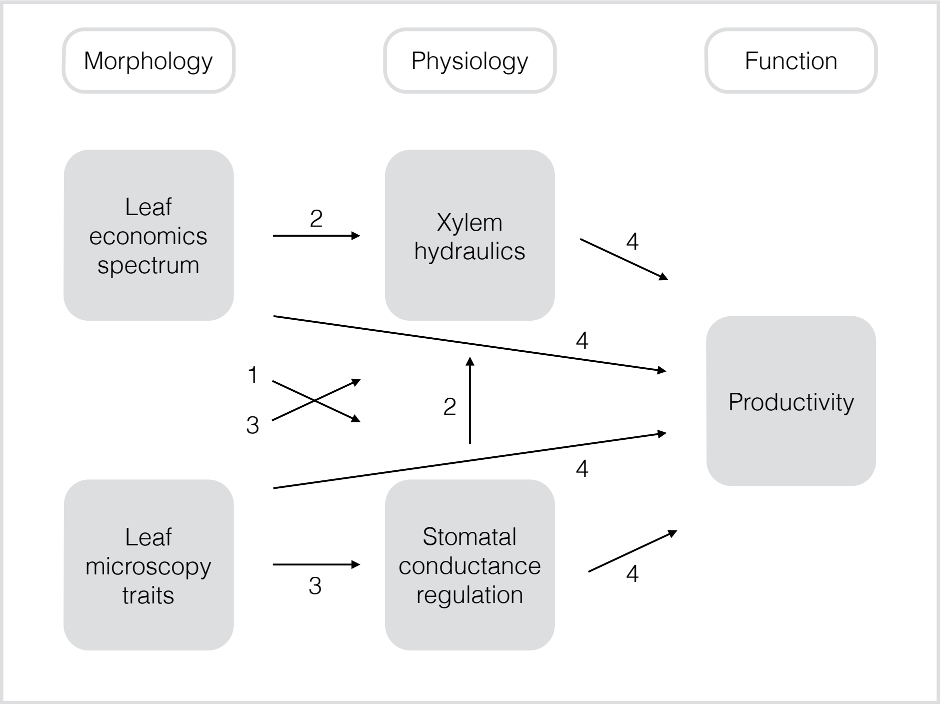

Figure 1

A simplified view on the relationships between ecological functions and trait complexes analysed in the thesis. The boxes represent the ecological functions and trait complexes and the arrows demonstrate the direction of the relationships. The numbers indicate the number of the manuscript and its main hypothesis, respectively. For the specific relationship, see also Table 1.

Table 2

Overview of the species included and the data analysed for the four studies.

Manuscript	Sites	Number of species	Leaf traits	Stomatal conductance traits	Xylem hydraulic traits	Leaf microscopy traits	Additional data
1	A&B	39[1]	✓	✓			
2	A&B	39[2]	✓	✓	✓		
3	A&B	all 40	✓	✓	✓	✓	
4	A	23[3]	✓	✓	✓	✓	Li et al. (2014)

1 excluding *Castanopsis carlesii*; 2 excluding *Quercus phillyraeoides*; 3 all broadleaved tree species planted in site A excluding *Castanopsis carlesii*

1.5.2 Hypotheses tested in the thesis

Figure 1 represents an overview of the hypotheses tested where the numbers stand for the summarized hypotheses for each manuscript.

1. *Rationale:* The LES captures nutrient investment strategies in leaves; therefore important physiological parameters should be linked to the LES. Leaf habit as a key trait should differentiate species in their physiological parameters.

Hypothesis: Parameters of g_S are linked to traits described by the LES, such as SLA and leaf dry matter content, and high g_S values correspond to species with tender leaves and high photosynthetic capacity. Evergreen species have lower mean and maximum g_S than deciduous species.

2. *Rationale:* The importance of the LES is also reflected in wood characteristics and in the resulting physiological constraints.

Hypothesis: Parameters of xylem hydraulics are related to leaf traits describing the LES. In particular, evergreen species have lower Ψ_{50} values compared to deciduous species. Finally, parameters of g_S regulation and parameters of xylem hydraulics should be related to each other.

3. *Rationale:* Leaf traits such as SLA, leaf dry matter content or leaf nitrogen concentration are the main traits used in functional plant ecology analyses. Further leaf traits describing microscopy features provide additional physiological parameters and thus predict plant water relations even better than LES traits.

Hypothesis: Microscopy leaf traits are connected to both parameters – parameters of g_S regulation and parameters of xylem hydraulics.

4. *Rationale:* Combining all plant functional traits analysed in the first three papers, the impact of functional composition on tree growth can be quantified. In the BEF-China experiment, topographic heterogeneity is very high, and thus the environmental impact can be expected to be large. In contrast, due to the young age of the tree individuals, interaction between them cannot yet be expected to be of considerable magnitude yet, thus abiotic effects should not play a very important role.

Hypothesis: Single variables out of environment, CWM and FD do explain tree growth, but in sum environmental predictors have a higher explanatory power for tree growth than CWM or FD.

The sequence of the hypotheses is reflected in the sequence of the manuscripts in the following chapters. Explicit hypotheses postulated in each manuscript further specify the four condensed hypotheses formulated here.

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Chapter 2

TRANSPIRATION AND STOMATAL CONTROL: A CROSS-SPECIES STUDY OF LEAF TRAITS IN 39 EVERGREEN AND DECIDUOUS BROADLEAVED SUBTROPICAL TREE SPECIES

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ORIGINAL PAPER

Transpiration and stomatal control: a cross-species study of leaf traits in 39 evergreen and deciduous broadleaved subtropical tree species

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Abstract

Key message **Using an extensive dataset for 39 subtropical broadleaved tree species, we found traits of the leaf economics spectrum to be linked to mean stomatal conductance but not to stomatal regulation.**

Abstract The aim of our study was to establish links between stomatal control and functional leaf traits. We hypothesized that mean and maximum stomatal conductance (g_s) varies with the traits described by the leaf economics spectrum, such as specific leaf area and leaf dry matter content, and that high g_s values correspond to species with tender leaves and high photosynthetic capacity. In addition, we hypothesized that species with leaves of low stomata density have more limited stomatal closure than those with high stomata density. In order to account for confounding site condition effects, we made use of a common garden situation in which 39 deciduous and evergreen species of the same age were grown in a biodiversity ecosystem functioning experiment in Jiangxi (China). Daily courses of g_s were measured with porometry, and the species-specific $g_s \sim$ vpd relationships were modeled. Our results show that mean stomatal conductance can be predicted from leaf traits that represent the leaf economics spectrum, with a positive relationship being related to leaf nitrogen content and a negative relationship with the leaf carbon: nitrogen ratio. In contrast, parameters of stomatal control were related to traits unassociated with the leaf economics spectrum. The maximum of the conductance $\sim$ vpd curve was positively related to leaf carbon content and vein length. The vpd at the point of inflection of the conductance $\sim$ vpd curve was lower for species with higher stomata density and higher for species with a high leaf carbon content. Overall, stomata size and density as well as vein length were more effective at explaining stomatal regulation than traits used in the leaf economics spectrum.

Keywords BEF-China · Biodiversity ecosystem functioning · Leaf economics spectrum · Leaf traits · Stomatal conductance · Subtropics

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Introduction

In higher terrestrial plants, transpiration and carbon assimilation are intimately linked because plants cannot assimilate carbon without simultaneously losing water (Jarvis and Davies 1998; Roelfsema and Hedrich 2005). Stomatal conductance (g_s) is strongly dependent on the environment and it is subject to diurnal changes that are mainly driven by photosynthetically active photon flux density (PPFD), ambient air temperature (T_a), and leaf-to-air vapor pressure deficit (vpd) (Waring and Landsberg 2011). For typical C3 plants, a diurnal course on a dry and sunny day is described by stomata opening at sunrise, with maximum conductance (g_{smax}) occurring in the early morning, followed by stomatal closure at noon (Larcher 2003; Lüttge and Hertel 2009). Stomatal opening is physiologically triggered by photosynthetically active radiation or blue light (Roelfsema and Hedrich 2005) and stomatal closure is induced by decreasing water potentials mediated by abscisic acid (ABA) (Acharya and Assmann 2009).

When photon flux density regulatory mechanisms are not limiting, the daily course of stomatal regulation is best described by $g_s \sim$ vpd curves (Jones 1998). Plants invariably close stomata at high vpd to keep leaf water potentials above a certain threshold of ψ to avoid cavitation of vessels and inactivation of metabolic processes (Whitehead 1998). As the threshold of ψ is highly species-specific (Jacobsen et al. 2007), species are also expected to differ in the shape of their $g_s \sim$ vpd curves. It should be noted that vpd is not the only driver of g_s, as the same vpd on two different days might be associated with a different stomatal conductance depending on the plant's water status (Siegert and Levia 2011; Strobl et al. 2011). During spells of similar climatic conditions, however, vpd explains a large proportion of variation in g_s. As such, Oren et al. (1999) recommended the use of a modified version of Lohammar's function and a reference conductance at vpd $= 1$ kPa to derive stomatal sensitivity from a linear regression of g_s to ln(vpd). In their analysis, they excluded g_s values below 1 kPa, as under such conditions g_s may not be measured with sufficient accuracy, and low vpd often occurs when irradiance is low (Martin et al. 1997). However, the inferred assumption of a continuous decline of g_s with increasing vpd beyond a threshold of vpd $= 1$ kPa may be inaccurate for species that reach g_{smax} only at vpd values higher than 1 kPa (pers. observation). Furthermore, Oren et al. (1999) noted that the log-linear relationship bears the risk of predicting unrealistic vpd values at $g_s = 0$, i.e., when stomata are fully closed. For these reasons, Leuning (1995) proposed to apply a hyperbolic function to relate g_s to vpd, while in later studies, Eamus et al. (2008) used a 3-parameter polynomial and Patané (2011) used a 3-parameter exponential function to fit the $g_s \sim$ vpd relationship when studying stomatal sensitivity in *Eucalyptus haemastoma* and *Lycopersicon esculentum*, respectively. However, not all of these parameters are physiologically meaningful to describe mechanistics, which is certainly desirable in comparative approaches across different species.

For the current study, we model $g_s \sim$ vpd curves by regressing the logits of g_s to g_{smax} in a generalized linear model on vpd and by using vpd in a quadratic regressor term as a measure of vpd for which g_s is down-regulated with increasing vpd, and which allows for the derivation of slopes and the point of inflection.

In addition to deriving parameters for stomata sensitivity, this approach can also be used to extract modeled values of g_{smax} and vpd at g_{smax}, both of which are key parameters describing a plant's basic hydraulic characteristics (Choat et al. 2006; Franks et al. 2009; Van Wittenberghe et al. 2012).

Most of the earlier models on stomata responses were developed to understand the carbon balance of leaves, single plants, and whole communities (e.g. Cowan 1982; Leuning 1995; Oren et al. 1999). The key parameters of a plant's carbon balance are photosynthetic capacity (A_{max}) and the CO_2 compensation point of assimilation in the presence of dark respiration (R_{dark}). Wright et al. (2004) found A_{max} and R_{dark} to be highly correlated to the leaf economics spectrum, which describes the two opposing strategies of either long-lived leaves with low mass-based carbon assimilation rates or short-lived leaves with high photosynthetic capacity per leaf mass. The leaf economics spectrum is characterized by increasing specific leaf area (SLA), leaf nitrogen (N), phosphorus (P) and cation content, and decreasing leaf dry matter content (LDMC) and carbon to nitrogen ratios (CN). As maximum conductance g_{smax} is proportional to A_{max} (Wong et al. 1979; Santiago and Kim 2009), —and mass-based A_{max} and R_{dark} covary with the leaf economics spectrum, mainly because of their proportionality to leaf area (Osnas et al. 2013)—g_{smax} should also be related to the leaf economics spectrum. While short-lived leaves with high mass-based A_{max} and rapid returns would be expected to have high stomatal conductance, long-lived leaves with low mass-based carbon assimilation rates and low returns on carbon and nutrient investments should display low stomatal conductance. There is some support for such a relationship as g_{smax} was found to be related to leaf N concentration (Schulze et al. 1994; Chaturvedi et al. 2013). In contrast, Santiago and Kim (2009) did not find a correlation between g_s and SLA. Still, multi-species studies are rare, and those that have been conducted have not often made an attempt to relate stomatal regulation to morphological or anatomical traits. In any case, large species-specific differences in stomatal conductance and stomatal regulation have been described in the past (Jacobsen et al. 2008; Hiromi et al. 2012). Aasamaa and Sõber (2011) compared stomatal sensitivities as a response to environmental factors in six temperate deciduous tree species and found a link between stomatal regulation and growth rates. Fast- and slow-growing species showed low and high stomatal sensitivity, respectively. The ability to attain high values of stomatal conductance has consequences at the whole plant level, as demonstrated by Nardini and Salleo (2000) for 11 Mediterranean woody species that showed a positive relationship between g_{smax} and shoot xylem hydraulic conductance. Using eight southeast Asian tree species, Juhrbandt et al. (2004) tested whether leaf traits were connected to maximal stomatal conductance. They found that leaf size and area-based leaf nitrogen content exhibited the highest correlations to g_{smax}. Interestingly, they did not encounter a significant relationship with SLA and only a weak one with leaf N per unit dry mass. Similarly, in a study of 16 tree species from a species-rich, lowland dipterocarp forest, Sales-Come and Hölscher (2010) found g_{smax} to only be related to the corresponding carbon isotope ratio $\delta^{13}C$; the relationship with SLA only emerged when the analysis was confined to dipterocarp species. All these equivocal findings might be explained by the strong dependence of g_s on environmental conditions and, in particular, vpd. This point was raised by Augé et al. (2000), who studied g_s in 11 temperate deciduous tree species and found environmental conditions such as vpd to be much more important than hydraulic and chemical traits. Thus, it may be assumed that using the parameters of the $g_s \sim$ vpd relationship provide more insight into the relationships between stomatal regulation and traits.

As the leaf economics spectrum also reflects the difference between deciduous and evergreen leaves, differences in stomatal conductance would also be expected between these two types of leaf habit. Indications for such differences are already provided by Sobrado (1998), who studied six tropical tree species and found g_s to be higher in deciduous than in evergreen species. Similarly, Zhang et al. (2013) encountered higher midday g_s in deciduous than in evergreen broadleaved tree species in a subtropical cloud forest in Southwest China. The comparison of six shrub and Chinese karst tree species by Liu et al. (2011) suggests that deciduous trees display more conservative stomatal control, thus avoiding drought stress more effectively. However, a more consistent understanding of how stomatal regulation might be related to leaf habit has not yet been achieved.

While a relationship between mean g_s or g_{smax} and traits of the leaf economics spectrum may be expected, values of vpd at g_{smax} or at the point of inflection may alternatively be related to stomata size or stomata density, not least because stomata density is generally thought to be positively related to a plant's ability to regulate transpiration (Carpenter and Smith 1975). Some evidence does, however, indicate that stomata size and density are independent from the traits that define the leaf economics spectrum, e.g., in a study of 120 subtropical species, Kröber et al. (2012) found that stomata density was inversely related to stomata size in a principal component analysis (PCA) and that the stomata density-stomata size axis was perpendicular to the axis of the leaf economics spectrum. However, there is only indirect evidence to suggest a relationship between stomata density and g_s. Miyazawa et al. (2006) manipulated stomatal density by exposing poplars to increased CO_2 concentrations, which resulted in reduced stomatal density, stomatal index (i.e., the ratio of stomata number to leaf epidermal cell number), and level of g_s.

Comparing stomatal control mechanisms across different tree species requires the use of a common environment that hosts trees of similar size and age, such as that encountered in a dendrological garden or in common garden situations such as those found in the tree plantations of biodiversity-ecosystem functioning (BEF) experiments. BEF-China represents the largest of these BEF experiments (Bruelheide et al. 2014); with a species pool of 40 tree species comprising an equal amount of deciduous and evergreen trees (Yang et al. 2013), it offers an ideal site on which to study species-specific differences in stomatal control.

Comparing 39 species in a common garden situation, we hypothesized that (1) conductance parameters would be linked to leaf traits represented in the leaf economics spectrum. In particular, we expected mean g_s and g_{smax} to be positively related to specific leaf area (SLA) and leaf nitrogen content (N), but negatively related to the leaf carbon to nitrogen ratio (CN). In accordance, we additionally hypothesized that (2) evergreen species would display lower mean g_s and g_{smax} values than deciduous species and that (3) traits related to stomata, such as stomata density or stomata size, would describe additional characteristics of stomatal control that are not captured by the leaf economics spectrum. Specifically, we expected species with a high density of stomata to be able to tolerate high vpd, and thus show higher vpd values at g_{smax} or at the point of inflection of the $g_s \sim$ vpd curve.

Materials and methods

Study site and project design

The study was carried out in the BEF China project in Southeast China, Jiangxi Province (http://www.bef-china.de). For the project, a total of 219,000 trees belonging to 42 different species were planted as saplings in 2008 and 2009 on a net area of 38 ha (Yang et al. 2013). The resulting diversity gradient spans six different levels of tree species richness, ranging from monocultures up to 24 tree species per plot. The tree species represent typical elements of the regional subtropical broadleaved forest. Leaf phenology type is equally distributed between evergreen and deciduous species (for species names see Table 1). A detailed description of the establishment procedure is given by Yang et al. (2013). The study site is located within subtropical China and the natural vegetation type is broadleaved laurophyllous mixed forest. Mean annual temperature is 16.7 °C and mean annual precipitation is 1,821 mm (mean from 1971 to 2000, Yang et al. 2013).

Measurement design

We randomly selected 12 high-diversity plots, each containing 16 or 24 species, which allowed us to compare as many species as possible across relatively short distances

Table 1 Tree species planted in the BEF-China experiment and included in this study

Species name	Family	Abbreviation	Leafhabit
Acer davidii Franch.	Aceraceae	Ad	d
Ailanthus altissima (Miller) Swingle	Simaroubaceae	Aa	d
Alniphyllum fortunei (Hemsl.) Makino	Styracaceae	Af	d
Betula luminifera Winkl.	Betulaceae	Bl	d
Castanopsis eyrei (Champion ex Bentham) Tutcher	Fagaceae	Ce	e
Castanopsis fargesii Franch.	Fagaceae	Cf	e
Castanea henryi (Skan) Rehd. et Wils.	Fagaceae	Ch	d
Castanopsis sclerophylla (Lindley & Paxton) Schottky	Fagaceae	Cs	e
Celtis biondii Pamp.	Cannabaceae	Cb	d
Choerospondias axillaris (Roxb.) Burtt et Hill	Anacardiaceae	Ca	d
Cinnamomum camphora (Linn.) Presl	Lauraceae	Cc	e
Cyclobalanopsis glauca (Thunberg) Oersted	Fagaceae	Cg	e
Cyclobalanopsis myrsinifolia (Blume) Oersted	Fagaceae	Cm	e
Daphniphyllum oldhamii (Hemsl.) Rosenthal	Daphniphyllaceae	Do	e
Diospyros japonica Siebold & Zuccarini	Ebenaceae	Dj	d
Elaeocarpus chinensis (Gardn. et Chanp.) Hook. f. ex Benth.	Elaeocarpaceae	Ec	e
Elaeocarpus glabripetalus Merr.	Elaeocarpaceae	Eg	e
Elaeocarpus japonicus Sieb. et Zucc.	Elaeocarpaceae	Ej	e
Idesia polycarpa Maxim.	Flacourtiaceae	Ip	d
Koelreuteria bipinnata Franch.	Sapindaceae	Kb	d
Liquidambar formosana Hance	Altingiaceae	Lf	d
Lithocarpus glaber (Thunb.) Nakai	Fagaceae	Lg	e
Machilus grijsii Hance	Lauraceae	Mg	e
Machilus leptophylla Hand.-Mazz.	Lauraceae	Ml	e
Machilus thunbergii Sieb. et Zucc.	Lauraceae	Mt	e
Manglietia fordiana (Oliver) HuY.W.Law	Magnoliaceae	Manf	e
Melia azedarach Linn.	Meliaceae	Ma	d
Meliosma flexuosa Blume	Sabiaceae	Mf	d
Nyssa sinensis Oliver	Nyssaceae	Ns	d
Phoebe bournei (Hemsl.) Yen C. Yang,	Lauraceae	Pb	e
Quercus acutissima Carruthers	Fagaceae	Qa	d
Quercus fabri Hance	Fagaceae	Qf	d
Quercus phillyreoides A. Gray	Fagaceae	Qp	e
Quercus serrata Murray	Fagaceae	Qs	d
Rhus chinensis Mill.	Anacardiaceae	Rc	d
Sapindus saponaria Linn.	Sapindaceae	Sd	d
Triadica cochinchinensis Loureiro	Euphorbiaceae	Sm	d
Triadica sebifera (L.) Small	Euphorbiaceae	Ss	d
Schima superba Gardn. et Champ.	Theaceae	Schs	e

Species names are in accordance with nomenclature in The Flora of China (http://flora.huh.harvard.edu/china)

d deciduous, *e* evergreen

and in a monogamous topographical setting. The sampling number per plot ranged between 11 and 23 species as not all planted species were represented with full-grown individuals in every plot. In total, we took 3,290 measurements of stomatal conductance in leaf tissue of 218 individuals from 39 distinct species, with 3 to 13 individuals being sampled per species.

Measurements of stomatal conductance

We recorded daily courses of stomatal conductance for all species in a particular plot by taking repeated measurements on the same leaf from the same individual. Figure 1 presents the results from five daily courses. Each sampled leaf was fully developed, undamaged, and fully

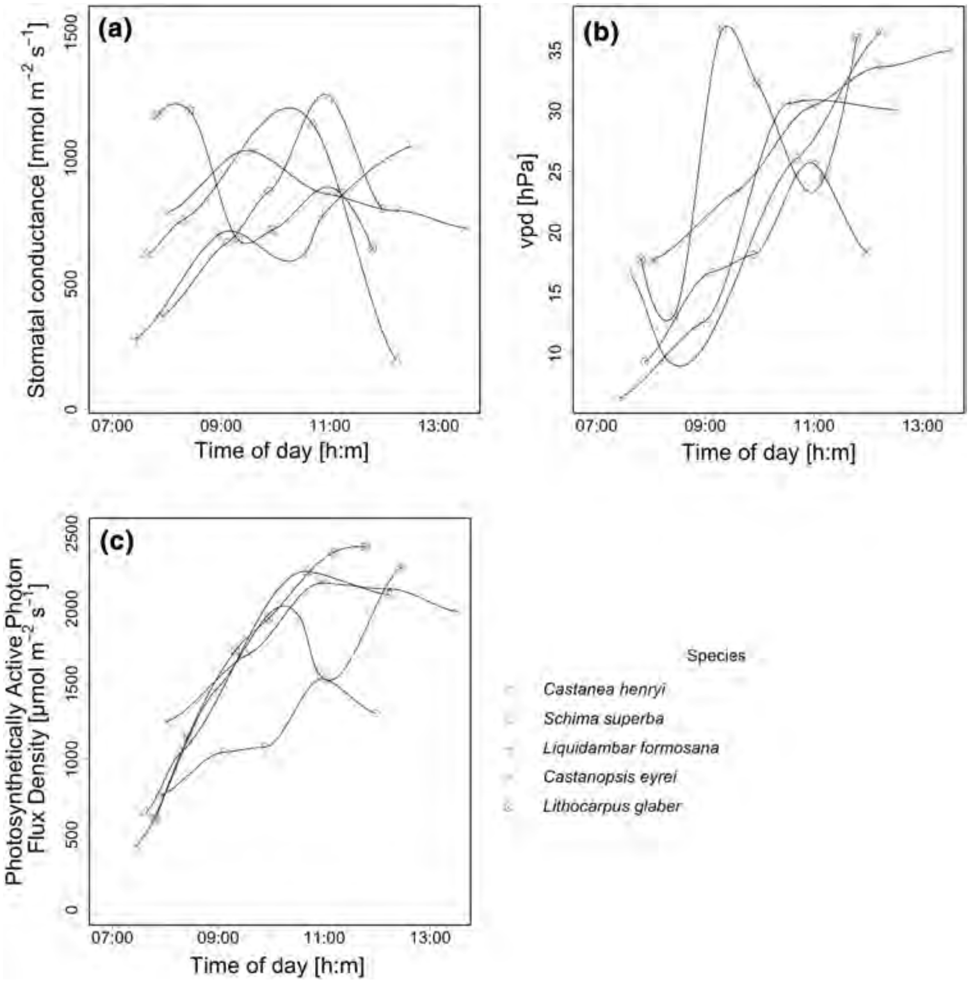


Fig. 1 Daily course of **a** stomatal conductance (g_s), **b** vapor pressure deficit (vpd), and **c** Photosynthetically active photon flux density (PPFD). Symbols refer to one individual each of five different species (*Castanea henryi*, *Schima superba, Liquidambar formosana*, *Castanopsis eyrei,* and *Lithocarpus glaber*), measured on five different days. Values from the same species were measured on the same leaf of the same individual on the same day. The *smoothing lines* were obtained by applying the loess function, a local polynomial regression fitting function

exposed to the sun each day, and leaves were marked with colored plastic ties to ensure the same leaf was measured each day. To provide comparable environmental conditions, all measurements were taken in summer during dry spells and only on sunny days. Stomatal conductance was measured using an SC1 porometer (Decagon), which is based on steady-state technology. It measures the gradient of relative humidity at two distances from the leaf and then calculates vapor flux from the stomata and stomatal conductance per area and time. Only stomatal conductance of the abaxial leaf side was measured as none of the species displayed adaxial stomata. For each measurement of stomatal conductance, we also recorded air temperature and relative humidity using a T2 thermo-hygrometer (Trotec) as well as photosynthetically active photon flux density (PPFD) using a HD2102.2 PAR Logger (DELTA OHM). The field studies took place in August to October 2010, May/June 2011 and August to October 2011 and only included continuous periods of sunny days to ensure radiation was not limiting photosynthesis and stomatal conductance. There were only 154 and 347 measurements with a PPFD <300 and <500 µmol m^{-2} s^{-1}, respectively, out of the total of 3,290 measurements. However, we made sure that these low-light values did not affect our findings by calculating the parameters of stomatal control (see below) by both using the full data set of available measurements and only those taken at PPFD >300 and >500 µmol m^{-2} s^{-1}.

Measurement of leaf traits

We measured 19 leaf traits (see Table 2 for a complete list including abbreviations) following the protocols of Cornelissen et al. (2003) unless stated otherwise. We classified these traits across five groups: (1) categorical leaf type: leaf margin (LeafMar), leaf pinnation (LeafPin), leaf habit (LeafHabit), and extrafloral nectaries (ExtfloNec); (2) quantitative leaf traits: leaf area (LA), leaf toughness (LT), specific leaf area (SLA), and leaf dry matter content (LDMC); (3) leaf element content: leaf calcium content (Ca), leaf magnesium content (Mg), leaf potassium content (K), leaf carbon content (LCC), and leaf nitrogen content (LNC); (4) stomata-related traits: stomata density (StoDens), stomata width (StoW), stomata length (StoL), and

Table 2 List of the leaf traits measured across the 39 tree species

Code	Trait	Analytical technique	Type	Units/categories
LeafHabit	Leaf habit	Literature	Binary	(0) evergreen; (1) deciduous
LeafPin	Leaf pinnation	Field observation	Binary	(0) pinnate; (1) simple
LeafMar	Leaf margin	Field observation	Binary	(0) dentate; (1) entire
ExtfloNec	Extrafloral nectaries	Field observation	Binary	(0) no; (1) yes
LA	Leaf area	Scanner	Continuous	mm^2
LDMC	Leaf dry matter content	Balance	Continuous	$mg\ g^{-1}$
SLA	Specific leaf area	Scanner, balance	Continuous	$m^2\ kg^{-1}$
LT	Leaf toughness	Leaf toughness device	Continuous	$N\ mm^{-1}$
StoDens	Stomata density	Microscopy	Continuous	$1\ mm^{-2}$
StoSize	Stomata size	Microscopy	Continuous	μm^2
StoInd	Stomata index	Microscopy	Continuous	Ratio
LNC	Leaf nitrogen content	CN analyzer	Continuous	$mg\ g^{-1}$
LCC	Leaf carbon content	CN analyzer	Continuous	$mg\ g^{-1}$
CN	Carbon to nitrogen ratio	CN analyzer	Continuous	$g\ g^{-1}$
Ca	Leaf calcium content	AAS analyzer	Continuous	$mg\ g^{-1}$
K	Leaf potassium content	AAS analyzer	Continuous	$mg\ g^{-1}$
Mg	Leaf magnesium content	AAS analyzer	Continuous	$mg\ g^{-1}$
Diamvein1	Diameter veins 1st order	Scanner	Continuous	cm
Diamvein2	Diameter veins 2nd order	Scanner	Continuous	cm
VeinLength	Length of veins in 1 mm^2	Scanner	Continuous	$cm\ cm^{-2}$

All traits were assessed on the individuals planted in the experiment. The table includes the trait abbreviations (code) used throughout the text

(5) quantitative leaf vein traits: diameter of veins of first order (DiamVein1), diameter of veins of second order (DiamVeins2), and vein length (VeinLength). Vein length was determined as the total length of first order veins in an area of 1 cm^2. From these measurements we calculated a further three traits: stomata size (StoSize), stomata index (StoInd), and carbon to nitrogen ratio (CN). We computed stomata size from stomata length and stomata width, assuming an elliptic stomata shape. Stomata index was computed as the product of stomata size and stomata density.

Five leaves from five individuals were sampled per species and measured for leaf area, leaf fresh weight, leaf dry weight, leaf nitrogen content, and leaf carbon content (elementar vario EL). We also calculated specific leaf area, leaf dry matter content, and carbon to nitrogen ratio. Leaf calcium, potassium, and magnesium content were analyzed by means of atom absorption spectrometry (AAS vario 6 analytik jena) for a set of 15 leaves per species (three individuals per species). Leaf vein patterns were analyzed according to modified methods described in Sack and Frole (2006), and stomata traits were assessed following the protocols of Gerlach (1984). Stomata were counted on three leaves from three individuals per species on a minimum of 50,000 mm^2. Length and width were measured on three stomata per replicate and stomata density was expressed as stomata number per mm^2. The observation was done with a light-optical microscope (Zeiss Axioskop 2 plus) and image analysis was carried out using the Axio Vision (Version 3.0) software. Data for stomatal density, length, and width of the guard cells were collected from the same leaves on which stomatal conductance was measured during the field campaigns.

Extra sets of 30 leaves originating from three individuals per species were used for measuring leaf toughness. Leaf toughness was measured as leaf tensile strength, using a modified tearing apparatus, developed and based on the descriptions of Hendry and Grime (1993). A fragment of 5 mm width was cut from the central part of each leaf (not including the midrib) along the longitudinal axis, which did not include main veins. This fragment was then positioned between the two clamps in the tearing apparatus. The two clamps, steered by an external wheel, tore apart the leaf fragment until it ruptured. A spring balance showed the maximal force needed to rupture the leaf fragment. All traits were assessed for all species with the same number of replicates per species. In addition, we determined the presence of leaf pinnation, entire leaf margin, and extrafloral nectaries. Leaf habit was compiled from The Flora of China (http://flora.huh.harvard.edu/china).

Statistical analyses

We analyzed the relationship of g_s to vpd by non-linear regression. Vpd was derived from air temperature and

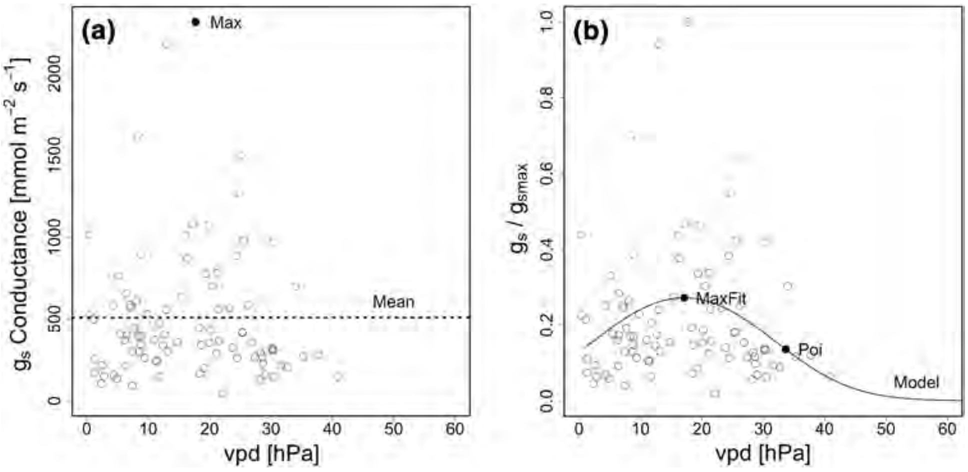


Fig. 2 Stomatal conductance as a function of vapor pressure deficit (vpd), taking *Liquidambar formosana* as an example. *Circles* show the observed values. **a** Absolute stomatal conductance (g_s) data were used to calculate maximum stomatal conductance and the vpd at maximum stomatal conductance as well as mean stomatal conductance. **b** Stomatal conductance was scaled by dividing g_s by the maximum stomatal conductance. The *regression line* was obtained by regressing the logits of g_s/g_{smax} to vpd and the quadratic term of vpd using a generalized linear model with a binomial error distribution. The physiological parameters of stomata control obtained from this regression are shown as *dots*

relative humidity following the August-Roche Magnus formula (Murray 1967):

$$e_s(T) = 6.1094^{17.625T/(T+243.04)},$$

where e_s = saturation water vapor pressure (hPa), and T = temperature (°C)

For every measurement of g_s, we obtained a vpd value (Fig. 1b). Then, for each species, all g_s data were plotted against vpd (Fig. 2a). This allowed us to combine the data from several days into one analysis. We then determined maximum stomatal conductance (ConMax) and vpd at ConMax (VpdMax) for all species. As VpdMax is the actually measured vpd at maximum stomatal conductance it may represent a threshold of vpd, at which the specific individual is limiting stomatal conductance due to high vpd. In addition, we calculated the mean of all conductance measurements per species (ConMean, Fig. 2a). Stomatal conductance data were then scaled per species by dividing observed values through the maximum value observed for that species (g_s/g_{smax}). For each species, we regressed the logits of g_s/g_{smax} to vpd and the quadratic term of vpd using a generalized linear model with a binomial error distribution (a, b and c represent regression parameters):

$$\ln\left(\frac{g_s}{g_{smax}}\right) = a\ \mathrm{vpd}^2 + b\ \mathrm{vpd} + c$$

Using the logits instead of regressing g_s directly to vpd made sure that modeled maximum values (ConMaxfit) did not exceed g_{smax} and that g_s approached 0 at high vpd.

From the model obtained, we derived conductance and vpd values for the following two points: (1) conductance and vpd at the maximum of the model: (ConMaxfit) and (VpdMaxfit), and (2) vpd at the second point of inflection of the curve: (VpdPoi, Fig. 2b). VpdPoi can be interpreted as the sensitivity of stomatal control, as lower values indicate less pronounced stomata closure.

All parameters derived from the $g_s \sim$ vpd curves were correlated to the species' traits in a PCA employing the FactoMineR package in R (http://cran.r-project.org/web/packages/FactoMineR/index.html). We employed linear models to test for bivariate relationships between the modeled parameters and means of leaf traits. The calculations were done with all measurements obtained. To exclude the possibility that low light values affected stomatal conductance, we repeated all measurements using only those taken at PPFD >300 and >500 μmol m^{-2} s^{-1}. For all statistical analyses, we used the software R version 3.0.0 (R Core Team 2013).

Results

Maximum stomatal conductance g_{smax} ranged between 850.3 and 2,979.5 mmol m^{-2} s^{-1} for *Cyclobalanopsis myrsinifolia* and *Elaeocarpus japonicus*, respectively. Mean stomatal conductance (g_s) varied from 1,221.8 to 291.4 mmol m^{-2} s^{-1} and was closely linked to maximum stomatal conductance values g_{smax} ($r^2 = 0.66$, $p < 0.0001$, $n = 39$). Figure 2 shows an example of how the relationship of stomatal conductance to vpd was modeled.

To compare differences in stomatal conductance and stomata regulation, the regression lines of all models of all species were transposed into one graph (Fig. 3). There were no significant differences in any conductance

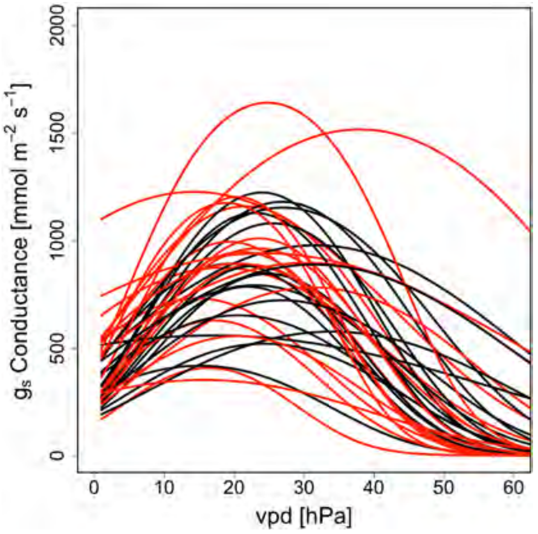


Fig. 3 Regression lines of models of stomatal conductance as a function of vapor pressure deficit (vpd) for all 39 species included in the study. Evergreen species are shown in *black*, deciduous species shown in *red*

parameter between deciduous and evergreen species (Table 3).

For the PCA, 28 leaf traits were used to ordinate the 39 species. The first three axes explained 46.55 % of the total variance, with eigenvalues of 5.51, 3.87 and 3.68 being produced, respectively. As traits representing the leaf economics spectrum, LNC, LeafHabit, and SLA correlated strongly with the first PCA axis (Fig. 4a; digital Appendix Table 5). Positive loadings on the first axis decreased in importance from LA, Mg, ExtfloNec to Ca. Negative loadings on the first axis included CN, LT, LeafPin, LCC, and VeinLength. The second axis was mainly characterized by stomata-related traits with StoW, StoSize and StoL showing positive loadings, and StoInd and StoDens showing negative loadings. The two physiological parameters ConMax and ConMean showed similar positive loadings on all three axes. The extracted physiological parameters ConMaxfit, VpdMaxfit, and VpdPoi showed strong loadings on the third axis (Fig. 4b). VpdMax displayed lesser loadings on all three axes. In the PCA, the range of species formed two clear groups on the first axis according to leaf habit (see Fig. 4c). No clear pattern in leaf habit was apparent for the second and the third axes (Fig. 4c, d; digital Appendix Table 6).

We found several leaf traits to be significantly related to physiological parameters of stomatal control (Table 4). Mean conductivity (ConMean) was positively and negatively related to leaf nitrogen content (LNC), and leaf carbon to nitrogen ratio (CN), respectively (Fig. 5a, b). The maximum of modeled conductances (ConMaxfit) was positively related to leaf carbon content (LCC) and vein length (Fig. 6a, b). Vpd at maximum conductance (VpdMaxfit) showed a negative correlation with specific leaf area (SLA) and stomata size (Fig. 7a, b) and a negative correlation with LCC (Fig. 7c). Vpd at the point of inflection (VpdPoi) significantly decreased with stomata density and stomata index (Fig. 8a, b) and increased with LCC (Fig. 8c). No relationships were recorded for any of the morphological leaf traits for maximum conductance (ConMax). We obtained essentially the same significant relationships when only using the measurements taken at PPFD >300 µmol m^{-2} s^{-1} (data not shown). The only difference was that ConMaxfit was no longer significantly related to vein length (Table 4). When taking only measurements at PPFD >500 µmol m^{-2} s^{-1}, four further relationships were lost. However, ConMean was still significantly related to LNC and CN, ConMaxfit to LCC, and VpdPoi to stomata index and LCC (data not shown).

Discussion

Our study covering 39 subtropical tree species revealed clear relationships between the parameters of stomatal control and other important functional leaf traits. As stomatal conductance under field situations is potentially influenced by a number of confounding environmental variables, our approach of modeling $g_s \sim$ vpd curves by regressing the

Table 3 Mean values (±standard deviation) for the parameters of stomatal control for deciduous and evergreen leaf habit

Parameter of stomatal conductance	Mean deciduous	Mean evergreen	SD deciduous	SD evergreen	*F* value	*p*
ConMean (mmol m^2 s^{-1})	749.8	699.4	243.1	188.1	0.5106	0.4794
ConMax (mmol m^2 s^{-1})	2,107.2	2,081.2	519.8	574	0.22	0.8828
VpdMax (hPa)	22.2	23.6	5.1	5.7	0.6868	0.4126
ConMaxfit	0.4439	0.4113	0.09802	0.05157	1.6221	0.2107
VpdMaxfit (hPa)	21.7	24.4	5.8	4.9	2.3164	0.1365
VpdPoi (hPa)	43.43	46.85	10.4	8.8	1.2037	0.2797

ConMaxfit is a relative unit, obtained from relating g_s to g_{smax}. *F* and *p* values refer to a one-factorial ANOVA comparing the mean values, with 37 degrees of freedom

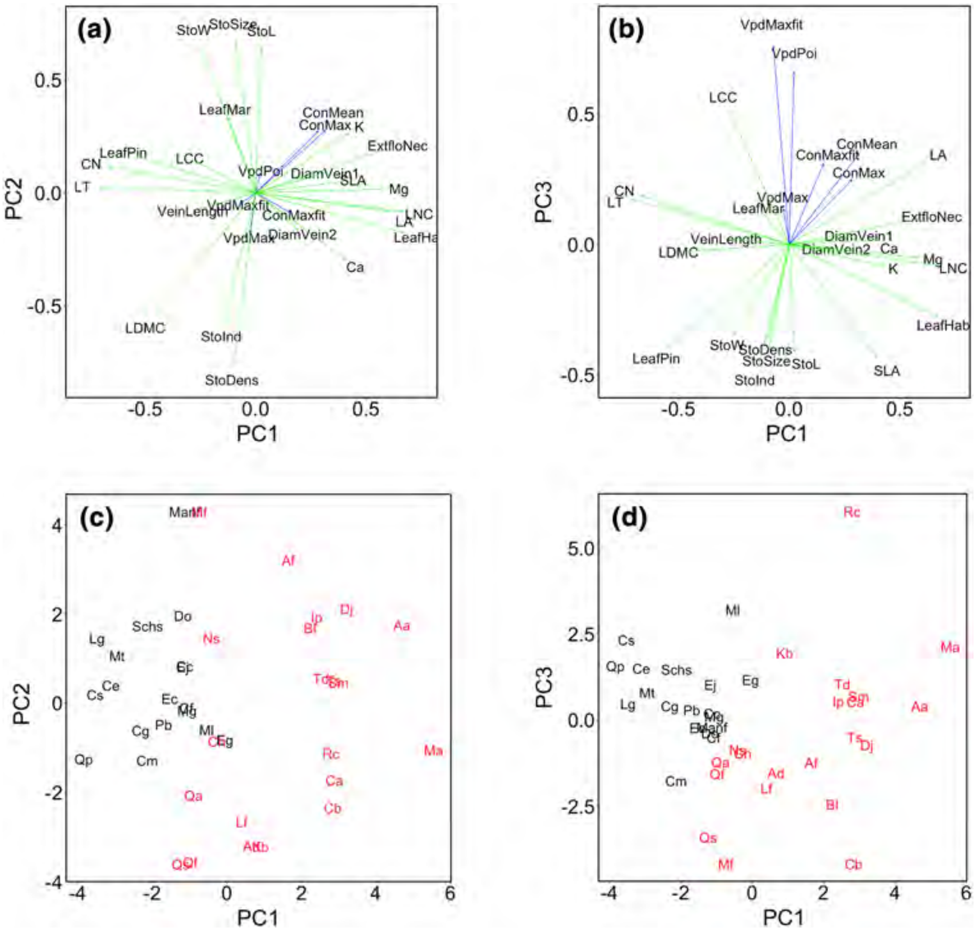


Fig. 4 Principal component analysis (PCA) biplots showing the relationships between the mean values of all chemical and morphological leaf traits (*green arrows*) and all parameters of stomatal regulation (*blue arrows*). **a**, **c** PCA axes 1 and 2, **b**, **d** PCA axes 1 and 3. **a**, **b** Loadings of the different traits. Eigenvalues: axis 1 = 5.51, axis 2 = 3.87, axis 3 = 3.68, with cumulative proportion of explained inertia 21.0, 34.5, and 46.5 %, respectively. See Table 2 for abbreviations of trait names. **c**, **d** Species scores in the PCA shown separately by leafhabit. Evergreen species are shown in *black* and deciduous in *red*. See Table 1 for abbreviations of species names

Table 4 Correlation matrix between the leaf traits and the parameters of stomatal control

	ConMean		ConMax		VpdMax		ConMaxfit		VpdMaxfit		VpdPoi	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
LA	0.16	0.33	0.10	0.56	0.09	0.57	0.19	0.24	0.22	0.18	0.28	0.09
LDMC	−0.20	0.21	−0.30	0.06	0.01	0.97	0.18	0.28	0.04	0.83	−0.04	0.82
SLA	−0.21	0.20	−0.27	0.10	0.09	0.58	−0.04	0.79	**−0.40**	**0.01**	−0.26	0.11
LT	−0.23	0.15	−0.18	0.26	0.12	0.45	−0.15	0.37	0.13	0.43	0.09	0.58
StoDens	−0.29	0.08	−0.23	0.17	0.06	0.72	−0.04	0.81	−0.22	0.18	**−0.33**	**0.04**
StoSize	0.07	0.69	0.05	0.77	−0.16	0.34	−0.11	0.49	**−0.33**	**0.04**	−0.16	0.34
StoInd	−0.21	0.20	−0.18	0.28	0.03	0.83	−0.01	0.95	−0.29	0.07	**−0.35**	**0.03**
LNC	**0.32**	**0.05**	0.18	0.28	0.25	0.12	0.26	0.12	−0.15	0.37	0.05	0.78
LCC	0.08	0.61	−0.10	0.53	0.06	0.71	**0.33**	**0.04**	**0.36**	**0.02**	**0.37**	**0.02**
CN	**−0.32**	**0.05**	−0.21	0.20	−0.23	0.15	−0.22	0.19	0.24	0.14	0.09	0.60
Ca	−0.09	0.58	0.06	0.73	−0.12	0.46	−0.07	0.66	−0.09	0.58	−0.14	0.39
K	0.04	0.81	0.14	0.38	0.03	0.84	−0.20	0.23	−0.06	0.72	0.13	0.42
Mg	0.00	0.98	0.09	0.60	−0.11	0.52	−0.03	0.86	−0.10	0.56	−0.11	0.51
DiamVein1	0.10	0.55	0.18	0.28	0.05	0.75	−0.05	0.76	−0.06	0.70	−0.01	0.97
DiamVein2	0.20	0.22	0.27	0.10	0.02	0.92	0.10	0.57	0.07	0.67	0.10	0.55
VeinLength	0.15	0.37	−0.01	0.94	−0.10	0.55	**0.34**	**0.04**	0.03	0.86	−0.04	0.82

The correlations were calculated from linear model analysis between functional leaf traits and the extracted physiological parameters. Significant relationships are shown in bold letters

ConMean mean g_s, *ConMax* mean g_{smax}, *VpdMax* vpd at g_{smax}, *ConMaxfit* modeled g_{smax}, *VpdMaxfit* vpd at modeled g_{smax}, *VpdPoi* vpd at the point of inflection of the $g_s \sim$ vpd curve

Fig. 5 Mean stomatal conductance (ConMean) as a function of **a** leaf nitrogen content and **b** leaf carbon to nitrogen ratio

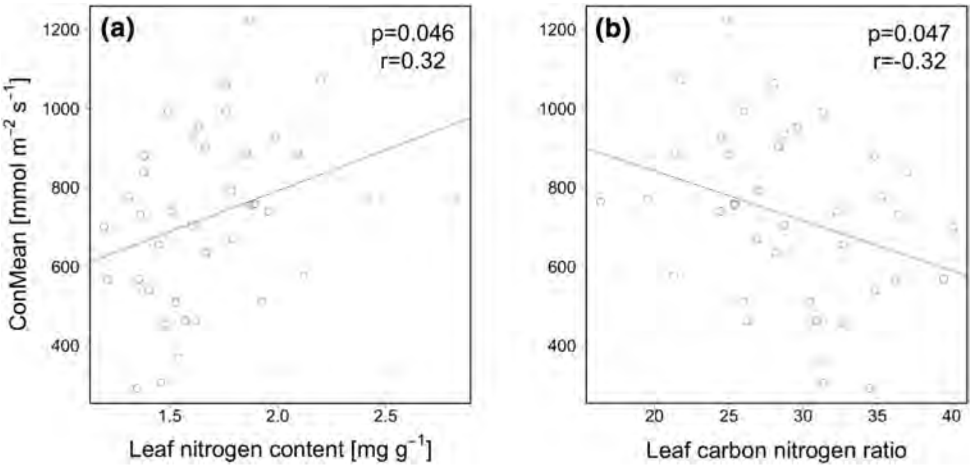
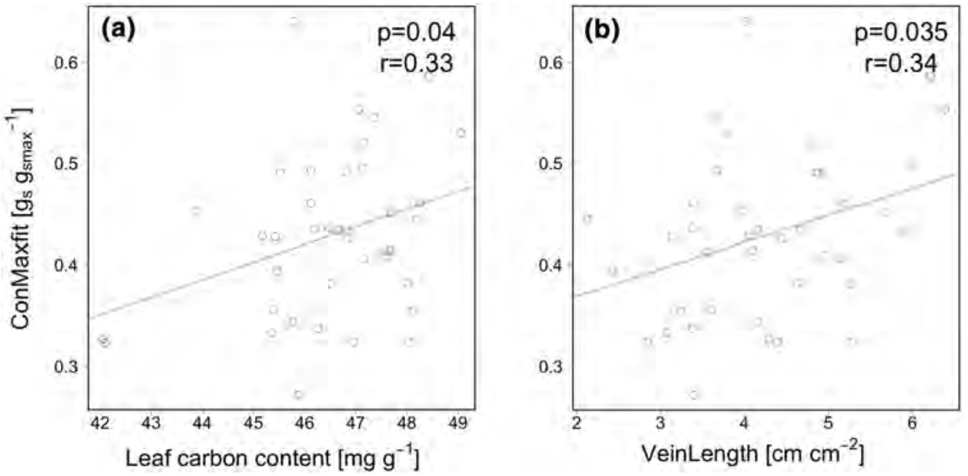


Fig. 6 Maximum of modeled stomatal conductance (ConMaxfit) as a function of **a** leaf carbon content and **b** vein length



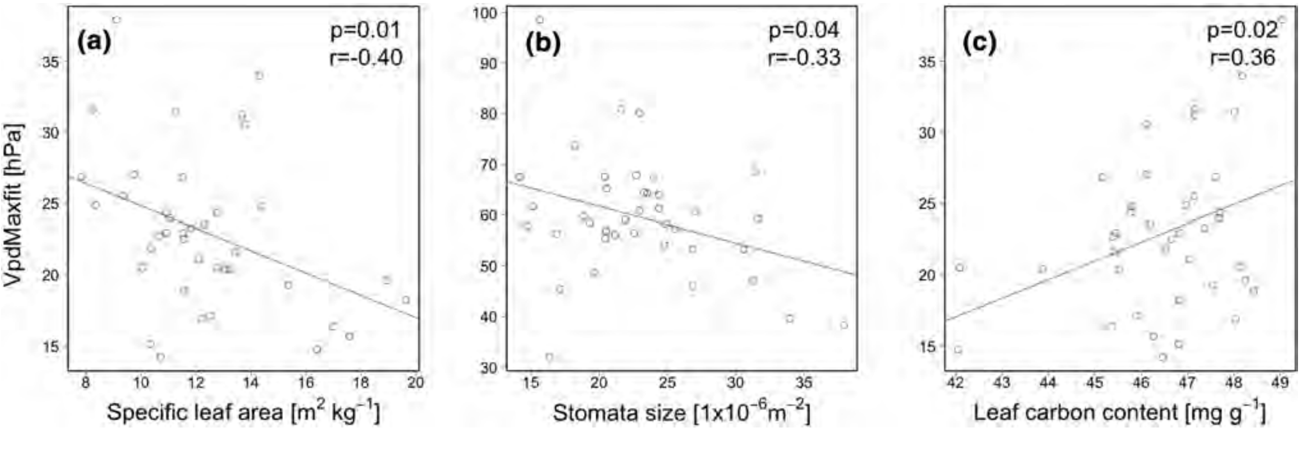


Fig. 7 Vapor pressure deficit (vpd) at the maximum modeled conductance (VpdMaxfit) as a function of **a** specific leaf area, **b** stomata size, and **c** leaf carbon content

logits of g_s to g_{smax} on vpd in a generalized linear model and then testing the parameters from the regression for trait relationships revealed patterns that had not previously been encountered. We were also able to eliminate a large proportion of environmental noise using the experimental setting of a common garden situation, with sampling sessions only being conducted on sunny days and multitude species being measured on the same day. However, using field data cannot fully exclude that our modeled $g_s \sim$ vpd relationship might have been affected by PPFD, as under

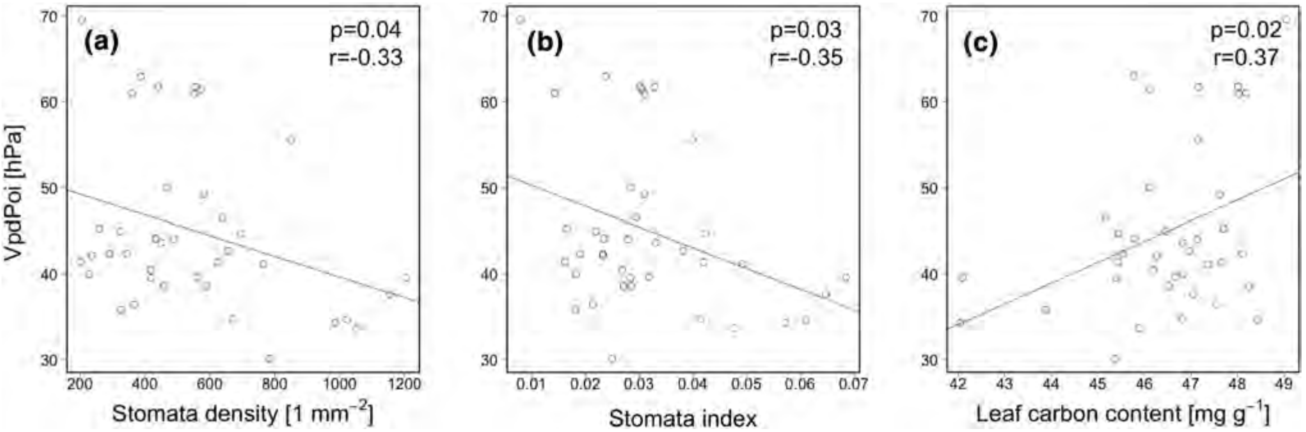


Fig. 8 Vapor pressure deficit (vpd) at the point of inflection of the $g_s \sim$ vpd curve (VpdPoi) as a function of **a** stomata density, **b** stomata index, and **c** leaf carbon content

field conditions PPFD covaries with vpd. Confining our analyses to values taken at PPFD >300 and >500 µmol m^{-2} s^{-1} did not fundamentally change our results. At these PPFD values many of our study species have reached 70–80 or >90 % of A_{max}, as was shown by Liu et al. (2012) for, e.g., *Schima superba* and *Castanopsis* spec.

The results partly confirm our first hypothesis of a link between conductance parameters and leaf traits of the leaf economics spectrum, as the mean and modeled maximum stomatal conductance showed significant relationships with particular leaf traits. Although mean g_s and g_{smax} were not related to SLA as hypothesized, we encountered a positive correlation between g_s and g_{smax} and leaf nitrogen content, and a negative correlation with the leaf carbon to nitrogen ratio. The positive correlation of stomatal conductance and leaf nitrogen content is in accordance with the global review of Schulze et al. (1994), who found clear links between leaf nitrogen content and g_{smax}. Similarly, our results confirm those of Juhrbandt et al. (2004), who reported significant relationships between g_{smax} and leaf area and leaf nitrogen content. As in our study, the authors did not find a relationship between g_s or g_{smax} and SLA, leaf potassium content or leaf calcium content. Elsewhere, Poorter and Bongers (2006) reported a close link between g_s and the main traits represented by the leaf economics spectrum, with g_s decreasing with increasing leaf lifespan.

In our study, the link between mean conductance parameters and SLA, leaf carbon and nitrogen content explains the clear segregation of deciduous and evergreen species encountered on the first PCA axis. However, our second hypothesis suggesting differences between deciduous and evergreen species could not be confirmed by a direct statistical comparison for any parameter of stomata control, although the tendency was the same as in the PCA. The missing statistical segregation between species of evergreen and deciduous leaf habit is explained by the high variance of all parameters and traits observed within the two groups and suggests that leaf habit is a poor predictor for traits on the leaf economics spectrum. In a comparative study, Brodribb and Holbrook (2005) proposed that leaf habit and leaf physiological strategies are not necessarily linked, and they further suggested that a wide range of combinations of functional traits are possible within both the deciduous and evergreen leaf habit.

We also found support for our third hypothesis suggesting additional characteristics of stomatal control are related to stomata density and stomata size. Species with large stomata displayed low values of vpd at maximum conductance (VpdMaxfit). This finding is a hint that not all physiologically meaningful information is captured by the leaf economics spectrum. However, somewhat surprisingly, species with high stomata density and high stomata index showed low values of vpd at the point of inflection of the $g_s \sim$ vpd curve.

In a leaf trait study on 53 rainforest species, Poorter and Bongers (2006) successfully predicted plant performance variables such as growth, survival, and light requirements. In their analysis, they demonstrated close coordination between various leaf traits, including physiological parameters such as stomatal conductance and the slow–fast continuum of nutrient investment in leaves. We only found studies that describe a positive relationship between stomata size and stomatal conductance (Aasamaa et al. 2001; Camposeo et al. 2011), suggesting that stomatal control may not be invariably related to important functional leaf traits, a view echoed by Wright and Sutton-Grier (2012), who further emphasize the limited explanatory power of the leaf economics spectrum in relation to their two greenhouse experiments.

Some physiological characteristics may, therefore, be related to stomata size and density, and smaller stomata have indeed been found to confer increased water-use efficiency in plants, as they allow for a quicker response to changing vpd (Cowan and Farquhar 1977; Hetherington and Woodward 2003; Franks and Farquhar 2007). In consequence, leaves with small stomata would allow for more fine-tuning of stomata control, and thus, would allow species to operate closer to the physiological limit at high vpd. With smaller stomata, plants may also be able to avoid xylem embolisms more effectively (Franks and Beerling 2009). Size and frequency of stomata may, therefore, be key parameters in terms of plant adaptation to prevailing environmental conditions.

Consistent with the findings of our study, Franks found stomata size and density to be inversely related (Franks and Beerling 2009), and Franks et al. (2009) found a similar relationship between density and stomatal pore length. In a paleobotanical context, Franks and Beerling (2009) showed that this negative relationship accounted for changes in global CO_2 levels. In paleobotany, stomatal density is considered a valid predictor for past CO_2 concentrations, as higher CO_2 concentrations result in lower stomatal density (Van Der Burgh et al. 1993; Mcelwain 2004; van Hoof et al. 2006). Franks et al. (2009) modeled different combinations of stomatal density and stomatal size and found that smaller stomatal size in combination with higher stomatal density was related to higher g_{smax}. Accordingly, Willmer and Fricker (1996) found that many species with high densities of small stomata are capable of very high stomatal conductance. The relationships between stomatal density and size are also encountered within single species. For example, Franks et al. (2009) described a trade-off between stomatal density and size within a single species (*Eucalyptus globulus*) and concluded that this trade-off limited the plasticity of g_{smax} in the species. In addition, Miyazawa et al. (2006) studied the stomatal development of new leaves in poplar trees and found a positive relationship between g_s and stomatal index.

Conclusion

In conclusion, our study confirms the close link between stomatal conductance and traits aggregated in the leaf economics spectrum. However, we also demonstrate that the leaf economics spectrum does not capture all information and aspects of stomatal regulation and that additional information can be derived from the modeled parameters of the conductance ∼vpd curve. Furthermore, leaf traits such as stomata size, density, and index as well as vein length may serve as simple proxies for some of these parameters and thus might confer additional information in comparative studies on plant functional traits.

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Conflict of interest The authors declare that they have no conflict of interest.

Appendix

See Tables 5 and 6

Table 5 Loadings of traits in the principal components analysis (PCA)

Traits	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
ExtfloNec	0.64	0.21	0.11	0.10	0.20
LA	0.67	−0.09	0.34	0.02	−0.51
LDMC	−0.50	−0.60	−0.03	0.38	−0.02
SLA	0.44	0.05	−0.48	−0.34	−0.05
LT	−0.78	0.03	0.19	−0.03	0.08
ConMean	0.35	0.31	0.39	0.62	0.38
ConMax	0.31	0.30	0.28	0.36	0.50
VpdMax	−0.03	−0.20	0.15	−0.23	−0.32
ConMaxfit	0.17	−0.10	0.35	0.56	−0.03
VpdMaxfit	−0.08	−0.05	0.84	0.04	−0.11
VpdPoi	0.02	0.10	0.73	−0.01	−0.25
StoL	0.03	0.72	−0.46	0.27	−0.31
StoW	−0.28	0.72	−0.38	0.27	−0.22
StoSize	−0.10	0.75	−0.44	0.28	−0.29
StoInd	−0.16	−0.63	−0.52	0.38	−0.06
StoDens	−0.11	−0.83	−0.40	0.18	0.02
LNC	0.74	−0.09	−0.09	0.33	−0.09
LCC	−0.30	0.15	0.56	0.10	−0.14
CN	−0.74	0.13	0.21	−0.32	−0.04
Ca	0.45	−0.33	0.02	−0.25	−0.03
K	0.47	0.29	−0.09	−0.25	0.05
Mg	0.65	0.02	−0.06	−0.37	−0.06
DiamVein1	0.32	0.09	0.03	−0.11	0.41
DiamVein2	0.21	−0.18	−0.02	0.13	0.61
VeinLength	−0.28	−0.08	0.02	0.62	−0.24
LeafHab	0.75	−0.20	−0.31	0.16	−0.10
LeafPin	−0.60	0.18	−0.44	−0.03	0.46
LeafMar	−0.14	0.37	0.14	−0.26	0.33

See Table 2 for full trait names

Table 6 PCA scores for all species

Species	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Acer davidii	0.39	−2.05	−3.53	−1.57	0.37
Ailanthus altissima	4.47	−0.13	1.88	0.18	−2.03
Alniphyllum fortunei	1.67	−1.06	0.31	2.35	0.57
Betula luminifera	2.65	−0.65	−1.65	2.55	0.48
Castanopsis eyrei	−3.02	1.83	0.93	1.17	−0.60
Castanopsis fargesii	−0.90	−0.91	0.32	−0.57	2.85
Castanea henryi	0.33	2.14	−1.52	1.89	1.22
Castanopsis sclerophylla	−3.50	2.09	1.55	−0.01	1.54
Celtis biondii	2.93	−3.05	−3.10	−1.45	1.29
Choerospondias axillaris	2.57	−0.65	0.32	−3.27	−2.72
Cinnamomum camphora	−1.09	−0.04	1.09	0.51	−0.46
Cyclobalanopsis glauca	−2.55	0.09	0.27	−0.50	−0.13
Cyclobalanopsis myrsinifolia	−1.75	0.80	−2.57	−0.21	−1.34
Daphniphyllum oldhamii	−1.56	−2.37	0.92	−0.73	−1.52
Diospyros japonica	2.90	−3.21	0.94	1.71	1.01
Elaeocarpus chinensis	−1.84	−0.88	0.17	0.29	−0.89
Elaeocarpus glabripetalus	−0.23	0.44	0.48	0.49	1.65
Elaeocarpus japonicus	−1.27	0.65	1.38	0.08	−0.25
Idesia polycarpa	2.29	−1.21	1.52	0.61	1.88
Koelreuteria bipinnata	1.12	2.85	−0.63	−3.37	−0.59
Liquidambar formosana	0.05	−1.84	−2.35	−1.72	−0.31
Lithocarpus glaber	−3.77	−1.72	1.19	−0.39	−0.53
Machilus grijsii	−1.21	−1.78	0.22	0.16	0.36
Machilus leptophylla	−0.99	−2.18	1.07	−0.98	1.78
Machilus thunbergii	−3.13	−0.48	0.25	−0.12	−2.45
Manglietia fordiana	−1.07	−1.11	1.82	2.20	−0.14
Melia azedarach	5.74	1.89	1.26	−1.71	0.98
Meliosma flexuosa	−0.51	−2.73	−2.11	0.73	−0.85
Nyssa sinensis	−0.44	0.21	−0.61	0.68	−2.30
Phoebe bournei	−1.55	−0.33	−0.43	0.37	1.92
Quercus acutissima	−0.28	3.54	−1.92	2.12	−1.03
Quercus fabri	−0.38	3.67	−3.02	1.01	−0.68
Quercus phillyraeoides	−4.03	0.28	0.60	−1.79	−0.85
Quercus serrata	−0.46	3.68	−2.30	−1.62	3.14
Rhus chinensis	2.45	3.07	3.63	−2.02	0.30
Sapindus saponaria	2.94	0.00	0.90	−1.15	−1.37
Schima superba	−2.14	−0.36	2.05	−0.41	1.76
Triadica cochinchinensis	2.51	2.06	0.80	2.96	−1.22
Triadica sebifera	2.64	−0.56	−0.14	1.53	−0.83

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Chapter 3

LINKING XYLEM HYDRAULIC CONDUCTIVITY AND VULNERABILITY TO THE LEAF ECONOMICS SPECTRUM – A CROSS SPECIES STUDY OF 39 EVERGREEN AND BROADLEAVED SUBTROPICAL TREE SPECIES

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RESEARCH ARTICLE

Linking Xylem Hydraulic Conductivity and Vulnerability to the Leaf Economics Spectrum—A Cross-Species Study of 39 Evergreen and Deciduous Broadleaved Subtropical Tree Species

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files. Requests can also be directed to Wenzel Kröber, wenzel.kroeber@botanik.uni-halle.de.

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Abstract

While the fundamental trade-off in leaf traits related to carbon capture as described by the leaf economics spectrum is well-established among plant species, the relationship of the leaf economics spectrum to stem hydraulics is much less known. Since carbon capture and transpiration are coupled, a close connection between leaf traits and stem hydraulics should be expected. We thus asked whether xylem traits that describe drought tolerance and vulnerability to cavitation are linked to particular leaf traits. We assessed xylem vulnerability, using the pressure sleeve technique, and anatomical xylem characteristics in 39 subtropical tree species grown under common garden conditions in the BEF-China experiment and tested for correlations with traits related to the leaf economics spectrum as well as to stomatal control, including maximum stomatal conductance, vapor pressure deficit at maximum stomatal conductance and vapor pressure deficit at which stomatal conductance is down-regulated. Our results revealed that specific xylem hydraulic conductivity and cavitation resistance were closely linked to traits represented in the leaf economic spectrum, in particular to leaf nitrogen concentration, as well as to log leaf area and leaf carbon to nitrogen ratio but not to any parameter of stomatal conductance. The study highlights the potential use of well-known leaf traits from the leaf economics spectrum to predict plant species' drought resistance.

Introduction

The worldwide leaf economics spectrum (LES) represents an important framework of trade-offs between key functional leaf traits [1]. It describes different strategies of carbon capture among vascular land plants, from that of short-lived leaves with high photosynthetic capacity per leaf mass, to long-lived leaves with low mass-based carbon assimilation rates. Mass-based photosynthetic capacity is positively related to mass-based leaf nitrogen concentration (LNC) and to specific leaf area [2] and is negatively related to leaf life span [1]. Many studies have confirmed the global validity of these trade-off patterns [3–5], and Osnas *et al.* [6] recently demonstrated that such relationships between traits in the LES result from relationships to leaf area and from normalizing area-proportional traits by leaf mass. Kröber & Bruelheide [7] have demonstrated that there are additional dimensions to plants' functional traits that are orthogonal to the LES. They found parameters of stomatal regulation (derived from stomatal conductance - vapor pressure deficit relationships), stomatal density and stomatal size to be independent from the LES.

So far, reported relationships between traits of the LES and those of plant organs other than leaves, such as wood or roots, are equivocal. For example, Baraloto *et al.* [8] found the main axes in leaf and wood traits to be decoupled, while Freschet *et al.* [9] provided evidence for a tight relationship between the main dimensions of the leaf, stem and root economics spectra. However, from a 'whole plant' perspective, tight relationships would be expected for those leaf and wood traits that determine a plant's tolerated minimum water potential, because the plant's water status links a multitude of physiological processes [10]. Choat *et al.* [11] and Poorter *et al.* [12] reported that leaves with high specific leaf area (SLA) were linked to stems with low wood density. Similar to wood density, hydraulic xylem properties would also be expected to be correlated across roots, stems and leaves. In particular, a high photosynthetic capacity of leaves, as expressed by high SLA, should be associated with high xylem hydraulic conductivity to facilitate sufficient water supply required for high stomatal conductance. Accordingly, in a study on ten tropical tree species in Panama, Sack and Frole [13] reported that leaf hydraulic resistance was strongly linked to leaf venation and mesophyll structure. The relationship between hydraulic conductivity and leaf venation was also confirmed across 43 species worldwide [14]. However, Sack *et al.* [15] argued that leaf hydraulic conductance might be mechanistically independent from the LES, but might be linked statistically as both hydraulic conductance and LES traits affect mass-based photosynthesis.

Besides being hydraulically efficient, another required feature of the vascular plumbing network is drought resistance. In this regard, species with stress-resistant leaves, as indicated by low SLA values, should be expected to have stress-resistant wood. Wood stress resistance is reflected in high wood density, which is thought to confer a higher tolerance from shade, wind, herbivores and drought [16]. In particular, drought resistance determined by measuring xylem vulnerability to cavitation should be correlated between leaves and wood, because cavitation is a persistent hazard under drought stress and affects leaves and wood [17]. Sustaining low water potentials requires high cavitation resistance of conduits, as derived from xylem vulnerability curves [18]. These curves allow quantifying the specific xylem hydraulic conductivity of the xylem (K_S) and the xylem pressure at which 50% loss of the maximum specific xylem hydraulic conductivity occurs (Ψ_{50}). Ψ_{50} is mainly determined by pit size and structure [19, 20]. However, low water potentials are transmitted throughout the whole plant, from the point where the water-pathway ends and the regulation of the water flow takes place to the xylem, where cavitation occurs. Thus, Ψ_{50} should be reflected in functional leaf traits and parameters of stomatal regulation.

Tree species that are able to endure severe drought periods have characteristic leaves. The leaves are tough and have a high leaf dry matter content (LDMC), allowing them to sustain low water potentials [21, 22]. Such species should likewise be characterized by low Ψ_{50} values. SLA is inversely related to LDMC, in that it decreases with drought resistance [23, 24], and it would be expected to scale negatively with Ψ_{50}. In addition, cavitation vulnerability should also be related to stomatal regulation, because cavitation-sensitive and -insensitive species would be expected to close their stomata at low and high vapor pressure deficits, respectively [25–27]. Such parameters of stomatal closure have recently been provided by Kröber & Bruelheide [7] for the same 39 species used also in the current study. The authors measured daily courses of stomatal conductance (g_s) with porometry in the same plots as in the present study, and modeled the species-specific $g_s \sim$ vapor pressure deficit (VPD) relationships. They found that mean g_s can be predicted from leaf traits that reflect the LES, with a positive relationship to LNC and a negative relationship to leaf carbon to nitrogen ratio. In contrast, the maximum of the $g_s \sim$ VPD curve was unrelated to traits of the LES and increased with leaf carbon concentration (LCC) and vein length. The VPD at which g_s was down-regulated, characterized by the point of inflection of the $g_s \sim$ VPD curve at high VPD, was lower for species with higher stomatal density and lower leaf carbon concentration. In addition to leaf trait measurements, we use these parameters of stomatal control from Kröber & Bruelheide [7] to predict xylem hydraulics.

The objective of our study was to quantify hydraulic conductivity and Ψ_{50} from xylem vulnerability curves, making use of the common garden situation of the BEF-China experiment. Comparing 39 broad-leaved tree species, we hypothesized that (1) leaf traits describing the leaf economics spectrum are related to specific xylem hydraulic conductivity and cavitation resistance. Accordingly, we expected that (2) evergreen species characterized by low SLA and high LDMC are more resistant to cavitation, i.e. have lower Ψ_{50} values than deciduous species, Finally, (3) we tested the hypothesis that parameters of stomatal regulation, such as maximum stomatal conductance, the vapor pressure deficit (VPD) at maximum stomatal conductance and VPD at which stomatal conductance is down-regulated, are related to high xylem hydraulic conductivity.

Materials and Methods

Study Site

The study was conducted in the BEF-China project, which is a biodiversity-ecosystem functioning experiment based in Jiangxi Province, southeast China (http://www.bef-china.de; 29.08–29.11 N, 117.90–117.93 E). The climate at the experimental site is subtropical with moderately cold and dry winters and warm summers. Based on data of meteorological stations established at the sites, mean annual temperature was 17.4℃ and mean annual precipitation was 1635 mm (Fig. 1). Across an area of 38 ha, 219,000 trees were planted at different levels of species richness [29]. The diversity gradient spans from monoculture to two, four, eight, 16 and up to 24 species per plot. The 39 tree species included in the study (see Table 1) are representative of the local natural broadleaved subtropical forest community [30, 31], and the trees assessed had already reached an age of four or five years at the time when our study was carried out. Using young even-aged trees in a common garden situation allowed for controlling for confounding factors, such as different ontological stage, but also allowed to sample leaves and branches at a standardized height above ground. No specific permissions were required for these locations and activities. The field studies did involve neither endangered nor protected species.

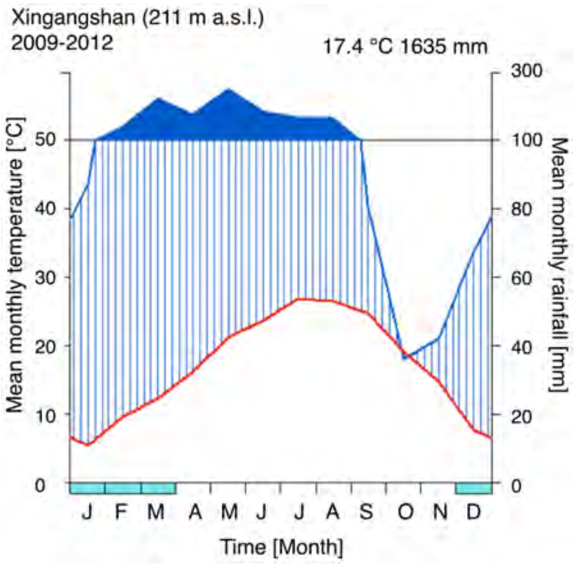

Figure 1. Climate diagram according to Walter & Lieth [28] of Xingangshan, the location of the experimental sites. Elevation: 211 m above sea level. Observation period was March 2009 to October 2012. Mean annual temperature was 17.4˚C and total annual precipitation was 1635 mm. Monthly precipitation below 100 mm is scaled 2:1 with mean monthly temperature (vertically hatched) and above 100 mm 15:1. Turquoise bars below the x-axis show the months where frosts can occur (when absolute monthly minimums are equal or lower than 0˚C). Climate data were recorded by a meteorological station established at the very center of the experimental site (Kühn, unpublished).

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Table 1. Tree species planted in the BEF-China experiment and included in this study.

Species name	Family	Abbreviation	Leaf habit
Acer davidii Franch.	Aceraceae	Ad	d
Ailanthus altissima (Miller) Swingle	Simaroubaceae	Aa	d
Alniphyllum fortunei (Hemsl.) Makino	Styracaceae	Af	d
Betula luminifera Winkl.	Betulaceae	Bl	d
Castanopsis eyrei (Champion ex Bentham) Tutcher	Fagaceae	Ce	e
Castanopsis fargesii Franch.	Fagaceae	Cf	e
Castanea henryi (Skan) Rehd. et Wils.	Fagaceae	Ch	d
Castanopsis sclerophylla (Lindley & Paxton) Schottky	Fagaceae	Cs	e
Celtis biondii Pamp.	Cannabaceae	Cb	d
Choerospondias axillaris (Roxb.) Burtt et Hill	Anacardiaceae	Ca	d
Cinnamomum camphora (Linn.) Presl	Lauraceae	Cc	e
Cyclobalanopsis glauca (Thunberg) Oersted	Fagaceae	Cg	e
Cyclobalanopsis myrsinifolia (Blume) Oersted	Fagaceae	Cm	e
Daphniphyllum oldhamii (Hemsl.) Rosenthal	Daphniphyllaceae	Do	e
Diospyros japonica Siebold & Zuccarini	Ebenaceae	Dj	d
Elaeocarpus chinensis (Gardn. et Chanp.) Hook. f. ex Benth.	Elaeocarpaceae	Ec	e
Elaeocarpus glabripetalus Merr.	Elaeocarpaceae	Eg	e
Elaeocarpus japonicus Sieb. et Zucc.	Elaeocarpaceae	Ej	e
Idesia polycarpa Maxim.	Flacourtiaceae	Ip	d
Koelreuteria bipinnata Franch.	Sapindaceae	Kb	d
Liquidambar formosana Hance	Altingiaceae	Lf	d
Lithocarpus glaber (Thunb.) Nakai	Fagaceae	Lg	e
Machilus grijsii Hance	Lauraceae	Mg	e
Machilus leptophylla Hand.-Mazz.	Lauraceae	Ml	e
Machilus thunbergii Sieb. et Zucc.	Lauraceae	Mt	e
Manglietia fordiana (Oliver) HuY.W.Law	Magnoliaceae	Manf	e
Melia azedarach Linn.	Meliaceae	Ma	d
Meliosma flexuosa Blume	Sabiaceae	Mf	d
Nyssa sinensis Oliver	Nyssaceae	Ns	d
Phoebe bournei (Hemsl.) Yen C. Yang,	Lauraceae	Pb	e
Quercus acutissima Carruthers	Fagaceae	Qa	d
Quercus fabri Hance	Fagaceae	Qf	d
Quercus phillyreoides A. Gray	Fagaceae	Qp	e
Quercus serrata Murray	Fagaceae	Qs	d
Rhus chinensis Mill.	Anacardiaceae	Rc	d
Sapindus saponaria Linn.	Sapindaceae	Sd	d
Triadica cochinchinensis Loureiro	Euphorbiaceae	Tc	d
Triadica sebifera (L.) Small	Euphorbiaceae	Ts	d
Schima superba Gardn. et Champ.	Theaceae	Schs	e

Species names are in accordance with nomenclature in The Flora of China (http://flora.huh.harvard.edu/china). d= deciduous, e= evergreen.

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Tree Species and Vulnerability Curves

We randomly chose three individuals per species in the high-diversity plots, with one individual per species being sampled per plot. This enabled us to minimize time between sample cutting and lab procession of samples because different species grew in close proximity to each other. Xylem conductivity and vulnerability and leaf stomatal conductance (see below) were measured on the same plots, but not explicitly on the same individuals. The sampling and measurements on xylem hydraulics were conducted in August - October 2012. This period was characterized by monthly mean temperatures of about 20℃ and a monthly precipitation of 40 mm (Fig. 1), which involved dry spells of several weeks, typically resulting in midday depressions of stomatal conductance. Samples were always taken in the early morning hours between 6 and 8 am, when relative humidity was still high (70–95% Rh) and temperatures were around 20℃. Measurements of leaf water potentials were made in spring 2012, using a PMS M1000 Scholander pressure chamber. These data showed that water potentials were well above -2 MPa, and for many species>-1 MPa. A twig with no leaves, buds or branches, around 15 cm in length and 5–15 mm diameter was cut and immediately immersed in water. We are aware that maximum vessel length of some of the species might be larger than 15 cm [32], which would result in overestimating specific xylem hydraulic conductivity (K_S) and the absolute value of the xylem pressure at which 50% loss of the maximum specific xylem hydraulic conductivity occurs (Ψ_{50}). However, it has been shown that extreme vessel lengths are very rare [33]. In any case, obtaining non-ramified twigs longer than 15 cm would have been impossible in most species. After transportation to the lab, the stem pieces were then placed into a double-ended pressure sleeve (PMS M1000 Scholander pressure chamber) in the laboratory following established protocols [18, 34] (Fig. 2). Xylem vulnerability was measured within at maximum four hours after cutting. Increasing the air pressure in the cavitation chamber was used to simulate increasingly negative xylem sap pressures [35]. Before the measurements were taken, each twig segment was treated for one hour with perfusion solution pressurized at 0.15 MPa in order to flush out air from older embolism events and any potential air entry into the xylem during the cutting and handling of samples. We used 10 mM citric acid perfusion solution, using filtered and demineralized water to prevent any blockages caused by microorganisms.

Specific xylem hydraulic conductivity (K_S) was measured as the mass of flow-through perfusion solution through the piece of wood per unit of time and per cross-sectional area of the twig. The measurements were started at ambient air pressure and repeated as pressure was increased in increments of 0.5 MPa up to 7 MPa, or to the pressure at which no more perfusion solution flow was encountered.

Measurements of Stomatal Conductance

Data on stomatal conductance were taken from [7]. For the measurements of stomatal conductance the authors had randomly selected twelve high-diversity

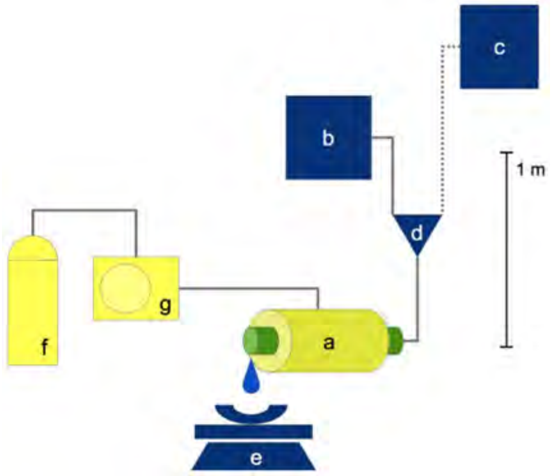

Figure 2. Simplified overview of the xylem hydraulics measurement installation. A) Pressure chamber with the stem segment, B) tank with the perfusion solution, C) flush tank, D) three-way stopcock, E) electronic scale, F) nitrogen pressure cylinder, and G) pressure gauge.

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plots (with 16 or 24 species). Eleven to 23 individuals of all species were measured per plot. In total, 3,290 measurements of stomatal conductance were made in August to October 2010, May/June 2011 and August to October 2011. Each of the 39 species was represented between at least three and at maximum 13 individuals, resulting in 218 individuals in total. This non-balanced sampling design resulted from measuring daily course of stomatal conductance, which required walking time to be minimal. The repeated measurements were taken on the same leaf, which was fully developed, undamaged and fully exposed to the sun. Daily courses of stomatal conductance were produced for all species in every plot. Stomatal conductance was recorded with an SC1 porometer (Decagon). Air temperature and relative humidity was measured simultaneously using a T2 thermo-hygrometer (Trotec). Vapor pressure deficit (VPD) was calculated following the August-Roche Magnus formula. The daily courses of all different individuals from all different daily courses were then aggregated to one $g_s \sim$ VPD relationship which included all data for one species. Mean and maximum stomatal conductance (g_{smax}) could then be estimated per species. The species-specific $g_s \sim$ VPD relationships were modeled by regressing the logits of g_s/g_{smax} to VPD and the quadratic term of VPD using a generalized linear model with a binomial error distribution. The parameters of the model allowed calculating the maximum stomatal conductance and the VPD at which the modeled stomatal conductance was maximal.

Trait Measurements

A total of 34 leaf and wood traits were assessed to analyze possible relationships with Ψ_{50} and K_S (see Table 2). To accomplish this, four total sets of samples were taken: 1) A set of five individuals with five leaves being sampled per individual for the traditional leaf traits, such as absolute area per leaf, leaf fresh-weight, leaf dry-weight, leaf nitrogen concentration (LNC) and leaf carbon concentration (LCC). The data were used to calculate specific leaf area (SLA), leaf dry matter content (LDMC) and carbon to nitrogen ratio (CN). We also determined leaf habit (deciduous/evergreen), leaf pinnation (pinnate or simple), leaf margin (entire or serrate) and recorded the presence or absence of extrafloral nectaries. 2) Another 30 leaves were sampled from three individuals per species to determine leaf tensile strength as a measure of leaf toughness. Leaf tensile strength was measured with a tearing apparatus modified after Hendry [36]. 3) The same leaves on which stomatal conductance was measured (see above), were taken to analyze the stomatal related traits. Stomatal traits were analyzed after Gerlach [37], with stomata being counted on a minimum area of 50,000 μm^2 on three leaves from three individuals per species. Stomata were counted on nail polish impressions made on leaf samples, which had been stored in 70% ethanol. Length and width of three stomata per replicate were measured, and stomatal density was expressed as stomatal number per area. The analysis was performed with a light-optical microscope (Zeiss Axioskop 2 plus) and using the Axio Vision (Version 3.0) software. 4) A sample was taken from each twig used in the cavitation sensitivity analysis for further xylem anatomical investigation. Twig sections were prepared for light microscopic inspection and, from an area of 4.4 mm2 per sample, every xylem vessel was analyzed. To determine xylem traits, we made use of XylemDetector that was implemented as part of the open-source package MiToBo (http://www.informatik.uni-halle.de/mitobo), an extension of the Java image processing software ImageJ. We measured the mean lumen area of conducting vessels (MEANAREA) and the mean roundness of conducting vessels (MEANROUND), which is a measure of how close the vessel shape is to a perfect circle, and ranges from 0 to 1. MEANROUND was calculated as:

$$meanround = \frac{4\pi a}{p^2}$$

where a is the area and p the perimeter of the lumen.

Following Sperry [38], we calculated hydraulically weighted conduit diameter (DHYD) from the lumen area data according to:

$$dhyd = 2\frac{\sum r^5}{\sum r^4}$$

where r are the circle radii calculated from the lumen areas.

Statistical Analyses

We plotted vulnerability curves that show the flow rates of perfusion solution through stem segments as a function of water potential [35]. A sigmoid, three-parameter regression was applied to the vulnerability data [39, 40]:

Table 2. List of the leaf traits measured across the 39 tree species.

Code	Trait	Analytical technique	Type	Units/categories
Ψ_{50}	Loss of 50% initial conductivity	Pressure Chamber	Continuous	MPa
K_S	Maximum conductivity	Pressure Chamber	Continuous	kg m^{-1} s^{-1} MPa^{-1}
b	Parameter b (Sigmoid Regression)	Pressure Chamber	Continuous	nondimensional
CONMEAN	Average stomatal condutance	Porometer	Continuous	mmol m^2 s^{-1}
CONMAX	Maximum stomatal condutance	Porometer	Continuous	mmol m^2 s^{-1}
VPDMAX	VPD at CONMAX	Porometer, Hygrometer, Thermometer	Continuous	hPa
CONMAXFIT	Fitted Max. stomatal conductance	Porometer, Hygrometer, Thermometer	Continuous	nondimensional
VPDMAXFIT	VPD at CONMAXFIT	Porometer, Hygrometer, Thermometer	Continuous	hPa
VPDPOI	VPD at point of inflection of fitted stomatal conductance	Porometer, Hygrometer, Thermometer	Continuous	hPa
SLA	Specific leaf area	Scanner, Balance	Continuous	m^2 kg^{-1}
LOG10LA	Decadic log (Leaf Area)	Scanner	Continuous	mm^2
LDMC	Leaf dry matter content	Balance	Continuous	mg g^{-1}
LT	Leaf toughness	Leaf toughness device	Continuous	N mm^{-1}
LEAFHABIT	Leaf habit	Literature	Binary	(0) evergreen; (1) deciduous
LNC	Leaf nitrogen concentration	CN Analyzer	Continuous	mg g^{-1}
LCC	Leaf carbon concentration	CN Analyzer	Continuous	mg g^{-1}
CN	Carbon-nitrogen ratio	CN Analyzer	Continuous	ratio
CA	Leaf calcium concentration	AAS Analyzer	Continuous	mg g^{-1}
K	Leaf potassium concentration	AAS Analyzer	Continuous	mg g^{-1}
MG	Leaf magnesium concentration	AAS Analyzer	Continuous	mg g^{-1}
LEAFPIN	Leaf pinnation	Field Observation	Binary	(0) pinnate; (1) simple
LEAFMAR	Leaf margin	Field Observation	Binary	(0) dentate; (1) entire
EXTRAFLO	Extrafloral nectaries	Field Observation	Binary	(0) no; (1) yes
STODENS	Stomata density	Microscopy	Continuous	1 mm^{-2}
STOSIZE	Stomata size	Microscopy	Continuous	μm^2
STOIND	Stomata index	Microscopy	Continuous	ratio
DIAMVEIN1	Diameter veins 1st order	Scanner	Continuous	cm
DIAMVEIN2	Diameter veins 2nd order	Scanner	Continuous	cm
VEINDENS	Length of veins per unit leaf area	Scanner	Continuous	cm cm^{-2}
WPOT	Water potential	Pressure Chamber	Continuous	MPa

Table 2. Cont.

Code	Trait	Analytical technique	Type	Units/categories
WOODDENS	Wood density	Balance	Continuous	g cm^3
MEANAREA	Mean area of conducting vessels	Microscopy	Continuous	μm^2
MEANROUND	Mean roundness of conducting vessels	Microscopy	Continuous	nondimensional
DHYD	Hydraulically weighted diameter of conducting vessels	Microscopy	Continuous	μm

All traits were assessed on the individuals planted in the experiment. The table includes the trait abbreviations (Code) used throughout the text.

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$$K_S = \frac{a}{1+e^{-(\frac{\Psi-\Psi_{50}}{b})}}$$

where K_S is the specific hydraulic conductivity of the xylem [kg m^{-1} s^{-1} MPa^{-1}], Ψ is the xylem pressure at which water flow was measured [MPa], a is the original maximum specific xylem hydraulic conductivity, b is the slope of the regression and Ψ_{50} is the xylem pressure at which 50% loss of the original maximum specific xylem hydraulic conductivity occurs. Fig. 3 shows an example of how the sigmoid model was fitted to predict the loss of specific xylem hydraulic conductivity (K_S) from water potential (please refer to the Figure S1 and Table S1 for raw data on xylem vulnerability). We made use of the FactoMineR package in R (http://cran.r-project.org/web/packages/FactoMineR/index.html) to correlate the physiological parameters to the species' traits in a PCA. K_S, Ψ_{50} and the slope b of the $K_S \sim \Psi$

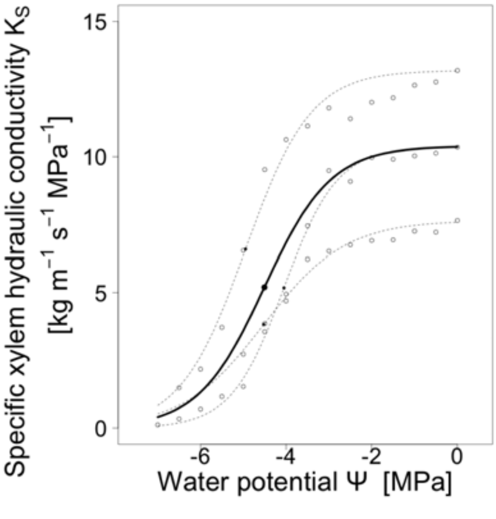

Figure 3. Conductivity rates as a function of decreasing water potential, taking *Castanea henryi* as an example. Outline dots show the measured values of three independent vulnerability curves. Dotted lines show the single regression functions obtained from the measured value per vulnerability curve, obtained from a 3-parametric sigmoid function. The bold line shows the regression lines constructed from the means of the parameters from the three single vulnerability curves. Filled dots represent Ψ_{50} values.

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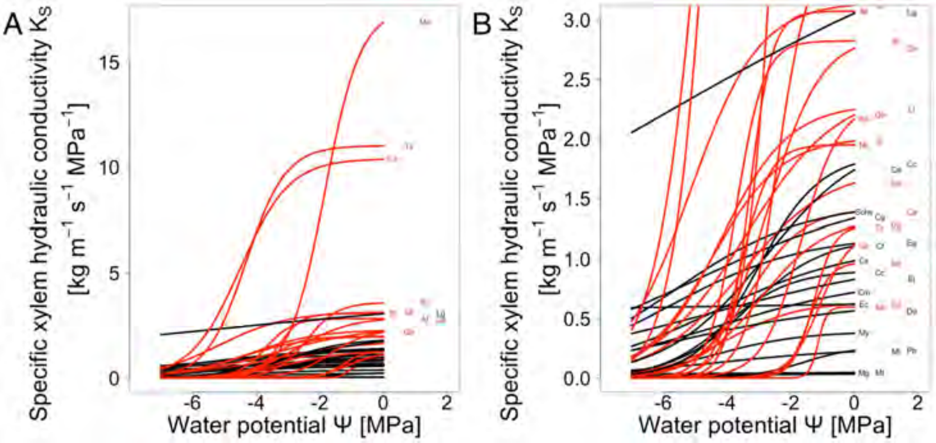

Figure 4. Plots of modeled specific xylem hydraulic conductivity K_S versus water potentials for all 39 species included in the study. A) and B) show the same data at different scale of K_S. Deciduous species are shown in red, evergreen species shown in black. For species abbreviations see Table 1. For details of calculation of regression lines, see Fig. 2 and Methods.

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relationship were tested for differences between deciduous and evergreen leaf habit by analysis of variance (ANOVA) and for bivariate relationships to leaf traits by linear regression models. As these tests performed multiple testing, they run the risk of error inflation and cannot be used to infer statistical significances. These tests were exploratory and had the purpose to identify possible candidate predictors and to show the direction of their effects. To further investigate the emerging significant relationships, we rerun all significant linear regressions by additionally including the interaction with leaf habit. For all statistics, R software version 3.0.2 was used.

Results

Fig. 4 shows the vulnerability curves for all 39 species included in the study. Values of Ψ_{50} ranged between −1.08 MPa and −6.6 MPa for *Celtis biondii* and *Lithocarpus glaber*, respectively, with an overall mean of −3.78 MPa (SD=1.48). Specific xylem hydraulic conductivity (K_S) was highest in *Melia azedarach*, *Triadica sebifera* and *Castanea henryi* (17.52, 11.01 and 10.40 kg m^{-1} s^{-1} MPa^{-1}, respectively) and lowest in *Machilus grijsii* (0.036 kg m^{-1} s^{-1} MPa^{-1}), with a overall mean of 2.44 kg m^{-1} s^{-1} MPa^{-1} (SD=3.31). K_S and Ψ_{50} were not correlated across all species ($p=0.512$). Evergreen species had significantly lower values of maximum hydraulic conductivity and lower Ψ_{50} values than deciduous species (Fig. 5).

Fig. 6 shows the principal components analysis (PCA) of all 34 leaf traits, including the parameters of stomatal control and xylem vulnerability for all 39 study species (Fig. 6 a, b). The species mean values of all traits are provided in Table S1. The first three PCA axes explained 43.3% of the total variance, with

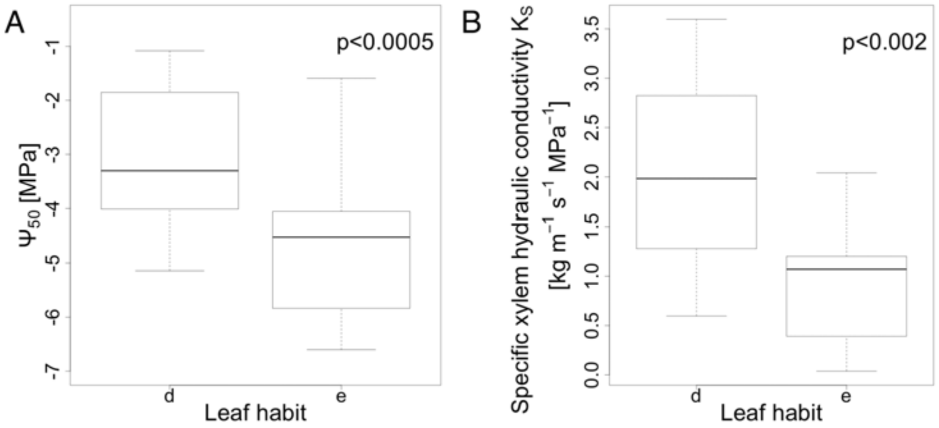

Figure 5. Boxplots characterizing the species set by leaf habit. A) xylem pressure at 50% loss of the maximum specific xylem hydraulic conductivity (Ψ_{50}) and B) specific xylem hydraulic conductivity of the xylem (K_S) as a function of leaf habit. d = deciduous, e = evergreen. The probability values were derived from an ANOVA.

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eigenvalues of 7.03, 4.2 and 3.48, respectively. While evergreen species tended to score higher on the first PCA axes than deciduous species, there was a large overlap between the two leaf habits (Fig.6 c, d). Positive scores on the first PCA axis reflected both decreasing xylem vulnerability and increasing values of traits of the leaf economics spectrum, such as leaf nitrogen concentration (LNC) and specific leaf area (SLA), as well as evergreen leaf habit and the logarithm of the area of a single leaf (Log10LA), while leaf toughness (LEAFT) and leaf carbon to nitrogen ratio (CN) showed negative loadings. Parameters of stomatal control were correlated with the second PCA axis, with positive loadings being recorded for stomatal index (STOIND), stomatal density (STOMDENS) and wood density (WOODDENS), and negative ones for the point of inflection of the $g_s \sim$ VPD curve (VPDPOI) and maximum stomatal conductance (CONMAX).

We found xylem vulnerability to be significantly related to numerous traits (Table 3, Fig. 7 & 8). Ψ_{50} was positively correlated with leaf area, LNC, hydraulically weighted diameter and leaf magnesium concentration, while it was only marginally significantly related to leaf calcium concentration, and negatively related to leaf toughness and carbon to nitrogen ratio. A similar pattern was found for maximum hydraulic conductivity (K_S), which showed a positive relationship to leaf area (Log10LA), leaf nitrogen concentration (LNC) and two morphological wood traits (i.e. the mean area of conducting vessels (MEANAREA), and the hydraulically weighted diameter) and a negative correlation with leaf carbon to nitrogen ratio (CN). The regression parameter b was not related to any of the traits studied. The regression equations from the significant linear models are shown in Table 4. There were no significant correlations between Ψ_{50} or K_S to any parameter of stomatal control.

Including leaf habit in the significant models resulted in two models with significant interactions with leaf habit, i.e. for leaf nitrogen concentration (LNC)

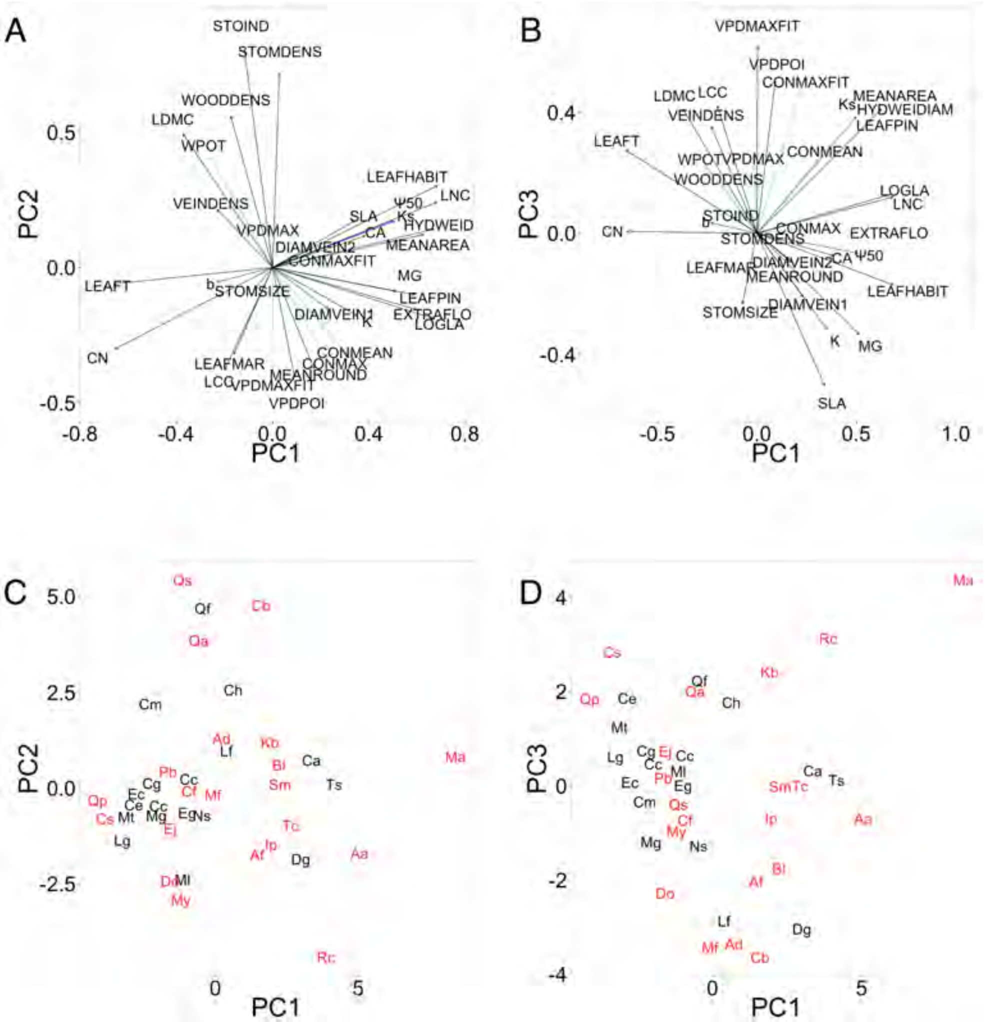

Figure 6. Principal component analysis (PCA) biplots showing the relationships between the mean values of all chemical and morphological leaf traits (black arrows), all parameters of stomatal regulation (green arrows) and all parameters of cavitation sensitivity (blue arrows). A) and C) PCA axes 1 and 2, B) and D) PCA axes 1 and 3. A) and B) loadings of the different traits. C) and D) species scores in the PCA shown separately by leaf habit. Deciduous species are shown in red and evergreen species in black. See Table 1 for abbreviations of species names. Eigenvalues: axis 1=7.03, axis 2=4.2, axis 3=3.48, with cumulative proportion of explained inertia 20.1%, 33.0% and 43.3%, respectively. See Table 2 for abbreviations of trait names.

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and the leaf carbon to nitrogen ratio (CN). While with increasing CN and decreasing LNC, K_S increased in evergreen species, deciduous species showed a decrease (Fig. 8 a, b).

Discussion

We found a large variation in specific xylem hydraulic conductivity and xylem vulnerability among our study species, which points to different complementary

Table 3. Correlation matrix of hydraulic xylem characteristics with numerical leaf traits and parameters of stomatal control.

	Ψ_{50}		K_S		b	
	r	p	r	p	r	p
CONMEAN	0.12	0.49	0.13	0.42	−0.12	0.49
CONMAX	0.02	0.92	0.02	0.91	−0.06	0.73
VPDMAX	−0.04	0.81	0.15	0.36	0.14	0.39
CONMAXFIT	0.14	0.41	0.26	0.12	−0.11	0.50
VPDMAXFIT	−0.13	0.43	0.18	0.26	−0.01	0.96
VPDPOI	0.13	0.44	0.13	0.44	−0.10	0.53
SLA	0.27	0.09	0.05	0.78	−0.17	0.30
LOG10LA	**0.34**	**0.03**	**0.10**	**0.04**	−0.20	0.22
LDMC	−0.02	0.91	−0.02	0.91	−0.03	0.85
LEAFT	−**0.47**	**0.00**	−0.23	0.16	0.02	0.90
LNC	**0.43**	**0.01**	**0.59**	**0.00**	−0.08	0.62
LCC	−0.23	0.17	−0.12	0.49	0.05	0.76
CN	−**0.43**	**0.01**	−**0.48**	**0.00**	0.05	0.75
CA	0.32	0.05	0.09	0.58	−0.16	0.35
K	0.16	0.33	0.05	0.75	−0.13	0.44
MG	**0.33**	**0.04**	0.02	0.91	−0.24	0.15
STOMDENS	0.09	0.58	0.07	0.65	−0.08	0.64
STOMSIZE	0.06	0.74	−0.06	0.71	−0.01	0.96
STOIND	0.11	0.52	0.01	0.69	−0.09	0.59
DIAMVEIN1	0.20	0.23	−0.09	0.59	−0.23	0.17
DIAMVEIN2	0.17	0.33	0.01	0.93	−0.14	0.42
VEINLENGTH	−0.15	0.37	−0.09	0.61	0.19	0.24
WPOT	0.09	0.57	−0.04	0.79	0.01	0.95
WOODDENS	−0.06	0.73	0.06	0.71	−0.13	0.42
MEANAREA	0.24	0.15	**0.85**	**0.00**	−0.11	0.52
MEANROUND	0.04	0.82	−0.01	0.94	−0.02	0.92
DHYD	**0.39**	**0.01**	**0.72**	**0.00**	−0.20	0.23

The correlations were calculated from linear model analyses between functional leaf traits and the extracted physiological parameters. Significant relationships are shown in bold letters. Ψ_{50}= xylem pressure at which 50% loss of the original maximum specific xylem hydraulic conductivity occurred, K_S= specific hydraulic conductivity of the xylem, b= slope of the three-parametric sigmoid model of the vulnerability curve, CONMEAN = mean g_s; CONMAX = mean $g_{s\ max}$; VPDMax = VPD at $g_{s\ max}$; CONMAXFIT = modeled $g_{s\ max}$; VPDMaxfit = VPD at modeled $g_{s\ max}$, and VPDPoi = VPD at the point of inflexion of the $g_s \sim$ VPD curve, MEANAREA = the mean area of conducting vessels, MEANROUND = the mean roundness of conducting vessels, and DHYD the hydraulically weighted diameter of conducting vessels.

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strategies of the species in the same subtropical forest community. Thus, our results conform to the findings of Böhnke *et al.* [41], who revealed a high and temporally constant level of functional diversity in the course of succession in these forests. In particular, variation in cavitation resistance could offer an explanation for species coexistence in the same community [42]. Our values on specific xylem hydraulic conductivity with a range of K_S between 0.036 and 17.52 kg m^{-1} s^{-1} MPa^{-1} and xylem vulnerability to cavitation Ψ_{50} between −1.08 and −6.6 MPa cover a large part of the total range recorded for such

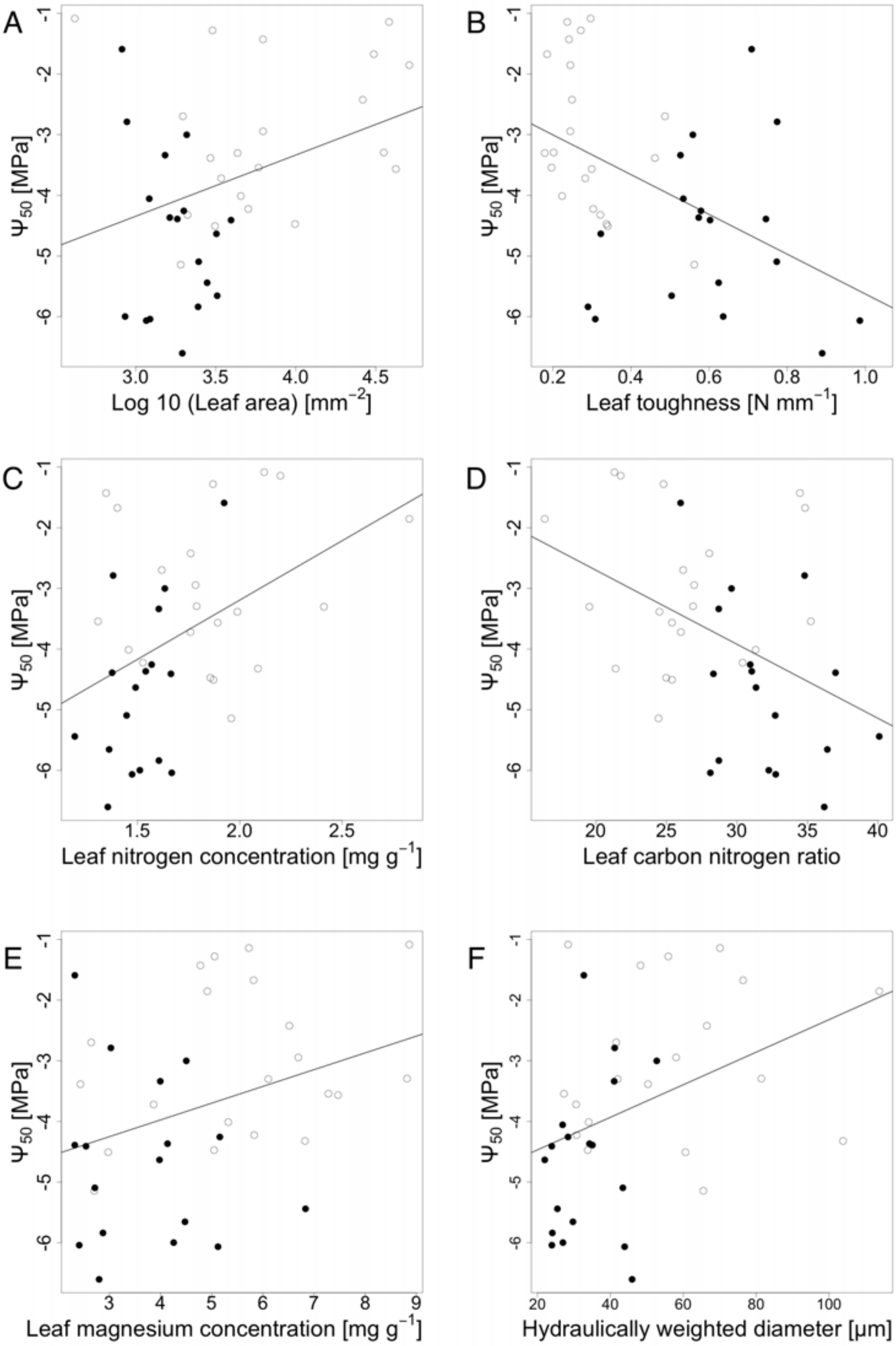

Figure 7. Ψ_{50} as a function of A) leaf area ($p=0.0315$, $r=0.34$), B) leaf toughness ($p<0.0003$, $r=-0.47$), C) leaf nitrogen concentration ($p<0.0075$, $r=0.43$), D) leaf carbon to nitrogen ratio ($p<0.0078$, $r=-0.43$), E) leaf magnesium concentration ($p<0.042$, $r=0.33$) and F) hydraulically weighted conduit diameter ($p<0.01$, $r=0.39$). Filled black dots represent species of evergreen leaf habit; empty dots represent species of deciduous leaf habit.

doi:10.1371/journal.pone.0109211.g007

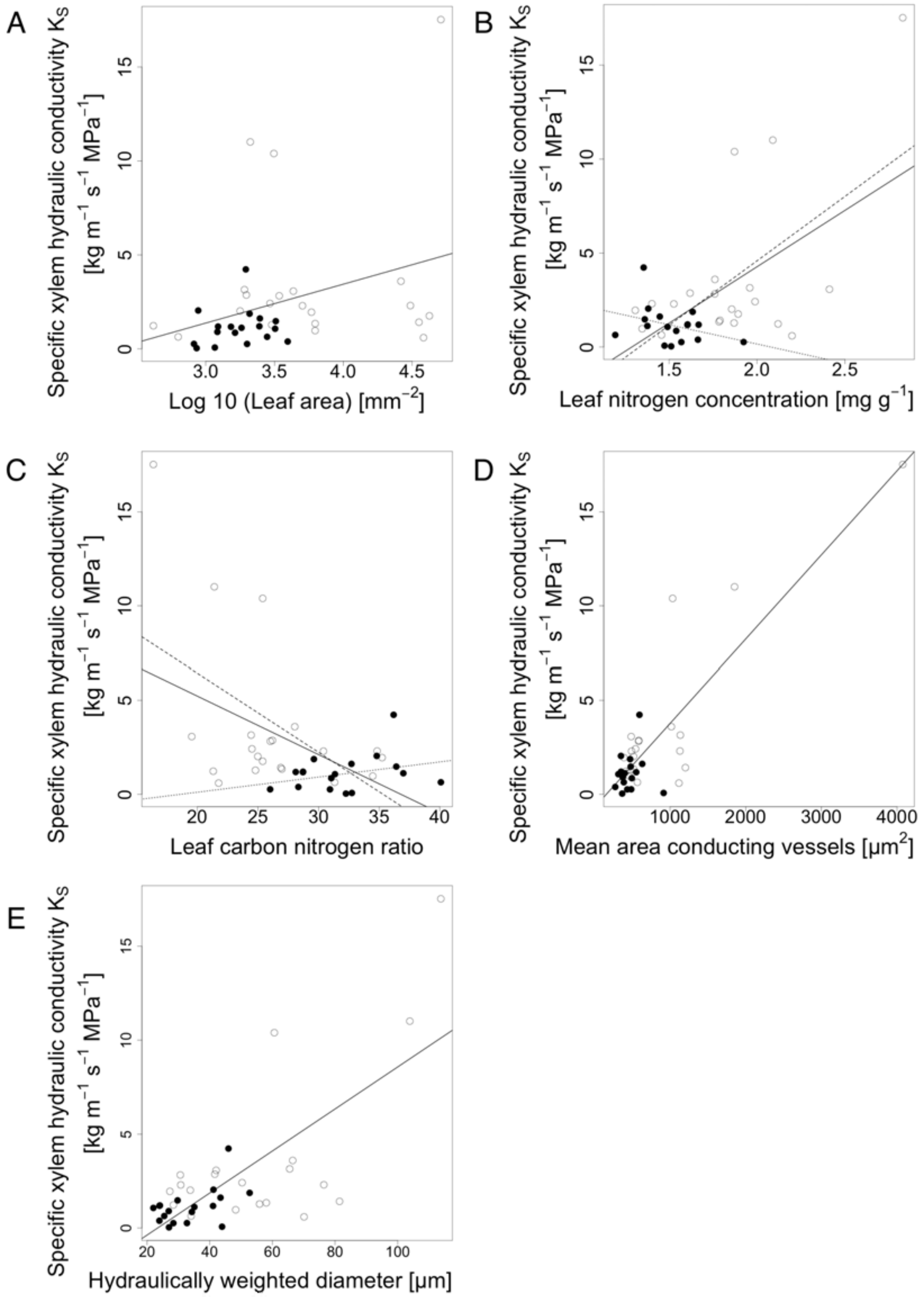
A
Specific xylem hydraulic conductivity K_S [kg m^{-1} s^{-1} MPa^{-1}]
0
5
10
15
3.0
3.5
4.0
4.5
Log 10 (Leaf area) [mm^{-2}]
B
Specific xylem hydraulic conductivity K_S [kg m^{-1} s^{-1} MPa^{-1}]
0
5
10
15
1.5
2.0
2.5
Leaf nitrogen concentration [mg g^{-1}]
C
Specific xylem hydraulic conductivity K_S [kg m^{-1} s^{-1} MPa^{-1}]
0
5
10
15
20
25
30
35
40
Leaf carbon nitrogen ratio
D
Specific xylem hydraulic conductivity K_S [kg m^{-1} s^{-1} MPa^{-1}]
0
5
10
15
1000
2000
3000
4000
Mean area conducting vessels [μm^2]
E
Specific xylem hydraulic conductivity K_S [kg m^{-1} s^{-1} MPa^{-1}]
0
5
10
15
20
40
60
80
100
Hydraulically weighted diameter [µm]

Figure 8. K_S as a function of A) leaf area ($p<0.044$, $r=0.1$), B) leaf nitrogen concentration ($p<0.00001$, $r=0.59$), C) leaf carbon to nitrogen ratio ($p<0.0019$, $r=0.48$), D) mean area of conducting vessels ($p<0.0001$, $r=0.85$), and E) hydraulically weighted diameter of conducting vessels ($p<0.0001$, $r=0.72$). Filled black dots represent species of evergreen leaf habit and empty dots represent species of deciduous leaf habit. In B) and C) K_S also showed significant interactions with leaf habits. Dotted lines represent species of evergreen and broken lines represent species of deciduous leaf habit. B) leaf habit $p=0.061$, interaction leaf nitrogen concentration and leaf habit $p=0.049$, C) leaf habit $p=0.026$, interaction leaf carbon to nitrogen ratio and leaf habit $p=0.031$.

doi:10.1371/journal.pone.0109211.g008

variables in other studies [43]. For example, Cavender-Bares *et al.* [44] described a range of maximum specific xylem hydraulic conductivity of between 1.75 and 5 kg m^{-1} s^{-1} MPa^{-1} for 17 oak species, while Maherali *et al.* [45] reported a mean of 1.36 kg m^{-1} s^{-1} MPa^{-1} of maximum specific xylem hydraulic conductivity (n species =87) and a mean of −3.15 MPa for Ψ_{50} values (n species =167). Slightly higher Ψ_{50} values of −1.2 up to −2.76 MPa were encountered for eight tree species from a tropical dry forest [46]. However, we have to consider that extreme values of K_S of more than 10 kg m^{-1} s^{-1} MPa^{-1} might be methodological artifacts, caused by some open vessels in these samples. However, the three species with extreme K_S also ranked high in values of the predictor traits for K_S, indicating that the relative rank in K_S in these species might be realistic. As vessel length of the species was not measured, and thus, in some species, specific xylem hydraulic conductivity might have been overestimated, comparisons to other studies should be done with caution. However, as Melcher *et al.* [33] pointed out, such an overestimation is probably not severe, as long vessels are also very rare.

Important leaf traits such as leaf nitrogen concentration (LNC) and leaf area (Log10LA) were highly correlated to Ψ_{50} and K_S. Thus, we can fully confirm our first hypothesis that leaf traits describing the leaf economics spectrum are related

Table 4. Regression equations for all significant correlations of Ψ_{50} and K_S to the functional traits.

Xylem parameter	Functional trait	Equation
Ψ_{50}	LOG10LA	$y=1.0090x-7.3717$
Ψ_{50}	LEAFT	$y=-3.2808x-2.3454$
Ψ_{50}	LNC	$y=1.9556x-7.1038$
Ψ_{50}	CN	$y=-0.12210x-0.25799$
Ψ_{50}	MG	$y=0.2763x-5.0792$
Ψ_{50}	DHYD	$y=0.0136x-5.005$
K_S	LOG10LA	$y=2.0699x-4.8375$
K_S	LNC	$y=6.003x-7.734$
K_S	CN	$y=-0.31132x+11.44974$
K_S	MEANAREA	$y=0.0011481x-0.7089940$
K_S	DHYD	$y=0.0569x-2.611$

The correlations were calculated from linear model analyses. Ψ_{50}= xylem pressure at which 50% loss of the original maximum specific xylem hydraulic conductivity occurred, K_S= specific hydraulic conductivity of the xylem, b= slope of the three-parametric sigmoid model of the vulnerability curve, CONMEAN = mean g_s; CONMAX = mean $g_{s\ max}$; VPDMax = VPD at $g_{s\ max}$; CONMAXFIT = modeled $g_{s\ max}$; VPDMaxfit = VPD at modeled $g_{s\ max}$, and VPDPoi = VPD at the point of inflexion of the $g_s \sim$ VPD curve, MEANAREA = the mean area of conducting vessels, and DHYD the hydraulically weighted diameter of conducting vessels.

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to specific xylem hydraulic conductivity and cavitation resistance. Interestingly, there were no significant correlations for some of the traits of the leaf economics spectrum, such as SLA and LDMC. Thus, leaf thickness and water content of leaves seem to have less importance for hydraulic characteristics than the leaves' absolute size and protein content. This contrasts with the findings reported by Willson *et al.* [47], who described a significant relationship between SLA and Ψ_{50} for the genus *Juniperus*. Thus, comparative studies confined to certain taxonomic levels, such as congeneric comparisons, might arrive at different conclusions than studies covering a wider range of taxa. Alternatively, SLA may have a differing level of importance with regard to the physiology of gymnosperms and angiosperms. Interestingly, our study did also not support a link between K_S and leaf vein density, which was predicted by the flux trait network suggested by Sack et al. [15]. However, there was only equivocal evidence for a significant relationship between K_S and leaf vein density in their reviewed studies [15].

Our results of a strong relationship of Ψ_{50} and K_S to leaf area, conform to those encountered for eight southern African tree species of a seasonally dry tropical forest by Vinya *et al.* [48], except that they reported a link to leaf area only for K_S, but not for Ψ_{50}. Our findings of a relationship of Ψ_{50} to Mg concentration and a marginal one to Ca concentration might indicate that the non-vein and non-sclerenchyma mesophyll density in the leaf are more relevant for cavitation resistance than overall leaf tissue density. As a central component of chlorophyll, Mg concentration is directly related to photosynthetic capacity, and thus, might capture this proportion of actively assimilating tissue in the leaf. In addition, as a cofactor of many enzymatic processes, Mg can be considered an indicator for the plant's nutrition status [49].

The absence of any direct relationship between SLA or LDMC with Ψ_{50} or K_S which is in accordance with Sack *et al.* [50], in combination with the large overlap in Ψ_{50} and K_S detected between deciduous and evergreen species, is not a conclusive result. It appears that evergreen and deciduous subtropical forest species form two ends of a gradient from cavitation resistance to cavitation avoidance, respectively. This view is supported by a recent study by Fu *et al.* [17], who investigated the relationship of stem hydraulics and leaf phenology in Asian tropical dry forest species. In particular, they found a negative relationship between leaf life span and K_S but no significant relationship between leaf life span and Ψ_{50}. In accordance with our results, Maherali *et al.*, Choat *et al.* and Chen *et al.* [27, 45, 51] also reported significant differences in Ψ_{50} and K_S between the different leaf habit groups. Such differences in hydraulic characteristics also translate to higher growth rates, as shown by Fan *et al.* [52] for 40 Asian tropical trees. However, some other studies failed to detect any differences, such as that of Markesteijn *et al.* [42], who attributed the substantial differences they encountered in Ψ_{50} and K_S to shade tolerance. They also argued that the distinction between pioneer vs. shade-tolerant species predicts hydraulic properties better than leaf habit, because there are considerable overlaps in strategies along the gradient of leaf longevity. As Givnish [53] pointed out, evergreen leaves can be advantageous under a wide range of ecological conditions, and the relationship of leaf habit with Ψ_{50} and K_S may therefore strongly depend on the system considered. We can also confirm our second hypothesis that evergreen species characterized by low SLA and high LDMC show lower Ψ_{50} and K_S values than deciduous species. Interestingly, we found leaf habit to significantly influence the relationships of K_S to leaf nitrogen concentration and carbon to nitrogen ratio, which might be explained by differences in basic leaf constructing principles. Deciduous species tend to invest high amounts of nitrogen to maximize photosynthetic assimilation per leaf mass, whereas in evergreen species, the focus is on increased leaf lifespan, which is reflected in higher leaf carbon concentration [54]. Furthermore, deciduous species show a more conservative stomatal control to avoid embolism, whereas evergreen species tend to have more cavitation-resistant vessels [7, 25–27, 55]. The underlying reason is that evergreen species are mostly diffuse-porous, which also explains the strong impact of the hydraulically weighted conduit diameter and mean area of conducting vessels on K_S, which is also well-known from the literature [19].

Contrary to expectations, this study did not find a significant link between xylem hydraulic conductivity and parameters of stomatal regulation. Neither the maximum stomatal conductance, the vapor pressure deficit at maximum stomatal conductance nor the vapor pressure deficit at which stomatal conductance is down-regulated was related to any parameter of the xylem vulnerability curves. As such, our third hypothesis has to be rejected, which implies that the ability of a very precise and fast stomatal regulation versus a retarded and inert stomatal regulation does not translate into cavitation resistance. Additional insights into the relationship between K_S and leaf stomatal regulation might be gained by calculating leaf-specific xylem hydraulic conductivity K_L, which would directly refer to the capacity of the vascular system of a stem to supply the water to that stem [56]. However, our results confirm those of Brodribb *et al.* [46], who found no correlation between Ψ_{50} and the leaf water potential at stomatal closure in eight tropical, dry forest trees. They concluded that xylem cavitation and stomatal closure are linked through complex indirect regulatory mechanisms and argue that this potential linkage is considerably flexible, especially with regard to different leaf phenology strategies, and that there may be carry-over effects of preceding embolism events on stomatal control. A further explanation of a lacking relationship between xylem vulnerability and stomatal regulation may be the different scale at which stomatal regulation is considered. At the level of whole trees, Litvak *et al.* [55] found a strong linear relationship between the sensitivity of tree-level sap flow to VPD and Ψ_{50} both within diffuse- and ring-porous species, which was not encountered for leaf-level transpiration rates. The authors argue that the tree-level transpiration sensitivity, in addition to stomatal regulation, also directly responds to drought-induced embolisms.

Several studies showed a trade-off between high hydraulic conductivity and cavitation resistance [57–60]. In contrast to these studies, we found Ψ_{50} and K_S to be unrelated. According to our current understanding of the causes of xylem embolism under drought conditions, there may indeed be no mechanistic link between these two hydraulic characteristics. As such, diameter and length of vessels may differ autonomously from pit structure and size [19]. While K_S is mainly driven by vessel diameter, Ψ_{50} depends on pit size and structure [19, 20]. The pit area hypothesis states that cavitation resistance is linked to the total area of inter-vessel pits per vessel [61, 62]. Thus, the risk of an embolism expanding between vessels rises with the maximum size of the pit membrane pore, which in turn is dependent on the associated pit membrane area per vessel. This was demonstrated by Hacke *et al.* [57], who reported a strong negative link between xylem vulnerability and pit membrane area per vessel, resulting in small pits potentially increasing hydraulic resistance and decreasing K_S. However, pit size may be of minor importance to K_S compared to that of vessel diameter, and the relationship of hydraulic conductivity and cavitation resistance might depend on the specific ecosystem considered. Tyree *et al.* [59] distinguished between frost- and drought-induced cavitation. In their meta-analysis, the trade-off between hydraulic conductivity and cavitation resistance was mainly related to frost-induced cavitation events. Although frosts occur in the Chinese subtropics, they are neither very strong, nor long-lasting [63]. Thus, cavitation in the forests of our study area will mainly be brought about by drought events, which may result in far fewer, or insignificant, trade-offs.

Since all our individuals have the same age, our species set provides a high comparability usually not found in comparative studies. We expect that some of our response variables will change with tree age, such as specific xylem hydraulic conductivity [64, 65]. In addition, future comparisons should take the sustained leaf area into account, as whole-tree leaf-specific hydraulic conductance (K_L) is known to decrease with tree age [66, 67].

Conclusion

For the studied subtropical forest community, we demonstrated a clear link of K_S and Ψ_{50} with functional traits, and particularly with leaf nitrogen concentration, log leaf area and leaf carbon to nitrogen ratio. Thus, easily measured leaf traits from the LES have the potential to predict plant species' drought resistance. However, current knowledge on xylem vulnerability and traits from other ecosystems do not allow generalizing from these results. In addition, our finding of an absence of any relationship between parameters of stomatal control raises the question whether stomatal control as characteristics that are an independent axis of the LES might be related to an axis of xylem characteristics that are independent of specific xylem hydraulic conductivity and xylem vulnerability.

Supporting Information

Figure S1. Raw data for the vulnerability curves of the 39 study species analyzed. Filled dots represent measured data, empty dots show estimated Ψ_{50} values and the broken lines represent the fitted models of xylem vulnerability. For species abbreviations see Table 1.
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Table S1. Trait raw data for the 39 study species analyzed. For trait codes and full species names, please see Tables 1 and 2.
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Author Contributions

Conceived and designed the experiments: WK SZ ME HB. Performed the experiments: WK ME. Analyzed the data: WK HB. Contributed reagents/materials/analysis tools: WK SZ ME HB. Wrote the paper: WK SZ ME HB.

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Chapter 4

LEAF MORPHOLOGY OF 40 EVERGREEN AND DECIDUOUS BROADLEAVED SUBTROPICAL TREE SPECIES AND RELATIONSHIPS TO FUNCTIONAL ECOPHYSIOLOGICAL TRAITS

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RESEARCH PAPER

Leaf morphology of 40 evergreen and deciduous broadleaved subtropical tree species and relationships to functional ecophysiological traits

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ABSTRACT

We explored potential of morphological and anatomical leaf traits for predicting ecophysiological key functions in subtropical trees. We asked whether the ecophysiological parameters stomatal conductance and xylem cavitation vulnerability could be predicted from microscopy leaf traits. We investigated 21 deciduous and 19 evergreen subtropical tree species, using individuals of the same age and from the same environment in the Biodiversity-Ecosystem Functioning experiment at Jiangxi (BEF-China). Information-theoretic linear model selection was used to identify the best combination of morphological and anatomical predictors for ecophysiological functions. Leaf anatomy and morphology strongly depended on leaf habit. Evergreen species tended to have thicker leaves, thicker spongy and palisade mesophyll, more palisade mesophyll layers and a thicker subepidermis. Over 50% of all evergreen species had leaves with multi-layered palisade parenchyma, while only one deciduous species (*Koelreuteria bipinnata*) had this. Interactions with leaf habit were also included in best multi-predictor models for stomatal conductance (g_s) and xylem cavitation vulnerability. In addition, maximum g_s was positively related to log ratio of palisade to spongy mesophyll thickness. Vapour pressure deficit (vpd) for maximum g_s increased with the log ratio of palisade to spongy mesophyll thickness in species having leaves with papillae. In contrast, maximum specific hydraulic conductivity and xylem pressure at which 50% loss of maximum specific xylem hydraulic conductivity occurred (Ψ_{50}) were best predicted by leaf habit and density of spongy parenchyma. Evergreen species had lower Ψ_{50} values and lower maximum xylem hydraulic conductivities. As hydraulic leaf and wood characteristics were reflected in structural leaf traits, there is high potential for identifying further linkages between morphological and anatomical leaf traits and ecophysiological responses.

INTRODUCTION

Plant functional traits have been successfully used to link biological diversity to ecosystem functioning (EF) of communities (Garnier *et al.* 2004; Díaz *et al.* 2007). However, the assessment of physiologically relevant traits often requires high expenditure of time and lab resources. For example, determination of xylem cavitation vulnerability as a measure of drought stress requires several hours per sample (Sperry *et al.* 1988; Kröber *et al.* 2014). Hence, there are only a few multi-species studies that connect leaf traits to more than a single ecophysiological response. However, establishing relationships between leaf traits and ecophysiological mechanisms across large species sets would certainly improve our understanding of ecosystem functioning (Sack & Holbrook 2006). For many leaf traits, the functional relevance is well known. For example, specific leaf area (SLA) was found to be closely linked to mass-based photosynthesis and respiration rates (Wright *et al.* 2004). In addition, leaves with low SLA are physically more robust, less prone to herbivory and tend to have a longer life span (Sterck *et al.* 2006). It should be considered that functional relationships of ecophysiological responses to leaf traits might not be universal, but are only valid in a specific context. For example, low SLA was found to be only associated with higher shade tolerance in evergreen species, but only weakly in deciduous trees (Lusk & Warton 2007). For many other leaf traits, links to ecophysiological functions still have to be established. In particular, our knowledge is limited on how morphological and anatomical leaf traits are related to physiologically relevant wood traits (Zanne *et al.* 2010; Böhnke *et al.* 2011).

Many structural leaf traits vary with the evergreen or deciduous leaf habit. In particular, evergreen leaves are often sclerophyllous and associated with a lower SLA, lower leaf nitrogen content and lower mass-based photosynthetic capacity (Reich *et al.* 1997; Shipley *et al.* 2006; Curtis & Ackerly 2008), which results in increased durability and leaf life span (Medina 1984). In contrast, deciduous leaves are often mesophyllous or hydrophyllous. Because of higher elasticity of leaf cells, deciduous species with mesophyllous leaves display more variation in cell volume but only tolerate small variation in leaf water potential

(Ψ) as compared to evergreen species with sclerophyllous leaves (Sobrado 1986). Since Orians & Solbrig (1977) published their seminal paper on the trade-off between photosynthetic capacity and leaf longevity, many studies at the global (Kikuzawa 1991; Reich *et al.* 1997; Cornelissen *et al.* 2005) or regional scale (Mulkey *et al.* 1995; Diemer 1998; Pringle *et al.* 2011) have supported this view. Meanwhile, the concept of the leaf economics spectrum (LES; Wright *et al.* 2004) that reflects this trade-off has become widely accepted in trait-based ecology. The LES describes a universal spectrum of leaf economics consisting of key chemical, structural and physiological properties. The spectrum runs from rapid to slow return on investment in nutrients and dry mass of leaves, and operates largely independently of growth form, plant functional type or biome (Wright *et al.* 2004). However, much less attention has been paid to other ecophysiological functions that are not reflected in the LES or morphological traits other than leaf longevity or leaf toughness. One example of such a key ecophysiological function is xylem vulnerability. Comparing four species, each of deciduous and evergreen leaf habit from Costa Rican dry tropical forests, Brodribb *et al.* (2003) did not find a close link between xylem vulnerability and leaf traits, and in particular, no link to leaf habit. In contrast, Kröber *et al.* (2014) encountered significantly lower values of maximum specific hydraulic conductivity and lower Ψ_{50} values (xylem pressure at which 50% loss of maximum specific hydraulic conductivity occurs) in evergreen than deciduous species. Accordingly, Ψ_{50} decreased with leaf nitrogen content and log leaf area and increased with leaf carbon-to-nitrogen ratio. However, structural leaf traits such as thickness of the epidermis or wax layers have not yet been tested for relationships to xylem cavitation resistance.

In another study, Kröber & Bruelheide (2014) established links between parameters of stomatal control and traits of the LES. There was a positive relationship of stomatal conductance (g_s) to leaf nitrogen content and a negative one to the leaf carbon-to-nitrogen ratio. In contrast, stomatal control parameters, which were derived from the conductance–vapour pressure deficit (vpd) curve, were not related to the LES but to stomatal traits. The stomatal traits, in turn, were unrelated to the LES. The vpd at the point of inflection of the conductance–vpd curve was the higher, the lower the stomata density and the higher the leaf carbon content. Furthermore, maximum conductance was positively associated with leaf carbon content and vein length. However, Kröber & Bruelheide (2014) only considered leaf traits related to stomata and veins and did not explore relationships to other anatomical and morphological traits.

The expectation of connections between leaf morphology and leaf functionality is based on the fundamental idea that leaf structure reflects functional coordination (Sisó *et al.* 2001; Sack *et al.* 2003). For example, a less densely packed leaf has more extensive intercellular spaces, which should facilitate CO_2 and H_2O diffusion, and thus be connected to stomatal or xylem conductance. However, Aasamaa *et al.* (2001) did not find the volume of intercellular space to be related to specific hydraulic conductivity. The leaf anatomy, in particular the regular arrangement of palisade mesophyll cells, affects light perception of deeper cell layers, and thus has consequences for photosynthetic efficiency (Smith & Hughes 2009). The regular arrangement of palisade mesophyll cells is thought to efficiently absorb incident radiation of high intensity, whereas spongy mesophyll results in higher scattering of light, which increases absorption at low light intensities (Beck 2010). The fact that thicker mesophyll requires increased gas exchange per leaf unit area explains why Sack & Frole (2006) found palisade mesophyll thickness to be negatively correlated with xylem hydraulic conductance in a set of ten tropical rain forest tree species. Furthermore, leaf hydraulic conductance was positively related to the ratio of palisade to spongy mesophyll thickness. This ratio of thickness of the two types of photosynthetically active tissues has been highlighted as being tightly correlated to leaf venation more than 50 yrs ago (Wylie 1946). The underlying mechanistic explanation is that with increasing ratio of palisade to spongy mesophyll thickness, the density of chloroplasts per unit leaf volume increases. This translates into higher photosynthesis rates per unit leaf area. This is only true up to a certain threshold of the palisade to spongy mesophyll thickness ratio, since some spongy mesophyll is required for intercellular gas diffusion. Popma *et al.* (1992) reported that lowland rain forest species in Mexico having leaves of high photosynthetic capacity had thicker palisade parenchyma and higher palisade to spongy parenchyma ratios. For five dipterocarp rain forest tree species, Kenzo *et al.* (2004) found leaf thickness and palisade layer thickness to be strongly correlated to photosynthetic capacity. Leaf hydraulic conductance was also linked positively to leaf thickness in temperate woody species (Sack *et al.* 2003). Finally, light absorption not only depends on the thickness and structure of the green mesophyll but also on epidermal traits. For example, Vogelmann *et al.* (1996a) reported that leaf epidermal cells can enhance photosynthesis through focusing light onto the underlying mesophyll.

The morphological and anatomical leaf structure is also expected to affect transpiration and drought resistance. In general, thin-leaved plants with high SLA are considered more vulnerable to drought stress than those with thick and robust leaves (Mediavilla *et al.* 2001; Li *et al.* 2009). As low SLA leaves transpire less water, they would be expected to have higher water use efficiency (WUE). However, in a study with six poplar species, Cao *et al.* (2012) found intrinsic WUE not to be related to SLA but only to increase with abaxial stomatal density and decrease with vessel lumen area. In contrast to SLA and mesophyll traits, the structure of the epidermis and the cuticle has received surprisingly little attention in comparative studies. As both a thick outer epidermal wall and a cuticle reinforced with wax layers should decrease transpiration during stomata closure, they would be expected to occur predominantly in leaves with high WUE. Accordingly, water permeability of epicuticular waxes has been reported to be positively correlated with plant sensitivity to vpd (Kerstiens 1997). However, linking water permeation across the cuticle directly to its structure or composition has turned out to be difficult (Riederer & Schreiber 2001) because the cuticle and epicuticular waxes also have additional functions, such as protecting the leaf from radiation, insect herbivory and microbial infection (Kerstiens 2006; Pruem *et al.* 2012).

Similarly, trichomes are thought to play an important role in leaf hydraulic properties. Similar to the cuticle, trichomes also have multiple functions: temperature insulation, reflection of radiation, increase in boundary layer thickness, and thus increase in the gas diffusion pathway, reduced ion leaching and trapping of moisture on the leaf surface (Gutschick 1999; Press 1999; Gates 2003).

In summary, relationships between ecophysiology and leaf morphology and anatomy are only partly established, although the need to compare these relationships across different life forms and habitats has been pointed out (Sack & Holbrook 2006). We set out to analyse these structure–function relationships using four ecophysiological key traits with high relevance for species performance and survival as functional responses. In addition, we investigated interrelationships among leaf microscopy traits, with particular emphasis on the species' evergreen or deciduous leaf habit. Thus, we asked which microscopy leaf traits were connected to: (i) stomatal regulation, expressed as the absolute maximum fitted stomatal conductance and to vpd at the maximum modelled stomatal conductance–vpd relationship, and (ii) maximum xylem specific hydraulic conductivity and xylem hydraulics, expressed as xylem pressure at which 50% loss of maximum specific hydraulic conductivity occurs (Ψ_{50}). To our knowledge, our study is the first that explores such functional–morphological relationships systematically across a large set of plant species.

MATERIAL AND METHODS

Plant material

We studied the leaf anatomy of 40 indigenous tree species (Table S4), which are all typical of the subtropical monsoon forest in southeast China, Jiangxi Province (http://www.bef--china.de). Warm and humid summers and dry winters with occasional frost events characterise the local subtropical climate. Mean annual precipitation at the study site is 1821 mm and mean annual temperature is 16.7 °C, measured from 1971 to 2000 (Yang *et al.* 2013). The sampled plants were all planted in the Biodiversity-Ecosystem Functioning experiment (BEF-China) as 1-year-old or 2-year-old saplings in 2008 and 2009. Total tree number in the experiment is 219,000, arranged in 566 plots with 400 tree individuals per plot on an area of 38 ha (Bruelheide *et al.* 2014). The studied 40 tree species represent 19 families (Table S4). According to their representation in the natural forests in a nearby nature reserve (Bruelheide *et al.* 2011), some families were planted with numerous species, *e.g.* Fagaceae (12 species), Lauraceae (five), Sapindaceae (three) and Elaeocarpaceae (three). In total, 19 and 21 species were evergreen and deciduous, respectively (Table S4).

Leaf sampling

Five leaves were sampled each from five randomly chosen individuals per species (in total 25 leaves) for leaf trait analyses (SLA and microscopy leaf traits), taking the five individuals from different plots. Only fully exposed healthy sun leaves with no visible damage from *e.g.* herbivory were sampled, with a focus on sampling at comparable plant heights. SLA was measured following Cornelissen *et al.* (2003). The microscopy traits were measured in accordance to Gerlach (1984), with at least three replicates per nominal trait and species and at least 30 replicates per numerical trait and species. A full list of the morphological traits studied is provided in Table S8.

The four functional leaf traits related to stomatal conductance and hydraulic xylem properties (CONMAXFITA, absolute maximum of fitted stomatal conductance; VPDMAXFIT, vpd at maximum of modelled g_s–vpd relationship; HYDCOND, maximum specific xylem hydraulic conductivity; PSI50, xylem pressure Ψ_{50}, at which 50% loss of maximum specific hydraulic conductivity occurs) were assessed in two preceding studies (Kröber & Bruelheide 2014; Kröber *et al.* 2014). Stomatal conductance was measured with a SC1 porometer (Decagon, Pullman, WA, USA), based on steady-state technology. Daily courses were measured on at least three individuals per species, always on the same leaf, in the high-diversity plots of the experiment. Then g_s–vpd relationships were aggregated by species. To derive parameters of stomatal control, we modelled these species-specific g_s–vpd relationships by regressing the logits of $g_s/g_{s\max}$ to vpd and the quadratic term of vpd using a generalised linear model with binomial error distribution. From these relationships, we extracted the absolute maximum fitted conductance as the maximum value of the fitted model and the vpd, thus the corresponding x-value for the maximum of the modelled g_s–vpd relationship for every species. By making use of logits we ensured that modelled maximum values (CONMAXFIT) did not exceed $g_{s\max}$, and that g_s approached zero at high vpd. For further details see Kröber & Bruelheide (2014).

A second study focused on xylem vulnerability. Using three samples per species, maximum specific xylem hydraulic conductivity and Ψ_{50} were investigated. Freshly sampled twigs were placed in a cavitation chamber (PMS 1505D-EXP; PMS Instrument Co., Albany, OR, USA) connected to a Scholander pressure chamber (PMS M1000), and air was flushed out from previous embolism events following established protocols (Sperry *et al.* 1988; Perez-Harguindeguy *et al.* 2013). To ensure that all air was evacuated from the xylem, the twig segments were flushed for 1 h with 10 mM citric acid perfusion solution, using filtered and demineralised water at 0.15 MPa. Maximum specific xylem hydraulic conductivity was measured as the mass of water through-flow per time, related to the length of the twig and to the cross-sectional area of the twig. Then, with increasing air pressure, we simulated decreasingly negative water potentials and determined the xylem cavitation sensitivity by applying a sigmoid, three-parameter regression to the vulnerability data, where K_S is the specific hydraulic conductivity of the xylem ($\mathrm{kg \cdot m^{-1} \cdot s^{-1} \cdot MPa^{-1}}$; thus it is related to cross-sectional area). Ψ is the xylem pressure at which water flow was measured (MPa); Ψ_{50} is defined as the point of xylem pressure at which 50% loss of the original maximum specific hydraulic conductivity occurs. For further information see Kröber *et al.* (2014).

Anatomical preparations

We applied traditional botanical microtechniques as described in Gerlach (1984). Formalin–acetic acid–70% alcohol (FAA; 5 ml 40% formaldehyde, 5 ml glacial acetic acid, 90 ml 70% ethanol) was used as fixing agent to prepare microscope slides. Transverse sections of leaves, enclosed in natural cork, were made with a Reichert slide microtome at a thickness of 20–30 µm. Safranin was used to red-stain lignified tissue and Astra blue or alcian blue to blue-stain non-lignified cell walls. First, the sections were placed in Astra blue or alcian blue for 5 min, washed in distilled water, placed in Safranin (1% Safranin in 50% alcohol) for 2–5 min and transferred to 50% alcohol. After dehydration through an alcohol series (50%, 70%, 92%

and 100% alcohol), the sections were placed in Histo-Clear® (distilled essential oils, food grade) or xylene and mounted in Euparal or Canada balsam. The embedded sections were dried in the laboratory oven at 60 °C overnight.

Light microscopy features

Slides were examined under a light microscope (Zeiss Axioskop 2; Zeiss, Jena, Germany) and digital photographs taken (Zeiss Axio Cam MRc). Measurements of leaf anatomy characteristics were made using the Axio Vision software (Zeiss, Rel. 4.8). Leaf transverse sections were prepared to measure thickness of the upper and lower epidermis, palisade and spongy parenchyma. In each leaf transverse section, ten measurements were made and averaged. In addition, we calculated the $\log_{10}$ ratio between thickness of palisade to spongy parenchyma (Table S8). The epidermis as a boundary tissue is build from one layer of cells; however, in several species, we found multilayer epidermal structures and defined the layer beneath the final layer as subepidermis. Density of the spongy parenchyma was assessed optically in three ordinal categories in relation to the frequency of intercellular spaces, and classified as: 0 = extensive intercellular space; 0.5 = some intercellular spaces; 1.0 = closely packed cells.

Scanning electron microscopy (SEM)

For sample preparation and investigation of the adaxial and abaxial leaf surfaces, we used the scanning electron microscope at the Jodrell Laboratory of the Royal Botanic Gardens, Kew (UK). Minute parts of leaves were fixed in 70% ethanol in small-sized caskets and dehydrated in an ethanol series (70%, 90% and 100%). To remove liquid in a controlled way, the dehydrated samples were subjected to supercritical drying in a Tousimus critical point dryer. This procedure avoids damage to delicate structures, such as breakage of cell walls through surface tension as the boundary of liquid–gas transition moves by. Supercritical drying employs high temperature and high pressure to avoid crossing phase boundaries. The fluid used was CO_2, which has equal density to the liquid and vapour phase at the critical point. In the Tousimus critical point dryer liquid CO_2 is heated to the critical point (31.04 °C), at which time pressure can be gradually released, allowing the gas to escape and leaving dried samples. Subsequently, the minute leaf samples were removed from the caskets and mounted on stubs using double-sided Sellotape. The stubs with the samples were put in the Quorum sputter coater Q150 T (Quorum Technologies, Lewes, UK) and coated with platinum under an argon gas atmosphere for 60 s. Subsequently, the platinum-coated samples on the stubs were subjected to Field Emissions Scanning Electron microscopy (Hitachi S-4700 II; Hitachi, Krefeld, Germany). The digital SEM images of the 40 species were used for description of leaf cuticle characters. The cuticle is basically a two-layered sheet of lipids, wax and cutin and hydroxyl fatty acids. In addition, the cuticle contains small amounts of other substances, such as phenolic compounds. We classified epicuticular waxes and cuticle characteristics following Ditsch & Barthlott (1997). Cuticle characteristics were recorded on both sides of the leaf, recording type of ornamentation of the outer surface of the cuticle (cuticular folding) and presence or absence of papillae, using the classification of Wilkinson (1979; see Table S8). Trichomes were classified as simple, stellate or bifurcate hairs.

Statistics

All numerical leaf traits were tested for differences between deciduous and evergreen leaf habit using ANOVA. To reveal trait interrelationships, the 17 binary, numeric or ordinal leaf traits listed in Table S8 were subjected to a principal coordinate analysis (PCoA). We calculated Gower's distance to simultaneously handle traits of different scales using the ade4 package of R (Dray & Dufour 2007; Pavoine *et al.* 2009). The correlations between traits and PCoA axes were obtained by *post-hoc* correlation using the envfit function in the vegan package (Oksanen *et al.* 2013). Particularly relevant relationships were confirmed with bivariate linear regressions and Pearson correlations. Additional analyses were carried out for relationships to the four selected key ecophysiological traits: CONMAXFITA, VPDMAXFIT, HYDCOND and PSI50. Differences in these four ecophysiological traits between the four categorical cuticle characteristics (cuticular folding and presence or absence of papillae, both on upper and lower leaf surface) were tested using separate one-factorial ANOVA for each ecophysiological variable. To test for relationships between the four key ecophysiological traits to morphological traits we used linear regression model selection based on information theory. In a first step, the best linear combination of main effects for the 14 non-nominal traits in Table S8 were identified using step-wise forward selection, according to the Akaike Information Criterion (AIC) in different models, each predicting one of the four ecophysiological traits. These analyses revealed that a maximum of three predictor variables was included in the best models. In a second step, we calculated all models with all possible combinations of these 13 traits, allowing models with up to three predictor terms. As we were particularly interested in the role of leaf habit, we also included all possible twofold interactions with leaf habit as possible model terms. The best model was identified using the dredge routine of the MuMIn package (Barton 2014). All statistical analyses and plots were conducted with the R software version 3.1.0 (R Core Team 2014).

RESULTS

Morphology and anatomy of leaves

Leaf thickness ranged from 105 μm (*Sapindus saponaria*; Fig. S1E) to 367 μm (*Manglietia yuyuanensis*; Fig. S1D), with a mean of 180 μm (Table 1). While the deciduous species had the lower range in leaf thickness (105–218 μm), the evergreen trees ranged from 137 μm to 367 μm. All leaves studied were dorsiventral. Differences between evergreen and deciduous species were especially evident in the number of layers of palisade parenchyma. More than half of evergreen species had leaves with multi-layered palisade parenchyma (*e.g. Daphniphyllum oldhamii*; Fig. S1A; *Castanopsis sclerophylla*; Fig. S1C), while only one deciduous species (*Koelreuteria bipinnata*) had two- to three-layered palisade parenchyma. For further details of the mean values of the numerical leaf traits and their distribution,

Table 1. Mean values of numerical leaf traits and their minima, maxima and SD. In addition, mean values are shown separately for deciduous and evergreen leaf habit, including the *P*-values from an ANOVA testing differences in trait values between leaf habits.

trait [unit]	mean	max	min	SD	mean deciduous	mean evergreen	*P*
CONMAXFITA [$\mathrm{mmol \cdot m^{-2} \cdot s^{-1}}$]	904.30	1915.99	336.07	315.35	953.81	846.53	0.30
VPDMAXFIT [hPa]	22.97	73.81	6.17	10.35	22.34	23.70	0.68
HYDCOND [$\mathrm{kg \cdot m^{-1} \cdot s^{-2} \cdot MPa^{-1}}$]	2.44	17.52	0.04	3.36	3.55	1.13	0.023
PSI50 [MPa]	−3.78	−1.09	−6.60	1.51	−3.05	−4.64	<0.001
UPPEREPI [μm]	13.41	24.83	5.50	4.40	13.83	12.94	0.53
PALIS [μm]	73.51	129.42	32.43	21.06	65.27	82.62	0.008
SPONGY [μm]	74.91	175.25	31.58	28.88	61.08	90.19	<0.001
LOG10RATIO [$\mathrm{\mu m \cdot \mu m^{-1}}$]	0.03	0.42	−0.22	0.13	0.056	0.011	0.27
LEAFTHICK [μm]	180.97	366.57	105.23	51.50	155.77	208.82	<0.001
SLA [$\mathrm{m^2 \cdot kg^{-1}}$]	12.28	19.64	7.84	2.80	13.56	10.86	0.001

see Table 1. The complete raw data trait set for all study species can be found in Table S5. Most deciduous species (76%) had leaves with mono-layered palisade parenchyma, which was often very extensive, especially in *Rhus chinensis* (Fig. S1F). Among the evergreen species, only *Phoebe bournei* and *Schima superba* had mono-layered palisade parenchyma. *Phoebe bournei* (Lauraceae) was also the species with the lowest leaf thickness among all evergreen species. The deciduous species *Meliosma flexuosa* and *Celtis biondii* displayed the smallest log ratio of thickness of palisade to spongy parenchyma, with −0.2 and −0.21, respectively. However, the species with highest ratios, *Ailanthus altissima* (0.27) and *Rhus chinensis* (0.41), were also deciduous.

Overall, leaf habit was a good predictor of leaf anatomical characteristics; we found the following traits were significantly higher in evergreen than in deciduous leaves: leaf thickness ($F = 14.15$, $P = 0.0006$; Fig. 1A), palisade mesophyll thickness ($F = 7.94$, $P = 0.0075$; Fig. 1B) and spongy mesophyll thickness ($F = 13.33$, $P = 0.0008$; Fig. 1C). Furthermore, SLA was significantly higher in deciduous than in evergreen species ($F = 11.82$, $P = 0.0014$; Fig. 1D). The two leaf habits did not differ in the log ratio of thickness of palisade and spongy mesophyll and thickness of the upper epidermis (Table 1).

Ornamentation of the outer surface of the cuticle largely consisted of striae or ridges, which are folds of the cuticle. As an example for cuticular folding with striae of random orientation on the adaxial leaf side, see *Ailanthus altissima* (Fig. S2E). This cuticular folding represented an important character in deciduous leaves in the set of analysed species, but was much less pronounced in evergreen leaves. Epicuticular waxes were mostly present as smooth or fine-warty films, especially on the lower (abaxial) side of the leaf in most species studied (*e.g.* Fig. S2A). There were only three evergreen species with epicuticular wax plates on the abaxial side of the leaf (*Manglietia yuyunanensis*, *Phoebe bournei* and *Quercus phillyraeoides*; Fig. S2B), while deciduous species showed a large variation in epicuticular waxes on the abaxial leaf side (*e.g. Acer davidii* and *Quercus serrata*; Fig. S2A, F). On the upper (adaxial) leaf side, evergreen species showed a wider variety of epicuticular waxes than deciduous species, in the form of smooth or fine-warty films (*Cinnamomum camphora*; Figs S1B, S2C, D), heaps of grains or filaments, plates (*Lithocarpus glaber*, *Quercus phillyraeoides*; Fig. S2B) or scales (*Cyclobalanopsis glauca*).

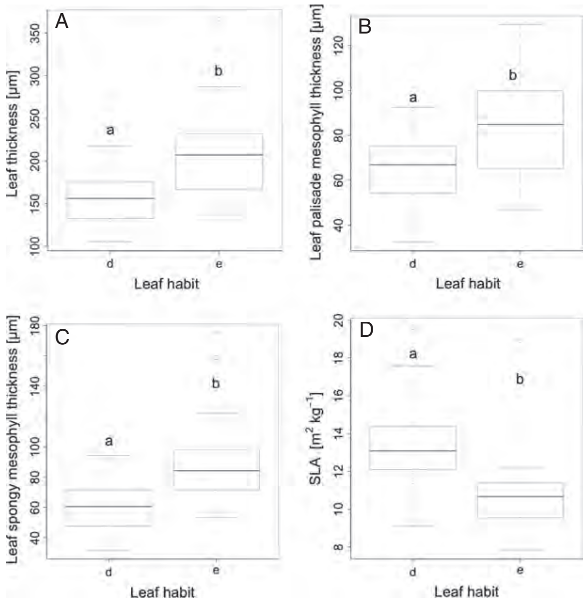

Fig. 1. Anatomical and morphological variables that significantly differ between evergreen and deciduous leaf habit. (A) leaf thickness, (B) leaf palisade mesophyll thickness, (C) leaf spongy mesophyll thickness, (D) specific leaf area (SLA). For statistics, see Table 1.

Papillae as projections of the epidermis cell wall were encountered on the abaxial leaf side of three deciduous species in the form of striate papillae (*Idesia polycarpa*; Fig. S3A; *Rhus chinensis*; Fig. S3D; *Triadica sebiferum*) and on the abaxial side of three evergreen species in the form of simple papillae (*Cyclobalanopsis myrsinifolia*, *Daphniphyllum oldhamii* and *Lithocarpus glaber*; Fig. S3B, F). The deciduous species *Rhus chinensis* showed both striate papillae and simple trichomes on the abaxial side, and additionally, trichomes on the adaxial side (Fig. S3C, D). ANOVAs on the effect of the presence of papillae on the four ecophysiological parameters did not reveal any significant relationship.

Trichomes occurred as simple, stellate or bifurcate hairs (Fig. S3E, F). In eight deciduous species, trichomes were present both on the adaxial and abaxial side, whereas trichomes did not occur in leaves of any of the evergreen species, In the studied evergreen species, adaxial trichomes were mainly a feature of Fagaceae and Lauraceae. Stellate hairs were mainly found in the Fagaceae but also in *Alniphyllum fortunei* (Styracaceae; Fig. S3E).

Interrelationships between morphological and anatomical leaf traits

The results of the principal coordinate analysis (PCoA) of the 14 leaf traits characteristic of the leaf anatomy are shown in Fig. 2A and 2B for axis 1 *versus* 2 and 1 *versus* 3, respectively. For more detailed information see Table S6 with all correlations of the 14 traits with the three PCoA axes, and Table S7 for coordinates of all species. The strongest significant positive correlations with the first PCoA axis were observed for number of palisade layers, leaf habit, thickness of the palisade mesophyll layer, leaf thickness and thickness of the spongy mesophyll layer, in order of decreasing importance. SLA and cover of trichomes on the adaxial side of the leaf (COVTRIUP) were significantly negatively correlated with the first axis. The second PCoA axis was, to a large extent, characterised by a significant positive relationship to cover of trichomes on the abaxial side of the leaf (COVTRILO), type of trichomes (TRICHOM) and the presence of excretory glands (EXCRET), as well as by a significant negative relationship to epidermal cell size (EPICLLSIZ) and presence of a column of sclerenchyma cells through the leaf (COLSCLER).

Leaf thickness was the variable with the most significant relationships to other anatomical and morphological leaf traits and increased with the number of layers of palisade mesophyll cells ($r = 0.55$, $P = 0.0002$), palisade parenchyma thickness ($r = 0.80$, $P < 0.0001$) and spongy parenchyma thickness ($r = 0.90$, $P < 0.0001$; Fig. 3A). Thickness of both mesophyll types was positively related to one another (Fig. 3C; $r = 0.63$, $P < 0.0001$). Both thickness of the palisade mesophyll layer and log ratio of palisade to spongy parenchyma thickness decreased with increasing SLA (Fig. 3B; $r = -0.46$, $P = 0.0027$; Fig. 3D; $r = -0.32$, $P = 0.0427$).

Relationships between morphological/anatomical and ecophysiological traits

Analysis of the relationships between the four ecophysiological key traits and cuticle characteristics did not reveal any significant relationships. However, ANOVA analyses revealed marginally significant differences in fitted absolute maximum g_s (CONMAXFITA) for different patterns of the upper epicuticular wax layer (EPIWAXUP; $P = 0.0787$).

The best multi-predictor models with at most three terms were able to explain a proportion of the variation in the four ecophysiological parameters of g_s and xylem cavitation vulnerability among species (r^2 between 0.30 and 0.39), except for maximum specific xylem hydraulic conductivity (HYDCOND), for which the coefficient of determination was only 0.18 (Table 2). Three of the four ecophysiological characteristics contained leaf habit and interaction with leaf habit (Table 2, Fig. 4). Absolute maximum of fitted g_s (CONMAXFITA) significantly increased with the $\log_{10}$ ratio of thickness of palisade to spongy parenchyma (LOG10RATIO; $P = 0.006$), with steeper slopes for deciduous than evergreen species ($P < 0.001$), but identical intercepts ($P = 0.84$; Fig. 4A). The vpd at the maximum of modelled g_s–vpd relationship (VPDMAXFIT) showed an increase with LOG10RATIO only in the presence of papillae (PAPILL; $P = 0.002$), while neither LOG10RATIO ($P = 0.71$) nor PAPILL ($P = 0.94$) alone had a significant effect (Fig. 4B). Both parameters of stem xylem hydraulics depended on the same leaf traits, which were leaf habit (LEAFHABIT) and density of the spongy parenchyma (DENSINTCEL). Maximum specific xylem hydraulic conductivity (HYDCOND) was lower in evergreen than deciduous

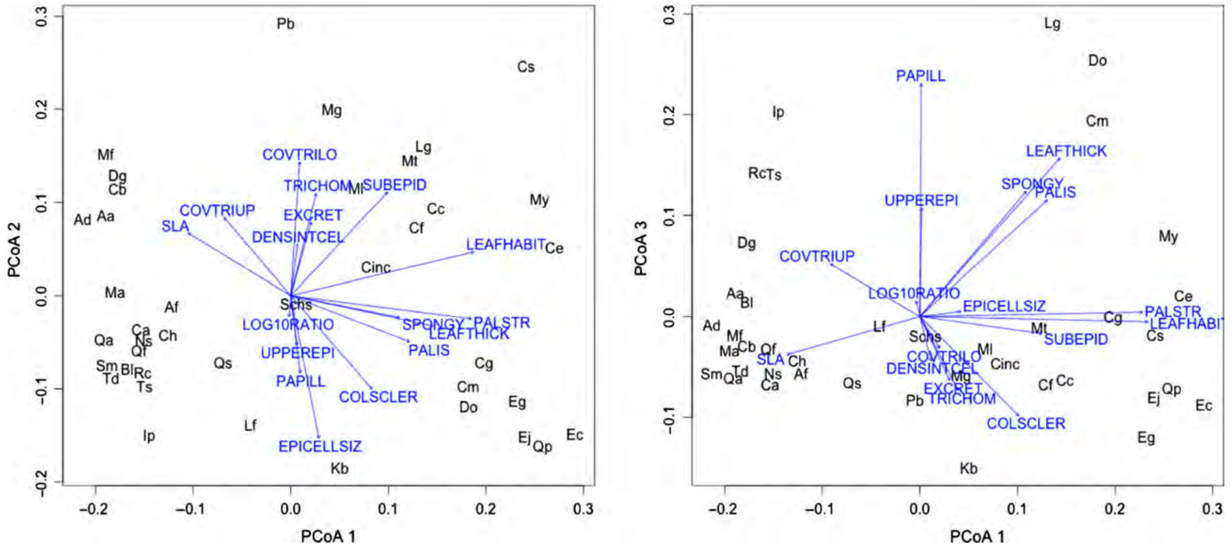

Fig. 2. Principal coordinate analysis (PCoA) biplots of the species mean values of the 14 binary, ordinal and numeric morphological leaf traits. (A) PCoA axes 1 and 2, (B) PCoA axes 1 and 3. See Table S8 for coding of trait names. Eigenvalues: axis 1 = 1.18, axis 2 = 0.582, axis 3 = 0.387, with cumulative proportion of explained variance 30.3%, 45.4% and 55.4%, respectively. Species abbreviations refer to genus and species epitheton. See Table S4 for full species names.

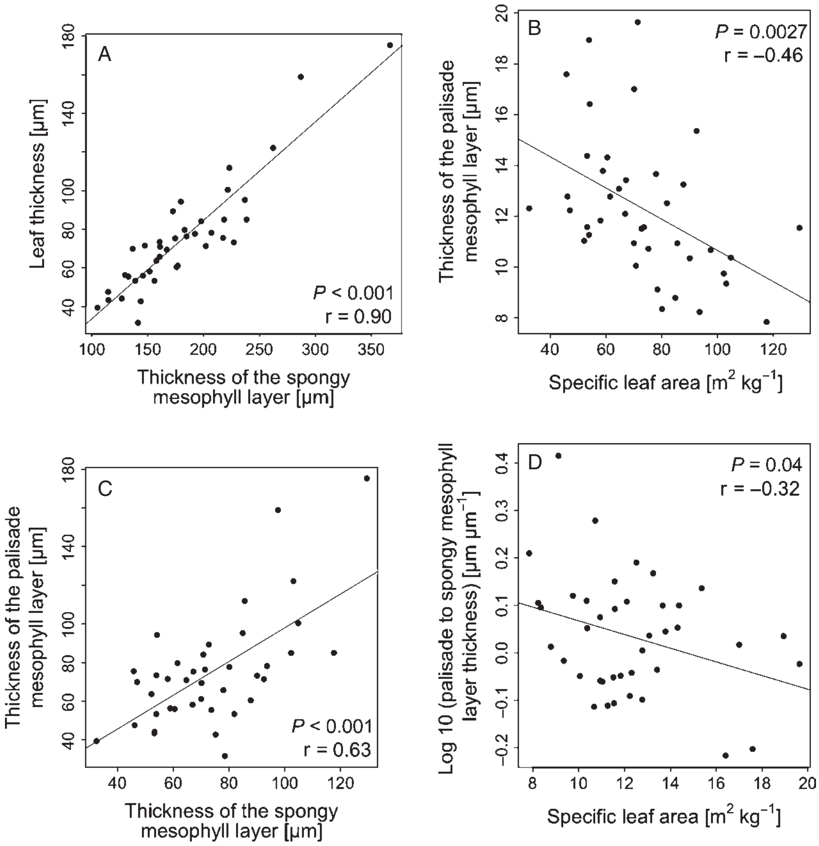

Fig. 3. Results of the strongest interrelationships among numerical anatomical leaf traits. For statistics, see text.

species ($P = 0.023$) and showed a tendency to decrease with increasing DENSINTCEL ($P = 0.15$). Xylem pressure at which 50% loss of maximum specific hydraulic conductivity occurred (PSI50) was higher in deciduous than evergreen species ($P < 0.001$), and increased with DENSINTCEL ($P = 0.02$; Fig. 4D).

DISCUSSION

Our study demonstrated that key ecophysiological traits of stomatal control and wood hydraulics were reflected in structural leaf traits. Interestingly, whether a leaf was evergreen or deciduous did explain a large proportion of the variation in morphological and anatomical leaf traits investigated. In addition, leaf habit was also among the best predictors for three of the four key hydrological traits. This justifies the use of leaf habit in ecophysiological models for woody species, as is the current practice in dynamic global vegetation models (DGVM; *e.g.* Woodward & Cramer 1996; Sitch *et al.* 2003). Just as leaf habit was related to leaf thickness, all leaf traits related to thickness of different layers also differed strongly between the two leaf habits. These interrelated traits also formed the first axis in the PCA analysis. Our findings confirm those in cross-species comparisons for different regions described in the literature. For example, in two studies with a comparable set of deciduous and evergreen study species, Roth *et al.* (1995) and Arambarri *et al.* (2006, 2008) described similar patterns in leaf structure for a montane forest in Venezuela and tree species from the lowest level of an Andine mountain forest of Tucumán (Argentina), respectively. For a set of 23 tree species native to seasonally dry tropical forest, Pringle *et al.* (2011) found differences in water availability were reflected in specific trait syndromes associated with evergreen and deciduous leaf habit.

Table 2. Results of the best model with at most three predictors selected using information theory, separately for the four ecophysiological traits: CONMAXFIT, absolute maxima of fitted stomatal conductance; VPDMAXFIT, vpd at maximum of modelled stomatal conductance–vpd relationship; HYDCCOND, maximum specific xylem hydraulic conductivity; PSI50, pressure at 50% loss of maximum specific xylem hydraulic conductance. For plots of models, see Fig. 4. For significances of parameter estimates, see text.

response	start AIC	final AIC	model of predictors	r^2	F-value	df	*P*
CONMAXFITA	449.78	441.88	CONMAXFITA ~ LOG10RATIO + LEAFHABIT + LOG10RATIO: LEAFHABIT	0.30	4.991	3; 35	0.0055
VPDMAXFIT	183.31	170.49	VPDMAXFIT ~ LOG10RATIO + PAPILL + LOG10RATIO: PAPILL	0.3827	7.234	3; 35	0.00067
HYDCOND	95.47	91.60	HYDCOND ~ LEAFHABIT + DENSINTCEL	0.1827	4.024	2; 36	0.02647
PSI50	32.91	17.90	PSI50 ~ LEAFHABIT + DENSINTCEL	0.3858	11.3	2; 36	0.00015

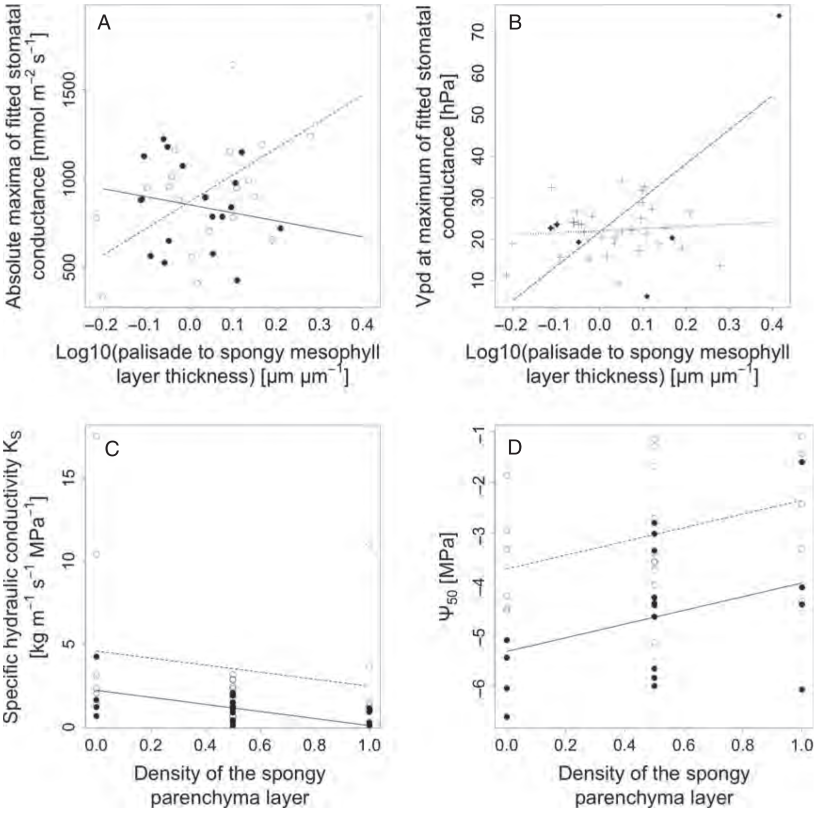

Fig. 4. The interrelationships between anatomical leaf traits and the four ecophysiological traits. (A) Absolute maxima of fitted stomatal conductance (CONMAXFITA) as a function of the log10 ratio of thickness of palisade to spongy parenchyma (LOG10RATIO) and LEAFHABIT and their interaction; (B) vpd at maximum of the modelled g_s–vpd relationship (VPDMAXFIT) as a function of LOG10RATIO and the presence of papillae (PAPILL) and their interaction ($P = 0.002$); (C) maximum specific xylem hydraulic conductivity (HYDCOND) as a function of leaf habit and density of spongy parenchyma (DENSINTCEL); (D) xylem pressure Ψ_{50}, at which 50% loss of the maximum specific hydraulic conductivity occurs (PSI50) as a function of leaf habit and DENSINTCEL. Regression lines were obtained from parameter estimates of the best multi-predictor models in Table 2. For significances of parameter estimates, see text. In plots (A), (C) and (D) white circles and dashed lines represent deciduous species and filled black points and solid lines represent evergreen species. In plot (B), crosses and dotted line represent species without papillae, whereas filled black diamonds and broken line represent species with papillae.

Our first PCoA axis was related to the leaf economics spectrum and includes several morphological and anatomical traits. In a set of laboratory-grown seedlings of 52 European woody species Castro-Díez *et al.* (2000) showed that evergreen and deciduous species significantly differed in leaf thickness and thickness of mesophyll and spongy parenchyma, which we have now confirmed. Burrows (2001) examined leaves of 39 species on the east coast of sub-humid New South Wales, Australia (35° S); however, the relationships between, for example, mesophyll distribution and the ecological habitat of the species were weak.

Species with traits that implied an adaptation to dry habitats often co-occurred with mesophyllous species and *vice versa* (Medina *et al.* 1990; Gibson 1996). One reason for this might be that not all leaf traits related to ecophysiology are also related to the leaf economics spectrum (LES). This is reflected in our findings that not all variation in traits was captured by the first PCoA axis, as a further 25.1% of the variation was explained with axes two and three. In our study, we found cover and type of trichomes, presence of excretory glands, epidermal cell size and presence of a column of sclerenchyma cells through the leaf were unrelated to the LES. Similarly, stomatal size and density have been demonstrated to be independent of the LES, and thus of leaf thickness and leaf habit (Beerling & Kelly 1996; Kröber *et al.* 2012). Along this line, Brodribb & Holbrook (2005) concluded that leaf habit and the specific physiological solutions are not inevitably connected. Although leaf habit was a strong predictor in our analyses, as it sets certain boundaries for leaf physiology, within evergreen and deciduous leaves there is still a wide range of further differentiation (Kikuzawa 1991; Aerts 1995). One trait that was unrelated to the LES but was the best predictor for g_s parameters was $\log_{10}$ ratio of palisade to spongy mesophyll layer thickness. This trait had already been suggested by Wylie (1946) as relevant for leaf hydraulics, and was confirmed to be also relevant to leaf hydraulic conductance (Sack & Frole 2006). The mechanistic explanation might be that with increasing $\log_{10}$ ratio, the amount of intercellular spaces decreases (Castro-Díez *et al.* 2000), which requires increased gas exchange, which in turn is reflected in higher maximum g_s and stomata that remain open at higher vpd. Another function of a high $\log_{10}$ ratio might be related to resistance to air pollutants. Dineva (2004) suggested that wide intercellular spaces at low palisade/mesophyll ratios allow faster penetration and absorption of toxicants into photosynthetically active tissues.

Our study also revealed a relationship between leaf traits and xylem hydraulics. This contradicts the results of Baraloto *et al.* (2010), who found the main dimensions of leaf and stem functional traits were unrelated across a wide range of rain forest tree species. However, in contrast to Ψ_{50}, maximum specific xylem hydraulic conductivity was only poorly explained by leaf traits and mainly depended on leaf habit, which confirms the results of Kröber *et al.* (2014). These authors found maximum hydraulic conductance and Ψ_{50} were mainly connected to leaf carbon to nitrogen ratio, which in turn was related to leaf habit. In their study, traits of xylem hydraulics were not related to traits of stomatal regulation, which is congruent with the findings of the present paper. In contrast, our observation that the Ψ_{50} increased with increasing density of the spongy parenchyma layer, was independent of leaf habit and thus of the LES, adds new aspects to the findings of Kröber *et al.* (2014). However, we do not have an explanation for why species with more drought-resistant xylem had less dense spongy parenchyma, as the opposite relationship of Ψ_{50} to density of the whole leaf would be expected (Niinemets 2001; Bacelar *et al.* 2004).

In contrast to expectations, leaf hydraulics were not significantly related to cuticle traits; however, with a larger species set and higher replication of the different types of cuticle structure, relationships might be detectable. Although cuticular waxes are generally considered as protection against water loss, Riederer & Schreiber (2001) stated that there is no simple link between the amount of wax and drought protection. Similarly, we also did not find a simple link between hydraulic traits and epicuticular wax structure. Thus, we must conclude that the ecological relevance of the different types of epicuticular waxes remains unsolved. Similarly, the presence of trichomes and especially papillae did not well reflect hydraulic functions, although their function to increase the leaf boundary layer, and thus the diffusion pathway for transpiration (Schuepp 1993), should confer higher drought resistance. The papillae found in *Idesia polycarpa, Cyclobalanopsis myrsinifolia* and *Daphniphyllum oldhamii* may have the same function as trichomes, as no trichomes were recorded for these species. An alternative explanation for papillae in contrast to trichomes might be that they reduce the adherence of water to the leaf surface, thus might facilitate gas exchange during precipitation events (Barthlott & Neinhuis 1997; Neinhuis & Barthlott 1997; Haworth & McElwain 2008).

For all results discussed, we should remember that all measurements were made on juvenile trees of a limited species set typical of subtropical forests in southeast China, which implies limitations. It is an open question whether the conclusions drawn are transferable to adult trees, as many such relationships vary with ontogeny (Lusk & Warton 2007). Furthermore, under different climate conditions, especially drought and other precipitation-related influences, the relationships between stomatal conductance, xylem hydraulics and morphological leaf traits may differ from the results obtained here. Finally, we should be aware that we could not provide mechanistic explanations for all observed relationships. In these cases, our analyses can only be regarded as explorative, reporting patterns that have not yet been described in the literature.

CONCLUSION

The aim of this study was to explore links between leaf anatomy and structure in relation to key ecophysiological traits. In addition to confirming the role of leaf habit in explaining leaf and xylem hydraulic traits, we identified further leaf traits that were unrelated to leaf habit and the leaf economics spectrum. In particular, the log ratio of palisade to spongy mesophyll layer thickness appeared to be a suitable predictor for stomatal regulation patterns and should be considered in future leaf trait analyses.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Leaf transverse sections.

Figure S2. Cuticular and epicuticular structures, analysed with SEM.

Figure S3. Trichomes and papillae, analysed with SEM.

Table S4. Tree species studied, assignment to family and order as well as leaf habit (d = deciduous; e = evergreen).

Table S5. Trait raw data of all measured traits on all species.

Table S6. PCoA correlations for all traits included in the PCoA (see Fig. 2).

Table S7. PCoA coordinates for all species included in the PCoA (see Fig. 2).

Table S8. Ecological relevance and encoding of morphological leaf traits.

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Chapter 5

EARLY SUBTROPICAL FOREST GROWTH IS DRIVEN BY COMMUNITY MEAN TRAITS VALUES AND FUNCTIONAL DIVERSITY RATHER THAN THE ABIOTIC ENVIRONMENT

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Early subtropical forest growth is driven by community mean trait values and functional diversity rather than the abiotic environment.

Summary

1. Functional diversity (FD) has been shown to be positively related to many ecosystem functions, including biomass production, but might have much lower effects than abiotic environmental factors or species-specific properties. Leaf and wood traits can be considered particularly relevant for tree growth, as they reflect the trade-off in invested resources into growth and persistence. We asked to which degree early forest growth in a large-scale tree diversity experiment (BEF-China) is driven by FD, ecoscape (11 environmental variables characterizing abiotic habitat conditions) and community weighted mean (CWM) values of species traits.

2. Crown diameter growth rates of trees were aggregated across 231 plots varying in richness between one and 23 tree species and related them to ecoscape as well as to FD and CWM, the latter two based on 41 plant functional traits. The effects of each of the three predictor groups were analysed separately by mixed model optimization and jointly by variance partitioning.

3. Numerous single traits predicted plot-level tree growth, both in the models based on CWMs and FD, but none of the selected ecoscape variables. In the best models, ecoscape and FD explained only 4% and 31% of variation in crown growth rates, respectively, while CWM trait values explained 42%. In total, the best models accounted for 51% of crown growth.

4. *Synthesis.* The marginal role of the selected ecoscape variables was unexpected, given the high topographic heterogeneity and large size of the experiment, as was the significant impact of FD, demonstrating that positive diversity effects do already occur in early stages of tree plantations.

Introduction

One of the most important aims in functional biodiversity research is to predict the importance of different facets of biodiversity for ecosystem functions (EF). It has been shown that many different EFs are positively related to producer diversity (Loreau *et al.* 2001; Hooper *et al.* 2005; Balvanera *et al.* 2014). A meta-analysis (Cardinale *et al.* 2011) revealed that 414 out of 574 independent experimental manipulations of species richness had a positive effect on producer biomass. This also applies to forests, which are the most important ecosystems globally, because of their wide geographical cover and the unique ecosystem goods they provide (Quijas *et al.* 2012). A review of worldwide inventories on forest growth and standing biomass demonstrated positive relationships with tree species richness in the majority of published studies (Scherer-Lorenzen 2013). For example, it was shown that biomass production in Swedish forests increased with tree species richness (Gamfeldt *et al.* 2013).

This biodiversity-focused research has often tried to minimize environmental variation and thus may have underestimated other major determinants of tree growth such as climate and soil conditions. The analysis of the pan-European tree-ring network showed that forest productivity is driven by temperature in high-elevation and high-latitude areas and by moisture at low elevation in central and southern Europe (Babst *et al.* 2013). Climate also determines tree growth at the microsite scale brought about by differences in slope, aspect, and inclination (Chen *et al.* 1999; Geiger, Aron & Todhunter 2003). In the northern hemisphere, south-facing slopes receive more solar irradiation than north-facing slopes (Warren 2010), resulting in positive effects on individual tree growth (Fralish 1994; Li *et al.* 2014). In consequence, each single variable such as slope aspect, slope inclination or altitude has the potential to significantly affect tree growth (Saremi *et al.* 2014). In addition, soil conditions have a strong impact on forest productivity (Grier *et al.* 1989), rendering soil type a key predictor in forest growth models (Landsberg & Waring 1997; Pinjuv, Mason & Watt 2006). This strong dependence of forest productivity on climate and soil conditions shows that any attempt to detect biodiversity signals on tree growth needs to be separated from effects of the abiotic environmental setting. The term 'ecoscape' has been suggested to summarize all local topographic, microclimatic and edaphic environmental factors (Bruelheide *et al.* 2014), which are potential co-drivers of biodiversity effects. In functional biodiversity research the ecoscape can be considered a type of null model against which additional biodiversity effects have to be compared (Díaz *et al.* 2007). In the current paper, we have analysed a large forest biodiversity experiment in this way, assuming the biodiversity treatments were only creating a large variation in functional diversities of tree communities across a heterogeneous landscape and beyond that simply ignoring all design aspects of the experiment. We believe that this novel approach of analysing a designed experiment as if it was a sample survey of plots across the landscape (Snedecor & Cochran 1989) is justified to give the null model of the ecoscape influencing tree growth the maximum chance to reveal itself. The selected set of ecoscape variables were related to topography, such as slope, aspect and elevation, and soil, such as pH value, carbon and nitrogen content in the topsoil, which may be considered relevant for tree growth at the time of establishing the experiment. At this early stage of the experiment we can assume that the ecoscape variables where themselves not yet or only minimally affected by the experimental biodiversity treatments. Obviously, in the longer term this may not hold and in particular microclimate and soil conditions might respond to biodiversity in feed-back loops, whereby, depending on plot productivity and tree richness, organisms modify their environment (Bruelheide *et al.* 2014).

Further key determinants of forest production are the tree species themselves. It is well known that tree growth varies by one order of magnitude among different tree species (Lieberman *et al.* 1985; Lambers & Poorter 1992). The impact of species identity has been found to be strong in relation to effects of tree species richness per se (Nadrowski, Wirth & Scherer-Lorenzen 2010; Lang *et al.* 2012a). One approach to better understand such species identity effects is to relate species-specific differences in growth to the species' functional traits (Díaz *et al.* 2007). It has been shown that a few key traits that describe the leaf economics spectrum (LES) (Wright *et al.* 2004), such as specific leaf area (SLA) and leaf nitrogen content can successfully predict tree growth of 53 rainforest species in Bolivia (Poorter & Bongers 2006). At the plot scale, productivity should depend on the mixture of species in the community. According to the mass-ratio hypothesis (Grime 1998) the most abundant or dominant species are expected to exert the highest impact on EF. This hypothesis provides the basis for using community weighted means (CWM) of trait values, obtained from averaging traits at the community level by weighting the species' traits by the species' relative abundance in that community (Ackerly *et al.* 2002). In grasslands, this approach has been successfully employed for predicting EF from CWM trait values (Garnier *et al.* 2004; Roscher *et al.* 2012). Thus, tree growth as one important EF might be predictable from CWM of a single key trait or from a combination of CWMs of different uncorrelated traits.

Representing overall plot means, CWM do not account for trait variation within plots. Thus, they do not capture the effect of functional diversity (FD), including both selection and complementarity effects (Loreau & Hector 2001). In particular, functional diversity may increase resource complementarity and facilitation among species in species-rich plots and thus increase forest productivity (Spasojevic & Suding 2012; Dias *et al.* 2013). For example, in southern New Zealand, nutrient-rich forest sites were not only characterized by species with high relative growth rates, but also had a higher variation in growth rates, which was related to a high variation in species-specific shade tolerances and resulted in a larger complementarity of light use (Coomes *et al.* 2009). Hence, within defined forest age classes, FD has been found to be positively related to aboveground biomass (Bu, Zang & Ding 2014). Similarly, in the Cedar Creek experiment, functional complementarity of grassland species resulted in higher C and N accumulation in soils (Fornara & Tilman 2007). Complementarity in resource use is expected to emerge in trait space, seen in a higher variation and dispersion of values of relevant traits (Lavorel *et al.* 2008). In principle, a trait can contribute to complementarity of a particular EF in the community either spatially via above- or belowground resource partitioning (Felten *et al.* 2009; Bessler *et al.* 2009) or temporally via differential resource use in different seasons (Dedeyn & Vanderputten 2005). Trait value distribution in the community can mathematically be expressed by FD measures, one of which is the regularity of the distribution of trait abundances (Villéger, Mason & Mouillot 2008), as designated by Rao's quadratic entropy (FDQ) (Rao 1982). It should be noted that in communities FD of a trait is not independent of CWM of the same trait, since trait variation is constrained by the mean (Dias *et al.* 2013). In consequence, both, FD and CWM of a single trait, explain some degree of variation in EF. Thus, separating FD from CWM poses a similar problem as separating ecoscape from biodiversity effects.

In summary, variation in productivity as important ecosystem function in forests, may be largely explained variation by ecoscape, variation in community weighted mean trait values (CWM) and functional diversity (FD). The contribution of each of these components on a certain EF can be visualized as a triangle where ecoscape, CWM and FD are the corners. The location of a particular plant community in this triangular space will depend on the relative impact of the abiotic environment, species-specific properties and biotic interactions. For example, aboveground net primary production (ANPP) in alpine grasslands was found to be dependent on both nutrient supply (quantified by a nitrogen nutrition index) and FD in vegetative height, which reflected light acquisition complementarity, but not by CWM of any trait (Díaz *et al.* 2007). Taking together all predictors, 44% of the total variation in ANPP was explained by abiotic conditions alone, and inclusion of FD did not improve the model's explanatory power. However, to our knowledge no attempt has been made to quantify the contribution of ecoscape, CWM and FD in forest communities. One important caveat that must be considered in the above context is that not all of the three explanatory corners in the mentioned triangle will usually have the same range of variation in any particular study and thus they will not have the same chance to influence variation in the dependent variable. In the present study, variation was particularly high in CWM and FD, because the plots came from a biodiversity experiment, which ensured a large range of species richness levels, and as a consequence, resulted in a large variation in CWM and FD. Nevertheless, the very large topographic and hydrological variation at the experimental site also ensured a high variation of ecoscape variables.

It might be argued that partitioning the effect of ecoscape, CWM and FD is only necessary in natural communities but not in designed experiments, as in those biodiversity is manipulated and environmental variation should be controlled for. To control environmental variation, experimental plots are often established in homogeneous environments, such as a flat piece of land with uniform land use history and soil properties. However, even comparatively low environmental heterogeneity can strongly affect EF as was demonstrated in the Sardinilla forest experiment in Panama (Healy, Gotelli & Potvin 2008). In presence of environmental heterogeneity, fully randomized experiments cannot exclude that certain plots are located in exceptional site conditions. For example, in the Sardinilla experiment, all six-species diversity plots were located at low water drainage conditions (Healy *et al.* 2008). With increasing size of the experiments, homogeneity will decrease. For that reason, many experiments have employed blocking, for example with respect to distance from the river in the Jena-Experiment (Roscher *et al.* 2004). However, blocking is only useful if there are few and clear gradients across the experimental site (Bruelheide *et al.* 2014). In heterogeneous environments, blocking is not feasible. Typically forests occur in such topographically heterogeneous environments, as in many regions of the world, forests have withdrawn from flat land, which often is more fertile than sloped land, and thus, is used for agriculture (Sandel & Svenning 2013). This means that in realistic forest biodiversity-EF experiments, ecoscape effects are often confounded with biodiversity and have to be accounted for in the same way as in natural communities.

The aim of our study was to partition the effects of 11 environmental variables characterizing the ecoscape and CWM and FD variables calculated from 41 species traits on one key ecosystem function in the early stage of a large forest biodiversity experiment (Bruelheide *et al.* 2014). Located in subtropical China, a set of 40 broadleaved tree species, native to the natural vegetation was planted in richness levels of 1, 2, 4, 8, 16 and 24 tree species. We used the results from one of two sites, which was planted in 2009 with 1 year-old saplings (Yang *et al.* 2013; Bruelheide *et al.* 2014). As measure of the trees' productivity we chose mean annual crown width growth between 2011 and 2012 as the variable that best reflected tree growth in this early stage of the experiment (Li *et al.* 2014). We expected that forest growth at this early stage was mainly dominated by abiotic conditions, which was based on the finding of Li *et al.* (2014) that growth of individual trees was related to aspect and soil nitrogen content but not to Shannon diversity of the local tree neighbourhood. In the present study, we used the crown width growth rate data of Li *et al.* (2014), but aggregated the data at the plot level. We then subjected the plot mean values to the analysis framework of Díaz *et al.* (2007). To disentangle the effects of the three groups of predictor variables mentioned above, a stepwise approach as suggested by these authors (Díaz *et al.* 2007) was used, sequentially fitting the influence of different predictors of the environment, CWM and FD. The objective of our study was to identify those single ecoscape-, CWM- and FD- predictors that best predicted plot-level tree growth. In particular, we hypothesized 1) that there are single variables from the three predictor groups (ecoscape, CWM and FD) that significantly explain tree growth, and 2) that, comparing the best predictors from the three groups, the ecoscape variables have the highest explanatory power for tree growth rate at the early stage of the experiment.

To our knowledge, our study is the first to disentangle the effect of ecoscape, CWM and FD in a biodiversity functioning experiment with trees. Our results are the first ones on trait-EF relationships from all forest diversity experiments worldwide.

Materials and Methods

STUDY SITE

We conducted our study in a field experiment (BEF-China) in South-East Subtropical China (29.08–29.11 N, 117.90–117.93 E). BEF-China is a large-scale biodiversity and ecosystem functioning experiment with tree species (Yang *et al.* 2013; Bruelheide *et al.* 2014). Prior to the experiment the sites were covered with conifer plantations of *Pinus massoniana* and *Cunninghamia lanceolata*, harvested at about 20-year intervals. After clear-cutting the conifer plantations, the aboveground plant biomass was removed from the experimental site (Yang *et al.* 2013). Using a pool of 40 species native to the regional broadleaved forest, the experiment was set up in 2008/2009, covering an area of 38 ha. The diversity gradient comprises monocultures and plots with 2, 4, 8, 16 and 24 species. Here we present the results of one of the two experimental sites (Site A). We evaluated data on tree growth measurements from 231 plots and 23 species planted at this site. As response variable we here analysed annual rates of crown width growth, calculated from two monitoring sessions in 2011 and 2012 (Li *et al.* 2014). We used crown diameter increment data of 23 species, of which 14 and 9 species were deciduous and evergreen, respectively, for which also an extensive list of functional traits was measured on the sites. All growth data were aggregated at the plot level by taking the arithmetic mean of absolute crown diameter increment across all individuals measured in a plot.

ASSESSMENT OF ECOSCAPE VARIABLES

A 5 m digital elevation model (DEM) was established based differential GPS measurements carried out in 2009. The DEM was used to derive plot mean values for elevation, aspect, mean slope, solar insolation, profile curvature and plan curvature as description of the environmental condition (Evans 1979; Zevenbergen & Thorne 1987; Dietrich & Montgomery 1998; Shary, Sharaya & Mitusov 2002). Sine and cosine transformations of the aspect were used to express eastness and northness, respectively (Roberts 1986). All calculations were made using ArcGIS 9.0 (ESRI Corp., Redlands, California, USA).

Soil variables were based on nine soil samples per plot, collected in 2010 by taking soil cores at a depth of 0-5 cm. The nine soil samples per plot were thoroughly mixed and one bulk sample per plot was analysed for total carbon (C) and total nitrogen (N) content. Prior to the chemical analyses, soil samples were air-dried and sieved (< 2 mm). For the C and N analyses, dry soil samples were ground with a ball mill and subjected to total C/N analysis based on gas chromatography (Vario EL, Elementar, Hanau, Germany). Minimum, maximum, mean values and standard deviation of all ecoscape variables is shown in Supplementary Information Table S1.

ASSESSMENT OF LEAF TRAITS

All traits were used to calculate community weighted means (CWMs) and functional diversity (FD) as predictive variables. These included a) traits connected to the leaf economics spectrum such as specific leaf area (SLA) and leaf nitrogen content (LNC), b) traits related to stomatal conductance such as maximum and mean stomatal conductance, c) traits related to xylem properties, such as specific hydraulic conductivity of the xylem (KS) and the xylem pressure at which 50% loss of the maximum specific hydraulic conductivity (Ψ_{50}) and d) leaf microscopic traits such as stomata density and the thickness of the palisade parenchyma. These trait datasets and the specific measurement protocols were published in three recent papers (Kröber & Bruelheide 2014; Kröber *et al.* 2014a; b).

STATISTICS

CWM value of traits was calculated according to (Garnier *et al.* 2004) and FD_Q (Rao's quadratic entropy) according to (Botta Dukát 2005). Both CWM and FD_Q were weighted by the frequency of the tree species in a plot. According to the design of the BEF-China experiment, all tree species in a plot were represented by the same number of trees. However, due to mortality, the number of trees per species on which growth rates were measured varied to some degree from equal proportions. Thus, we made sure that the same proportions of trees that were used in calculating plot means of crown diameter growth rates were also used for calculating CWM and FD. All predictor variables were scaled by mean and standard deviation, which allowed for an interpretation of the effect sizes with regard to their importance on crown width growth rate. In an initial analysis we tested each single predictor with single linear models for their impact on crown growth. We then tested the trait complexes in combination according to (Díaz *et al.* 2007) for their explanatory power in predicting crown growth. Since many different trait combinations can equally explain plant growth (Marks & Lechowicz 2006a; b), we tested all possible combinations of the predictor variables and then selected the best model that had a maximum of five predictor variables based on Akaike's Information Criterion corrected for small-sample-sizes (cAIC), using the MuMIn package in R (Barton 2014). The independent effect of each predictor variable in the final model on crown growth was assessed by plotting the residuals of crown width growth rates against every predictor variable. Finally, to test the impact of the three variable complexes ecoscape, CWM and FD, we applied variance partitioning with all the significant predictors, using the vegan package in R (Oksanen *et al.* 2013). For all statistical analyses, we used the software R version 3.1.0 (R Core Team 2014).

Results

Contrary to our expectations, environmental factors had no significant effects on plot means of annual crown width growth rates (Table 1). The best out of the selected ecoscape predictors was slope inclination (SLOPE), which however, had only a marginally significant effect on crown width (CW) growth rates (p=0.091). Testing for combinations of all ecoscape variables in all possible multi-predictor models, the minimal model only retained altitude and eastness, both of which with a negative impact on tree growth (Table 3). This means that plot mean of tree crown diameter growth at the plot level was larger at low elevations (valleys and foot slopes) and on slopes facing westwards than eastwards (Fig. 1). However, the minimal ecoscape model only explained 3.8% of the total variation in crown width growth rates (Table 3).

Table 1

Impact of ecoscape on crown growth. The effect of ecoscape predictors for crown width growth rate, assessed as plot mean values between 2011 and 2010. All environmental variables are scaled by mean and standard deviation, thus the estimates show the direction and magnitude of impact on CW growth rates. DEM: digital elevation model

Abbreviation	Predictor	Source	Estimate	r^2	p
ALT	altitude	DEM	-0.05	0.01	0.11
SLO	slope	DEM	-0.38	0.01	0.09
SOLAR	solar radiation	DEM	0.00	0.00	0.31
CURV X	profile curvature	DEM	0.02	0.00	0.50
CURV Y	plan curvature	DEM	0.00	0.00	0.94
NORTH	aspect northness	DEM, cosine of slope	-0.47	0.00	0.81
EAST	aspect eastness	DEM, sine of slope	-3.62	0.01	0.11
PH	soil pH (KCl)	Soil sampling, pH electrode	0.80	0.00	0.92
N	soil nitrogen content	Soil sampling, total CN analyzer	13.99	0.00	0.71
C	soil carbon content	Soil sampling, total CN analyzer	-0.28	0.00	0.88
CN	soil carbon nitrogen ratio	Soil sampling, total CN analyzer	-0.31	0.00	0.62

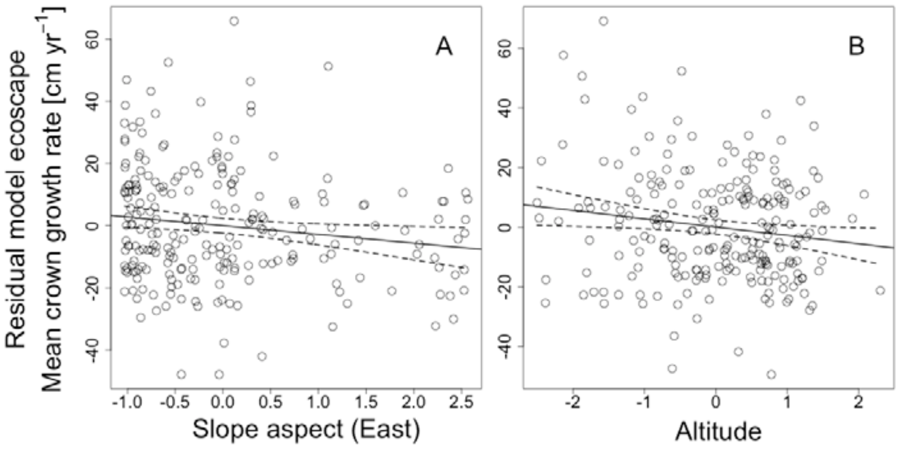

Figure 1

Mean annual crown width growth rate as predicted by the ecoscape multi-predictor model. The residuals from all other terms in the model are plotted against A) slope aspect (East) and B) altitude. Every dot represents one plot. All predictor variables are scaled by mean and standard deviation, thus the slope of the regression shows the direction and magnitude of impact on CW growth rates. The panels have been arranged in the sequence of decreasing order of effect sizes. For statistical details see Table 3.

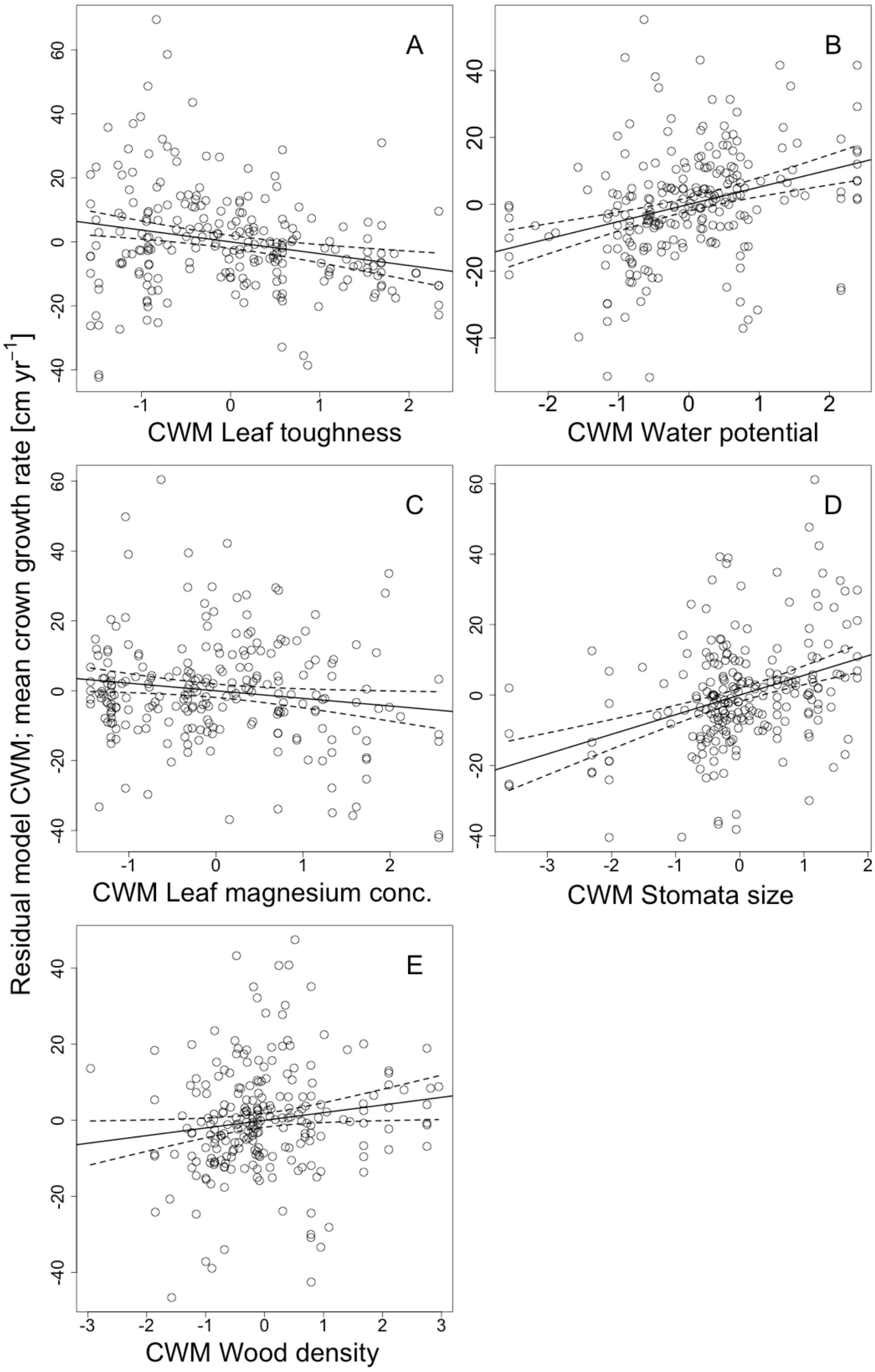

Figure 2

Mean annual crown width growth rate as predicted by the CWM multi-predictor model. The residuals from all other terms in the model are plotted against A) leaf toughness, B) water potential, C) leaf magnesium content, D) stomata size and E) wood density. Every dot represents one plot. All predictor variables are scaled by mean and standard deviation, thus the slope of the regression shows the direction and magnitude of impact on CW growth rates. The panels have been arranged in the sequence of decreasing order of effect sizes. For statistical details see Table 3.

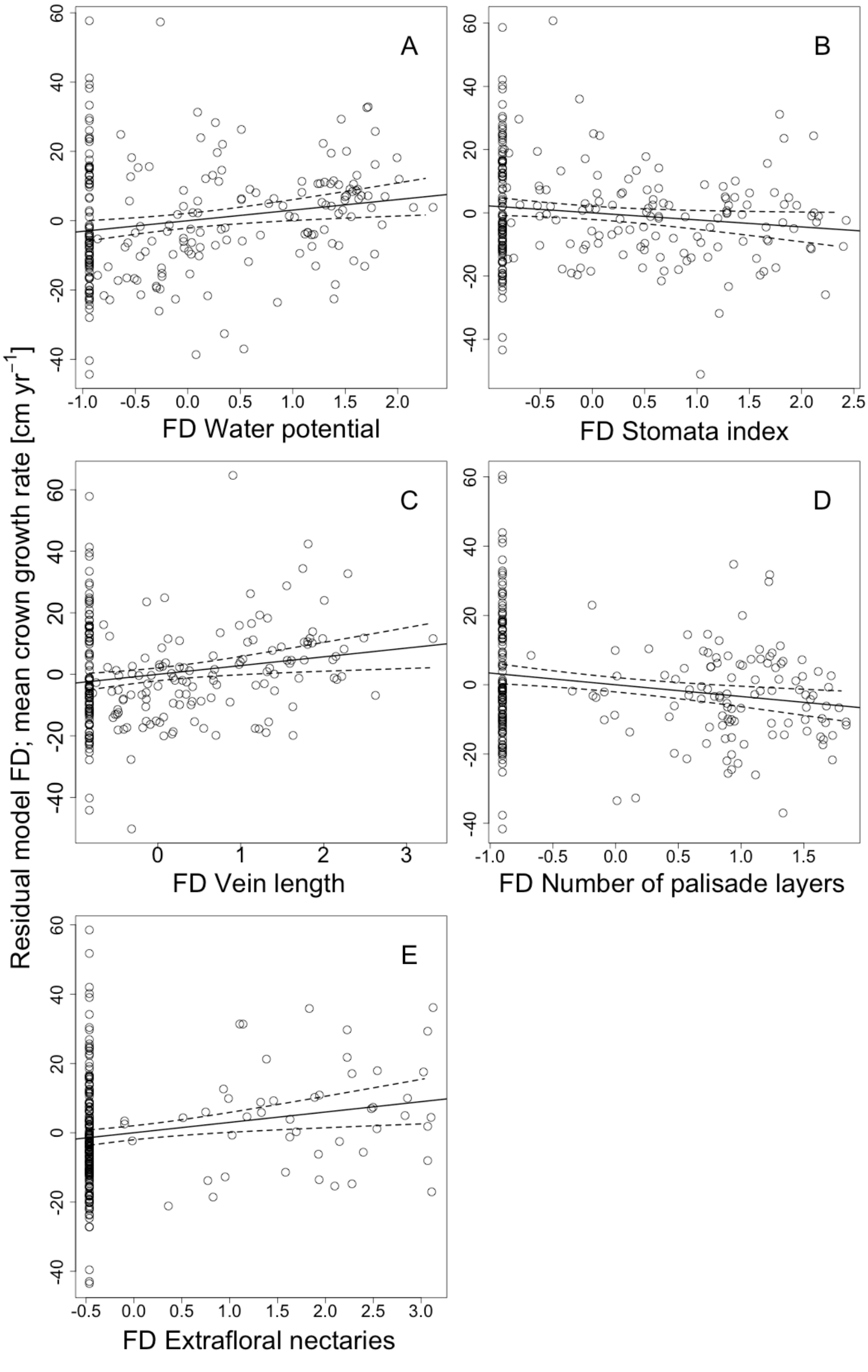

Figure 3

Mean annual crown width growth rate as predicted by the FD multi-predictor model. The residuals from all other terms in the model are plotted against A) water potential, B) stomata index, C) leaf vein length, D) number of palisade parenchyma layers and E). presence of extrafloral nectaries. Every dot represents one plot. All predictor variables are scaled by mean and standard deviation, thus the slope of the regression shows the direction and magnitude of impact on CW growth rates. The panels have been arranged in the sequence of decreasing order of effect sizes. For statistical details see Table 3.

In contrast, numerous variables of CWM and FD showed significant effects, also evident in the inclusion of many CWM and FD predictors in the minimal multiple regression models (Table 3). In total, 25 and 15 out of 41 variables produced significant single predictor models for CMW and FD, respectively (Table 2). The best single CWM predictor for CW growth rates was number of palisade parenchyma layers (PALSTR, r^2=0.24), while the best FD predictor was the presence of extrafloral nectaries (EXTRAFLORAL, r^2=0.10), a trait only encountered in four of the 23 species (i.e. *Diospyros japonica, Melia azedarach, Triadica cochinchinensis* and *T. sebifera*, Supplementary Information Table S2). Many significant CWM predictors were typical traits of the LES, such as specific leaf area (SLA), leaf nitrogen, potassium and magnesium content (LNC, K, MG) and the leaf carbon to nitrogen ratio (CN). However, except for magnesium these variables had lower estimates compared to morphological and anatomical variables such as leaf toughness, leaf dry matter content (LDMC), leaf thickness, presence of a subepidermis, number of palisade parenchyma layers and presence of a column of sclerenchyma cells through the leaf (Table 2). In the minimal multi-predictor model (Table 3), some of these variables such as water potential (WPOT), stomata size (STOMSIZE) and wood density (WOODDENS) had positive effects on crown width growth rates, while leaf toughness (LEAFT) and leaf magnesium content (MG) had negative effects (Fig. 2).

Significant FD variables were essentially a subset of the significant CWM variables, except for wood density, leaf area (LA), leaf calcium content (CA), the ratio of palisade to mesophyll layer thickness (LOG10RATIO) and presence of extrafloral nectaries (EXTRAFLORAL), for which only FD but not CWM had a significant effect on CW growth rate. In addition, there were two variables, hydraulic conductance (K_S) and vein length (VEINLENGTH), for which FD had a higher explanatory power than CWM.

Interestingly, the minimal multi-predictor model for the FD-growth relationship included variables with both positive (WPOT, VEINLENGTH, EXTRAFLORAL) and negative effect sizes, such as stomata index (STOIND) and number of palisade layers (PALSTR, Fig. 3).

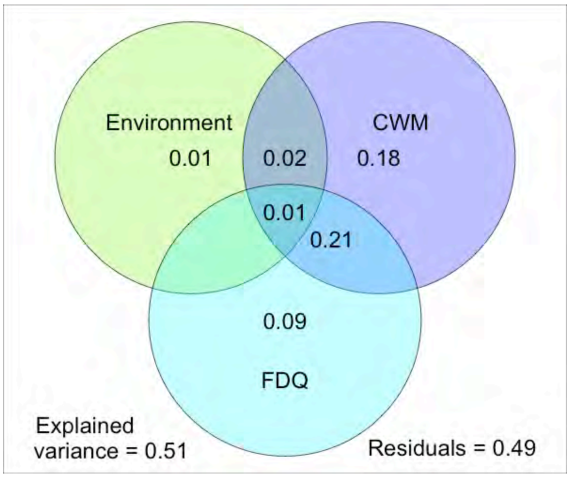

Figure 4

Plot of the partitioned variance explained by the three different variable complexes, green = environment, purple = CWM, light blue = FD, values below 0.01 not shown. For statistical details see Table 3.

In combination, the three best multi-predictor models of ecoscape, CWM and FD explained 51% of variation of plot-level crown width growth rates (Fig. 4). CWM explained most variation, both in terms of exclusive impact on tree growth that was not captured by ecoscape or FD and in terms of shared variance with ecoscape and FD. For example, out of the 31% variance in growth explained by FD, two thirds (i.e. 22%) were also shared by CWM predictors. There was almost no variation left that was exclusively explained by ecoscape (1%).

Table 2

Impact of CWM and FD on crown growth. The effect of community weighted means (CWM) and functional diversity (FD) of single traits for crown width (CW) growth rate, assessed as plot mean values between 2011 and 2010. All variables are scaled by mean and standard deviation, thus the estimates show the direction and magnitude of impact on CW growth rates.

Abbreviation	Predictor	Source		CWM			FD	
			Estimate	r^2	p	Estimate	r^2	p
PSI50	loss of 50% initial flowrate	scholander pressure bomb	0.84	0.00	0.50	-0.40	0.00	0.75
K_S	maximum flowrate	lab measurements	2.46	0.02	0.05	5.19	0.08	0.00
B	Parameter b (Sigmoid Regression)	scholander pressure bomb	-6.20	0.11	0.00	-3.44	0.03	0.01
CONMEAN	average stomatal conductance	steady state porometer	5.27	0.08	0.00	2.66	0.02	0.03
CONMAX	maximum stomatal conductance	steady state porometer	3.99	0.05	0.00	1.21	0.00	0.33
VPDMAX	vpd at CONMAX	steady state porometer	-0.82	0.44	0.51	-0.32	0.00	0.80
CONMAXFIT	relative fitted max. stomatal conductance	steady state porometer	2.32	3.57	0.06	1.62	0.01	0.19
CONMAXFITA	absolute fitted max. stomatal conductance	steady state porometer	5.00	0.07	0.00	2.73	0.02	0.03
VPDMAXFIT	vpd at CONMAXFIT	steady state porometer	1.73	0.01	0.16	0.25	0.00	0.84
VPDPOI	vpd at point of inflection of fitted stomatal conductance	steady state porometer	0.50	0.00	0.69	0.29	0.00	0.81
WOODDENS	wood density	lab measurements	0.42	0.00	0.74	4.42	0.06	0.00
WPOT	water potential	scholander pressure bomb	7.74	0.17	0.00	4.68	0.06	0.00
LA	leaf area	lab measurements	1.10	0.00	0.38	4.55	0.06	0.00
LDMC	leaf dry matter content	lab measurements	-7.24	0.15	0.00	2.09	0.01	0.09
SLA	specific leaf area	lab measurements	4.05	0.05	0.00	0.66	0.00	0.59
LEAFT	leaf toughness	leaf toughness device	-7.65	0.17	0.00	-0.40	0.00	0.75
STOMDENS	stomata density	microscope	-3.40	0.03	0.01	1.80	0.01	0.15
STOIND	stomata index	microscope	-2.95	0.02	0.02	1.82	0.01	0.14
LNC	leaf nitrogen content	CN analyzer	2.88	0.02	0.02	-1.99	0.01	0.11
LCC	leaf carbon content	CN analyzer	0.62	0.00	0.62	0.73	0.00	0.56
CN	leaf carbon nitrogen ratio	CN analyzer	-3.48	0.03	0.00	-0.80	0.00	0.52
CA	leaf calcium content	atom absorption spectrometer	-1.55	0.01	0.21	3.35	0.03	0.01

K	leaf potassium content	atom absorption spectrometer	4.30	0.05	0.00	-0.19	0.00	0.88
MG	leaf magnesium content	atom absorption spectrometer	6.78	0.13	0.00	1.27	0.00	0.31
DIAMVEIN1	diameter veins 1st order	microscope	2.41	0.02	0.05	0.79	0.00	0.53
DIAMVEIN2	diameter veins 2nd order	microscope	3.88	0.04	0.00	2.75	0.02	0.03
UPPEREPI	upper epidermis thickness	microscope	-1.28	0.00	0.30	0.78	0.00	0.53
PALIS	palisade parenchyma thickness	microscope	-3.73	0.04	0.00	2.70	0.02	0.03
SPONGY	spongy parenchyma thickness	microscope	-3.88	0.04	0.00	1.42	0.01	0.25
LOG10RATIO	log ratio of the palisade to spongy parenchyma thickness	microscope	0.28	0.00	0.82	2.81	0.02	0.02
LEAFTHICK	leaf thickness	microscope	-5.33	0.08	0.00	1.57	0.01	0.21
SUBEPID	presence of a subepidermis	microscope	-5.32	0.08	0.00	-3.96	0.04	0.00
EPICELLSIZ	ratio of the cell size of upper and lower epidermis	microscope	4.58	0.06	0.00	-2.37	0.02	0.05
PALSTR	number of palisade parenchyma layers	microscope	-9.10	0.24	0.00	-1.01	0.00	0.41
EXCRET	presence of excretory glands	electron microscope	-0.11	0.00	0.93	0.05	0.00	0.97
DENSINTCEL	density of spongy parenchyma	microscope	1.33	0.01	0.28	2.35	0.02	0.06
COLSCLER	presence of a column of sclerenchyma cells through the leaf	microscope	-5.30	0.08	0.00	-3.93	0.04	0.00
PAPILL	presence of papillae	electron microscope	-3.06	0.03	0.01	0.36	0.00	0.77
EXTRAFLORAL	presence of extrafloral nectaries	observation	1.85	0.01	0.13	5.96	0.10	0.00

Table 3

Multi-predictor model coefficients for ecoscape, CWM and FD. Results of the minimum multi-predictor models for ecoscape, community weighted means (CWM) and functional diversity (FD) and the overall model combining these three multipredictor models. All variables are scaled by mean and standard deviation, thus the estimates show the direction and magnitude of impact on CW growth rates.

Model	r^2	Significant predictors	Abbreviation	Estimate	p
ecoscape	0.04	altitude +	ALT	-0.08	0.0129
		aspect (east)	EAST	-6.03	0.0125
CWM	0.44	leaf toughness +	LEAFT	-14.5	<0.001
		leaf magnesium content +	MG	-11.1	<0.001
		stomata size +	STOMSIZE	7.2	<0.001
		wood density +	WOODDENS	2.9	0.0103
		water potential	WPOT	13.0	<0.001
FD	0.31	extrafloral nectaries +	EXTRAFLORAL	3.93	0.0011
		number of palisade layers +	PALSTR	-8.41	<0.001
		stomata index +	STOIND	-11.46	<0.001
		veinlength +	VEINLENGTH	9.27	<0.001
		water potential	WPOT	12.14	<0.001
combined	0.51	altitude + aspect (east) + leaf toughness + leaf magnesium content + stomata size + wood density + water potential + extrafloral nectaries + number of palisade layers + stomata index + veinlength + water potential			

Discussion

Using the combined information of selected ecoscape variables, community weighted means and functional diversity, we could account for 51% variability of crown width growth rates. Against expectation, no single variable did explain crown diameter growth to a sufficient degree. The largest proportion of variance explained by a single variable was 24% (number of palisade parenchyma layers). Nevertheless, our first hypothesis was confirmed by identifying some single key variables for tree growth, of which however, the most powerful ones were all based on community mean trait values. In contrast, ecoscape variables turned out to be weak predictors for crown growth and explained less than 4%, while functional diversity explained up to 31% and community weighted means up to 42% of crown growth. Thus, we have to reject our second hypothesis of a dominant impact of ecoscape on tree growth at this early stage of the experiment.

The low importance of ecoscape was unexpected, given the high topographic heterogeneity and large size of the experiment. Altitude had a negative impact on tree growth, which was opposite to the findings on initial tree survival at the same site (Yang *et al.* 2013). Similarly, altitude affected productivity in the Sardinilla experiment (Healy *et al.* 2008). In Sardinilla, the single environmental variable with the highest impact on productivity was slope inclination, followed by water drainage quality. Total variance of productivity explained by ecoscape in Sardinilla was 35%. However, the Sardinilla plots only differed 8 m in altitude, while our site A varied by 170 m, with a total experiment size of 26.7 ha vs. 8 ha in Sardinilla (Bruelheide *et al.* 2014). Interestingly, many ecoscape variables with reported effects in the literature did not have any significant impact on plot mean crown diameter growth in the BEF-China experiment, such as pH. Soil pH is well-known to limit nutrient availability (Lambers, Chapin & Pons 2008) and was found to limit tree growth in primeval forests in the Changbai Shan in Northeastern China (Yang, Hu & Bu 2009). Increasing soil pH, in addition to increasing elevation, showed negative effects on above ground biomass increment in tropical Andean forests (Unger, Homeier & Leuschner 2012).

The negative effect of higher elevation might be explained by a temperature gradient, with lower temperatures at higher elevation being in particular disadvantageous in winter and spring, but also by downslope growth locations being more sheltered from wind. However, we have no hint that elevation has indirect effects via differing soil conditions, as all these did not result in significant models. The fact that higher crown width growth rates were observed in plots on westward-facing slopes might result from the fact that on slopes with that compass direction morning dew lasted longer and vpd was lower in the morning, thus allowing the trees to have a prolonged period of gas exchange, and in consequence, higher rates of carbon assimilation, before stomata closure occurs at increasing vpd values.

With respect to community weighted means (CWM), we found traits of the leaf economics spectrum (LES) to affect tree growth, with a positive effect of specific leaf area (SLA) and leaf magnesium content (MG). Nonetheless, morphological and anatomical traits such as leaf toughness and thickness, number of palisade parenchyma layers as well as presence of a subepidermal layer had a higher explanatory power than typical LES traits. The number of palisade parenchyma layers had already been identified as a good proxy for maximum stomatal conductance (CONMAX) (Kröber, Heklau & Bruelheide 2014a), and thus increasing tree growth would have been expected with increasing number of palisade parenchyma layers. The best 5-predictor model for CWM variables comprised two traits of leaf morphology (leaf toughness and stomata size) and one of plant hydraulics (water potential), while only MG was included as a typical LES trait and wood density as a key trait of the wood economics spectrum (Freschet *et al.* 2010; Baraloto *et al.* 2010; Martínez-Cabrera *et al.* 2011). This complex of morphological, anatomical and physiological traits supports the idea that integrating more and novel functional traits might increase the predictability of ecosystem functioning, and thus, also the reliability of products relying on these relationships, such as dynamic vegetation models (Scheiter, Langan & Higgins 2013). As the different variables in the multi-predictor model explained additional variation in crown width growth rates, they were not fully collinear to each other, showing that the leaf and wood economics spectrum did not perfectly match (Baraloto *et al.* 2010). Interestingly, both wood density and magnesium content were related to tree growth, as crown width growth rates increased with decreasing Mg contents and increasing wood density. The comparably low explanatory power of LES traits on tree growth in the single predictor models and their contrasting role in the multi-predictor model challenges the assumption of a universal positive growth-LES effect on tree growth. Trees might behave differently than herbaceous plants, where strong positive growth-LES relationships have been described (Grime & Hunt 1975; Poorter & van der Werf 1998). The low predictive power for tree growth has been recently demonstrated in a meta-analysis that estimated size-standardized relative growth rates for 278 tree species from 27 sites around the world and found no significant relationship to SLA and wood density (Paine *et al.* 2014). Another variable in the best multi-predictor model was stomata size, which enables species to attain maximum stomatal conductance at low vpd values (Kröber & Bruelheide 2014). Furthermore, tree crown growth was positively related to xylem water potential measured in the field, showing that species grew the better, the more they were able to keep their water status at moderate levels. This was also reflected in leaf toughness, which had the highest explanatory power in the multi-predictor model and can be interpreted as a key defence trait against herbivores (Kursar & Coley 2003). In our model, species grew the better, the less they invested in physical defence.

We found several FD traits to explain variance in crown growth additional to the CWM effects. The significance of FD at this early stage shows that effects of complementarity in resource use already have emerged. In principle, the traits with significant FD effects on tree growth can be thought to operate through spatial complementarity, such as wood density and leaf area. The joint occurrence of species with low- and high-investment in wood allows a community to quickly build up tall canopies with fast-growing species and at the same time to form a second layer of more slowly growing, durable-wood species. In Iberian forests, canopy trees with denser wood had lower maximum height and wider crown widths (Poorter *et al.* 2012b). Since wood density and physiological strategies of trees are closely related (Santiago *et al.* 2004), a wide range of wood density in a plot might increase the total amount of resources captured in this plot. Leaf area plays a central role in leaf trait relationships because the mass-normalized traits in the leaf economics spectrum are proportional to leaf area (Osnas *et al.* 2013). Thus, leaf area might represent a sum variable that captures variance of various other variables in the LES, but also of morphological traits such as palisade parenchyma thickness (PALIS) and the palisade to mesophyll ratio (LOG10RATIO). Particularly, in young plantations, large-leaved species can quickly increase the stand's leaf area index, while species with small leaves follow a more invariable investment strategy. Studies on crown filling in the BEF-China experiment are still on-going, but results from natural forests revealed that diverse plots have a higher crown overlap than species-poor plots (Lang *et al.* 2012b). Species with small leaves also tend to be evergreen (Kröber *et al.* 2014a), and thus might also be complementary to large-leaved deciduous species in time. Traits that potentially contribute to temporal complementarity were all related to plant water relations, such as specific hydraulic conductance of the xylem (K_S), xylem water potential, leaf vein -length and -diameter and leaf stomatal conductance. FD in these water flux-related traits can increase growth rates if some species display high carbon assimilation rates under optimal humid conditions, while others continue with carbon sequestration in dry spells, which frequently occur in summer at the experimental site (Zhou *et al.* 2011; 2013). Interestingly, FD of some traits also had negative effects on crown width growth rates, such as slope of the xylem vulnerability curve (B), the presence of a subepidermis and the presence of columns of sclerenchyma cells in the leaf. In principle, negative estimates of FD can only be interpreted as corroborating the CWM signal of these traits, which was also negative in all these cases. These traits promote growth when their values are near to the possible minimum, where CWM and FD show a strong covariation (Dias *et al.* 2013). In addition, some traits might act through facilitation, i.e. enhancing the growth of other species' individuals through their presence in a certain species. Such a trait probably was the presence of extrafloral nectaries, which was the FD trait in the single predictor models with the highest impact on crown width growth. Interestingly, the presence of one of the four species with such nectaries in a plot increased overall plot mean crown growth rates. Extrafloral nectaries have previously been shown to have large effects on plant performance through ant-plant mutualism as ants attracted by extrafloral nectaries have been found to reduce infestation levels of herbivores (Oliveira 1997). However, the presence of extrafloral nectaries must not be beneficial for the target plant itself (Kersch-Becker, Buss & Fonseca 2013; Pereira & Trigo 2013), but might have positive effects at the community level (Koptur 1992). The multi-predictor model for the FD-growth relationship included presence of extrafloral nectaries as a variable representing facilitation and variables with positive and negative slope pointing to temporal complementarity, while most variables pointing at spatial complementarity did not enter the model.

Combining ecoscape, CWM and FD in the overall model confirms that ecoscape does only contribute a surprisingly small fraction to explain tree growth. This forms a contrast to the analyses at the single tree level carried out on the same site and using the same crown width growth data used by us (Li *et al.* 2014). This indicates that individual trees respond much stronger to topography and soil as does plot mean growth. However, the overall contribution of soil variables such as C and N content on individual tree growth was also low at the single tree level (Ying Li, unpublished data). A further reason for the discrepancy to the single tree-level data was that species were considered a random factor, thus assigning all trait differences between species to random variation (Li *et al.* 2014), while we accounted for these differences in CWM functional traits. Similarly, also FD was only partially represented in the single-tree models of Li *et al.* (2014) by including Shannon diversity of the local neighbourhood, which however was found not to contribute to explaining crown growth. Thus, FD might have captured more unexplained variation than Shannon diversity. In addition, FD effects might only play out if scales larger than the immediate neighbourhood are involved, i.e. whole plots with 400 tree individuals each.

We also have to consider that the ecoscape variables included in our study did not sufficiently reflect resource supply. On the one hand, many variables showed only a very low amount of variation among plots, such as soil pH. On the other hand, soil variables such as nitrogen content and carbon to nitrogen ratio might reflect the pre-planting conditions of the conifer plantations of *Pinus massoniana* and *Cunninghamia lanceolata*, which might have levelled out differences among plots. Finally, not all key ecoscape variables have been included, such as e.g. phosphorus supply.

Although our overall model explained 51% of variation in plot-level crown growth, a substantial amount of variation in the growth-trait relationship remained unexplained, which is typical of tree growth studies (Steege 2003; Poorter *et al.* 2008; Martínez-Vilalta *et al.* 2010; Wright *et al.* 2010; Rüger *et al.* 2012). Possible causes for unexplained variation might be negative biotic interactions such as pathogens and herbivores that reduced potential growth rates. Thus, field studies probably arrive at different conclusions than greenhouse trials, which assess potential growth rates under exclusion of biotic interactions and show strong relationships to functional leaf traits (Böhnke & Bruelheide 2013). Moreover, juvenile trees may allocate resources to the expansion of their root system for several years without substantial aboveground growth, especially in dry or nutrient-poor forests (Poorter *et al.* 2012a).

Conclusion

We have to be aware of the early stage of our experiment and that the system is neither stable nor in equilibrium, as the trees continue to grow and tree-tree interactions become increasingly intense. At present, forests growth is still dominated by CWM effects, but an increasing impact of FD at the expense of CWM effects can be expected. In contrast, the role of the ecoscape is unforeseen. A distinction can be made between ecoscape variables that are temporally invariable (such as slope, aspect, elevation) or dynamic (such as microclimate, content of soil organic matter and nutrients, soil reaction, etc.). On the one hand, invariable abiotic site conditions are not important at present. On the other hand, the dynamic ecoscape variables will be increasingly affected by tree growth. Thus, we expect that tree growth feeds back on this aspect of the ecoscape. In particular with respect to biodiversity, the dynamic ecoscape can have the form of a positive feed-back loop, whereby a higher variation of organisms that depend on higher tree richness modify the abiotic environment to their own favour. Therefore, it might be that diversity creates conditions that are amenable to more diversity. In this respect, the process might be similar to niche construction models for of single species (Odling-Smee, Laland & Feldman 2003). If biodiversity acts through modifying the ecoscape, an increase in importance of the dynamic ecoscape components can be expected. Thus, following the experiment over the next years will be very exciting!

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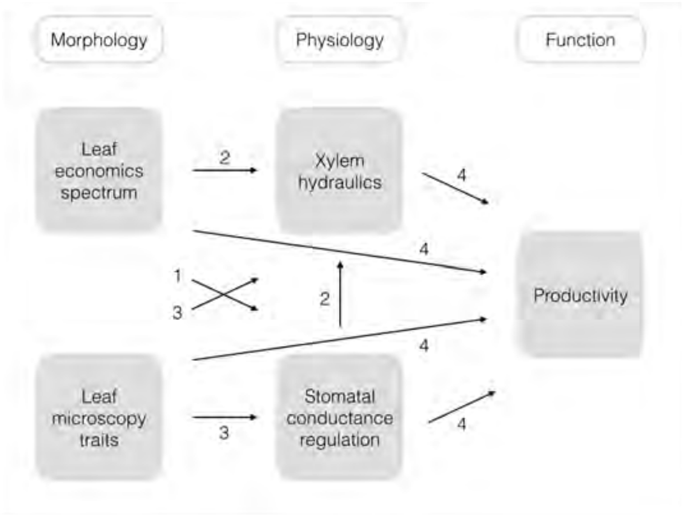
Chapter 6

6.1 Discussion

In a nutshell, the four research papers that make up this thesis introduced new parameters for stomatal control, increased the knowledge on xylem vulnerability and revealed new morphological proxies for physiological leaf traits. In addition, it successfully employed a framework to predict tree growth from plant functional traits at the plot level.

The new findings resulting from this thesis are added to the conceptual view of Figure 1 in the Introduction forming Figure 2.



1 Some but not all parameters of stomatal control were affected by the LES.

2 Xylem hydraulics are linked to the LES but not to stomatal control.

3 Leaf microscopy traits predict leaf physiology, however leaf habit had the strongest impact.

4 CWM and FD of plant functional traits have much higher influence on productivity than environment.

Figure 2

Recapitulation of the conceptual framework in the Introduction. The main results of the four papers are added to specify the hypothesized relationships.

6.1.1 Stomatal conductance regulation

The method of incorporating several daily courses of stomatal conductance (g_S) measurements into one analysis by plotting g_S versus vapour pressure deficit (vpd) has proved to be a feasible approach. The systematic setting of the experiment and a standardized measurement protocol allowed to control for a large amount of confounding influences on g_S.

Referring to the first hypothesis proposed in section 1.5.2 of the Introduction, this thesis could partly confirm the assumption that parameters of g_S are linked to traits described by the leaf economics spectrum (LES) (Wright et al. 2004). Mean g_S increased with leaf nitrogen concentration and decreased with the leaf nitrogen-to-carbon ratio whereas vapour pressure deficit (vpd) at the maximum modelled stomatal conductance decreased with SLA. However, maximum g_S and vpd at maximum g_S were not related to any leaf traits.

Interestingly, traits not represented in the LES were significantly linked to parameters of g_S, too. Specifically stomata size decreased with vpd at the maximum modelled conductance, whereas stomata density decreased with the vpd at the point of inflection of the g_S – vpd curve and vein density increased with the maximum of modelled g_S. Mechanistically it should be expected that stomatal traits are related to parameters of stomatal control; however, different authors report different directions of impact. Most publications describe a positive link between maximum g_S and stomata size and stomata density (Hetherington and Woodward 2003; Juhrbandt et al. 2004; Franks and Casson 2014). Conversely, recent research suggests a negative relationship of maximum g_S to stomata size or stomata density (Drake et al. 2013; Raven 2014). In general, larger stomata size trades off with lower stomata densities, which in consequence leads to delayed increase in g_S under non-optimal conditions (Aasamaa et al. 2001; Lawson et al. 2010). All these studies outline the critical role of the temporal dimension (Drake et al. 2013). The complexity of the regulatory influences at different points in time and their scale-dependency may explain the inconclusive outcome of studies that attempt to assess stomatal regulation from different perspectives (Franks et al. 2009; Lawson and Blatt 2014).

Against expectation, there was no difference in parameters of g_S between leaf habits, although the results from the principal component analysis indicated divergent strategies in g_S regulation. The reason behind might be that in the BEF-China experiment each species set in both groups of leaf habit type used a wide range of physiological strategies that were largely overlapping with regard to g_S regulation. This assumption is supported by the fact that the total range of maximum g_S values across all species was the tenfold of the numerical difference between the mean of maximum g_S values within each type of leaf habit, illustrating the clear overlap in maximum g_S values.

6.1.2 Xylem hydraulics

Specific xylem hydraulic conductivity and cavitation sensitivity were positively related to leaf nitrogen concentration and leaf area and, in turn, negatively related to the leaf carbon-to-nitrogen ratio, all traits representing the LES. Therefore, leaf area, leaf nitrogen concentration and leaf carbon-to-nitrogen ratio may serve as a proxy for xylem hydraulics in screening studies across a large set of species. The next step should now be to validate the encountered relationships between leaf traits and physiology over large geographic scales by using worldwide trait databases. In a comparable approach of relating leaf physiology to leaf traits globally, the first study using data from the TRY database (Kattge et al. 2011) found the maximum rate of carboxylation to be positively related to leaf nitrogen content (Kattge et al. 2011).

As expected, species with evergreen leaf habit displayed lower values of specific xylem hydraulic conductivity and lower Ψ_{50} values compared to species of deciduous leaf habit. These results are in contrast to those obtained by many preceding studies that did not find any evidence of lower cavitation sensitivities in evergreen species, although these studies had postulated such a relationship (Nardini and Salleo 2000; Brodribb et al. 2003; Markesteijn et al. 2011). Similarly, in a global meta-analysis, Maherali et al. (2004) could not find any differences in cavitation sensitivities between leaf habit types. One reason for this outcome, which is in contrast to the results of this study, was that the species set was not balanced with regard to leaf habit and climate type since Maherali et al. (2004) compared evergreen species from the tropics with temperate summergreen species. Maherali et al. (2004) concluded that future studies should compare tree species differing in leaf habit but originating from the same climate type. The results of this thesis fulfil this goal.

In contrast to traits captured by the LES, no parameter of g_S was linked to specific xylem hydraulic conductivity or to cavitation sensitivity. Referring back to the second hypothesis, stating that xylem hydraulics is related to traits described by the LES, on the one hand, and to parameters of g_S, on the other hand, the results confirmed only the former hypothesis (see Figure 2). In consequence, the initial assumption that high sensitivity to cavitation may be related to fast and precise g_S regulation had to be rejected.

This outcome is unexpected since mechanistically trees have to down-regulate g_S in order to prevent xylem cavitation (Jones and Sutherland 1991). In addition, some authors report on possible feedback-mechanisms of xylem hydraulics that constrain maximum g_S. These mechanisms are reflected in the way stomata sense and then prevent xylem cavitation under extreme conditions and thus under high cavitation risk (Nardini and Salleo 2000).

Regarding the specific set of tree species in the BEF-China experiment, it might be assumed that g_S regulation and xylem hydraulics have evolved under selection pressures acting at different time scales: g_S regulation being mainly affected by diurnal characteristics (Vialet-Chabrand et al. 2013) whereas xylem hydraulics being mainly affected by seasonal droughts (Markesteijn et al. 2011; Choat et al. 2012; Blackman et al. 2014).

Shedding more light on the diurnal characteristics of the link between xylem hydraulics and g_S regulation may be achieved by considering specific hydraulic conductance, i.e. hydraulic conductance to sustained leaf area (Bond and Kavanagh 1999; Sack et al. 2002; Pivovaroff et al. 2014). Additional sap flow measurements (Cermák et al. 2004; Clausnitzer et al. 2011; Reyes Acosta and Lubczynski 2014) and acoustic detection of xylem cavitation events (Jackson and Grace 1996; Johnson et al. 2012; Ponomarenko et al. 2014) may detect hidden features in seasonal characteristics of the link between xylem hydraulics and g_S regulation, since these techniques enable data collection over an extended period of time. The idea to incorporate further trait complexes into a general plant functional trait framework has resulted in a world-wide “fast-slow plant economics spectrum” (Reich 2014). Peter Reich suggests expanding the concept of economic traits such as the LES into two additional dimensions: first - from leaves over to stems and roots, and second - from nutrient use over to carbon and water use. The present thesis has undergone the first steps within this framework by incorporating water relation traits such as g_S regulation and by incorporating stem traits such as specific xylem hydraulic conductivity and cavitation sensitivity.

6.1.3 Leaf microscopy traits

The cell density of the spongy parenchyma and the ratio of palisade to spongy mesophyll thickness were the most important microscopy leaf traits that predicted physiological parameters of xylem hydraulics and g_S regulation. Accordingly, the thesis fully confirmed the third hypothesis that microscopy leaf traits are connected to g_S regulation and xylem hydraulics (see Figure 2).

Leaf thickness and all other structurally thickness-related traits differed between leaf habits. Strong differences between evergreen and deciduous leaf habits may be due to the longevity and the nutrient use strategy: leaf longevity is believed to be shorter when initial resource investment per leaf is low and the decrease in the initial net photosynthesis rate is high (Kikuzawa 1991). The fact that even leaf microscopy traits were strongly differentiated by leaf habit underlines the dominant influence of leaf duration strategy on several aspects of leaf physiology (Larcher 2003; Lambers et al. 2008). However, literature analysing physiological functionality of internal leaf structure in cross-species studies is scarce, and not all relationships have been understood so far (Grossoni et al. 1998; Oguchi et al. 2005; Niinemets et al. 2006). Accordingly, not for all microscopy leaf traits and physiological parameters a simple mechanistic explanation could have been found. For example, the link between fitted absolute maximum g_S and the structures of the epicuticular waxes on the adaxial side of the leaves was only weak.

Since the leaf layer-related parameters predicted leaf physiology well, further research on these structures may be rewarding. Nobel (1980) introduced the ratio of mesophyll surface area per unit leaf area (A_{MES}/A) as an informative anatomical parameter and proposed to investigate the changes in water use efficiency which are expected to vary with this ratio. The author discusses the challenges and expected relationships between internal leaf anatomy and leaf physiology and concludes that trade-offs determine the final leaf structure. These trade-offs will include maximizing internal mesophyll surface in order to optimize gas exchange per unit leaf area (Smith and Nobel 1977) versus maximizing cell density and layer number in order to optimize photosynthesis reaction output per unit leaf mass (Mediavilla et al. 2001). A further study with more focus on internal leaf anatomical details such as the parameter A_{MES}/A via an in-depth analysis of scanning electron microscope images like those produced for the third paper, is therefore suggested of this thesis.

In order to further investigate the link between leaf internal structure and water related leaf physiology (Warren and Adams 2006), cross-species studies should analyse specific details of the venation architecture such as the mean luminal area of the largest xylem vessels, the area of the midrib xylem cross section or the area of lateral ribs per unit length of leaf cross section (Aasamaa et al. 2005) and relate these to leaf hydraulic conductance (Flexas et al. 2013b) and leaf water potential (Nardini et al. 2012).

6.1.4 Biodiversity, traits & ecosystem functioning

Following the framework proposed by Diaz et al. (2007) to predict ecosystem functioning, trait values aggregated in community weighted mean (CWM) values explained most of the variation in tree growth of the forest plots; functional diversity (FD) scored medium impact whereas environment, in contrast to expectation, had only very limited impact. With 4% of explained variance in tree crown diameter increment, environment had only minor influence on tree growth. In turn, CWM and FD explained 42% and 31% respectively, with a share of 21% of jointly explained variance.

With respect to the fourth hypothesis, the first part of the hypothesis stating that single variables out of environment, CWM and FD explain tree growth was confirmed. However, the second part of the hypothesis claiming that environmental factors exert dominant influence compared to CWM and FD has to be rejected (See Figure 2).

At present, most of the current BEF research has been done on sets of known traits in grassland experiments (Zavaleta et al. 2010; Petersen et al. 2012; Roscher et al. 2014). This thesis could show that further traits describing g_S regulation, xylem hydraulics and leaf anatomy increase the statistical power in predicting ecosystem functioning from community features. So far, the impact of environment, of CWM and of FD have not been analysed in combination in tree BEF experiments (Healy et al. 2008; Hector et al. 2011). This thesis is a first attempt of closing this gap. The results point out plant functional traits of particular importance in the BEF research and, in general, underline the indispensable role of biodiversity for ecosystem functioning.

The CWM trait with the highest impact on tree growth in the BEF-China experiment was leaf toughness. The tenderer the leaves were, the higher was the mean crown increment growth per year and per plot. This phenomenon may be due to various reasons: for example, evergreen tree species tend to have tougher leaves and grow more slowly than deciduous tree species (Aerts 1995). However, independently from leaf habit, the more resources a plant invests in leaf toughness, the less resources are available for overall plant growth. A species that is able to grow fast without sacrificing too many resources on leaf toughness may increase the resource use efficiency of a community. According to the Production Ecology

Equation (Monteith and Moss 1977; Binkley et al. 2004; Richards et al. 2010) introduced in section 1.4.3

$$p = r * f * e$$

where *p* is biomass production, *r* is resource supply, *f* is fraction of resources captured and *e* is the efficiency of resource use (*p* & *r* being area normalized), this will lead to an increase in *e*.

A high value in FD may describe an increased ability of a plant community to capture resources. The presence of extrafloral nectaries and the number of palisade layers were the traits where FD had the highest predictive power for tree crown diameter increment on the experimental plots. Thus, combining species with and without extrafloral nectaries in a community might decrease the degree of herbivory. Similarly, combining species with different number of palisade layers might increase the amount of light captured per unit area in the community. In consequence, the total amount of captured resources will be higher, which in turn will result in an increased factor *f* in the Production Ecology Equation.

CWM and FD could explain 32% and 41% variation in tree crown growth. This is a hint that CWM and FD indeed contribute to a significant amount to *f* & *e* in the BEF-China experimental plots (Bruelheide et al. 2014). This is particularly true since further effects acting on *f* & *e* such as stem density, canopy layer structure and successional stage stayed constant in all the experimental plots in the BEF-China experiment.

The remaining factor *r* describes the environmental settings a community has to cope with. Eventually, biotic effects in the experiment will also lead to a variation in *r* in a way that plant communities will alter their own environment such as soil conditions or microclimate. The future development of the environmental conditions on the experimental plots of BEF-China will show whether the plant species assemblages are able to change their abiotic environment and how they do it.

High productivity in forests can be the result of optimum resource supply (Herbert and Fownes 1999; Unger et al. 2012). In addition, high productivity may result from communities containing either highly productive species or highly diverse species (Morin et al. 2011; Zhang et al. 2012). To detect the impact of the latter statistically, communities have to be compared that both vary in CWM and FD values (Binkley et al. 2004). However, a clear segregation of effects resulting from CWM versus effects resulting from FD should be done with care since CWM and FD are mathematically linked (Dias et al. 2013).

The abiotic environment has been thought to be a main driver of ecosystem functioning; however, recent research has shown that the impact of functional diversity may reach similar magnitude of impact compared to species-specific properties and environment (Tilman et al. 2012). The results of the fourth study imply that functional diversity has higher predictive power than abiotic environment, and it is expected that this relation will increase with time and eventually may even surpass effects of species-specific properties.

The regression framework (Díaz et al. 2007) applied in the fourth paper turned out to be a reliable method for predicting tree growth, even though it related trait values, environment and mean crown growth increment only by model selection algorithms. In contrast, relating these parameters through a mechanistic approach, for example, by choosing specific traits which are known to be related to specific ecosystem functions, may offer an alternative approach that may lead from correlative to causal relationships of biodiversity and ecosystem functioning (Grace et al. 2014).

6.1.5 Conclusion

Figure 2 sums up the results of the four manuscripts of this thesis. Paper 1 introduced a method to calculate new parameters of g_S regulation and revealed links between the LES and g_S regulation. Paper 2 extended the functional perspective from g_S regulation to xylem physiology and reported links between xylem hydraulics and the LES but not to g_S regulation. Paper 3 added several leaf internal structural traits to the analysis and showed relationships between these and g_S regulation and xylem hydraulics, respectively. And finally, Paper 4 merged all traits in CWM and FD plot values together with environmental parameters and successfully predicted tree crown increment.

Limitations of the results

One limitation of this work is that all trait measurements were done on juvenile tree individuals. Thus, the transfer of the findings to mature systems should be made with care keeping in mind that some trait relations may be altered in adult individuals. However, many traits are known not to change between juvenile and adult phases dramatically but remain conservative over the main life cycle episodes of a plant (Díaz et al. 2004; Perez-Harguindeguy et al. 2013).

All trait values have been analysed from an interspecific perspective, ignoring intraspecific trait variation which can reach considerable extent and can alter ecophysiological relationships (Ackerly et al. 2000; Chown et al. 2004; Albert et al. 2010b; Albert et al. 2010a; Bolnick et al. 2011; Baudis et al. 2014). In the future, the easy access, the established infrastructure and the unique planting design of the BEF-China experiment may allow for efficient investment of the high amounts of time and work necessary to generate the large sample size and to assess intraspecific trait variability of its 40 broadleaved experimental tree species.

Regarding the relationship between biodiversity and ecosystem functioning, only one property of one function – tree crown growth as an aspect of productivity – was analysed. It is expected that further ecosystem functions such as decomposition (Gessner et al. 2010), nutrient cycling (Nielsen et al. 2011) and water cycling (Mace et al. 2012) may be related to trait complexes different from those identified in this thesis as relevant for productivity. Many further ecosystem functions are worth being analysed from a functional perspective: the complete gas exchange process - by including CO_2 physiology (Tomas et al. 2013; Flexas et al. 2013a); photosynthetic capacity and regulation (Bauerle et al. 2012; Lawson and Blatt 2014; Flexas et al. 2014) and plant defence strategies against herbivores and pathogens (Mithöfer and Boland 2012; Scala et al. 2013).

Outlook on future research

In future BEF studies, specific emphasis should be put on the selection of traits for the assessment in order to optimize sampling efficiency and to avoid unwanted data redundancy. The results from this thesis emphasize the importance of the LES traits; but suggest to test in addition traits such as the palisade to spongy mesophyll thickness ratio, xylem cavitation sensitivity and vpd at the maximum modelled stomatal conductance. The encountered interrelationships between physiological trait complexes of g_S regulation and xylem hydraulics should be extended to further species from different biomes and ecosystems.

Additional traits such as the traits related to stomata regulation analysed in this thesis should be incorporated into future functional diversity assessments.

Building on the knowledge gained in this work, future studies that will focus on the analysis of ecosystem functioning will have an advantage: they will be able to choose relevant traits basing on the mechanistic relationships to the target function rather than simply use correlative regression frameworks with unwanted data redundancy. Concerning the study presented in the fourth publication, functional diversity can be expected to have an increasing impact on ecosystem productivity through time at the expense of influences from specific species and environmental effects in the BEF-China experiment. Tilman et al. (2012) reported results of eleven grassland experiments and showed that biodiversity loss entailed a decrease in productivity of a magnitude comparable to changes in relevant non-diversity related aspects. This thesis suggests that similar findings may be expected in the future from the BEF-China experiment and other forest BEF experiments. To confirm these expectations, the analyses conducted in the thesis should be repeated over time.

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Castanopsis eyrei (Champion ex Bentham) Tutcher

Castanopsis fargesii Franch.

Castanea henryi (Skan) Rehd. et Wils.

Castanopsis sclerophylla (Lindley & Paxton) Schottky

Celtis biondii Pamp.

Choerospondias axillaris (Roxb.) Burtt et Hill

Cinnamomum camphora (Linn.) Presl

Cyclobalanopsis glauca (Thunberg) Oersted

Cyclobalanopsis myrsinifolia (Blume) Oersted

Daphniphyllum oldhamii (Hemsl.) Rosenthal

Diospyros japonica Siebold & Zuccarini

Elaeocarpus chinensis (Gardn. et Chanp.) Hook. f. ex Benth.

Elaeocarpus glabripetalus Merr.

Elaeocarpus japonicus Sieb. et Zucc.

Idesia polycarpa Maxim.

Koelreuteria bipinnata Franch.

Liquidambar formosana Hance

Lithocarpus glaber (Thunb.) Nakai

Machilus grijsii Hance

Machilus leptophylla Hand.-Mazz.

Machilus thunbergii Sieb. et Zucc.

Manglietia fordiana (Oliver) HuY.W.Law

Melia azedarach Linn.

Meliosma flexuosa Blume

Nyssa sinensis Oliver

Phoebe bournei (Hemsl.) Yen C. Yang,

Quercus acutissima Carruthers

Quercus fabri Hance

Quercus phillyreoides A. Gray

Quercus serrata Murray

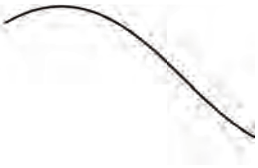
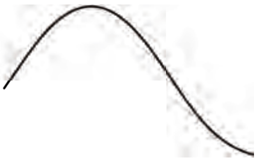
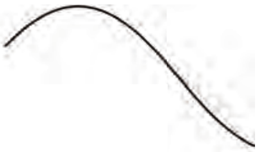
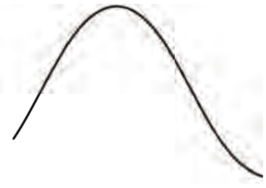
Rhus chinensis Mill.

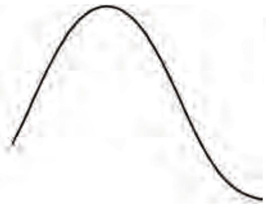
Sapindus saponaria Gaertn

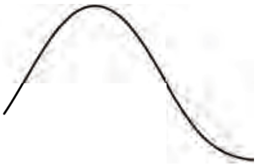
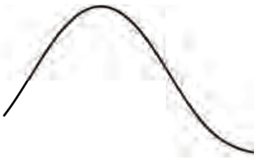
Triadica cochinchinensis Loureiro

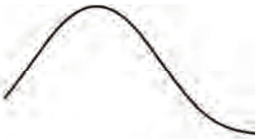
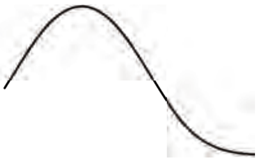
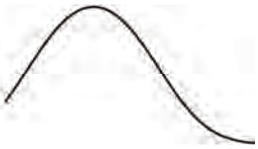
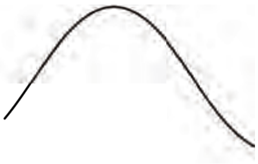
Triadica sebifera (L.) Small

Schima superba Gardn. et Champ.

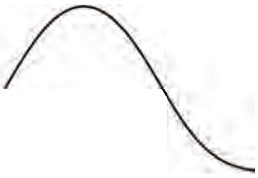


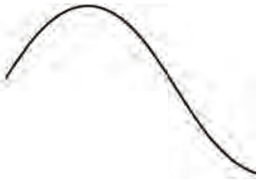


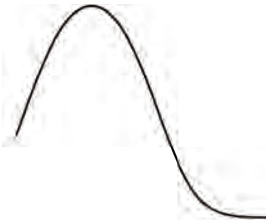
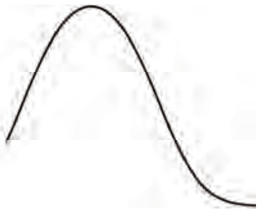






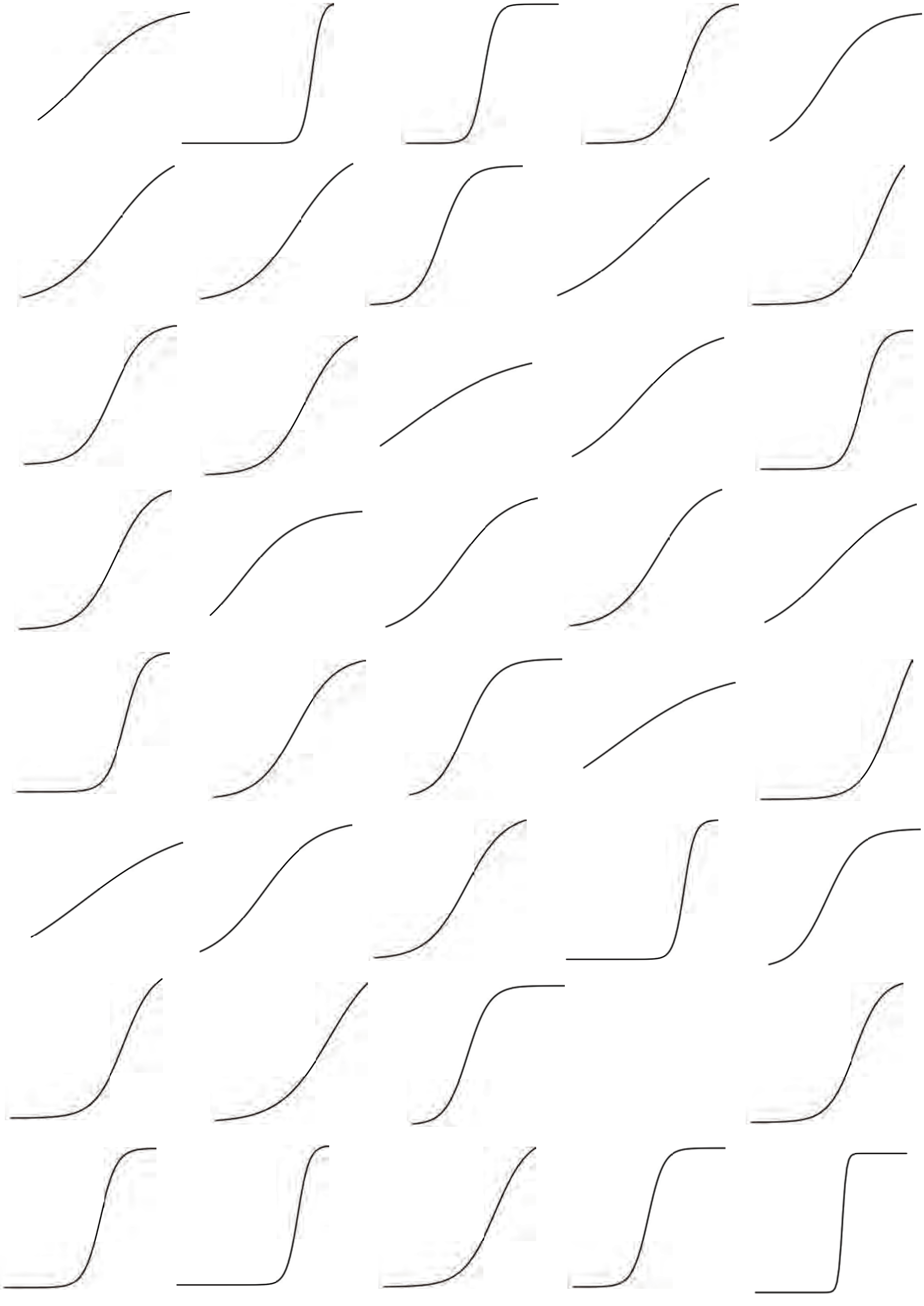


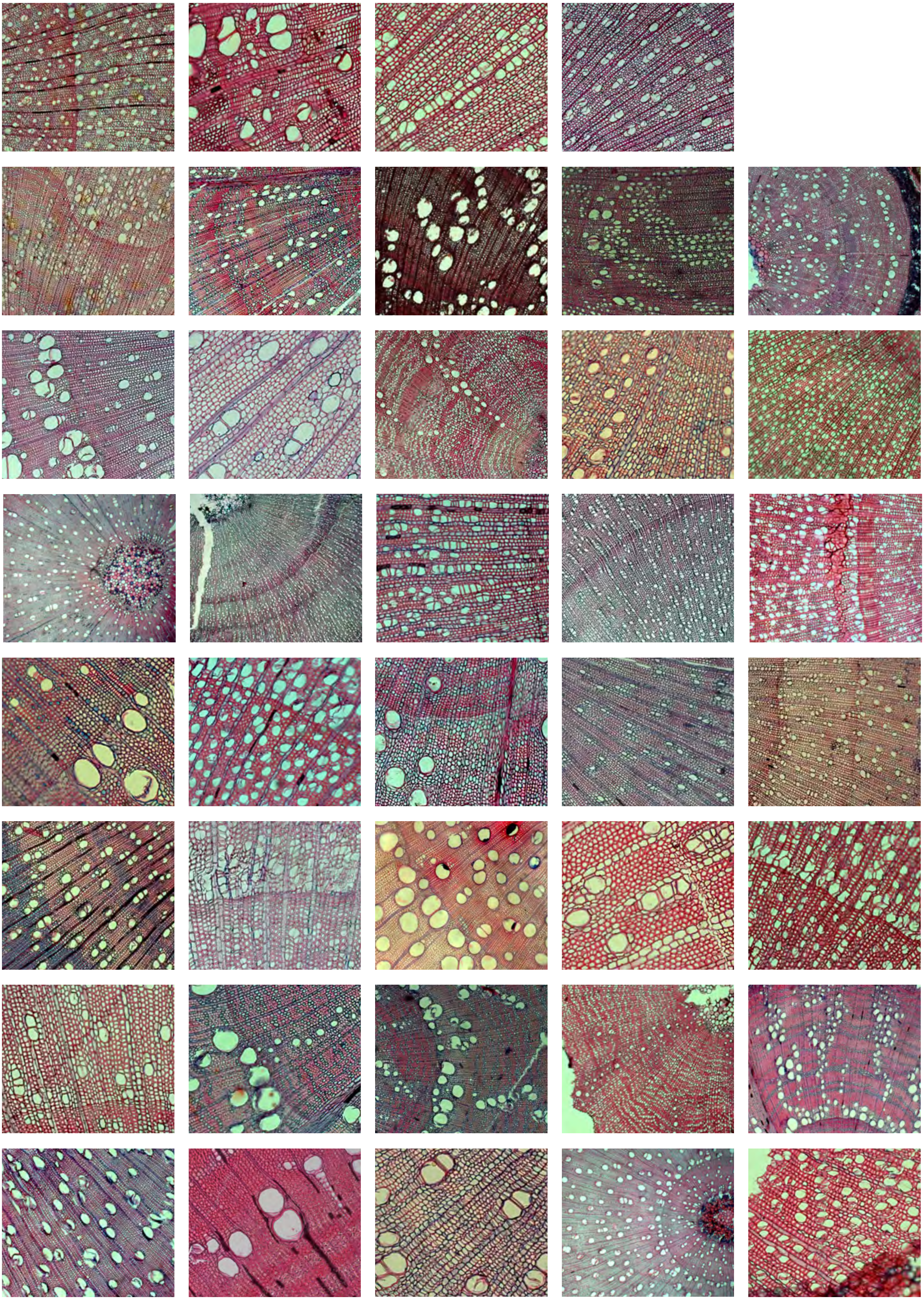


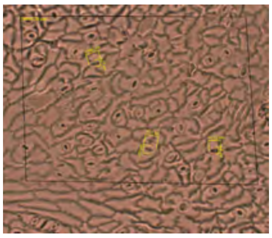
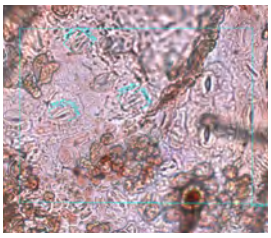
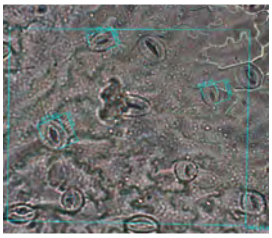
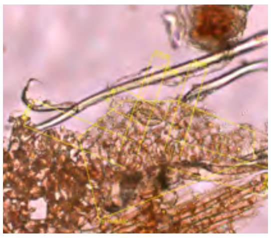
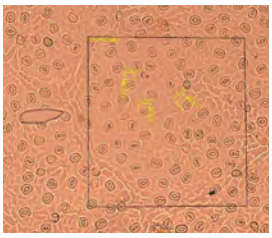
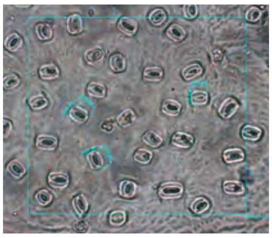
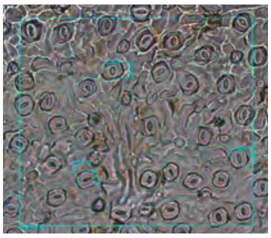
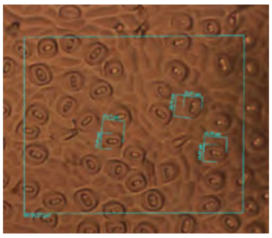
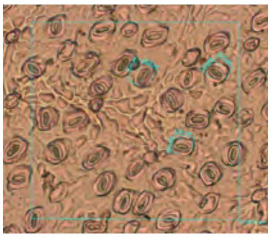
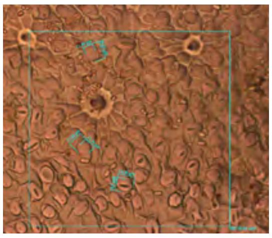
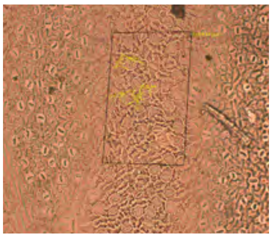
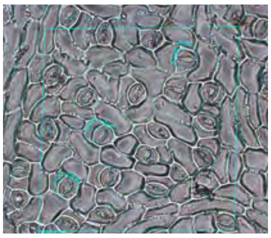
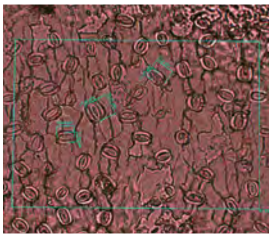
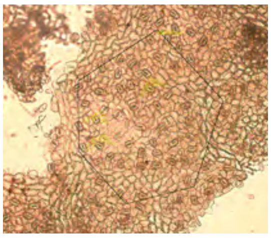
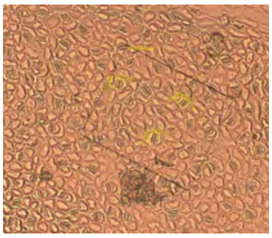
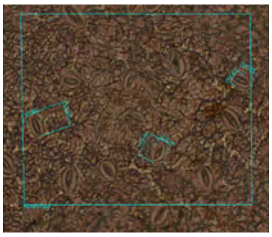
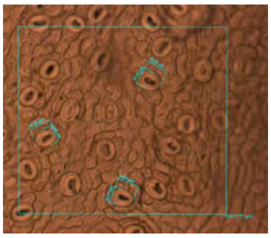
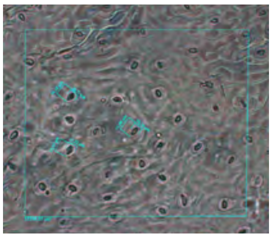
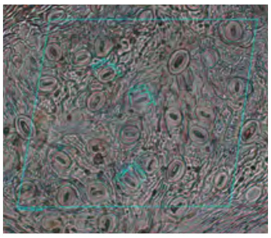
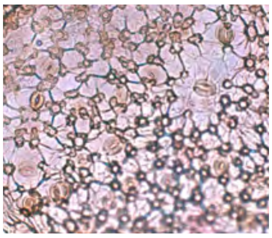
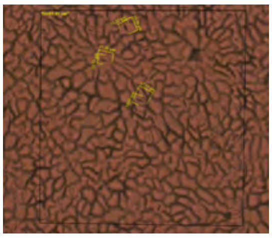
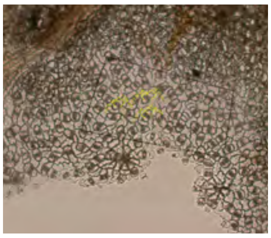
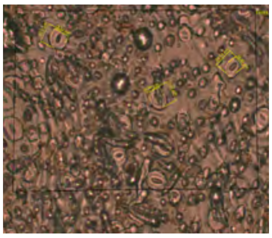
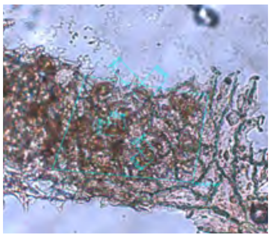
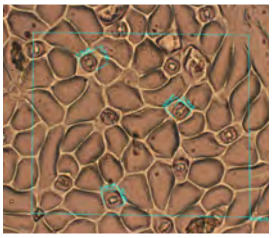
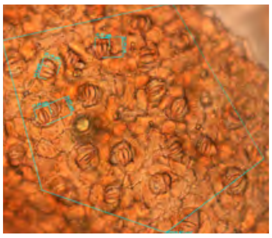
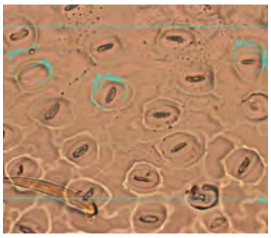



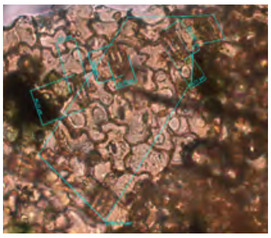
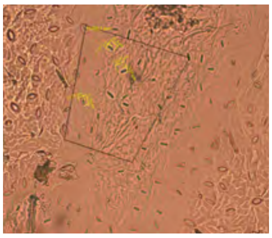
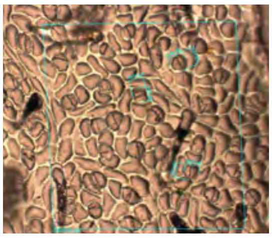
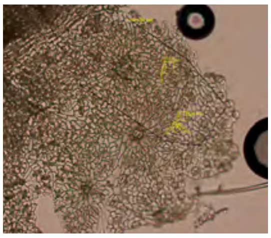
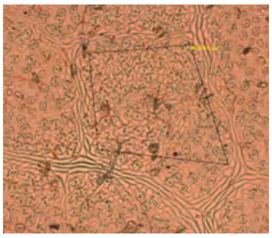
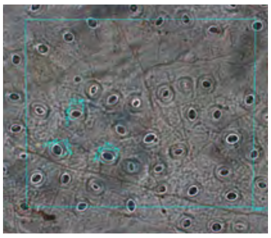
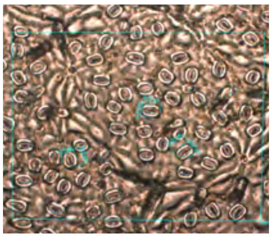
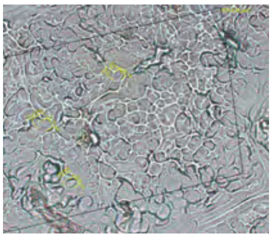
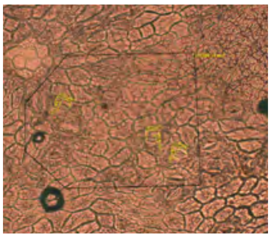
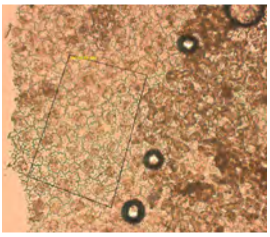
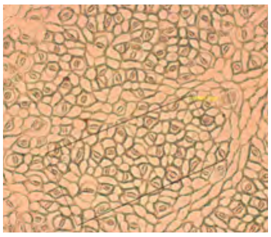
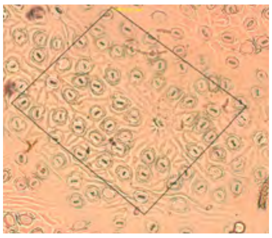


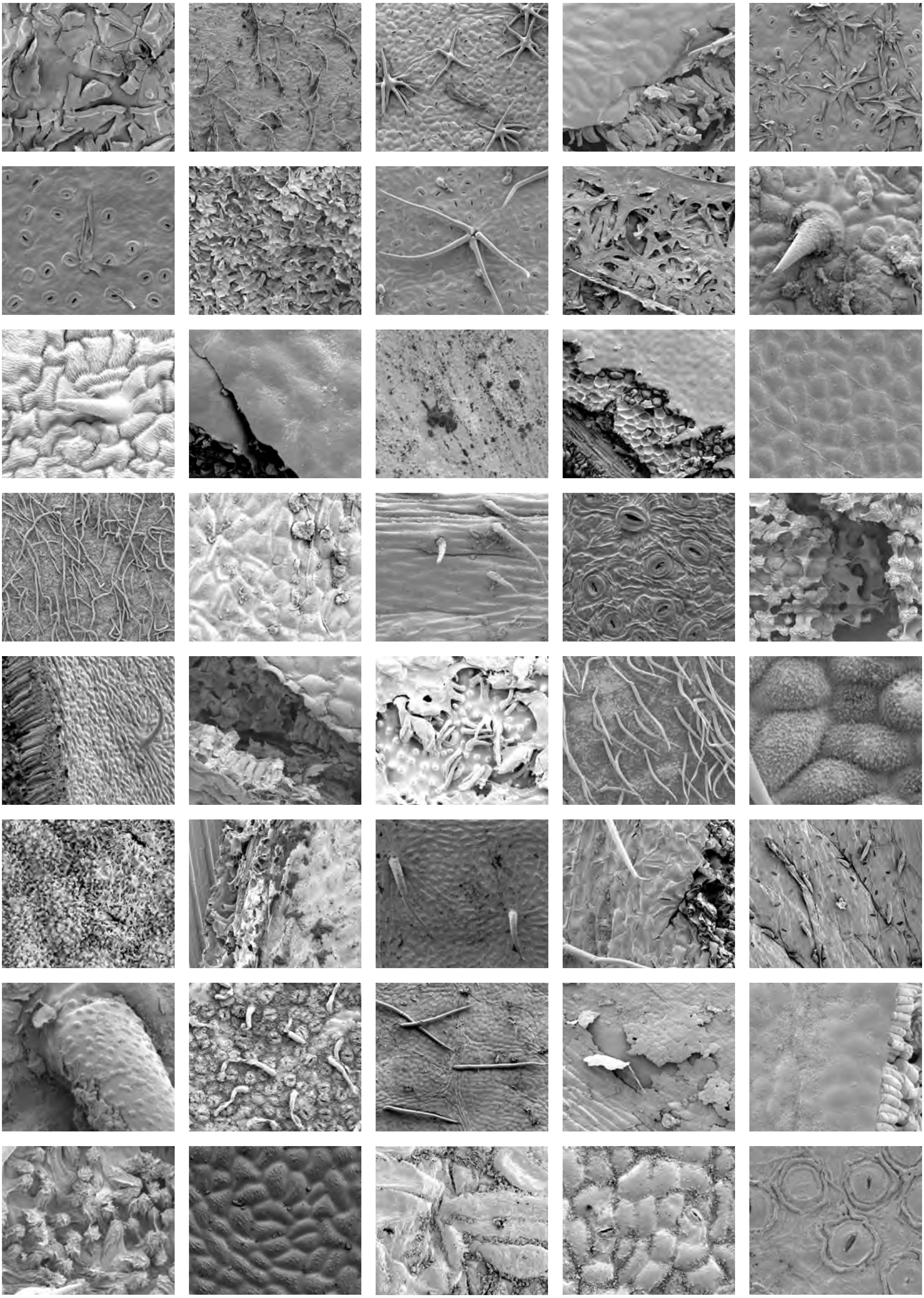


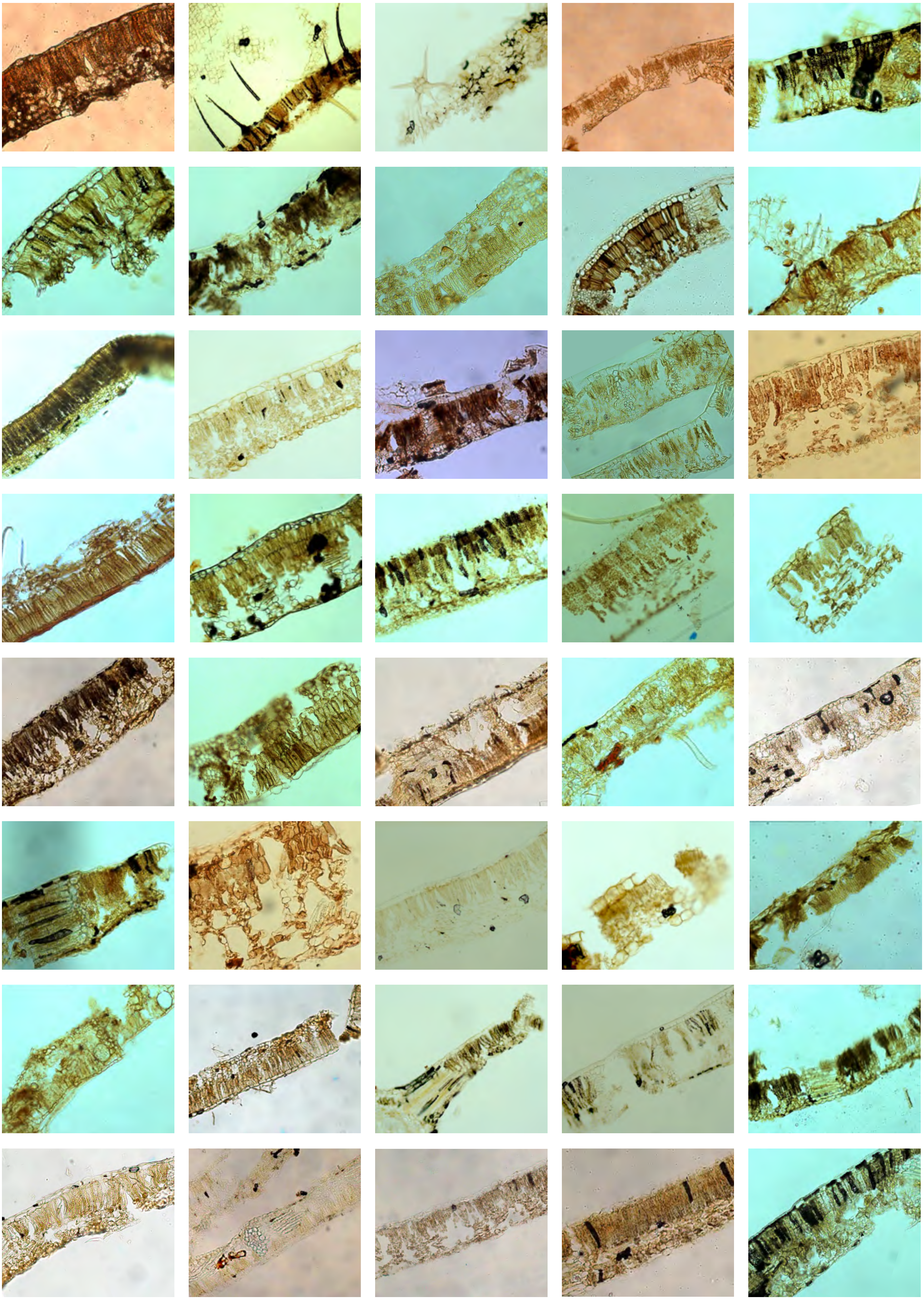









Explanations to the illustration pages

1	Species names of the 40 broadleaved tree species of BEF-China
2	Leaf photographs taken for measurements of specific leaf area
3	Stained leaf scans taken for vein trait analyses
4	Stomatal conductance-vapor pressure deficit curves
5	Xylem sensitivity to cavitation curves
6	Images of wood cross sections, taken for the assessment of wood microscopy traits
7	Microscope images of leaf surfaces, taken for the assessment of stomatal traits
8	Scanning electron microscope images, taken for the analysis of leaf surface microstructures
9	Leaf cross sections, taken to investigate leaf anatomy

6.3 Thanks

Nicht nur für die Betreuung der vorliegenden Arbeit möchte ich Helge Bruelheide danken. Er war auch ein großartiger Mentor, der mich fit gemacht hat für alles was die Wissenschaft ausmacht. Großen Anteil haben aber auch alle, die mich bei meinen umfangreichen Feld- und Laborarbeiten unterstützt haben: Heike Heklau, Merten Ehmig, Isa Plath, Katja Grotius und alle Mitdoktoranden im BEF-China Projekt. Alle meine Koautoren haben mich produktiv bei den Manuskripten unterstützt, Danke dafür.

Dem gesamten Fachbereich Geobotanik danke ich für die positive und motivierende Arbeitsatmosphäre am Institut. Für das Korrekturlesen der Arbeit und für chinesische Übersetzungshilfe bedanke ich mich bei Lydia Hantsch, Lotte Korell, Sylvia Haider und Xiaojuan Liu.

Das Kinderzimmer hat mich in ganz besonderer Weise motiviert und unterstützt! Danke an Maria Hofmann, Lydia Hantsch, Eva Breitschwerdt, Mathias Baudis und David Eichenberg!

Vielen lieben Dank auch an alle meine Freunde die mich auf meinem Weg zur Promotion begleitet haben. Meine Familie und insbesondere meine Eltern und meine Geschwister haben mir erst die Freiheit gegeben mich so ausführlich mit dieser Arbeit zu beschäftigen. Johanne, wie schön dass wir das Projekt Promotion nun gemeinsam abschließen können.

6.4 Appendix

6.4.1 Curriculum vitae

Wenzel Kröber	geb. 30.10.1982 in Halle (Saale)

PROMOTION

03/2010 – heute	Martin-Luther-Universität Halle-Wittenberg*, Halle (Saale)* Doktorand im internationalen Biodiversity and Ecosystem Functioning BEF-China Projekt am Institut für Biologie, Fachbereich Geobotanik, Thema „Trait relations in subtropical tree species“

AUSBILDUNG

10/2004–02/2010	Martin-Luther-Universität Halle-Wittenberg*, Halle (Saale)* Diplomstudium der Biologie und Psychologie, Abschlussnote 1,1, Thema der Diplomarbeit: „Functional leaf trait diversity in subtropical Chinese forests“ Prüfungsfächer: Geobotanik, mol. Ökologie, Naturschutz, Psychologie
08/1995–04/2003	Elisabeth-Gymnasium*, Halle (Saale)*, Abiturnote 1,5

BERUFLICHE ERFAHRUNG

04/2010–04/2014	Wissenschaftlicher Mitarbeiter, *Martin-Luther-Universität Halle-Wittenberg,* ▪ Lehrveranstaltungen (Seminare, Vorlesungen, Praktika) ▪ Betreuung mehrerer Bachelorarbeiten ▪ Entwicklung und Erprobung von Software zur Drohnensteuerung ▪ Einsatz von Drohnen zur Baumvermessung ▪ Umgang mit verschiedener Messtechnik zur Vermessung und ökologischer Datenaufnahme: Differentielles GPS, Klimastationen, PAR- und Photosynthese Messungen, Porometrie etc.
08/2006–03/2010	Wissenschaftliche Hilfskraft, *Martin-Luther-Universität Halle-Wittenberg* ▪ Gründung und Leitung des Diplomandenseminars Geobotanik ▪ Biodiversitätsforschung ▪ Forschungsreisen nach China, Südafrika und in die Mongolei ▪ Molekularbiologische Arbeit im Labor: DNA-Extraktion, DNA-Sequenzierung, Proteinanalyse

AUSZEICHNUNGEN UND STIPENDIEN

11/2010	Kurt-Mothes-Preisträger 2010, *Martin-Luther-Universität Halle-Wittenberg, Halle (Saale)* Forschungspreis des Halle Institute of Science and Technology für die beste Abschlussarbeit der Naturwissenschaftlichen Fakultät I
01/2008–06/2008	Stipendiat „Special student“, *University of Stellenbosch, Stellenbosch, South Africa*
06/1996	Preisträger Jugend Forscht*, Magdeburg* Erster Preis Wettbewerb Sachsen-Anhalt, Projekthema “Entwicklung des hallischen Binnenhafen”

AUSLAND

China:	Forschungsaufenthalte, *Shanghai, Beijing, Xingangshan, Gutianshan* 08/2012 – 11/2012, 04/2012, 08/2011 – 11/2011, 03/2011 – 05/2011, 08/2010 – 11/2010, 04/2010 – 05/2010, 02/2009 – 03/2009, 06/2008 – 09/2008, 08/2007 –09/2007, 06/2006 – 07/2006
Grönland	Forschungskooperation Eberhard-Karls-Universität Tübingen, *Illulisat* 06/2011 – 07/2011
Mongolei	Exkursion Georg-August-Universität Göttingen, *Ulan Bator* 06/2007 – 07/2007
Südafrika	Exkursion University of Pretoria, *Pretoria, Johannesburg* 04/2007 – 05/2007 University of Stellenbosch, *Stellenbosch, Cape Town, South Africa* Auslandssemester, Kurs Ökologie erfolgreich teilgenommen 01/2008 – 07/2008
Frankreich	Lycée René Cassin, *Mâcon, France* Schüleraustausch, Première Scientifique (11. Klasse, naturwissenschaftlicher Zweig, inklusive Abiturprüfungen), erfolgreich absolviert 08/1999 – 07/2000

SPRACHEN

Deutsch:	Muttersprache
Englisch:	verhandlungssicher
Französisch:	verhandlungssicher
Chinesisch:	Grundkenntnisse

WEITERBILDUNGEN

2014	„Grundlagen der Personalführung“, Martin-Luther-Universität Halle-Wittenberg
2013	„Mikroökonomie für Fortgeschrittene“, Martin-Luther-Universität Halle-Wittenberg
2013	„Externes Rechnungswesen II“, Martin-Luther-Universität Halle-Wittenberg
2013	„Umweltökonomik“ , Martin-Luther-Universität Halle-Wittenberg
2013	„Unternehmerische Kompetenz“, Martin-Luther-Universität Halle-Wittenberg
2011	„Rhetorik für Lehrende“, Martin-Luther-Universität Halle-Wittenberg

ENGAGEMENT UND INTERESSEN

04/2003–06/2004	Zivildienst (anschließend Anstellung) Nationalparkranger auf Föhr, Schutzstation Wattenmeer, Nationalpark Schleswig-Holsteinisches Wattenmeer
2002–2006	Wahlhelfer Europawahl 06/2004, Landtagswahl Sachsen-Anhalt 04/2002 und 03/2006 in Halle

Halle (Saale), den 5.12.2014

Wenzel Kröber

6.4.2 List of publications

Kröber W, Plath I, Heklau H, Bruelheide H (2014) Relating stomatal conductance to leaf functional traits. JOVE under review

Nadrowski K, Pietsch K, Baruffol M, Both S, Gutknecht J, Bruelheide H, Heklau H, Kahl A, Kahl T, Niklaus P, Kröber W, Liu XJ, Mi XC, Michalski S, von Oheimb G, Purschke O, Schmid B, Teng F, Welk E, Wirth C (2014): Tree species traits but not diversity mitigate stem breakage in a subtropical forest following a rare and extreme ice storm. PLOS ONE 9:e96022

Schuldt A, Assmann T, Bruelheide H, Durka W, Eichenberg D, Härdtle W, Kröber W, Michaliski S, Purschke O (2014) Functional and phylogenetic diversity of woody plants drive herbivory in a highly diverse forest. New Phytol 202:864–873

Eichenberg D, Ristok C, Kröber W, Bruelheide H. (2014) Plant polyphenols – implications of different sampling, storage and sample processing in biodiversity-ecosystem functioning experiments. Chem Ecol 30:676–692

Böhnke M, Kröber W, Welk E, Wirth C, Bruelheide H (2013) Maintenance of constant functional diversity during secondary succession of a subtropical forest in China. J Veg Sci 25:891–911

Yang X, Bauhus J, Both S, Fang T, Härdtle W, Kröber W, Ma K, Nadrowski K, Pei K, Scherer-Lorenzen M, Scholten T, Seidler G, Schmid B, von Oheimb G, Bruelheide H (2013) Establishment success in a forest biodiversity and ecosystem functioning experiment in subtropical China (BEF-China) Eur J Forest Res 132:593–606

Kröber W, Böhnke M, Welk E, Wirth C, Bruelheide H (2012). Leaf trait-environment relationships in a subtropical broadleaved forest in South-East China. PLOS ONE 7:e35742

Schuldt A, Bruelheide H, Durka W, Eichenberg D, Fischer M, Kröber W, Härdtle W, Ma K, Michalski S, Palm W-U, Schmid B, Welk E, Zhou HZ, Assmann T (2012) Plant traits affecting herbivory on tree recruits in highly diverse subtropical forests Ecol Letters 15: 732–739

Böhnke M, Kreißig N, Kröber W, Fang T, Bruelheide H (2012) Wood trait-environment relationships in a secondary forest succession in South-East China Trees 26:641–651

Lang AC, Härdtle W, Bruelheide H, Kröber W, Schröter M, von Wehrden H, von Oheimb G (2012) Horizontal, but not vertical canopy structure is related to stand functional diversity in a subtropical slope forest Ecol Res 27:181–189

Bruelheide H, Böhnke M, Both S, Fang T, Assmann T, Baruffol M, Bauhus J, Buscot F, Chen XY, Ding B-Y, Durka W, Erfmeier A, Fischer M, Geißler C, Guo D, Guo L-D, Härdtle W, He J-S, Hector A, Kröber W, Kühn P, Lang AC, Nadrowski K, Pei K, Scherer-Lorenzen M, Shi XZ, Scholten T, Schuldt A, Trogisch S, von Oheimb G, Welk E, Wirth C, Wu Y-T, Yang X, Zeng X, Zhang S, Zhou HZ, Ma K, Schmid B (2011) Community assembly during secondary forest succession in a Chinese subtropical forest Ecol Monogr 81:25–41

6.4.3 Eigenständigkeitserklärung

Hiermit erkläre ich, dass die Arbeit mit dem Titel „Stomatal control, xylem hydraulics and leaf morphology in the 40 BEF-China tree species: Trait interrelationships, functional diversity and tree growth prediction“ bisher weder bei der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Ferner erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche von mir kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle (Saale), den 5.12.2014

Wenzel Kröber

"Poets and moralists, judging from our English trees and fruits, have thought that there existed an inverse proportion between the size of the one and the other, so that their fall should be harmless to man. Two of the most formidable fruits known, however, the Brazil Nut (*Bertholletia*) and the Durian, grow on lofty trees, from which they both fall as soon as they are ripe, and often wound or kill those who seek to obtain them. From this we may learn two things: first, not to draw conclusions from a very partial view of nature; and secondly, that trees and fruits and all the varied productions of the animal and vegetable kingdoms, have not been created solely for the use and convenience of man."

A. R. Wallace (1856). From a letter to Sir William Jackson Hooker; printed in Volume 8 of Hooker's Journal of Botany, 1856.