Patterns of intraspecific and intraindividual trait variation in trees along experimental diversity gradients

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"There is nothing like looking, if you want to find something. You certainly usually find something, if you look, but it is not always quite the something you were after".

— J.R.R. Tolkien, "The Hobbit".

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SUMMARY

The use of functional traits in ecology has enhanced our understanding of tree strategies and the ecological processes affecting ecosystem functioning in forests. However, while most research in the field focused on species-level trait differences, recent studies have emphasized the importance of examining variation within species (intraspecific) and even within individual trees (intraindividual). For instance, this variation can play a crucial role in how trees mediate species interactions at local scales, including tree-tree interactions or tree-mycorrhizal fungi interactions. Consequently, understanding trait variation patterns along species diversity gradients is vital to addressing functional changes in forests under the ongoing loss of species.

This thesis explores the patterns of intraspecific and intraindividual leaf trait variation in trees as response to tree and mycorrhizal fungal diversity. Using leaf-level data from several thousands of leaves and spectroscopy to predict leaf functional traits, I studied trait variation along experimental diversity gradients in subtropical China (‘BEF-China’ experimental platform) and temperate Germany (‘MyDiv’ experiment). Overall, I aimed to assess changes individual trees’ strategy in response to species diversity, study changes in intraspecific and intraindividual trait variation with tree species diversity, understand the contribution of intraspecific and intraindividual variation to community functional diversity, and explore the relationship between intraindividual variation and trait covariation.

First, by working in the MyDiv experiment I aimed at understanding how tree species richness in combination with mycorrhizal fungal diversity drives changes in functional traits and in intraindividual variation. I found that specific leaf area increased with tree species richness, while the carbon-to-nitrogen ratio decreased with arbuscular mycorrhizal fungal richness. Intraindividual variation in traits from the leaf economics spectrum decreased with increasing tree species richness, suggesting a role of intraindividual variation in tree-to-tree interactions. Last, I could not find differences between two groups of trees associated preferably with a type of mycorrhizal fungi (arbuscular mycorrhiza trees and ectomycorrhiza trees) regarding their acquisitive versus conservative strategy.

Second, by working in the BEF-China experimental platform I aimed at elucidating how intraspecific trait variation and intraindividual trait variation respond to tree species richness and how they contribute to the functional diversity of forest stands. Intraspecific trait variation decreased with tree species richness to generate niche complementarity between conspecifics, while there was a negative effect of tree species richness on intraindividual variation of stomatal morphology. Further, the organization of trait variation within individuals and populations significantly contributes to functional diversity of forests, particularly in mixed stands.

Last, by using data from the BEF-China experimental platform, I explored the relationship between intraindividual trait variation and trait covariation, and assessed how this relationship is affected by tree species diversity. I found that intraindividual trait variation increases with trait covariation, meaning that trees with more coordinated traits show greater variability in leaf traits. This relationship was influenced by the identity of the nearest neighbor. These results suggest that intraindividual leaf trait variation requires strong trait coordination to prevent maladaptive phenotypic syndromes. Additionally, I found that intraindividual variation in leaf calcium was negatively correlated with the neighborhood's Shannon diversity.

The primary patterns found in this thesis highlight the significance of tree species diversity in driving intraspecific and intraindividual trait variation. Across two different experiments, I observed that species diversity influences intraindividual trait variation. Further, even though its role in tree-tree interactions remains uncertain, high intraindividual variation may arise in low-diversity stands in order to improve water use efficiency or as a result of higher differentiation between sun and shade leaves. Intraspecific variation was influenced by tree species diversity as well, likely due to resource partitioning. Specifically, populations in monocultures exhibited a conservative strategy with high variability among conspecifics, whereas conspecifics in mixed stands converged toward similar acquisitive traits. In addition, mycorrhizal fungal diversity also influenced trait expression, suggesting that a greater number of fungal species enables trees to access more nutrients, particularly nitrogen.

The thesis concludes that intraindividual and intraspecific trait variation are important but often overlooked aspects of trait-based ecology. The findings reveal that tree species exhibit variation at various levels of biological organization, adjusting traits in response to species diversity and fungal communities. Recognizing these sources of variation may be key to understanding the assembly of ecological communities and plant-plant interactions. Since functional traits respond to environmental drivers, but simultaneously affect ecosystem functioning, future research should focus on linking trait variation across multiple levels of biological organization to biodiversity-ecosystem functioning.

ZUSAMMENFASSUNG

Die Verwendung funktioneller Merkmale in der Ökologie hat unser Verständnis der Baumstrategien und der ökologischen Prozesse, die das Funktionieren von Ökosystemen in Wäldern beeinflussen, verbessert. Während sich die meisten Forschungsarbeiten auf diesem Gebiet jedoch auf die Unterschiede zwischen den einzelnen Arten konzentrierten, haben neuere Studien die Bedeutung der Untersuchung von Variationen innerhalb der Arten (intraspezifisch) und sogar innerhalb einzelner Bäume (intraindividuell) hervorgehoben. Diese Variation kann zum Beispiel eine entscheidende Rolle dabei spielen, wie Bäume auf lokaler Ebene Arteninteraktionen eingehen, einschließlich Baum-Baum-Interaktionen oder Baum-Mykorrhiza-Interaktionen. Folglich ist das Verständnis der Muster der Merkmalsvariation entlang von Gradienten in der Artenvielfalt von entscheidender Bedeutung, um funktionelle Veränderungen in Wäldern angesichts des fortschreitenden Artenverlusts zu verstehen.

In dieser Arbeit werden die Muster der intraspezifischen und intraindividuellen Variation von Blatteigenschaften bei Bäumen als Reaktion auf die Vielfalt von Bäumen und Mykorrhizapilzen untersucht. Unter Verwendung von mehreren Tausend Blättern und Spektroskopie zur Vorhersage von funktionellen Blatteigenschaften habe ich die Merkmalsvariation entlang experimenteller Diversitätsgradienten im subtropischen China (Versuchsplattform „BEF-China“) und im gemäßigten Deutschland (Experiment „MyDiv“) untersucht. Insgesamt wollte ich die Veränderungen in der Strategie einzelner Bäume in Abhängigkeit von der Artenvielfalt bewerten, Veränderungen in der intraspezifischen und intraindividuellen Merkmalsvariation mit der Baumartenvielfalt untersuchen, den Beitrag der intraspezifischen und intraindividuellen Variation zur funktionellen Vielfalt der Gemeinschaft verstehen und die Beziehung zwischen intraindividueller Variation und Merkmalskovariation erforschen.

Zunächst wollte ich durch meine Arbeit im MyDiv-Experiment verstehen, wie der Baumartenreichtum in Kombination mit der Mykorrhizapilz-Diversität Veränderungen in den funktionellen Merkmalen und in der intraindividuellen Variation bewirkt. Dabei habe ich herausgefunden, dass die spezifische Blattfläche mit dem Reichtum an Baumarten zunahm, während das Kohlenstoff-Stickstoff-Verhältnis mit dem Reichtum an arbuskulären Mykorrhizapilzen abnahm. Die intraindividuelle Variation bei Merkmalen, die zur

Blattökonomie (leaf economic spectrum) beitragen nahm mit zunehmendem Baumartenreichtum ab, was auf eine Rolle der intraindividuellen Variation bei Interaktionen zwischen Bäumen hindeutet. Schließlich konnte ich zwischen zwei Gruppen von Bäumen, die vorzugsweise mit einer Art von Mykorrhizatypassoziiert sind (arbuskuläre Mykorrhiza-Bäume und Ektomykorrhiza-Bäume), keine Unterschiede in Bezug auf ihre Nährstofförkonomie feststellen.

Zweitens wollte ich durch meine Arbeit in der BEF-China-Versuchsplattform herausfinden, wie die intraspezifische Merkmalsvariation und die intraindividuelle Merkmalsvariation auf den Baumartenreichtum reagieren und wie sie zur funktionellen Vielfalt von Waldbeständen beitragen. Die intraspezifische Merkmalsvariation nahm mit dem Baumartenreichtum ab, um Nischenkomplementarität zwischen Artgenossen zu erzeugen, während ich einen negativen Effekt des Baumartenreichtums auf die intraindividuelle Variation der Stomata-Morphologie feststellte. Darüber hinaus trägt die Organisation der Merkmalsvariation innerhalb von Individuen und Populationen erheblich zur funktionellen Vielfalt von Wäldern bei, insbesondere in Mischbeständen.

Schließlich habe ich anhand von Daten aus der BEF-China-Versuchsplattform die Beziehung zwischen intraindividueller Merkmalsvariation und Merkmalskovariation betrachtet und untersucht, wie diese Beziehung durch die Baumartenvielfalt beeinflusst wird. Dabei habe ich herausgefunden, dass die intraindividuelle Merkmalsvariation mit der Merkmalskovariation zunimmt, was bedeutet, dass Bäume mit stärkerkoordinierten Merkmalen eine größere Variabilität bei funktionellen Blatteigenschaften aufweisen. Diese Beziehung wurde durch die Identität des nächsten Nachbarbaumes beeinflusst. Diese Ergebnisse legen nahe, dass die intraindividuelle Variation von Blatteigenschaften eine starke Merkmalskoordination erfordert, um maladaptive phänotypische Syndrome zu verhindern. Darüber hinaus habe ich festgestellt, dass die intraindividuelle Variation des Blattkalziumgehaltes negativ mit der Shannon-Diversität der benachbarten Bäume korreliert war.

Die in dieser Arbeit gefundenen primären Muster unterstreichen die Bedeutung der Baumartenvielfalt für die intraspezifische und intraindividuelle Merkmalsvariation. In zwei verschiedenen Experimenten konnte ichzeigen, dass die Artenvielfalt die intraindividuelle

Merkmalsvariation beeinflusst. Auch wenn die Rolle der intraspezifischen Variation bei den Interaktionen zwischen Bäumen noch ungewiss ist, könnte eine hohe intraindividuelle Variation in Beständen mit geringer Artenvielfalt entstehen, um die Wassernutzungseffizienz zu verbessern oder Ergebnis einer stärkeren Differenzierung zwischen Sonnen- und Schattenblättern sein. Die intraspezifische Variation wurde auch durch die Baumartenvielfalt beeinflusst, was wahrscheinlich auf Ressourcenpartitionierungzurückzuführen ist. Insbesondere Populationen in Monokulturen wiesen eine konservative Strategie mit hoher Variabilität unter den Artgenossen auf, während Artgenossen in gemischten Beständen zu ähnlichen akquisitiven Merkmalen konvergierten. Darüber hinaus beeinflusste auch die Vielfalt der Mykorrhizapilze die Ausprägung der funktionellen Merkmale, was darauf hindeutet, dass eine größere Diversität von Mykorrhizapilzen den Bäumen den Zugang zu mehr Nährstoffen, insbesondere Stickstoff, ermöglicht.

Insgesamt kommt die Arbeit zu dem Schluss, dass intraindividuelle und intraspezifische Merkmalsvariationen wichtige, aber oft übersehene Aspekte der merkmalsbasierten Ökologie sind. Die Ergebnisse zeigen, dass Baumarten auf verschiedenen Ebenen der biologischen Organisation variieren und ihre Merkmale in Abhängigkeit von der Artendiversität und den Mykorrhizapilzgemeinschaften anpassen. Die Berücksichtigungdieser Variationsquellen kann der Schlüssel zum Verständnis des Aufbaus ökologischer Gemeinschaften und der Interaktionen zwischen Pflanzen sein. Da funktionelle Merkmale auf Umweltfaktoren reagieren, sich aber gleichzeitig auf das Funktionieren des Ökosystems auswirken, sollte sich künftige Forschung darauf konzentrieren, die Merkmalsvariation auf verschiedenen Ebenen mit dem Funktionieren der Biodiversität und des Ökosystems zu verknüpfen.

CHAPTER 1

GENERAL INTRODUCTION

Trait variation across scales

With ca. 2 million of species living on earth (Costello et al., 2012), biodiversity encompasses a large number of organisms differing in their ecological strategy (Díaz et al., 2016; Carmona et al., 2021; Junker et al., 2023). Understanding these strategies is one of the most persistent and fundamental challenges in ecology (Westoby et al., 2002). In plants, one of the most diverse groups on earth, differences in ecological strategies are enormous and explain adaptations to different environments. As an example, there are important differences between alpine plants that are characterized by small sizes and physiological adaptations that allow them to survive long periods in cold environments and covered by snow, and tropical trees that can grow tall and tend to be strong competitors for light (Díaz et al., 2016). That is why, ecologist have developed several frameworks that allow to describe plant ecological strategies, for example r/K strategist theory (MacArthur & Levins, 1967), CSR (competitive/stress-tolerant/ruderal) framework (Grime, 1979), and Raunkiaer's life forms (Raunkiaer, 1934). However, in the last two decades, the use of functional traits has emerged as a new promising tool to understand plant ecological strategies with great potential to explain community dynamics and ecosystem functioning (Díaz & Cabido, 2001; Westoby et al., 2002; Violle et al., 2007). Functional traits are morphological, biochemical, physiological, structural, phenological, or behavioral characteristics that can be measured at the individual level and directly or indirectly affect overall plant fitness (Lavorel et al., 1997; Violle et al., 2007). Among all of them, some functional traits have been especially useful to define plant ecological strategies. This is the case, for example, for specific leaf area, which is closely related to leaf lifespan and the strategy of the plant in terms of the acquisition of resources (Wright et al., 2004). As a result, functional traits have been widely used (1) to describe patterns of biodiversity (Díaz et al., 2016; Testolin et al., 2021; Weigelt et al., 2021), (2) to better understand ecological processes such as the sorting of species depending on the environment (McGill et al., 2006; Pavoine & Bonsall, 2011; Götzenberger et al., 2012) or biotic interactions (Kraft et al., 2015), and (3) to elucidate the biodiversity-ecosystem functioning relationship (Díaz & Cabido, 2001).

Most of the patterns of functional trait variation have been described considering contrasts between species' trait values (Wright et al., 2004; Díaz et al., 2016). Therefore, species were usually described by functional trait measurements collected from few individuals from one or few populations and averaged at the species level. However, there is substantial variation within species that typically arises from genetic differences, but also it can be the result of phenotypic plasticity (Schlichting, 1986; Pigliucci, 2005; Valladares et al., 2007). That is why, there are, for example, dissimilarities between different population from the same species or between individuals belonging to the same population. Indeed, Siefert et al. (2015) showed that, on average for some of the most commonly used functional traits, intraspecific variation represented about one quarter of the total trait variability across ecological communities. Further, recent studies have highlighted the importance of considering intraspecific trait variation when studying the patterns of ecological strategies (Violle et al., 2012; Wong & Carmona, 2021; Chacón-Labella et al., 2023). Intraspecific variation occurs as a response to both the abiotic environment and biotic interactions (Westerband et al., 2021). Indeed, a growing body of literature focused on understanding the phenotypic variation as response to environmental gradients (Benavides et al., 2021; Kühn et al., 2021), to multiple abiotic factors such as temperature (Albert et al., 2010; Scheepens et al., 2010) or water availability (Hajek et al., 2016; Welles & Funk, 2021), or to changing environments (Valladares et al., 2014; Matesanz & Ramírez-Valiente, 2019). In contrast, the patterns of intraspecific trait variation in response to species interactions have been much less studied, which, however, could be crucial to understand species coexistence (Hart et al., 2016). For instance, the same species can shift its trait expression in order to adopt a more competitive strategy (Carmona et al., 2019), as a way to avoid competition with other plant species (Mason et al., 2011; Wilson & Stubbs, 2012) or in response to diversity as a result of higher niche partitiioining (Davrinche & Haider, 2021). Further, Bolnick et al., (2011) suggested that the trait variability within a population could be crucial to understand the patterns of intraspecific competition, and should be highest in monospecific communities as a way to reduce intraspecific competitive interactions. Therefore, these shifts and the trait variability within populations emerge as potential mechanisms involved in species interactions.

Recently, ecologists went beyond in the study of trait variation by considering the changes in functional traits within the same individual (hereafter referred to as intraindividual trait variation; Herrera, 2009). Intraindividual trait variation occurs in modular organisms, such as plants (but also others like corals; García-Cárdenas et al., 2023), whose body plan is based on copies of the same organ and/or structure (De Kroon et al., 2005). Therefore, as a result of phenotypic plasticity, the same individual can produce different leaves (Winn, 1996; Valladares & Niinemets, 2008; Møller et al., 2022), flowers (March-Salas et al., 2021) or fruits (Sobral et al., 2019) with the same genetic information, but different expression of it (Herrera et al., 2022). For example, if we focus on leaves, which are the main organ for photosynthesis, the same plant can exhibit differences between the top or sun leaves, which are exposed directly to the sunlight, and the bottom or shade leaves, which tend to receive less direct light (Escribano-Rocafort et al., 2016). In this case, there is spatial specialization within the same plant (Laurans et al., 2024), with sun leaves showing high photosynthetic rates and a drought tolerance strategy and shade leaves showing lower photosynthetic rates and are shade tolerants. This variability tends to be larger in trees, as they have high modularity and a long lifespan (Escribano-Rocafort et al., 2016), but it also important in perennial herbs, as reported for *Onobrychis viciifolia* (March-Salas et al., 2021) or *Helleborus foetidus* (Herrera et al., 2015). Further, intraindividual trait variation has been observed to be target of natural selection (Herrera et al., 2022) and to influence ecosystem functioning (Proß et al., 2023; Sobral, 2023). Intraindividual trait variation can also overcome intraspecific trait variation in some cases (Herrera et al., 2015; Møller et al., 2024), a finding that had been previously neglected. Therefore, there is a growing need to understand the drivers of intraindividual trait variation to better understand ecological strategies in plants. These environmental drivers can be light (Møller et al., 2024), nutrient (Davrinche & Haider, 2024) or water availability (Møller et al., 2022, 2024), but also biotic drivers such as herbivory or plant-plant interactions (Herrera, 2017). Speaking of the latter, intraindividual trait variation could decrease in response to local diversity (Proß et al., 2021), and, even though there are not yet enough studies on this topic, it has been suggested that intraindividual variability could reduce niche overlap between conspecific neighbours that directly interact. That is why, there is urge to understand if, as it happens with intraspecific trait variation, intraindividual trait variation is also a mechanism that could reduce competitive interactions.

Functional diversity and trait variation

Understanding the diversity of functional traits (i.e. functional diversity) remains important as it positively influences ecosystem stability (de Belllo et al., 2021), ecosystem productivity (Bongers et al., 2021) and other ecosystem functions as well as the provisioning of ecosystem services (Cadotte et al., 2011). Functional diversity is often measured by using a range of different indices that reach higher values as the differences between trait values increase. As we have seen in the previous section, both intraspecific and intraindividual trait variation can represent substantial proportions of trait variation. Consequently, assessments failing to account for intraspecific and intraindividual trait variation may inaccurately estimate functional diversity occurring in reality. For instance, metrics of functional diversity of communities considering intraspecific and intraindividual trait variation should have larger values than those relying on species' mean values only (Wong & Carmona, 2021). This difference may be less evident at a global scale, where the variation occurring within species may be neglectable compared to the differences between species living in completely different biomes and, thus, with extremely contrasting ecological strategies. However, at local scales, such as a forest stand or a grassland, the functional diversity of communities is significantly higher when intraspecific trait variation is taken in account (Puglielli et al., 2024). In addition, these differences are expected to be even higher if intraindividual variation is also considered (Palacio et al., 2019). As a result, it remains important to assess the contribution of intraspecific and intraindividual trait variation to functional diversity. Specifically, as the extent of both intraspecific and intraindividual trait variation may change in response to species interactions, it remains unclear how functional diversity and the contributions of intraspecific and intraindividual trait variation to it change in response to species interactions. Based on the idea that plants may enhance niche partitioning with neighbouring plants, Escudero et al. (2021) suggested that the contribution of intraspecific trait variation to functional diversity of communities should be higher in species-poor communities compared to species-rich ones. However, evidence is still missing on this topic.

Patterns of trait covariation in plants

In the framework of trait variation, traits do not vary independently, but instead co-vary to optimize some functions at the cost of others (Armbruster et al., 2014). For example, there are well-studied patterns of covariation in plants such as the relationship between time of reproduction and plant longevity, which is strongly related to plant height (Westoby et al., 2002; Wright et al., 2004; Díaz et al., 2016) or the covariation between the investment of carbon in the roots and the ability of collaboration with fungal partners (Bergmann et al., 2020; Weigelt et al., 2021). However, the most-studied covariation pattern is the leaf economics spectrum (LES), which describes a trade-off between “conservative” leaves that have a longer lifespan and higher survival probability in response to abiotic and biotic hazards, and cheaply constructed “acquisitive” leaves which have higher photosynthetic rates (Wright et al., 2004). As a result of these patterns of covariation, there is a limited number of trait designs. For instance, species with higher nitrogen content in the leaves (which is strongly related to photosynthetic activity) cannot have a long lifespan (Osnas et al., 2013). Therefore, the functional variability across traits in ecological communities increases as a result of decreasing trait covariation (Dwyer & Laughlin, 2017; Gross et al., 2024). The same patterns of trait covariation also appear at the intraspecific level (Gorné et al., 2020), which suggest that the patterns of trait covariation and the relationship with trait diversity could be conserved across levels of biological organization (Boucher et al., 2013). However, we are still lacking empirical knowledge on what is happening at the intraindividual level. Studies using leaf-level data suggest that patters of trait covariation in leaves correspond to those described comparing only species’ mean values (Proß et al., 2021), but still do not delve into the relationship between trait covariation and intraindividual trait variation.

Trait variation and ecosystem functioning in forests

In the current scenario of biodiversity loss (Barnosky et al., 2011; Hooper et al., 2012), assessing the role of intraspecific and intraindividual variation on species interactions, as well as their changes in response to diversity and their contribution to functional diversity, could contribute to our understanding on the relationship between biodiversity and ecosystem functioning (Chacón-Labella et al., 2023). This could be especially relevant in forests, as we have already seen that trees show high variation in the expression of traits due to their longevity and modularity (Laurans et al., 2024). Many tree species are declining worldwide (Betts et al., 2017; BGCI, 2021; Boonman et al., 2024) and forests are suffering strong degradation leading to the loss of local diversity (Grantham et al., 2020). Further, even though forest cover has increased in some parts of the globe (Hansen, 2013), many of these forest stands correspond to tree plantations which are used to reduce harvesting pressure on natural forests and as a nature-based solution to mitigate climate change and restore degraded land, but are usually composed of single fast-growing tree species (Messier et al., 2022). As a result, the decline of tree species diversity should also affect their phenotypic variability in terms of differences between species (García-Valdés et al., 2018), but, as we have previously seen, this could also affect the trait variation occurring within species. In order to better understand the responses of intraspecific and intraindividual trait variation to the loss of species, which may be key to understand changes in ecosystem functioning, tree diversity experiments have emerged as an important tool (Bruelheide et al., 2014, Vanhellemont et al., 2016). Typically, tree diversity experiments aim at studying the effect of biodiversity on specific ecosystem functions in forests. For instance, tree diversity experiments have already shown that tree diversity promotes productivity (Huang et al., 2018), stability in productivity (Schnabel et al., 2021) and resistance to drought (Fichtner et al., 2020; Sachsenmaier et al., 2024), among others. Nevertheless, apart from their importance to understand ecosystem functioning, tree diversity experiments also provide an interesting setting to study changes in intraspecific and intraindividual trait variation by providing different scenarios of tree-tree interactions (Trogisch et al., 2021). For example, Davrinche & Haider (2021) and Proß et al. (2024) used tree diversity experiments to study changes in the expression of leaf functional traits and patterns of intraindividual leaf trait variation, respectively. Last, forest degradation does not only affect trees, but also has an effect on other organisms interacting with trees which are of special interest for forest functioning. For instance, mycorrhizal fungi, which improve soil nutrient uptake (Kaschuk et al., 2009; Smith & Smith, 2011), are also affected as a result of human activities (Ma et al., 2021), with potential effect on tree functioning, including tree's ecological strategy and the expression of traits (Fei et al., 2022). That is why, novel tree diversity experiments that consider not only the effect of tree diversity, but the diversity of mycorrhizal fungi (Ferlian et al., 2018) can also be useful to understand the patterns of trait variation in forests.

Outline of the thesis

This thesis aims at elucidating the patterns of intraspecific and intraindividual trait variation in response to biodiversity. To address this knowledge gap, I used leaf-level data collected along experimental gradients of diversity in tree diversity experiments. Thus, I studied different facets of trait variation (intraindividual trait variation, intraspecific trait diversity, individual mean trait values) in response to two drivers: tree taxonomic diversity and mycorrhizal fungal diversity. Specifically, the main objectives were to (Figure 1): (1) assess individual trees' changes from a slow-growth, conservative strategy to a fast-growth, acquisitive strategy in response to species diversity (**Chapter 2**), (2) understand changes in intraspecific trait variability with tree species diversity (**Chapter 3**), (3) study patterns of intraindividual trait variability in response to tree species diversity (**Chapter 2**, **Chapter 3**, **Chapter 4**), (4) understand the contribution of intraspecific and intraindividual variability to community functional diversity (**Chapter 3**), and (5) explore the relationship between intraindividual variation and trait covariation (**Chapter 4**). Specifically, I hypothesized that: (1) there is a shift from a conservative leaf strategy to an acquisitive leaf strategy with higher tree and mycorrhizal fungal diversity, (2) intraspecific trait variability decreases with tree species richness, (3) intraindividual trait variability decreases with tree species richness, (4) the contribution of intraspecific and intraindividual variability to functional diversity of forest stands is expected to decrease with increasing tree richness, and (5) intraindividual trait variability is limited by trait covariation. I investigated these questions in the BEF-China tree diversity experiment, in subtropical China, and in the MyDiv tree diversity experiment, in central Germany. As my approach implied the collection and the processing of a large dataset, I used leaf spectroscopy, a high-throughput method, together with statistical tools for prediction (including both convolutional neural networks and partial least square regression), for the estimation of leaf functional traits from measured samples.

In **Chapter 2**, "Tree and mycorrhizal fungal diversity drive intraspecific and intraindividual trait variation in temperate forests: Evidence from a tree diversity experiment", I collected leaf-level data from MyDiv, a tree diversity experiment that relies on the combination of gradients of tree species richness and mycorrhizal associations to study the patterns of individual mean trait values and intraindividual trait variation in response to tree and mycorrhizal fungal diversity.

In **Chapter 3**, “Intraspecific and intraindividual trait variability decrease with tree species richness in a subtropical tree biodiversity experiment”, I collected leaf-level data along an experimental tree species richness gradient in BEF-China, in order to understand how intraspecific diversity within populations and intraindividual trait variation change with tree species richness. Further, I aimed to explore their importance for the functional diversity of a community.

In **Chapter 4**, “Within-individual leaf trait variation increases with phenotypic integration in a subtropical tree diversity experiment” I used a large leaf-level dataset to explore the relationships between intraindividual trait variation and the trait covariation occurring at the individual level and the effect of the identity of the closest neighbor and neighborhood diversity on this relationship. Further, I studied how this relationship was modulated by the surrounding tree species diversity.

Finally, in **Chapter 5**, I propose a synthesis of the presented studies and their results, bringing together the effects of tree and mycorrhizal fungal diversity and comparing the results from a subtropical (BEF-China) and a temperate (MyDiv) tree diversity experiment. I use my results to interprent how trait variation enables to mediate tree species interactions, and how these mechanisms could be relevant for the functioning of forests. Last, I discuss the possibility of moving beyond established approaches in trait-based ecology by considering novel frameworks that take in account both intraspecific and intraindividual variation.

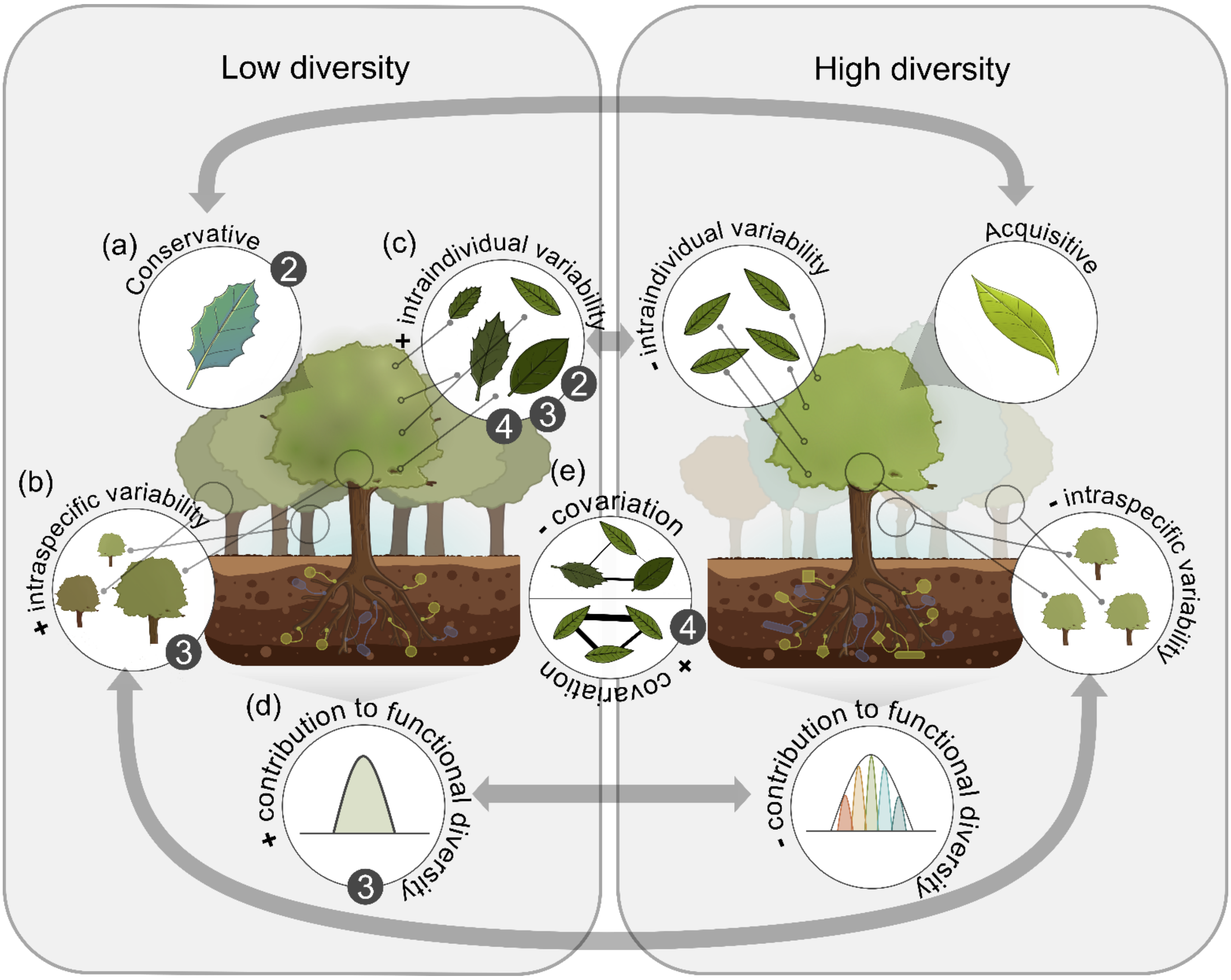

FIGURE 1 Graphical overview of the primary research questions and hypotheses that were addressed in this thesis. The figure presents two scenarios, a low diversity scenario (characterized by low tree species richness and low diversity of mycorrhizal fungi) and a high diversity scenario (characterized by high tree species richness and high diversity of mycorrhizal fungi). In this thesis, I expected to find (a) a shift from a conservative leaf strategy to an acquisitive leaf strategy with higher tree and mycorrhizal fungal diversity. Regarding (b) intraspecific and (c) intraindividual trait variability, I expect them to decrease with increasing tree richness "+" and "-" indicate higher and lower, respectively. (d) The contribution of intraspecific and intraindividual variability to functional diversity of forest stands is expected to decrease with increasing tree richness. Finally, I expect that (e) intraindividual trait variability is limited by trait covariation, and thus, intraindividual trait variability is higher when trait covariation is low. Different tree species are represented by different crown colours, while the diversity of arbuscular mycorrhizal fungi (in green) and ectomycorrhizal (in blue) is shown through the number of different polygon shapes. The numbers on the circles correspond to the chaprtes in which the research questions are studied.

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CHAPTER 2

Tree and mycorrhizal fungal diversity drive intraspecific and intraindividual trait variation in temperate forests: Evidence from a tree diversity experiment

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ABSTRACT

1. The study of tree species coexistence is crucial to understand the assembly of forest communities. In this context, trees adjust their traits in response to the interactions with other trees and, specifically, as a result of the competition for resources. Further, mycorrhizal fungal diversity and associations are important drivers of ecosystem functioning in forests, but their role as drivers of intraspecific trait variation has been disregarded. Here, we studied intraspecific trait variation of trees in response to tree and mycorrhizal fungal diversity.

2. We sampled 3200 leaves from 640 trees belonging to 10 native, deciduous species in a tree diversity experiment in Central Germany. This experiment relies on the combination of gradients of tree richness and mycorrhizal associations. To handle large amounts of leaf samples, we acquired leaf-level spectral data and used deep learning to predict values for five leaf traits from the leaf economics spectrum (LES): specific leaf area, leaf dry matter content, carbon to nitrogen ratio, carbon content and phosphorus content. For every tree, we calculated the mean value for every trait and two multi-trait functional indices (functional richness and functional dispersion) based on values for individual leaves. Finally, we used sequencing-based data to assess the richness of mycorrhizal fungi associated with the trees.

3. We found that tree and mycorrhizal fungi richness had an effect on different leaf functional traits. Specifically, tree richness positively affected specific leaf area and, additionally, had a negative effect on the functional indicies, which revealed that the phenotypic diversity within the tree crown decreased with tree species richness. In addition, leaf carbon to nitrogen ratio decreased with increasing arbuscular mycorrhizal fungal richness in both arbuscular and ectomycorrhizal tree species. Finally, we did not find differences between arbuscular and ectomycorrhizal trees regarding their location within the LES.

4. Our results suggest that trees modify their strategy in response to local tree diversity, not only by shifting trait values but also by shifting the variability intraindividually. In addition, higher mycorrhizal fungal diversity does not seem to lead to higher complementarity, but instead, tree and mycorrhizal fungi affect different aspects of leaf traits.

Keywords: arbuscular mycorrhiza, ectomycorrhiza, intraindividual trait variation, intraspecific trait variation, leaf economics spectrum, plant–plant interactions

INTRODUCTION

Biotic interactions shape species diversity in local communities through processes of competition and facilitation among others (Brännström et al., 2012; Chesson, 2000; Ricklefs, 2010). Referring to classical coexistence theories, each species is characterized by a particular niche framed by abiotic and biotic factors, where it avoids to be outcompeted by other local species (Grinnell, 1917). As plants need a common base of resources, this suggests that competition is the main type of plant–plant interaction (Hughesdon, 1927; Wright et al., 2014), and similarities in the resource uptake pathways of plants lead to a strong niche overlap and consequently higher competition for resources (Adler et al., 2018). However, such explanations for plant species coexistence have been criticized for being too simplistic (Escudero & Valladares, 2016), and there is growing demand to consider the variation within species when studying plant–plant interactions (Valladares et al., 2015). The reason behind is that species are not static entities, but instead are flexible, and plants adjust their functional traits (i.e. morphological, physiological or phenological characteristics that influence growth, reproduction or survival; Violle et al., 2007) via: (1) genetic adaptation (Baron et al., 2015) and (2) phenotypic plasticity, that is the ability of genotypes to express alternative phenotypic syndromes (Jump & Peñuelas, 2005; Stotz et al., 2022; Valladares et al., 2007). By adapting and shifting functional traits, individuals are able to reduce competition for limiting resources (Burns & Strauss, 2012; Roscher et al., 2018).

Differences in resource-use strategies are reflected by the expression of dissimilar functional traits (Suding et al., 2003). Across all functional traits in plants, leaf traits can be reliable proxies for resource-use strategies as summarized by the leaf economics spectrum (LES; Wright et al., 2004). The LES reflects a trade-off between a leaf's lifespan and its maximum photosynthetic rate (Díaz et al., 2016; Wright et al., 2004). In general, leaves with high values for traits related to photosynthetic activity, like high specific leaf area and high nitrogen concentration, are typically associated with higher resource acquisition, faster growth rates, and a lower investment in leaf construction and protective tissues. Therefore, the LES describes the resource-use strategy of plants which range from an acquisitive, growth-related strategy to a long-lived, conservative strategy (Pietsch et al., 2014; Poorter et al., 2009; Reich et al., 1997, 1999; Scheepens et al., 2010). Although the LES originally described differences between species, there is evidence that the gradient of the LES also occurs within species at the individual level (classically referenced as intraspecific trait variation; Fajardo & Siefert, 2018; Niinemets, 2015). It even reflects the diversity of alternative phenotypic syndromes within the same plant (Intraindividual trait variation; Herrera, 2017), and it can therefore be assumed that plants adjust their LES traits in order to mediate biotic interactions. This is especially true in the case of trees because, as a result of their longevity, it is crucial for them to adjust to local conditions within their lifespan instead of adapting through generation turnover like short-lived plants as forbs and grasses (Trogisch et al., 2017). As an increasing number of species in a community typically results in higher complementarity in the use of resources (Barry et al., 2019), trees in rich communities tend to show higher values for acquisitive-related traits compared to those in monospecific communities (Davrinche & Haider, 2021; Deschamps et al., 2023; Felix et al., 2023). Further, recent studies suggest that the extent of intraindividual trait variation could help to cope with micro-environmental conditions (March-Salas et al., 2021; Møller et al., 2022) but also with biotic interactions. Therefore, trees in monospecific communities have been suggested to display highly different leaf traits within their crown, probably to avoid competition with interacting conspecific neighbours (Proß et al., 2021).

Apart from plant–plant interactions, organisms from other trophic levels which are directly interacting with trees may act as drivers of leaf trait expression and variation (Tedersoo et al., 2020). Among the different plant interactions with other guilds, mycorrhizal associations, which improve soil nutrient uptake, may be among the most important ones (Kaschuk et al., 2009; Smith & Smith, 2011). Due to the variety of different nutrient uptake processes, the diversity of mycorrhizal associations seems to increase resource niche partitioning between plants (Bever et al., 2010; Hazard & Johnson, 2018; Klironomos et al., 2000; Wagg et al., 2015). Among all the different mycorrhizal types, there are two which are dominant, especially in trees: (1) arbuscular mycorrhizal fungi (intracellular symbioses dominated by Glomeromycota; AMF), which is the most abundant mycorrhizal type occurring in ca. 74% of angiosperm species (Trappe, 1987), and (2) ectomycorrhizal fungi (intercellular symbioses; EMF), which is a common type among temperate tree species. In the case of AMF, several studies suggested that more diverse AMF communities can improve nutrient uptake by providing plants access to different resource pools (Horsch et al., 2023; Jansa et al., 2005;

Wagg et al., 2015) and, besides the fewer studies carried out, similar results have been found for EMF communities (Leake, 2001).

Even though trees can interact with different types of mycorrhizal fungi simultaneously (Heklau et al., 2021, 2023), species have a preferred type of mycorrhizal partner (Brundrett & Tedersoo, 2018) and, thus, they are classified depending on the mycorrhizal host types as arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) trees. In addition, due to evolutionary differences between host plant species as well as differences in the strategy of the mycorrhizal fungal types, AM and EM trees show strong differences in their resource-use strategy (Shi et al., 2020). Specifically, AM trees typically employ a more acquisitive strategy in terms of economic traits (Averill et al., 2019). Further, AM trees tend to show a greater extent of intraspecific variation in traits from the LES compared to EM trees (Shi et al., 2020), even though there are exceptions in the case of some EM trees (Niinemets, 2015). Therefore, this suggests that AM and EM trees could show dissimilarities in their response to plant and mycorrhizal fungal diversity described above, and it is expected that AM trees show higher intraspecific variation in response to the diversity of species (of both trees and fungi) compared to EM trees.

Tree diversity experiments manipulate the number of species while standardizing confounding factors like varying tree density or abiotic conditions (Bruelheide et al., 2014). Therefore, they are useful set-ups to examine intraspecific changes of functional traits in trees in response to species richness. To explore the effect of tree species richness, mycorrhizal fungal richness and mycorrhizal types on LES traits, we studied the trait values and intraindividual trait diversity from ca. 640 trees representing 10 native deciduous tree species, five of them known to be primarily associated with arbuscular mycorrhizal fungi and the other five with ectomycorrhizal fungi, in the MyDiv tree diversity experiment located in Central Germany (Ferlian et al., 2018). In contrast to other tree diversity experiments, here a treatment of species richness is combined with different plot compositions of tree species differing in their host mycorrhizal type (either AM or EM). We hypothesized that (1) as nutrient partitioning is expected to be enhanced by the richness of species (both tree and fungal), trees increase the mean values of acquisitive-related traits in response to tree and mycorrhizal fungal richness, and (2) intraindividual trait diversity is highest in monocultures to accomplish niche differentiation between individual trees and decreases with increasing tree and mycorrhizal fungal richness. Due to the nature of AM plants, which seem to be more responsive with respect to intraspecific trait-environment responses, (3) these responses to tree and mycorrhizal fungal richness are expected to be stronger in AM than EM tree species.

MATERIALS AND METHODS

Study site

This study was conducted in the MyDiv experiment, which is a biodiversity–ecosystem functioning (BEF) experiment located at the Bad Lauchstädt Experimental Research Station of the Helmholtz Centre for Environmental Research–UFZ in Saxony-Anhalt, Germany (51°23′ N, 11°53′ E; Figure 1a). The climate is temperate with a mean annual temperature of 8.8°C (monthly mean temperatures ranging from 0.8°C in January to 17.3°C in July) and mean annual precipitation of 484 mm (data obtained from Altermann et al., 2005 and https://www.worldclim.org/). The soil type is described as haplic Chernozem, which is very fertile and characterized by a stable aggregate structure, high water-retention and base saturation as well as high bioturbation rates (Altermann et al., 2005). The experiment was established in 2015 and is comprised by 80 plots (Figure 1b). It includes a set of 10 native deciduous angiosperm tree species, with five species each being primarily associated with arbuscular mycorrhizal fungi (AM; Acer pseudoplatanus L., Aesculus hippocastanum L., Fraxinus excelsior L., Prunus avium L. and Sorbus aucuparia L.) or with ectomycorrhizal fungi (EM; Betula pendula Roth., Carpinus betulus L., Fagus sylvatica L., Quercus petraea Liebl., Tilia platyphyllos Scop.) (Table S1; Ferlian et al., 2018). The design is based on the combination of different numbers of tree species and trees of the same or different host mycorrhizal types. Tree species were planted following a tree species richness gradient from monospecific plots (one species) over two-species mixtures up to four-species mixtures (Ferlian et al., 2018). The two- and four-species mixtures comprised only AM- or only EM-associated tree species or a balanced combination of AM- and EM-associated species (Figure 1). Every level of tree richness and mixture of mycorrhizal host types was replicated 10 times, thus allowing to have a comprehensive set of possible combinations (see Ferlian et al., 2018 for details). In every plot, 140 trees were planted at a distance of 1 m in a regular grid to mix species to the greatest extent possible (Figure 1c). In order to avoid edge effects, every plot has a 1.5 m buffer area consisting of the outermost tree rows and a core area of 8 × 8 m. A plastic cover was placed on the ground to prevent the growth of understory vegetation.

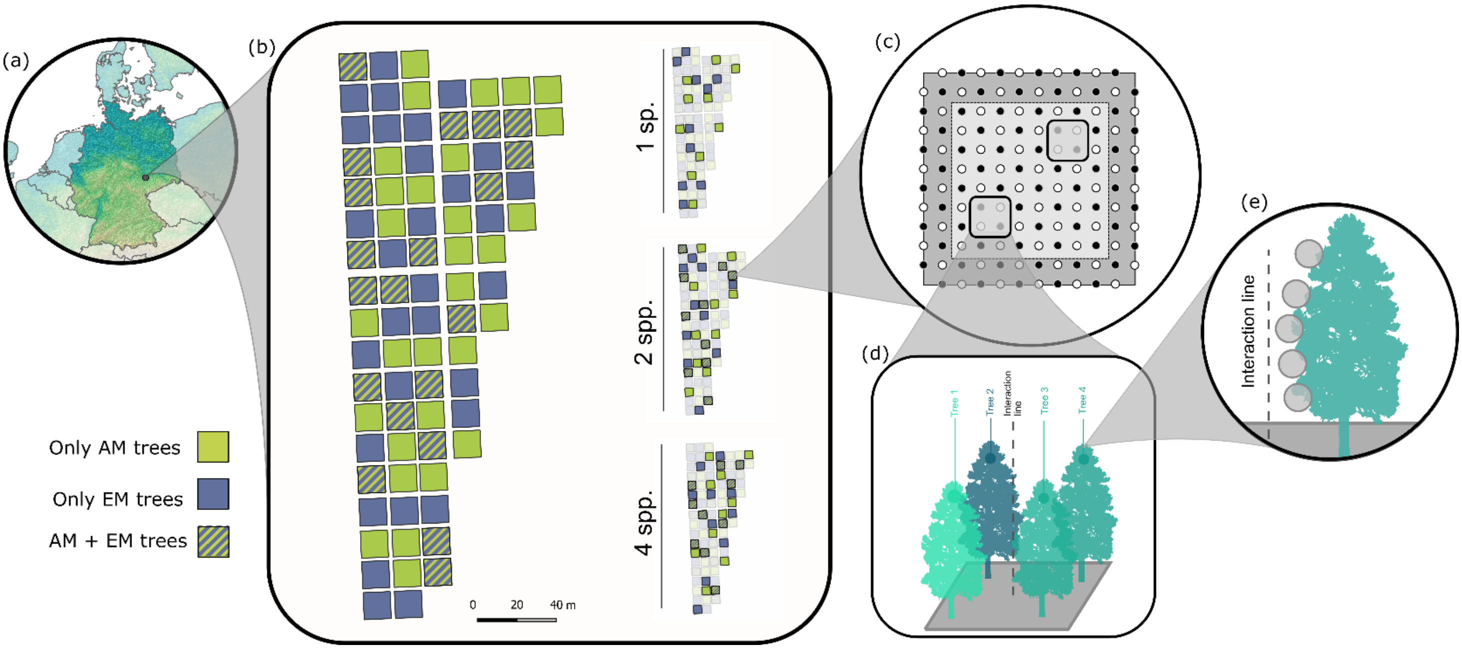

FIGURE 1 Location of (a) the MyDiv experiment in Germany, (b) plots in the experiment (adapted from Ferlian et al., 2018), (c, d) design of the tree species quadrats and (e) description of leaf sampling. As shown in (c), two tree species quadrats (TSQ) per plot were sampled in the inner part of the plots (light grey background) in order to avoid edge effects. Leaves from each individual within a TSQ were collected from the side pointing towards the focal line where trees interact (d, e). Five leaves were sampled from different heights of the tree, ranging from the lowest part of the crown to the top of the canopy.

Field sampling

As an extension of the tree-species pair design described in Trogisch et al. (2021) to study interactions among two directly neighbouring trees, sampling followed the tree-species quadrats design, which aims to study the interaction between four trees (hereafter, referenced as tree-species quadrat, TSQ; Figure 1d). In every plot, two TSQs were sampled, which resulted in 160 sampled TSQs containing a total of 640 sampled trees.

Sampling took place from mid to late August 2021. From each tree, we collected leaves along the interaction line between the TSQ partners, that is the focal point where the joint interaction of the four individuals is expected to be maximal (Figure 1d). In order to cover the trait variation of the whole tree individual, we sampled at five different heights. At each height, we cut one fully developed leaf free from mechanical or pathogen damage. This resulted in a total of 3200 collected leaves. Immediately after collection, leaves were conserved in sealable plastic bags with a moistened tissue. The samples were transported in an isothermal bag equipped with cooling bags to prevent desiccation. In the laboratory, the samples were temporarily stored at 6–8°C. In addition to this sampling (hereafter, referred to as ‘regular’ sampling), we collected a so-called calibration set that we used to train models for leaf trait prediction based on spectroscopy. This independent calibration set included leaf samples from 20 trees of each of the 10 species, resulting in a total of 200 sampled individuals. The leaves were collected at random heights and orientations within the tree crown across all species richness and mycorrhizal host type combinations. To ensure sufficient material for the laboratory analyses, a different number of leaves per tree was sampled, according to the size of the species-specific leaves (see Table S1).

Laboratory analyses

For the samples of the calibration set, we determined five morphological and chemical leaf traits, representing different dimensions of plant growth strategy and being key components of the LES (Reich, 2014; Wright et al., 2004; Figure 2): specific leaf area (SLA; leaf area/leaf dry mass; Kazakou et al., 2006; Niinemets & Kull, 1994; Reich et al., 1992; Reich et al., 1997; Reich et al., 1999), leaf dry matter content (LDMC; leaf dry mass/leaf fresh mass; Niinemets, 1999; Niinemets, 2001; Poorter et al., 2009; Poorter & Bergkotte, 1992; Westoby et al., 2002), carbon to nitrogen ratio (C:N; Niinemets et al., 2007; Pérez-Harguindeguy et al., 2003), carbon content (C) and phosphorus content (P; Hevia et al., 1999; Raaimakers et al., 1995; Tuohy et al., 1991). Leaf trait selection was based on previous analyses on the identification of independent and orthogonal sources of leaf trait variation (Figure S1) and the ability to obtain high-quality leaf trait predictions (see Section 2.5). Immediately after sampling, the fresh leaves of the calibration samples were weighed and scanned with a resolution of 300 dpi. The leaf area of the scans was analysed with the WinFOLIA software (Regent Instruments, Quebec, Canada). To determine the dry weight, we dried the leaves for 72 h at 60°C and weighed them again. Resulting from the leaf dry mass and the leaf fresh mass, both LDMC and SLA were calculated. Then, we grounded the dried leaves into a homogenous powder. To determine the P content, we used a spectrophotometric assay with the acid molybdate technique. We analysed C and N contents with an elemental analyser (Vario EL Cube, Elementar, Langenselbold, Germany) and calculated the C:N ratio.

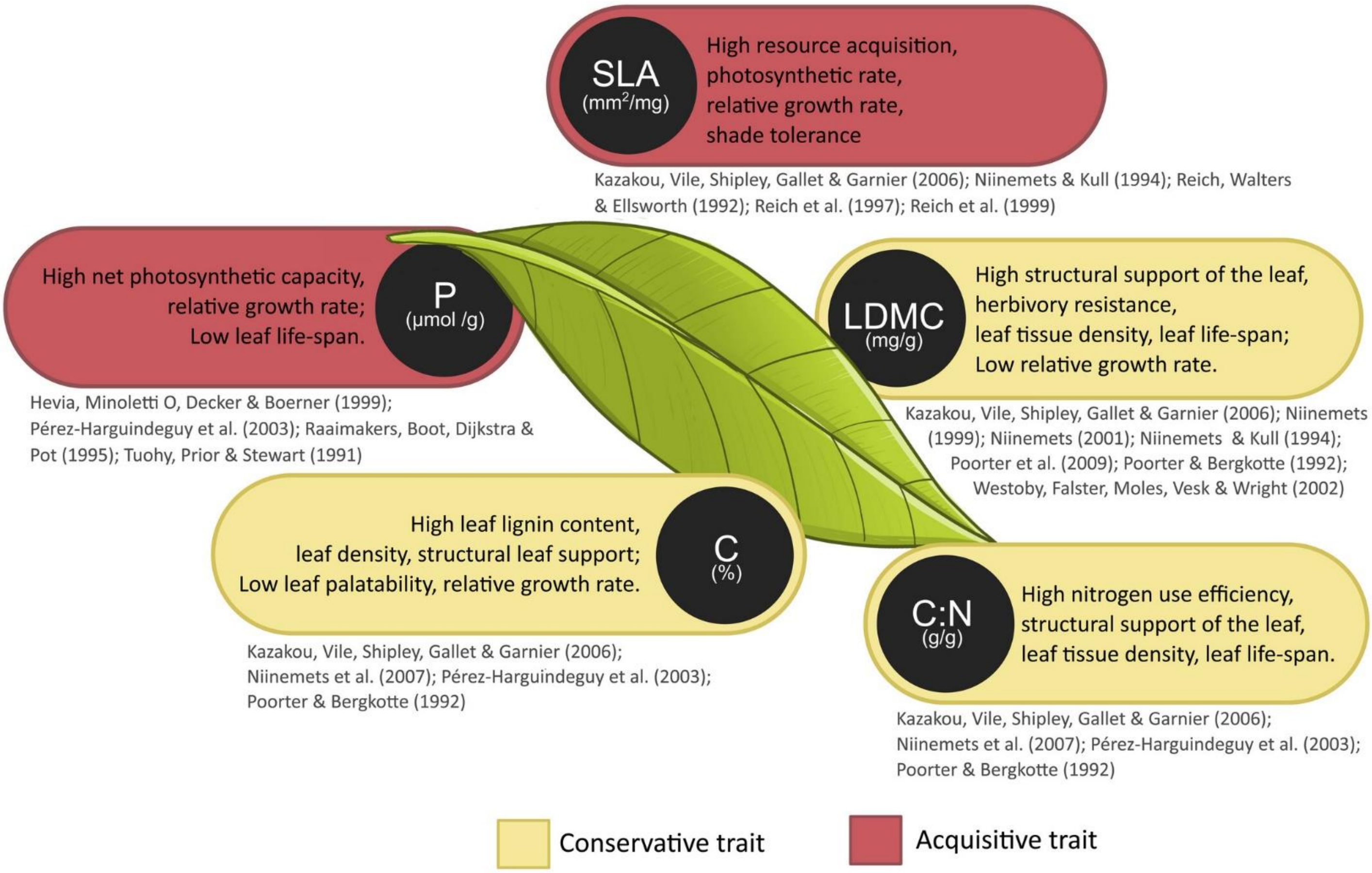

FIGURE 2 Leaf traits included in our study, ecological function of each trait and literature describing them. Yellow and red colour indicate that a trait is indicator of a conservative or an acquisitive strategy, respectively (according to the leaf economics spectrum LES; Wright et al., 2004).

Near-infrared reflectance spectroscopy

Current chemical analyses on single leaves are usually limited due to the lack of sufficient material from individual leaves. Thus, we used visible–near infrared spectrometry (Vis-NIRS), a technique of massive-phenotyping, to predict individual leaf trait values based on calibration models (Escudero et al., 2021; Foley et al., 1998). As reflectance depends on the size, density and shape of leaf tissues and their chemical compounds (Asner et al., 2014; Costa et al., 2018; Serbin et al., 2014), Vis-NIRS can be used for estimating morphological traits and leaf nutrients. For all leaves (regular and calibration samples), we acquired reflectance spectra with a portable Vis-NIRS device (ASD "FieldSpec4" Wide-Res Field Spectroradiometer, Malvern Panalytical Ltd, Almelo, Netherlands) in the laboratory immediately after collection. Reflectance was measured across the full range of the solar radiation spectrum (250–2500 nm), by taking three repeated measures on the adaxial side of each leaf while avoiding main veins. The equipment was optimized regularly with a calibration white panel (Spectralon, Labsphere, Durham, New Hampshire, USA). For each measurement, 10 spectra were averaged internally to reduce noise. Outlier removal of spectral data was performed by using the Local Outlier Factor (LOF) method (Breunig et al., 2000) as in Li et al. (2023) and, additionally, a visual inspection of the spectra was performed (see Figure S2a).

Leaf traits prediction

Leaf traits prediction and consecutive statistical analyses were conducted in the R environment with R version 4.1.3 (R Core Team, 2021). As deep learning has recently emerged as a promising tool in trait-based ecology (Perry et al., 2022; Vasseur et al., 2022), we used a convolutional neural network (CNN) approach for leaf trait prediction based on the spectral data. First, input spectra were augmented from 2501 to 12,906 features by using transformations based on a combination of standard normal variates and Savitzky–Golay derivatives (Figure S3; Passos & Mishra, 2021). Samples within the calibration set were split into a training and a test set which accounted for a proportion of 70% and 30%, respectively. Then, a CNN composed of one convolutional layer followed by three dense layers was fitted to train the samples for every trait (see Figure S3). In order to avoid overfitting, batch normalization was applied after the convolutional layer (Vasseur et al., 2022). Hyperparameter tuning for every CNN was performed independently for every trait, by adjusting the number of filters, their size for the convolutional layers, and the number of nodes in the dense layers (see Table S2). For model optimization, an Adam algorithm and a loss function based on the mean squared error were used (Passos & Mishra, 2022). CNNs were trained using a Keras framework and a TensorFlow backend as implemented in the 'Keras' package (Kalinowski, 2023). We tested the predictive ability of the CNNs by assessing the coefficient of determination for the predicted and measured values in the test set (R^2test) and in the whole calibration set (R^2model). As technical problems occurred during the laboratory analysis of leaf phosphorus concentration, we only had a reduced number of calibration samples for this trait. Therefore, to ensure the training of the CNN for this trait, we used additional and comparable samples from four deciduous species (Fagus sylvatica, Fraxinus excelsior, Quercus robur, Tilia cordata) collected by Proß et al. (2023) in the nearby Kreinitz experiment. This addition of samples aimed to represent the broadest trait space possible, in order to better reflect possible variation in our samples, as recommended in Burnett et al. (2021). Mean R^2model of the trained CNNs for each trait was 0.83 ± 0.10 (mean ± standard deviation), with a maximum R^2model for SLA (0.94) and minimum for phosphorus (0.71; see Figure S4). Eventually, these trained CNNs were used for predicting trait values of leaves from the regular set of samples. After leaf trait prediction, we excluded ca. 175 predicted trait values for every trait as they were lying outside a 95% confidence interval around the predicted values' distribution (Figure S2b).

Mycorrhizal fungal richness

In order to quantify mycorrhiza fungal richness associated to the trees, we used the metrics of arbuscular mycorrhizal and ectomycorrhizal fungi abundance measured in Ferlian et al. (2021; see Figure S5). To assess AMF and EMF richness, 200 root samples, one per plot and tree species, were taken in November 2019. In total, Ferlian et al. (2021) collected root samples from all species in all plots (excluding 12 samples that could not be assigned reliably to the correct tree species and, therefore, were excluded). They collected rootlets for every sampled tree and harvested those rootlets with 10 EM root tips in the case of EM trees or 10 lateral roots in the case of AM trees. Fungal species were identified by using Illumina sequencing (see Ferlian et al. (2021) for details). Based on these data, for every tree occurring in a plot, we calculated rarefied richness of AMF and EMF (hereafter, referenced as AMF and EMF richness) as implemented in the ‘vegan’ package. AMF and EMF reads per sample were rarefied to the minimum number of reads in every mycorrhizal type (Figure S5). Additionally, to avoid potential collinearity between predictors in further analyses, AMF and EMF richness where rescaled between 0 and 1 for every tree species, with 0 being the minimum richness of a specific fungal type (AMF or EMF) associated to a specific tree species, and 1 the highest richness of the same fungal type associated to the tree species.

Statistical analyses

In order to identity the main sources of trait variation, differences between mycorrhizal types, and to better understand changes in trait variation in further analyses, we first performed a principal component analyses of the predicted traits in every leaf. We used the prcomp function from the 'stats' package.

To characterize changes in the resource-use strategy of individual trees, we calculated the mean value of every trait in every tree. We tested the interacting effect of species richness, mycorrhizal fungal richness (AMF richness and EMF richness), and host type (either AM or EM trees) on mean trait values per tree by performing linear mixed-effects models. The models included the mean trait value as response variable, and the interaction effect of the log2-transformed species richness with both AMF and EMF richness and with the host type (AM or EM tree species) as fixed effects. Tree species identity as well as TSQ nested in plot, in turn nested in species composition were added as crossed random effects. We first fitted a "beyond optimal" model, which included all the fixed effects. By including subsets of the predictors, all possible models that varied in their fixed effects (including the intercept only model) were fitted using a maximum likelihood estimator. For all these models, the Akaike information criterion corrected for small sample sizes (hereafter referenced as AICc) was calculated. We selected all models with ΔAICc lower than 2 as competing models holding similar information, and followed the principle of parsimony to prioritize the simplest model with the smallest number of predictors among all competing models (Burnham & Anderson, 2004; Richards et al., 2011). Finally, we assessed the significance of factors by comparing models with and without each factor selected in each model using a likelihood ratio test (Zuur et al., 2009). We used diagnostic plots of the residuals to study the assumptions of normality, homoscedasticity and linearity in our models: residuals versus fitted values plots, histograms of the residuals, and Q-Q plots for the deviance of the residuals (Figure S7). In order to avoid biases in the estimation of mean trait values, for every trait, only trees for which there was information available for the five leaves were considered for the analyses. Therefore, the final number of trees ranged between 499 and 521, depending on the trait considered (Table S3).

In order to assess the trait diversity for each tree, we used two functional indices which reveal complementary indices in the functional hypervolume: (1) functional richness (FRic) and (2) functional dispersion (FDis). FRic aims at detecting reductions of the niche space occupied by individuals (Botta-Dukát & Czúcz, 2016; Cornwell et al., 2006), while FDis describes whether the distribution of leaves in a trait space of a tree is clustered or dispersed (Laliberte & Legendre, 2010). To calculate these indices, we first obtained a leaf-by-leaf trait distance matrix per tree by using Euclidean distance and, next, for every tree we computed both indices through a principal coordinate analysis (PCoA) based on this distance matrix. In order to test the interacting effect of species richness, AMF richness, EMF richness and host mycorrhizal type (either AM or EM trees) on the functional indices, we followed the same approach as described above for the mean trait values. We also checked the normality, linearity and homoscedasticity of the residuals by using diagnostic plots (Figure S8). Again, to avoid biases in the assessment of the functional indices, for the analyses we only used trees for which there were less than four missing values across all leaves and traits.

RESULTS

The first three axes of the PCA explained more than 80% of the total variation in our dataset (35% explained by the first axis, 25% explained by the second axis, and 21% explained by the third axis; see Figure 3). The first axis was strongly related to LDMC, leaf C, and SLA (with loadings 0.66, 0.54, and −0.46, respectively). The second axis was mainly related to leaf C:N, followed by SLA and leaf C (with loadings 0.70, −0.54, and −0.42 respectively). Finally, the third axis of the PCA was related to leaf P and C:N (with loadings 0.82 and −0.47, respectively). In this PCA, AM and EM trees were clearly separated along the second axis, with AM trees displaying higher values for leaf C:N. In contrast, the two groups were not divided along the first and third PCA axes.

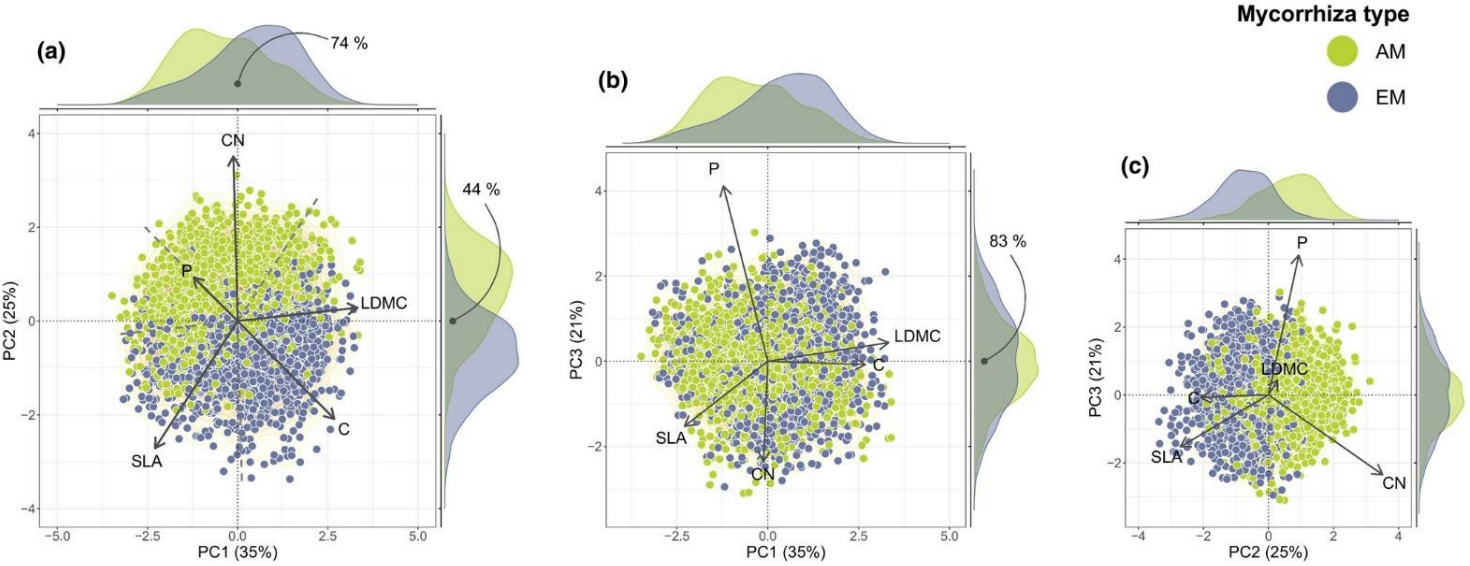

FIGURE 3 Main axes of a principal component analyses (PCA) for five leaf functional traits, including plots for (a) the first against the second component, (b) the first against the third component and (c) the second against the third component. Based on the loadings of every PCA axis, the first axis represents the variation in growth strategy, the second the variation in nitrogen content and the third one the variation in P content. The size of every plot is proportional to the portion of the variance explained by every combination of two axes (60% for the first and the second component, 55% for the first and the third component, and 45% for the second and the third component). The colour of the symbols represents the host mycorrhizal type (green: AM trees, blue: EM trees). Density plots for the distribution of the two mycorrhizal types in the main axes of the PCA are included in the margins of the PCA plots with the percentage of shared area between density distributions indicated on them.

Changes in mean values were found for SLA, C:N and C in the simplest models (Figure 4), although the drivers of these changes were different in every case (Figure S9a, c, d; Table S4). In the case of SLA, there was a significant effect of tree richness ($p = 0.01$; Table 1), suggesting that there was an increase in mean SLA with tree richness (Figure 4a). The simplest model for C:N suggests that AMF richness had a significant negative effect on mean C:N ($p = 0.04$; Figure 4b). In addition, we found that AM trees had higher values of C:N compared to EM trees ($p < 0.01$). Accordingly, the results for C revealed that EM trees displayed higher values of this trait compared to AM trees ($p = 0.02$; Figure 4c). Finally, among our results we did not find any effect of our predictors on mean LDMC and P in the simplest models.

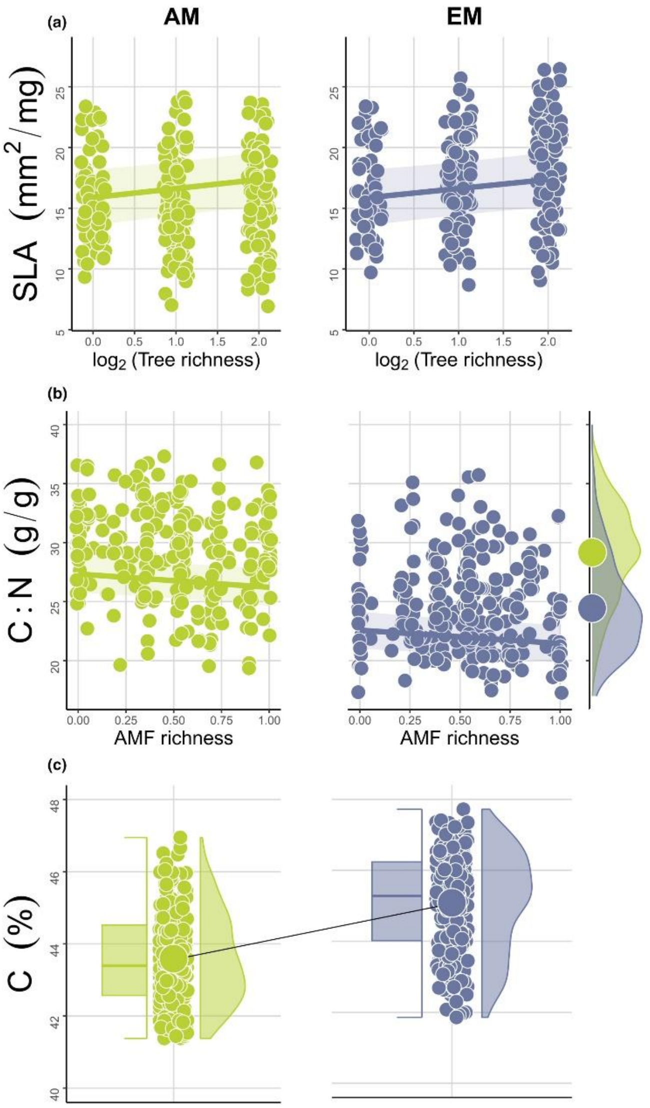

FIGURE 4 Main effects obtained in the simplest models for the mean values of (a) SLA, (b) C:N and (c) C content for AM and EM trees (in green and blue, respectively). Shaded areas in (a) and (b) represent the confidence intervals at 95%. Density plot (b) and raincloud plot in (c) indicate differences between AM and EM trees in terms of C:N and C, respectively. Large dots in (b) and (c) indicate the mean values of AM (green) and EM (blue) trees.

TABLE 1 Summary of the simplest linear mixed models. Estimates (standard errors) and significance assessed with likelihood-ratio tests are shown. The acronyms correspond to the different predictors (TR = tree richness; AMF = AMF richness; EMF = EMF richness; and MT = host mycorrhizal type), and interactions between predictors are indicated by "X".

	TR	AMF	EMF	MT	TR X AMF	TR X EMF	TR X MT	AMF X EMF	AMF X MT	EMF X MT	TR X AMF X EMF	TR X AMF X MT	TR X EMF X MT	AMF X EMR X MT	TR X AMR X EMR X MT	R^2m	R^2c
Functional traits																	
SLA	0.279(0.4)*															0.02	0.71
LDMC																0.00	0.80
C:N		-4.762(1.353)*		-1.123(0.545)**												0.27	0.66
C				1.358(0.557)*												0.18	0.77
P																0.00	0.69
Functional indices																	
	-0.002(0.001)**															0.02	0.03
FDis	-0.003(0.001)*															0.01	0.07

For both functional indices, we found an effect of species richness (Figure S10; Table S5) with a significant decrease in different properties of the hypervolume along the tree richness gradient ($p < 0.01$ and $p = 0.04$ for FRic and FDis, respectively; Figure 5).

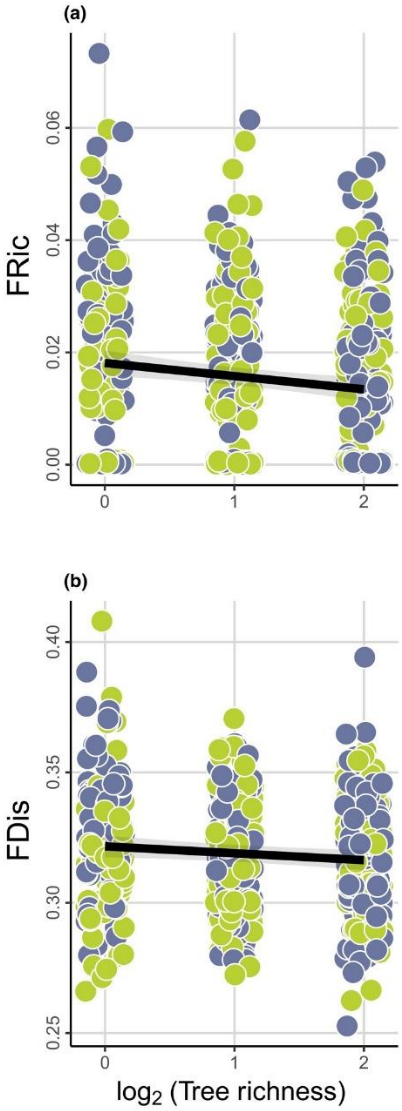

FIGURE 5 Main effects obtained in the simplest models for (a) functional richness (FRic) and (b) functional dispersion (FDis) of individual trees. Grey areas represent the confidence intervals at 95% and symbols the observed values for the two host mycorrhizal types (green: AM trees, blue: EM trees).

DISCUSSION

By using five traits of the LES from 485 to 514 tree individuals, depending on the trait, in a tree diversity experiment in Central Germany, we investigated how mean trait values and intraindividual trait diversity of AM and EM trees vary in response to the interacting effect of tree and mycorrhizal fungal richness. We found a consistent decrease of trait diversity within the canopy with increasing tree richness, whereas tree richness only had an effect on trees' mean SLA. Moreover, a key result was that aboveground traits, such as C:N, can also be affected by soil fungal richness.

Assuming that niche differentiation is determined by both tree richness and mycorrhizal fungal richness, we expected that both components interacted in driving intraspecific responses in our experiment. However, we found that tree and mycorrhiza diversity acted on different leaf traits. First, effects of tree richness on the growth strategy of trees were observed for SLA, which is related to the photosynthetic rate of the tree and, therefore, is one of the main proxies for a fast growth strategy (Reich et al., 1997; Wright et al., 2004). The increase of SLA in response to plant diversity has been described before and seems to respond to the complementarity in the use of resources (Felix et al., 2023). Thus, the decrease in competition for resources in mixtures would allow trees to show a more acquisitive strategy. Despite the lack of significant effects for other traits, we observed negative trends of LDMC in response to tree diversity (see Figure S9b), which also seem to be aligned with the changes from a conservative to an acquisitive strategy as a result of increasing complementarity in mixtures (Davrinche & Haider, 2021). Also, in the specific case of trees, SLA is strongly dependent on the availability of the light within the canopies. Therefore, a higher canopy stratification can lead trees to maximize photosynthesis in light-limited environments by decreasing leaf toughness and increasing SLA (Roberts & Paul, 2006; Williams et al., 2020). Indeed, in the case of our experimental site, the higher levels tree diversity are associated to a higher stratification that may contribute to explain the changes in SLA observed here (Ray et al., 2023). In addition, the decrease of C:N in response to AMF richness could be related to a better supply of nitrogen to the tree when the richness of these fungi is high as suggested by Powell and Rillig (2018). As AMF are efficient in nutrient uptake in dry soils (Querejeta et al., 2003) this effect could be especially noticeable in our study site where the precipitation is rather low (mean annual precipitation of 484 mm). However, this remains speculative as we are lacking empirical evidence regarding the improvement of soil nitrogen uptake by AMF in our experimental site. Further, it is remarkable that this pattern for C:N was encountered both for AM and EM tree species, which suggests that, despite the higher host-specificity of AMF in our study site (Ferlian et al., 2021), EM trees are not only linked to AMF through dual mycorrhization (see Figure S12; Heklau et al., 2021; Teste et al., 2019), but may also benefit from AMF richness. For instance, the dual mycorrhization of EM trees might be especially beneficial during dry periods (Querejeta et al., 2009), which might explain the advantage of these trees during droughts shown in Sachsenmaier et al. (2024) for our study site. Last, EMF richness did not have any significant effect on the traits studied, which suggest that even though EMF diversity can affect the uptake of nutrients (Khokon & Meier, 2023), this does not necessarily have an effect on the leaf strategy of the tree. All these changes in leaf functional traits, even though seem to be rather small and not significant for all traits, could have an effect on ecosystem functioning. First, while our approach of leaf trait prediction allows processing large sample sizes, it might underestimate the responses of leaf traits. Indeed, this may be the case of C and P, for which the higher impreciseness of the prediction (R^2 test is 0.65 and 0.66, respectively) might fail to detect a correlation with the predictors (Burnett et al., 2021). Further, intraspecific shifts in leaf traits, as the ones detected for SLA and N, may have an effect on different facets of ecosystem functioning (e.g. leaf herbivory, light capture) even if the variation within species is small compared to the variability found among them (Chacón-Labella et al., 2023; Williams et al., 2020). That is why, the effects of intraspecific trait variation on ecosystem functioning deserve further attention to better understand diversity effects in forests.

Recent studies have shown that the ability of plants to display different trait syndromes in repeated organs of the same individual may have important ecological and evolutionary implications (Herrera, 2017; Sobral, 2023; Sobral & Sampedro, 2022). Indeed, our approach, which suggests that for evaluating the growth strategy of trees not only shifts in mean trait values should be considered but also the changes in the phenotypic variability of leaves within the canopy (Escribano-Rocafort et al., 2017; Proß et al., 2021), reveals that the intraindividual diversity of leaf traits changes as a result of tree-tree interactions. Also, these changes in intraindividual diversity rarely act on single traits, but occur in different dimensions of the trait space (see Figure S11). Our results suggest that higher intraindividual diversity could be of great importance in monospecific communities where only intraspecific interactions are present. As an explanation for this, we propose that intraindividual trait diversity may help to reduce niche overlap between conspecific neighbours that directly interact (Castro Sánchez-Bermejo et al., 2023). Additionally, in the case of species-rich communities and as an extension of the framework proposed for intraspecific trait variation (Helsen et al., 2017), the reduced intraindividual trait diversity may contribute to niche packing (i.e. high phenotypic similarity between conspecifics from the same population; Violle et al., 2012), which is an adaptative strategy to reduce niche overlap between heterospecific neighbours. Even though we suggest that our results explain the importance of intraindividual trait variation for avoiding niche overlap, we should also consider two complementary hypotheses to explain our results, which are: (1) intraindividual trait diversity in leaves may improve plant performance in intraspecific interactions by, for example, improving light capture (Møller et al., 2022), and (2) intraindividual trait diversity could be a mechanism to cope with unpredictability of the environment (March-Salas et al., 2021) in monospecific communities, as these tend to show less environmental stability (Mori et al., 2017).

Previous literature supports that AM and EM tree species differ in their strategy for resource acquisition and suggest that AM trees have a fast and acquisitive strategy, while EM trees show a slow conservative strategy (Deng et al., 2023; Shi et al., 2020; Tedersoo et al., 2020; Tedersoo & Bahram, 2019). Specifically, it has been suggested that the higher acquisitiveness of AM trees could rely on the better mobilization and use of nutrients in their inorganic form (Averill et al., 2019; Phillips et al., 2013). Nevertheless, our results provide limited support to such general assumptions, and only for leaf C content, we found a significant difference which suggests that EM species invested more in leaf support. Rather, among the species included in the experiment, it seems that growth strategies are species-specific within the host mycorrhizal types, and there are AM trees with more acquisitive strategies (e.g. Fraxinus excelsior; see Figure S13), while others have a conservative strategy (e.g. Sorbus aucuparia). The same applies to EM species (Tilia platyphyllos, as an example, has an acquisitive strategy, while Fagus sylvatica has a more conservative strategy). Instead, as reflected by the PCA, host mycorrhizal types seem to mainly differ in C:N. Thus, EM trees seem to be more efficient in capturing nitrogen (Fellbaum et al., 2012; Tedersoo & Bahram, 2019), which explains the higher nitrogen content in the leaves resulting in the lower C:N ratio in EM as compared to AM trees (see Figure S14). While the main differences found between AM and EM trees suggest differences in N uptake, our results give only limited support to the differences in leaf P. However, we had expected differences in leaf P between the two host mycorrhizal types, because AM trees are described as efficient P capturers as a result of the symbiosis with AMF (Rosling et al., 2016). A possible explanation for the absence of this relationship in our study might be the soil type at the MyDiv experimental site, which was a nutrient-rich Chernozem, and the former land use, which was intensive agricultural use with high P fertilizer application (Ferlian et al., 2018). Finally, contrary to other studies on AM and EM trees, our experimental design did not include any gymnosperm species, which are EM trees in most cases (Averill et al., 2019) and show a conservative strategy in their leaf strategy (Díaz et al., 2016). Thus, our study suggests that the common belief in ecology of EM trees being more conservative than AM trees is not necessarily true, and, in the case of the temperate forests of central Europe, the higher conservativeness of EM trees could arise from the differences between gymnosperms and angiosperms instead of the mycorrhizal type.

CONCLUSIONS

Our work has novel implications to understand the assembly of forests and, specifically, how trees modify their resource-use strategy in response to biotic interactions, not only by shifting their trait values, but also the diversity of phenotypic syndromes within individual trees. In addition, we found little empirical support for our hypotheses, which related trees' resource-use strategy to the interactive effect of tree and mycorrhizal fungal richness via complementarity in the use of resources (Barry et al., 2019). In contrast, our study shows that tree and mycorrhizal diversity act on different traits and, therefore, suggest that tree diversity is not enough to explain all intraspecific responses in forests but better knowledge on the tree-mycorrhiza interactions is needed to more comprehensively understand how trees respond to biotic interactions.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

Code and data for the data analysis of this study are available at the Zenodo repository: https://zenodo.org/doi/10.5281/zenodo.10654726.

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SUPPORTING INFORMATION

FIGURE S1 Principal component analyses and correlations between nine leaf functional traits.

FIGURE S2 Data cleaning process for the spectral and predicted trait data.

FIGURE S3 Analytical framework used to generate convolutional neural networks (CNNs) to predict leaf traits from spectral data based on the calibration set.

FIGURE S4 Correlation between predicted and measured traits values to test the quality of convolutional neural networks (CNNs) to predict leaf traits from spectral data.

FIGURE S5 Violin plots for AMF richness and EMF richness in the different mycorrhiza treatments in the MyDiv experiment.

FIGURE S6 Rarefaction of arbuscular mycorrhizal fungal (AMF) and ectomycorrhizal fungal (EMF) richness

FIGURE S7 Diagnostic plots for assumptions of normality, homoscedasticity and linearity in the linear mixed-effects models for the mean values of traits.

FIGURE S8 Diagnostic plots for assumptions of normality, homoscedasticity and linearity in the linear mixed-effects models for functional richness (FRic) and functional dispersion (FDis).

FIGURE S9 Competing models for the drivers of the mean value of SLA, LDMC, C:N, C, and P.

FIGURE S10 Competing models for the drivers of indices of intraindividual trait diversity.

FIGURE S11 Results for the drivers of the variance of SLA, LDMC, C:N, C and P.

FIGURE S12 Boxplots for the AMF and EMF richness associated with every tree species.

FIGURE S13 Two main axes of a principal component analyses (PCA) for five leaf functional traits for 10 tree species.

FIGURE S14 Results for the drivers of the mean of nitrogen leaf content.

TABLE S1 Tree species in the MyDiv experiment and their mycorrhizal type.

TABLE S2 Layers and hyperparameters used for building a convolutional neural network for every trait.

TABLE S3 Species included in our study and number of individuals for every trait and functional indices (FDis and FRic) included in the analyses.

TABLE S4 Model selection for the analyses of the drivers of the mean value for five functional traits.

TABLE S5 Model selection for the analyses of the drivers of the multi-trait functional indices.

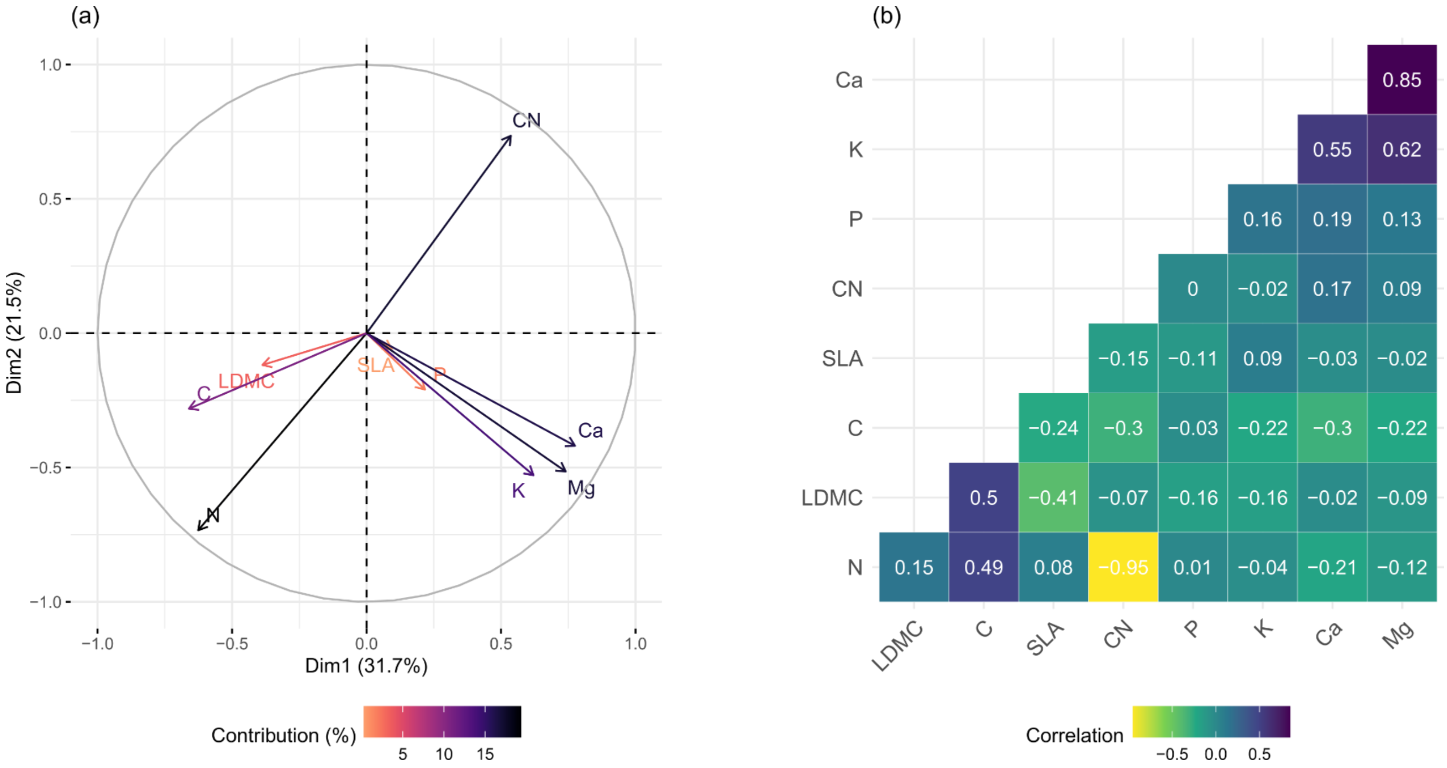

FIGURE S1 (a) principal component analysis (PCA) and (b) pairwise correlations between nine leaf functional traits measured in our calibration dataset. Colors in (a) indicate the contribution (in percentage) of the traits to the first PCA axis.

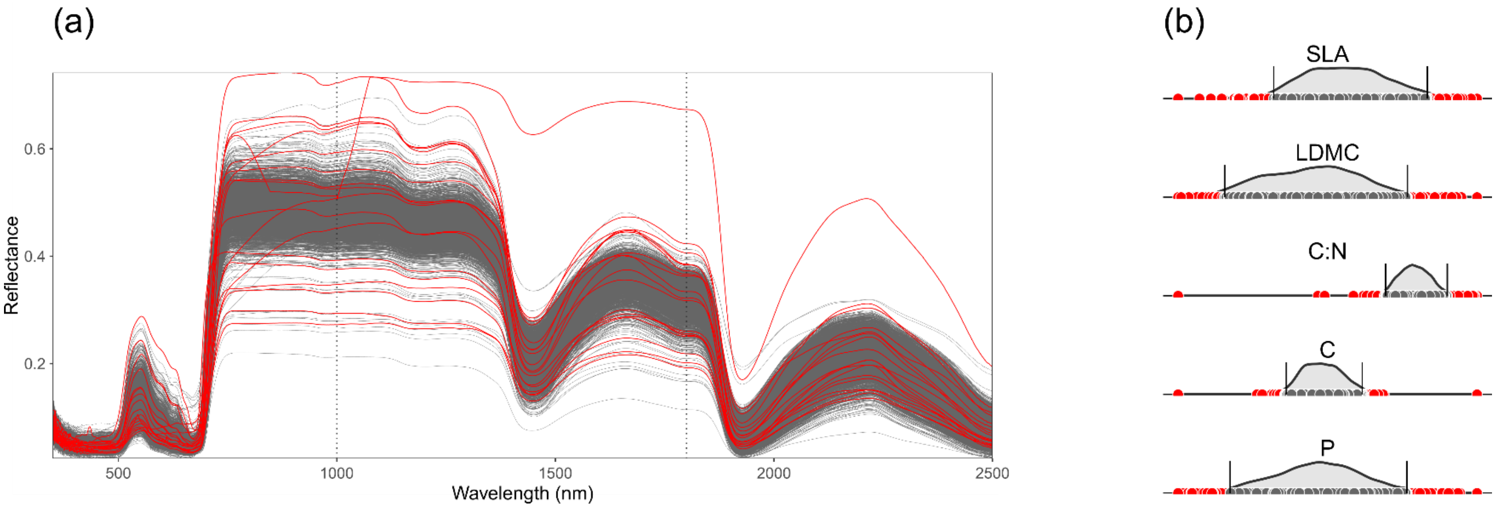

FIGURE S2 Excluded data (in red) from our (a) spectra and (b) predicted trait data. All spectral data excluded had a Local Outlier Factor (LOF) higher than 2 (Li et al., 2023), but no spectral data was excluded during the process of visual inspection. Data excluded in trait prediction was out of a 95% confidence interval for every trait.

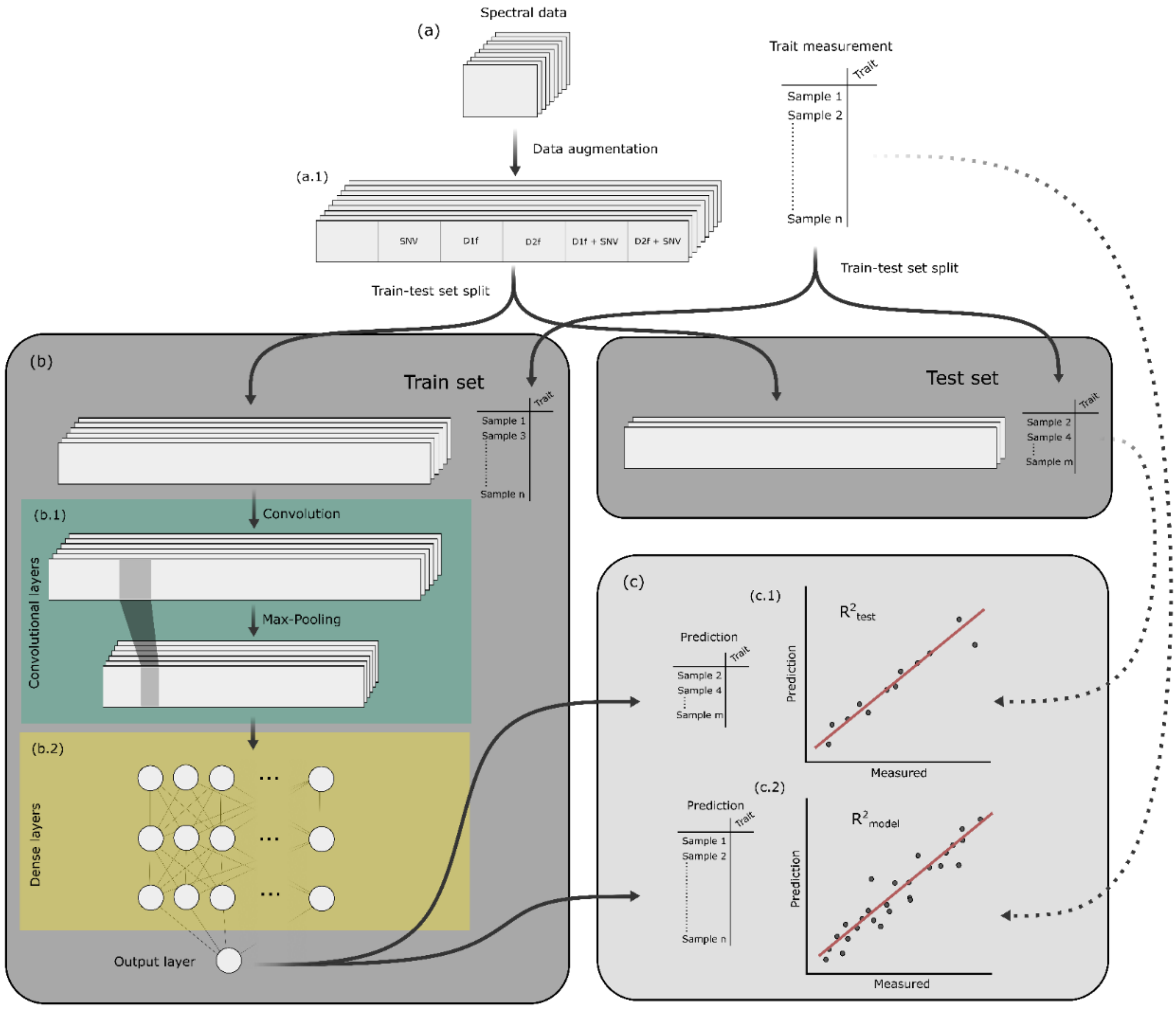

FIGURE S3 Analytical framework used to generate convolutional neural networks (CNNs) to predict leaf traits from spectral data based on the calibration set. (a) Spectral data were obtained for every tree and measurements of leaf functional traits were performed in the laboratory. A data augmentation procedure was used (a.1) by generating synthetic spectra based on standard normal variates and Savitzky-Golay derivative. Additionally, samples within the calibration set were split into a training and a test set which accounted for a proportion of 70% and 30%, respectively. Split was performed perpendicular to the main axes of a principal component analysis of the spectra to ensure the diversity of samples in both sets (Griffith & Anderson, 2019). (b) CNNs were composed by: (b.1) convolutional layers which perform convolutional operations followed by a max-pooling of values; (b.2) four dense layers with nodes which receive an input and produce an output via an activation function. (c) To test the predictive ability of the CNNs, we obtained the coefficient of determination for the predicted and measured values (c.1) in the test set (R^2_{test}) and (c.2) in the whole calibration set (R^2_{model}).

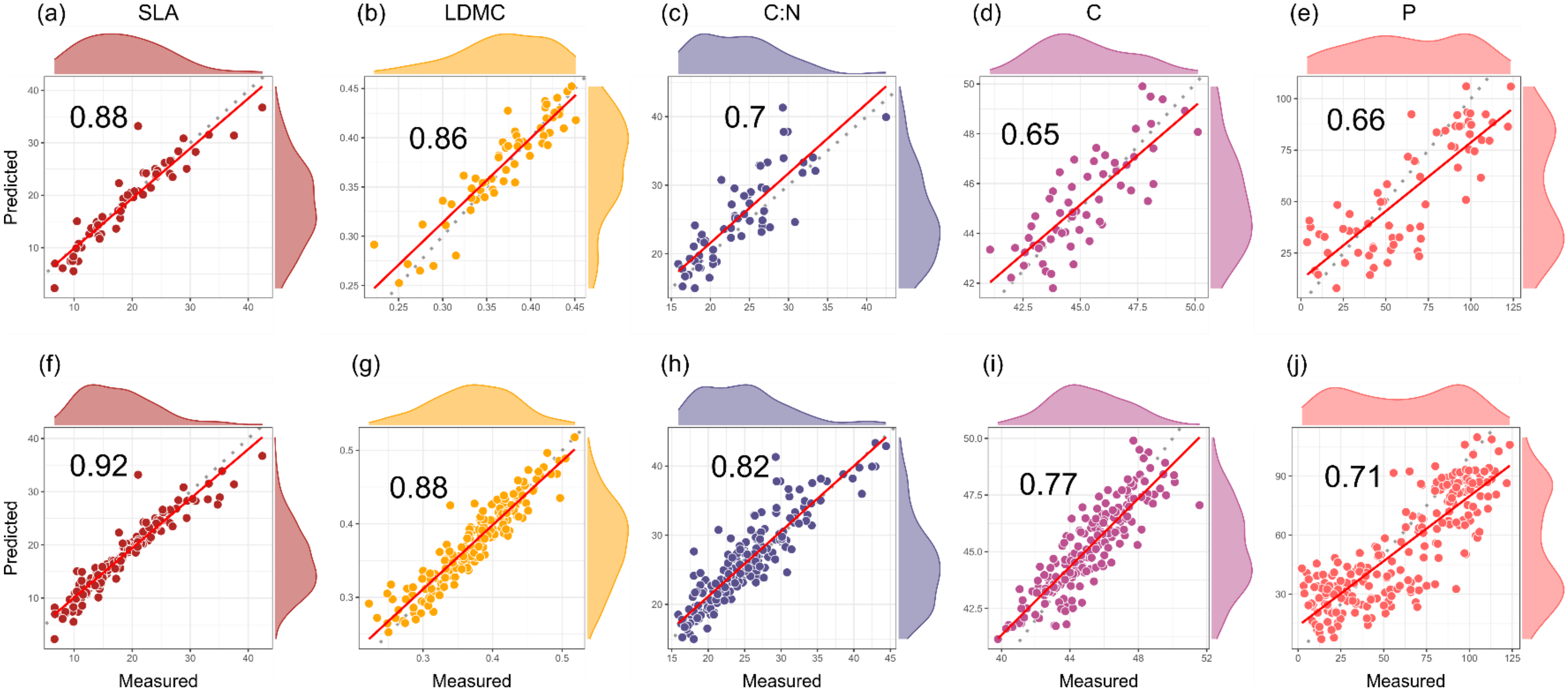

FIGURE S4 Correlation between predicted and measured traits values in (a, b, c, d, e) the test set (R^2_{test}) and in (f, g, h, I, j) the whole calibration set (R^2_{model}) for SLA, LDMC and C:N, C, P. Numbers inside the panels indicate the coefficient of determination.

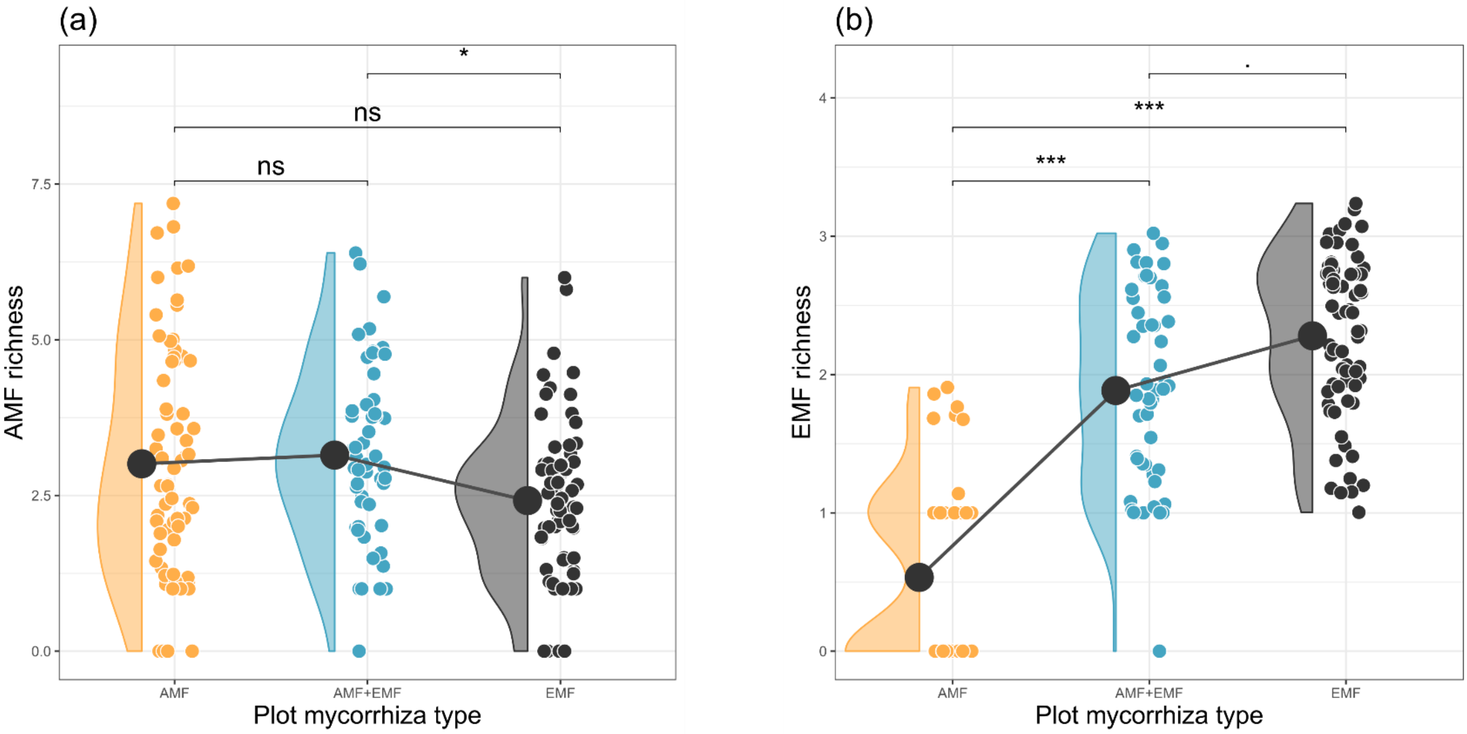

FIGURE S5 Violin plots for (a) arbuscular mycorrhizal fungal (AMF) richness and (b) ectomycorrhizal fungal (EMF) richness in the different mycorrhiza treatments in the MyDiv experiment (AMF in yellow, AMF + EMF in blue and EMF in black). The richness of mycorrhizal fungi had been previously rarefied to the minimum number of reads obtained through next-generation sequencing techniques applied to root samples of trees in the experiment. Asterisks indicate significant differences ($p < 0.05$) according to post-hoc Tukey tests (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; and ns, not significant) based in generalized linear mixed-models to study the effects of the treatments of the MyDiv experiment (based on different tree richness and host mycorrhizal types combinations). In addition, the linear mixed-models revealed that the richness of AMF and EMF was not affected by tree richness.

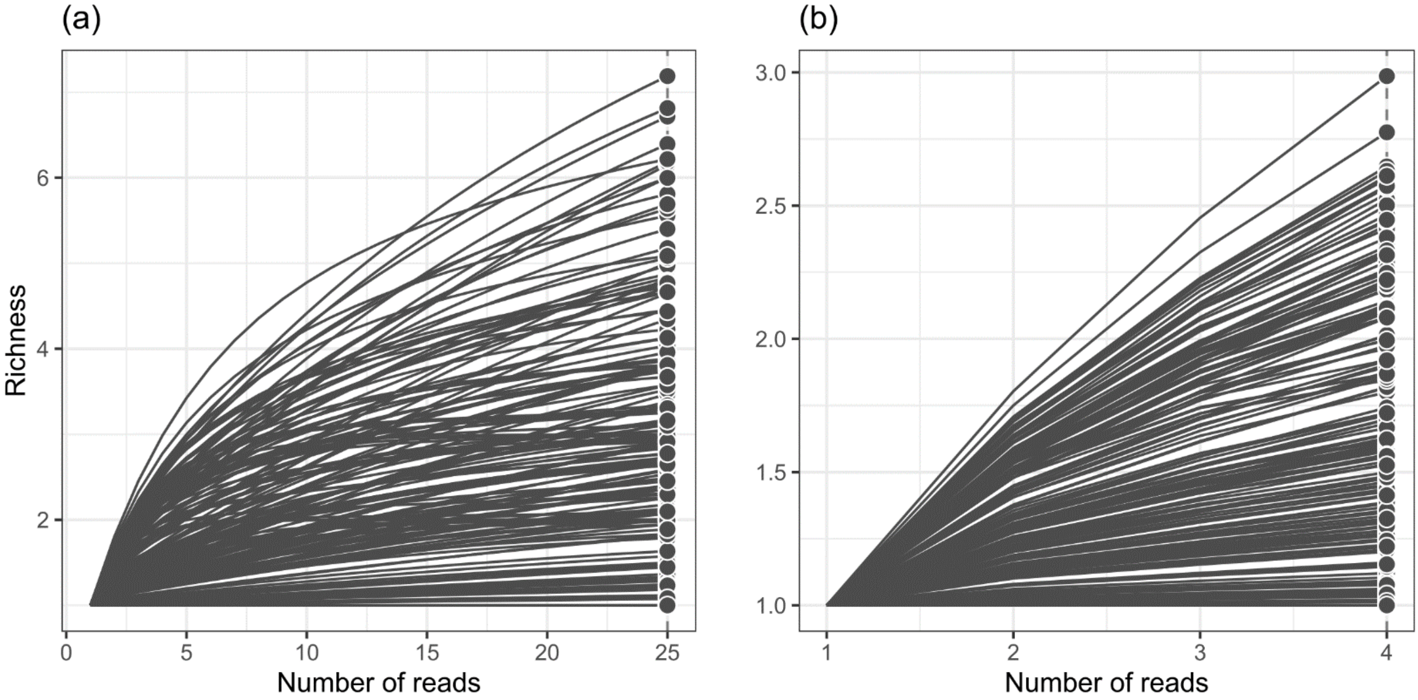

FIGURE S6 Rarefaction curves of (a) arbuscular mycorrhizal fungal richness and (b) ectomycorrhizal fungal richness to the minimum number of reads obtained through next-generation sequencing techniques applied to root samples of trees in the experiment.

FIGURE S7 Diagnostic plots for assumptions of normality, homoscedasticity and linearity in the linear mixed-effects models for the mean values of traits.

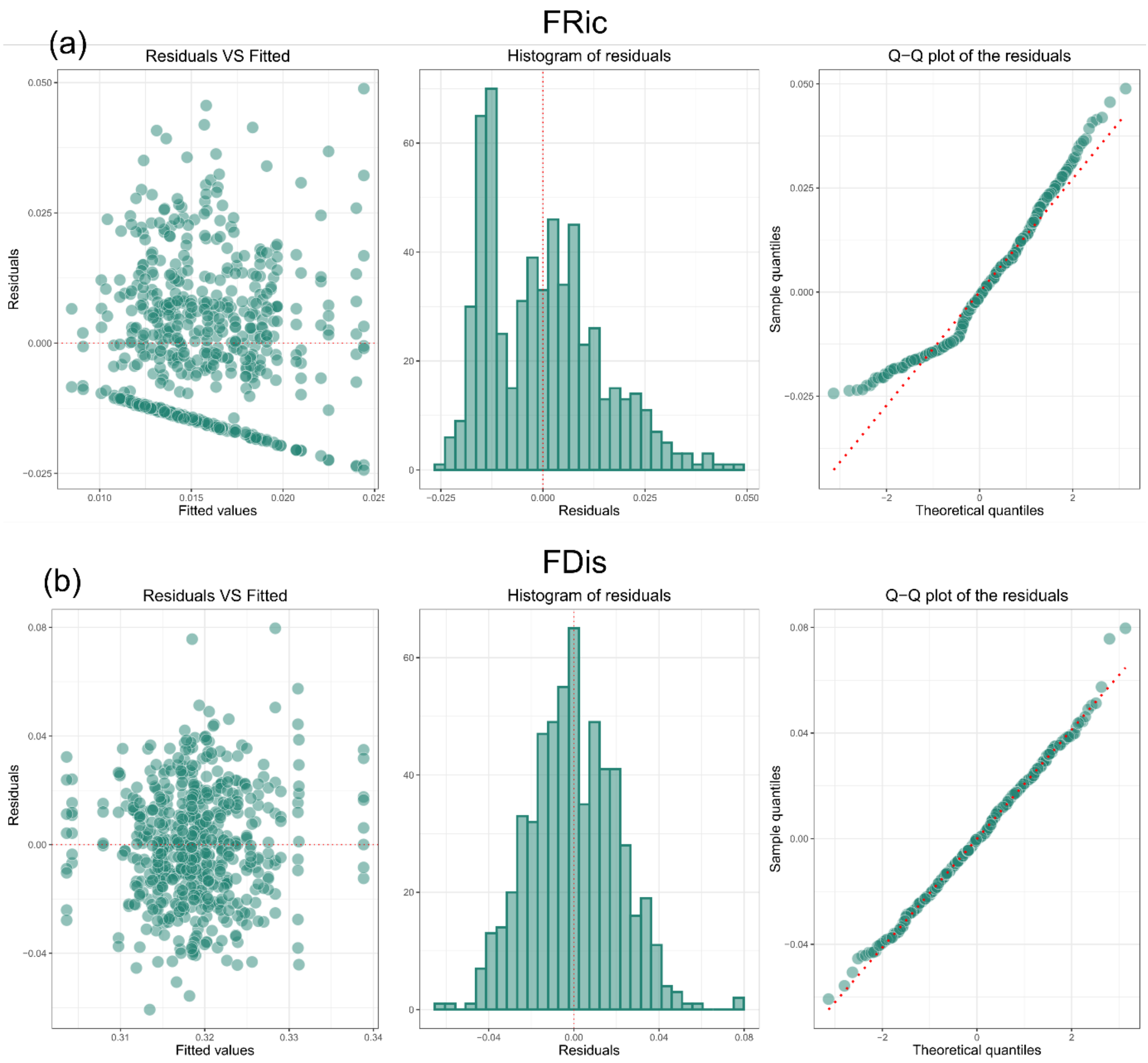

FIGURE S8 Diagnostic plots for assumptions of normality, homoscedasticity and linearity in the linear mixed-effects models for functional richness (FRic) and functional dispersion (FDis).

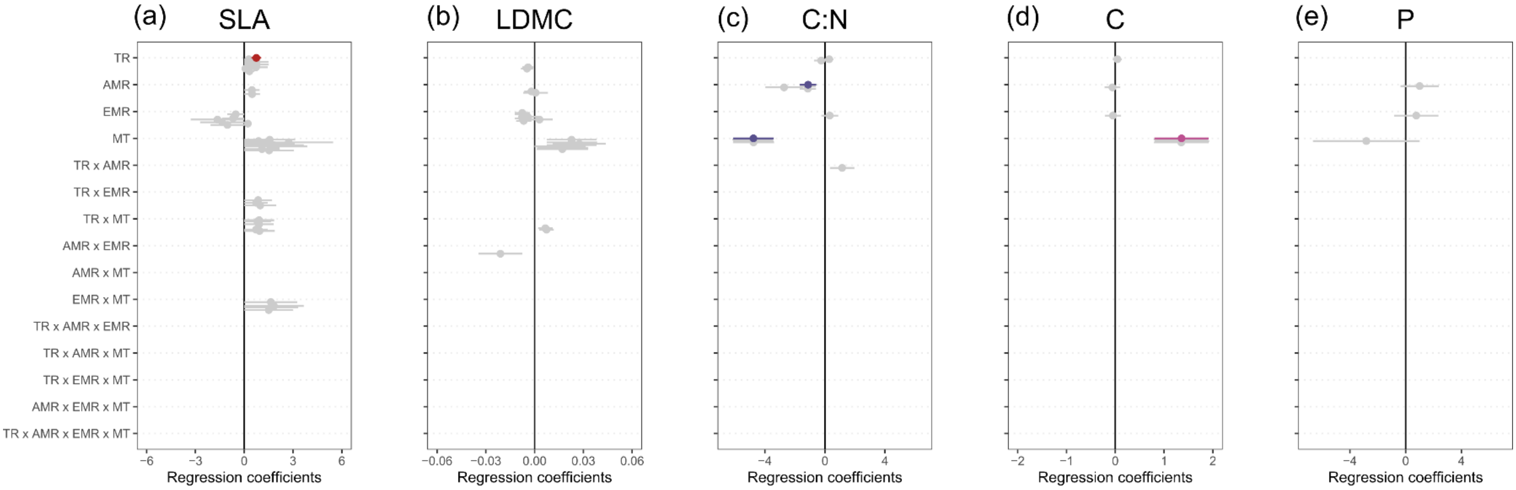

FIGURE S9 Effects in the simplest model (color) and competing models (grey; ΔAICc < 2) for the mean value of (a) SLA, (b) LDMC, (c) C:N, (d) C and (e) P, with 95% confidence intervals. The acronyms correspond to the different predictors (TR = tree richness; AMR = AMF richness; EMR = EMF richness; and MT = host mycorrhizal type), and interactions between predictors are indicated by "x". For host mycorrhizal type positive and negative coefficients indicate higher and lower values of EM trees compared to AM trees, respectively.

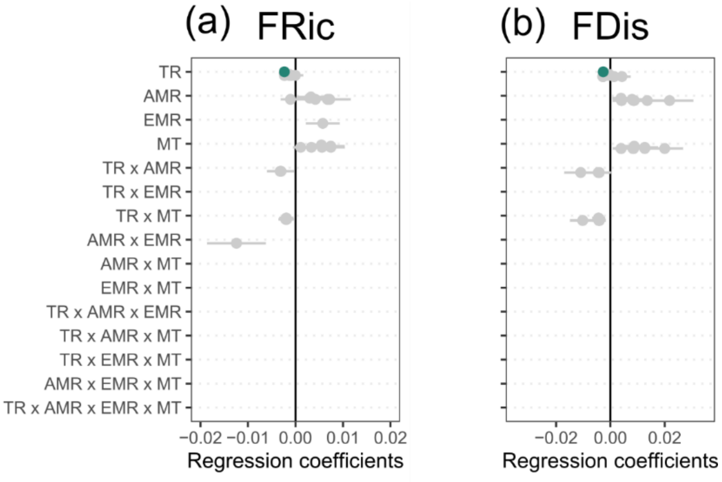

FIGURE S10 Effects in the simplest model (green) and competing models (grey; ΔAICc < 2) for (a) functional richness and (b) functional dispersion (FDis), with 95% confidence intervals. The acronyms correspond to the different predictors (TR = tree richness; AMR = AMF richness; EMR = EMF richness; and MT = host mycorrhizal type), and interactions between predictors are indicated by “x”. For host mycorrhizal type positive and negative coefficients indicate higher and lower values for EM trees compared to AM trees, respectively.

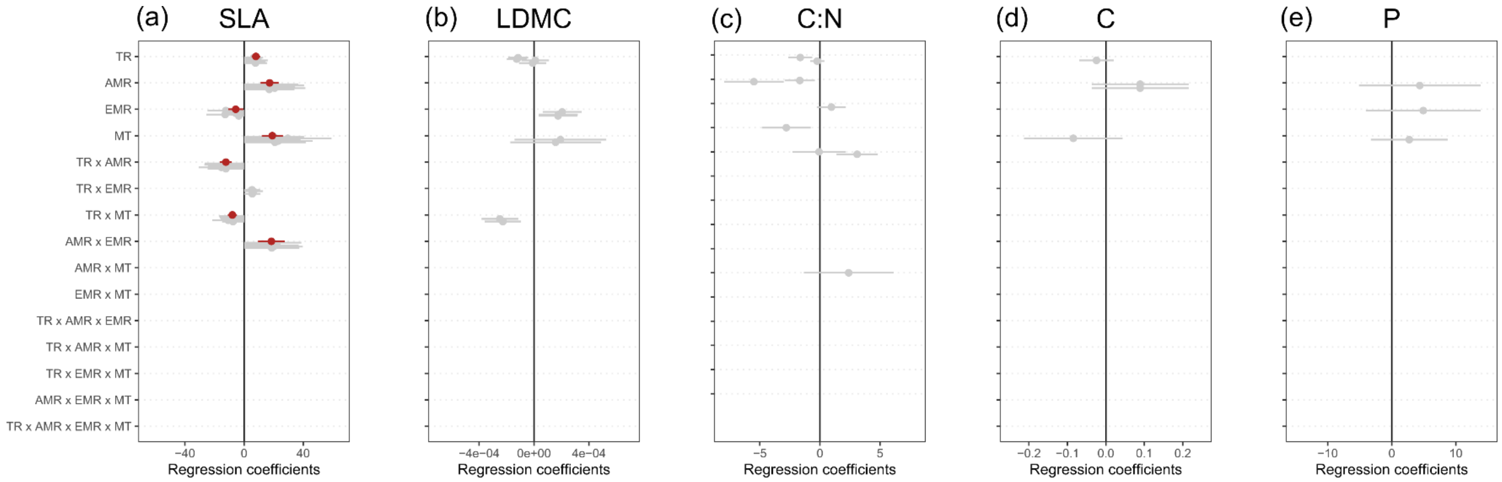

FIGURE S11 Effects in the best model (color) and competing models (grey; ΔAICc < 2) for the variance of (a) SLA, (b) LDMC, (c) C:N, (d) C and (e) P, with 95% confidence intervals. Variance was estimated for every trait in every tree. The acronyms correspond to the different predictors (TR = tree richness; AMR = AMF richness; EMR = EMF richness; and MT = host mycorrhizal type), and interactions between predictors are indicated by "x". For host mycorrhizal type positive and negative coefficients indicate higher and lower values of EM trees compared to AM trees, respectively.

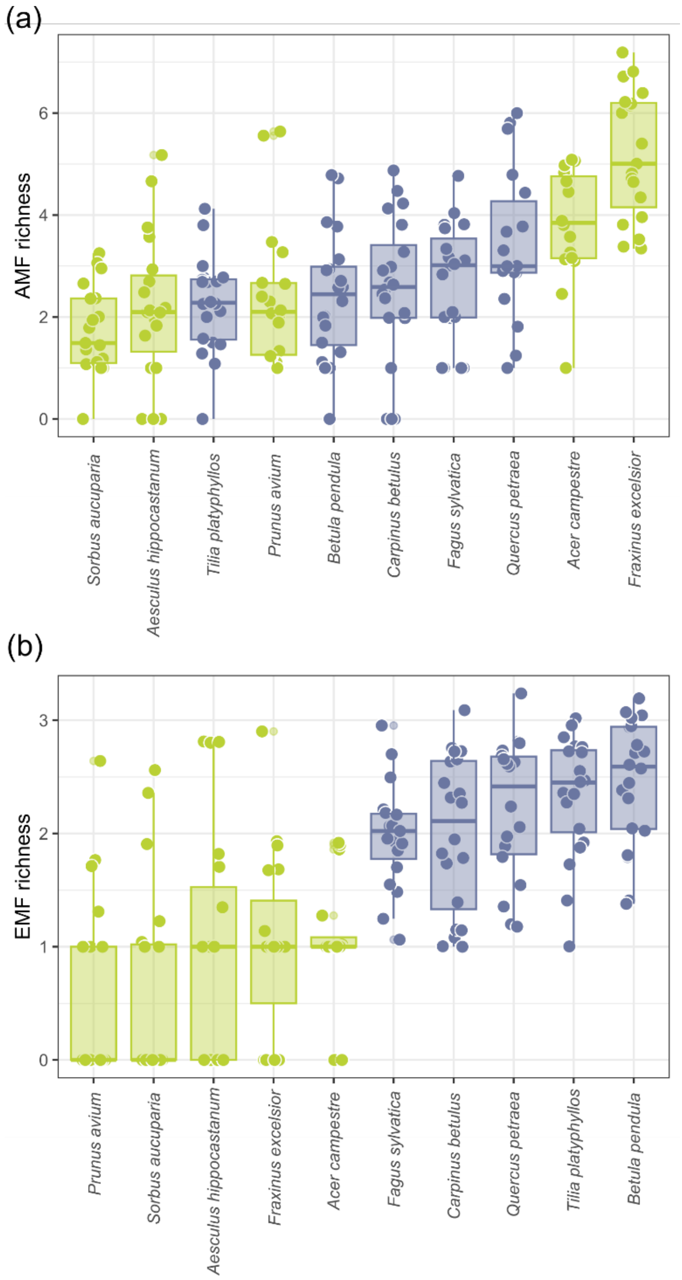

FIGURE S14 Boxplots for (a) AMF and (b) EMF richness (rarefied to the minimum number of reads obtained through next-generation sequencing techniques) associated to every tree species. The boxplots are ordered in the x-axis in (a) and (b) according to the mean value of AMF and EMF richness associated with every species, respectively. AM and EM trees are represented in green and blue, respectively.

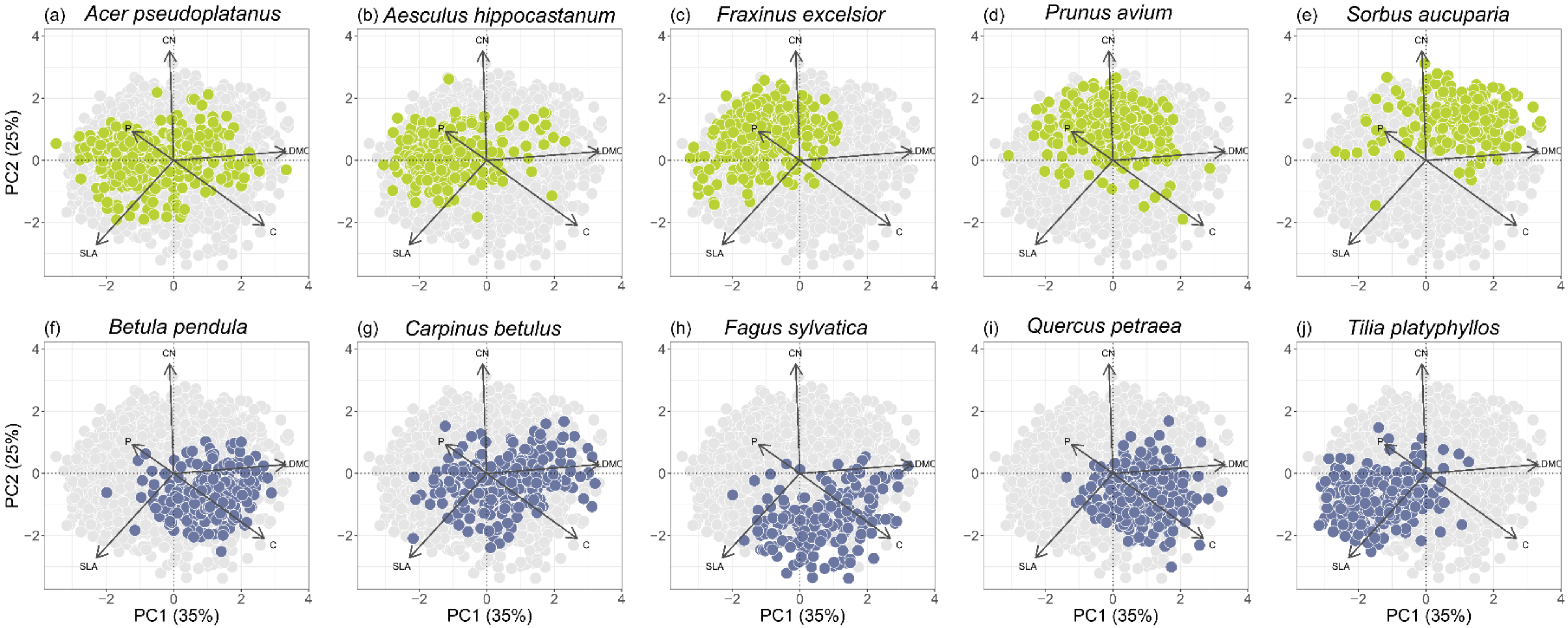

FIGURE S13 Two main axes of a principal component analyses (PCA) for five leaf functional traits for (a) *Acer pseudoplatanus*, (b) *Aesculus hippocastanum*, (c) *Fraxinus excelsior*, (d) *Prunus avium*, (e) *Sorbus aucuparia*, (f) *Betula pendula*, (g) *Carpinus betulus*, (h) *Fagus sylvatica*, (i) *Quercus petraea*, (j) *Tilia platyphyllos*. AM and EM trees are represented in green and blue, respectively.

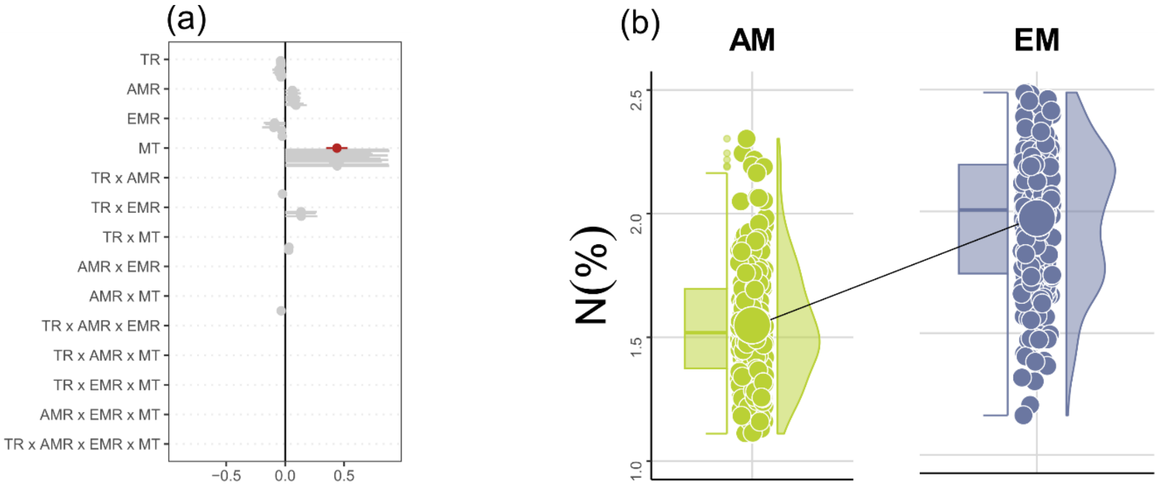

FIGURE S14 Effects in the best model (color) and competing models (grey; ΔAICc < 2) for the (a) mean of N, with 95% confidence intervals and main effects in response to AMF richness and the interaction between EMF richness and mycorrhiza type. The acronyms correspond to the different predictors (TR = tree richness; AMR = AMF richness; EMR = EMF richness; and MT = host mycorrhizal type), and interactions between predictors are indicated by "x". For host mycorrhizal type positive and negative coefficients indicate higher and lower values of EM trees compared to AM trees, respectively. Green and blue colours in (b) indicate AM and EM trees, respectively.

TABLE S1 Tree species in the MyDiv experiment and their mycorrhizal type with the number of leaves per individual tree which were collected for the calibration set of samples

Species	Family	Mycorrhizal type	Num. Leaves per ind. for calibration set
Acer pseudoplatanus L.	Sapindaceae	AM	3
Aesculus hippocastanum L.	Sapindaceae	AM	2
Fraxinus excelsior L.	Oleaceae	AM	2
Prunus avium L.	Rosaceae	AM	3
Sorbus aucuparia L.	Rosaceae	AM	2
Betula pendula Roth.	Betulaceae	EM	5
Carpinus betulus L.	Betulaceae	EM	3
Fagus sylvatica L.	Fagaceae	EM	3
Quercus petraea Liebl.	Fagaceae	EM	3
Tilia platyphyllos Scop.	Malvaceae	EM	3

TABLE S2 Layers and hyperparameters used for building a convolutional neural network for every trait

Layer	Hyperparameter	SLA	LDMC	C:N	C	P
1 dimension convolutional layer	Number of filters	2	2	1	2	1
1 dimension convolutional layer	Kernel size	50	2	25	50	100
Batch normalization layer	-					
Max-pooling layer	Pool size	2	2	2	2	2
Layer flatten	-					
Layer dense	Number of nodes	128	64	64	64	64
Layer dense	Number of nodes	32	16	16	16	16
Layer dense	Number of nodes	8	4	4	4	4
-	Epochs	5000	7000	1000	1000	100
-	Validation split	0.2	0.2	0.2	0.2	0.2

Note: -, no hyperparameter to tune.

TABLE S3 Species included in our study and number of individuals for every trait and functional indices (FDis and FRic) included in the analyses.

Species	Family	Host mycorrhizal type	Tree richness treatment	Mycorrhiza diversity treatment	SLA	LDMC	CN	C	P	FDis/FRic
Acer pseudoplatanus L.	Sapindaceae	AM	1	Mono	14	9	15	16	15	16
			2	Mono	15	14	15	16	15	16
			2	Mix	2	3	4	4	4	4
			4	Mono	8	8	11	11	11	11
			4	Mix	5	6	6	6	6	6
Aesculus hippocastanum L.	Sapindaceae	AM	1	Mono	14	10	14	13	11	14
			2	Mono	12	9	13	13	13	13
			2	Mix	6	4	8	6	8	8
			4	Mono	12	12	11	12	9	12
			4	Mix	6	5	5	5	5	6
Fraxinus excelsior L.	Oleaceae	AM	1	Mono	16	15	16	14	13	16
			2	Mono	16	16	16	13	13	16
			2	Mix	6	7	7	7	6	7
			4	Mono	13	11	13	7	12	13
			4	Mix	5	7	7	5	6	8
Prunus avium (L.) L.	Rosaceae	AM	1	Mono	14	15	13	15	12	15
			2	Mono	16	16	15	12	15	16
			2	Mix	4	4	2	4	4	4
			4	Mono	15	14	15	14	14	16
			4	Mix	6	6	6	6	6	6
Sorbus aucuparia L.	Rosaceae	AM	1	Mono	5	10	9	14	15	16
			2	Mono	14	15	11	14	15	16
			2	Mix	6	7	3	8	7	8
			4	Mono	12	13	9	12	13	14
			4	Mix	4	6	3	6	5	6
Betula pendula Roth	Betulaceae	EM	1	Mono	16	16	13	10	4	16
			2	Mono	15	15	14	7	15	15
			2	Mix	7	8	8	7	8	8
			4	Mono	16	16	15	8	13	16
			4	Mix	8	8	6	6	6	8
Carpinus betulus L.	Betulaceae	EM	1	Mono	8	11	15	16	9	16
			2	Mono	13	10	16	15	10	16
			2	Mix	6	8	8	8	6	8
			4	Mono	15	16	16	16	11	16
			4	Mix	6	6	8	8	6	8
Fagus sylvatica L.	Fagaceae	EM	1	Mono	6	4	10	12	4	14
			2	Mono	10	9	15	14	13	16
			2	Mix	5	6	6	3	3	7
			4	Mono	8	14	12	14	11	15
			4	Mix	3	6	6	5	4	6
Quercus petraea (Matt.) Liebl.	Fagaceae	EM	1	Mono	9	16	15	13	16	16
			2	Mono	14	16	14	15	15	16
			2	Mix	6	7	5	7	6	7
			4	Mono	12	12	11	11	12	12
			4	Mix	8	8	7	8	8	8
Tilia platyphyllos Scop.	Malvaceae	EM	1	Mono	13	12	10	8	14	15
			2	Mono	12	8	14	13	13	16
			2	Mix	5	7	4	7	6	7
			4	Mono	13	12	15	13	14	16
			4	Mix	5	7	4	6	8	8
Total					**485**	**500**	**514**	**503**	**488**	**589**

TABLE S4 Model selection for the analyses of the drivers of the mean value for five functional traits. The simplest models which include a subset of predictors in the rest of the competing models are marked in bold. The acronyms correspond to the different predictors (TR = tree richness; AMF = AMF richness; EMF = EMF richness; and MT = host mycorrhizal type), and interactions between predictors are indicated by "x". Whether the predictor is present is indicated by "+".

Trait	TR	MT	AMF	EMF	TR X MT	TR X AMF	TR X EMF	MT X AMF	MT X EMF	AMF X EMF	TR X MT X AMF	TR X MT X EMF	TR X AMF X EMF	MT X AMF X EMF	TR X MT X AMF X EMF	df	ΔAICc	R^2m	R^2c
SLA	+	+			+											9	0.00	0.10	0.73
	+															**7**	**0.62**	**0.02**	**0.71**
	+	+		+	+				+							11	1.00	0.10	0.73
	+	+														8	1.01	0.11	0.73
	+	+	+		+											10	1.10	0.10	0.73
	+	+		+					+							10	1.34	0.11	0.73
	+	+		+			+		+							11	1.45	0.11	0.73
	+		+													8	1.68	0.02	0.71
	+	+		+	+		+		+							12	1.71	0.11	0.74
	+	+		+	+											10	1.86	0.11	0.73
	+			+			+									9	1.98	0.02	0.71
LDMC		+		+												8	0.00	0.06	0.79
				+												7	0.19	0.00	0.79
		+														7	0.63	0.07	0.79
																6	**0.87**	**0.00**	**0.80**
		+		+					+							9	1.35	0.07	0.79
		+	+	+												9	1.86	0.06	0.79
		+	+	+				+		+						11	1.87	0.07	0.79
	+	+		+	+											10	1.90	0.08	0.79
	+	+			+											9	1.98	0.09	0.80
C:N		**+**	**+**													**8**	**0.00**	**0.27**	**0.66**
	+	+	+													9	1.10	0.27	0.66
	+	+	+			+										10	1.22	0.27	0.66
		+	+	+												9	1.78	0.26	0.66
C		**+**														**7**	**0.00**	**0.18**	**0.77**
	+	+														8	1.86	0.18	0.77
		+	+													8	1.92	0.18	0.77
		+		+												8	1.97	0.17	0.77
P																**6**	**0.00**	**0.00**	**0.69**
			+													7	1.53	0.00	0.69
		+														7	1.54	0.01	0.70
				+												7	1.83	0.00	0.69

Note: ΔAICc, delta of Akaike information criterion; R^2m, marginal R^2; R^2c, conditional R^2.

TABLE S5 Model selection for the analyses of the drivers of the multi-trait functional indices. The simplest models which include a subset of predictors in the rest of the competing models are marked in bold. The acronyms correspond to the different predictors (TR = tree richness; AMF = AMF richness; EMF = EMF richness; and MT = host mycorrhizal type), and interactions between predictors are indicated by "x". Whether the predictor is present is indicated by "+".

	TR	MT	AMF	EMF	TR X MT	TR X AMF	TR X EMF	MT X AMF	MT X EMF	AMF X EMF	TR X MT X AMF	TR X MT X EMF	TR X AMF X EMF	MT X AMF X EMF	TR X MT X AMF X EMF	df	ΔAICc	R^2m	R^2c
FRic	+															7	0.00	0.02	0.03
	+	+	+					+								10	0.68	0.03	0.04
	+	+	+		+			+								11	1.11	0.03	0.04
	+	+	+			+		+								11	1.42	0.03	0.04
	+	+														8	1.68	0.02	0.03
	+		+													8	1.83	0.02	0.03
	+	+			+											9	1.85	0.02	0.04
	+		+	+						+						10	1.86	0.02	0.03
	+	+	+		+	+		+								12	2.00	0.03	0.04
FDis	+	+			+											9	0.00	0.01	0.07
	+	+	+		+											10	0.49	0.03	0.07
	+	+	+		+			+								11	0.75	0.03	0.07
	+															7	1.15	0.01	0.07
	+	+	+		+	+		+			+					13	1.35	0.04	0.08
	+	+														8	1.44	0.02	0.07
	+		+													8	1.55	0.01	0.07
	+	+	+		+	+										11	1.71	0.03	0.08
	+	+	+		+	+		+								12	1.79	0.03	0.08
	+	+	+					+								10	1.80	0.02	0.07
	+	+	+													9	1.82	0.02	0.07

Note: ΔAICc, delta of Akaike information criterion; R^2m, marginal R^2; R^2c, conditional R^2.

CHAPTER 3

Intraspecific and intraindividual trait variability decrease with tree species richness in a subtropical tree biodiversity experiment

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ABSTRACT

Phenotypic variability within tree species responds to local tree species richness. However, we lack evidence on how different sources of trait variation shape tree-tree interactions. Along a diversity gradient from one to eight tree species, we sampled 4,568 leaves from 381 trees to study changes in intraspecific and intraindividual leaf trait variability and assessed their contribution to community functional diversity. Intraspecific variability decreased with tree species richness, while intraindividual variability barely responded. Functional overlap between conspecific trees increased with tree species richness and through intraindividual variation, but was reduced through intraspecific variability, meaning that intraspecific variability may reduce intraspecific competitive interactions while intraindividual variability could arise due to varying microenvironmental conditions within the canopy. Last, intraspecific and intraindividual variability explained high community functional richness and divergence, respectively, especially in mixtures. Our findings emphasize that fine-scale variability influences tree-tree interactions and can be a driver of local functional diversity.

Teaser

Trait variability within tree species decreases with species diversity and explains high forest functional diversity.

INTRODUCTION

Plant trait-based ecology focuses on phenotypic differences as a way to understand ecological and evolutionary processes (1–3). While the field has typically focused on differences between species, substantial trait variation occurs at different levels of biological organization (among populations, between individuals within the same population, or within individuals; 4, 5) which could be important to understand adaptations to the environment (6) and species coexistence (7). For instance, in response to competition, individuals are able to shift the trait expression to adopt a more conservative strategy in the use of resources (8) or to prevent local competitive exclusion by increasing dissimilarities with other individuals (9). However, while these shifts have been widely studied, less attention has been paid to the variability of traits within the same population, hereafter referred to as intraspecific trait variability (i.e. the extent of the differences between the trait values of individuals from the same population of a species).

Intraspecific trait variability within populations may reduce intraspecific competition by allowing individuals from the same species (conspecifics) to exploit alternative resources (10, 11). Therefore, given that conspecifics acquire and use resources in a similar way, intraspecific trait variability is expected to be larger in species-poor communities. Further, intraspecific trait variability depends not only on intraspecific competition but also on niche availability (12). That is why, when the number of species in a community increases (which commonly results in resource partitioning; 13), trees tend to become more dissimilar from the heterospecific neighbors. As a result, species may adopt a so called niche packing strategy characterized by the exploitation of a specific resource in a specific manner, resulting in lower intraspecific trait variability compared to monocultures (14). For example, conspecific trees in monocultures have been found to produce leaves with different specific leaf areas (SLA) to exploit different sections of the light gradient; by contrast, in mixed communities, as species specialize in exploiting specific parts of the canopy space, conspecifics tended to produce leaves with similar SLA (15). As a result, limited intraspecific variability may act as a mechanism that would allow species to exploit different niches, resulting in species complementarity in species-rich communities (16). In fact, recent studies found that intraspecific leaf variability of plant populations decreased with increasing plant diversity (14, 17, 18). However, these results contrast with other studies and with the responses found for other tree organs other than leaves (19–22). For instance, Yang et al. (22) suggested that inferior competitor species may express higher intraspecific trait variability, especially in seedling roots, to reduce the chances of being outcompeted when growing with a strong competitor species. These contrasting results among studies propose that there is no general direction of change of intraspecific trait variability in response to species diversity, but that it likely depends on the specific interaction partners as well as the plant organ studied.

Scaling down in the levels of biological organization, intraindividual variability, i.e., the extent of different trait values across different repeated architectural units of the plant body structure (e.g. leaves from the same plant; 23–25), could also matter for plant-plant interactions. Such plastic responses are especially noticeable in trees because they have a great potential to express intraspecific and intraindividual trait variability due to their longevity and extensive modularity (26). For instance, as the light interception by leaves is a key factor in competition (27), trees express different leaf phenotypes within individuals to adjust to light exposure (e.g. leaves directly exposed to sunlight show higher photosynthetic rates and lower drought tolerance than shade leaves; 27, 28). Additionally, it has been suggested that plants can experience intraindividual changes in eco-physiological traits that may eventually lead to enhanced water-use efficiency (29) or cope with environmental unpredictability (30). As tree composition affects crown packing and, therefore, light exposure, leaves also respond to the surrounding diversity (15). In fact, intraindividual leaf variability in trees was observed to decrease with local tree species diversity (15, 31) and it has been suggested that, similarly to intraspecific variability, high intraindividual leaf variability could support intraspecific complementarity (functional complementarity between conspecifics). This would imply that, under scenarios of high intraindividual variability, conspecifics may tend to be dissimilar in their leaves by exploiting new niches. Nevertheless, this role of intraindividual variation in interactions between trees and the mechanisms involved remain still unclear. For instance, these changes can be useful for trees growing in monocultures, where canopy density and structural diversity are lower and therefore temperatures less buffered compared to mixtures (32). That is why, in order to clarify whether intraindividual variability generates intraspecific complementarity, it is important to understand the patterns of intraindividual variability along tree species diversity gradients as well as its effect on how different conspecifics overlap in their traits.

Functional diversity (i.e. the extent of phenotypic differences in a community) is one of the most common tools in trait-based ecology (33) and can reveal key facets of ecosystem functioning (e.g. net primary productivity, biochemical cycles) and community assembly (e.g. environmental filtering, limiting similarity; 1, 34). Functional diversity estimations typically consider a single mean trait value for each species; this strategy reduces the amount of trait measurements but neglects trait variation within species (2). Nevertheless, intraspecific trait variability can account for a non-negligible proportion of the total trait variability within and across ecological communities (4, 35). Further, approaches considering intraindividual trait variability have shown that the sum of the variation occurring intraspecifically and intraindividually may be even larger than the differences between species in the case of some leaf traits such as SLA or leaf nitrogen content (5, 36). This shows the importance of studying species traits beyond single mean trait values to quantify community functional diversity, especially at local scales and in species-poor communities (37–40). Therefore, it has been suggested that the different sources of trait variation occurring within species, from the variability between populations to the intraindividual variability, could affect community functional diversity (41). In recent years, different methods to incorporate variability into functional diversity metrics have been developed (37, 42–45). The use of these methods allows testing the notion that community functional diversity is higher when considering intraspecific or intraindividual variability (41, 46), as well as understanding how this effect changes with species richness.

Here, we studied the patterns of intraspecific and intraindividual leaf trait variability in a tree diversity experiment in subtropical China (BEF-China; 47). By using leaf spectroscopy, we estimated seven morphological and chemical leaf traits in 381 tree individuals from eight species along a tree species richness gradient with monocultures and mixtures of 2, 4 and 8 tree species. We assessed population functional trait variability by using two functional indices that measure different facets of the functional space (functional richness and functional divergence) at the intraspecific (mean trait values of individual trees) and the intraindividual level (leaf trait values within an individual tree), respectively. Further, we assessed intraspecific overlap as the shared trait space between the trait distributions of conspecific trees belonging to the same population. Specifically, we aimed (1) to determine how tree species richness affects intraspecific and intraindividual leaf trait variability (Fig. 1A), and (2) to assess the direct and indirect effects (via intraspecific and intraindividual variability) of tree species richness on intraspecific trait overlap. Further, we used a framework that allows including hierarchical sources of trait variation on community functional diversity, from the population level to the leaf level, passing through the individual level (37, 48), and null models to identify which sources of variation within species affect functional diversity (Fig. 1B). With this, we aimed (3) to characterize the influence of intraspecific and intraindividual variability on the functional diversity of a community across levels of tree species richness. We hypothesized that:

H1: Intraspecific and intraindividual variability decrease with increasing tree species richness (Fig. 1A).

H2: Intraspecific overlap increases with tree species richness along a spectrum from high intraspecific complementarity in monocultures to low intraspecific complementarity in mixed communities (H2a; Fig. 1A). Intraspecific overlap increases with tree species richness via a decreasing intraspecific and intraindividual variability (H2b).

H3: As the organization of intraspecific and intraindividual trait variability is expected to contribute to community functional diversity, the observed functional diversity of tree communities is expected to be higher than the functional diversity measured from null models that randomize different sources of trait variation (H3a; Fig. 1B). Moreover, the deviations of observed functional diversity from null models decrease with tree species richness due to the expected responses of intraspecific and intraindividual trait variability with the number of species in a community (H3b; see H1). Last, these differences are expected to be highest in null models that randomize trait variation at the lowest level of biological organization measured (i.e. leaf level), as these randomizations imply random intraspecific and intraindividual variability (H3b).

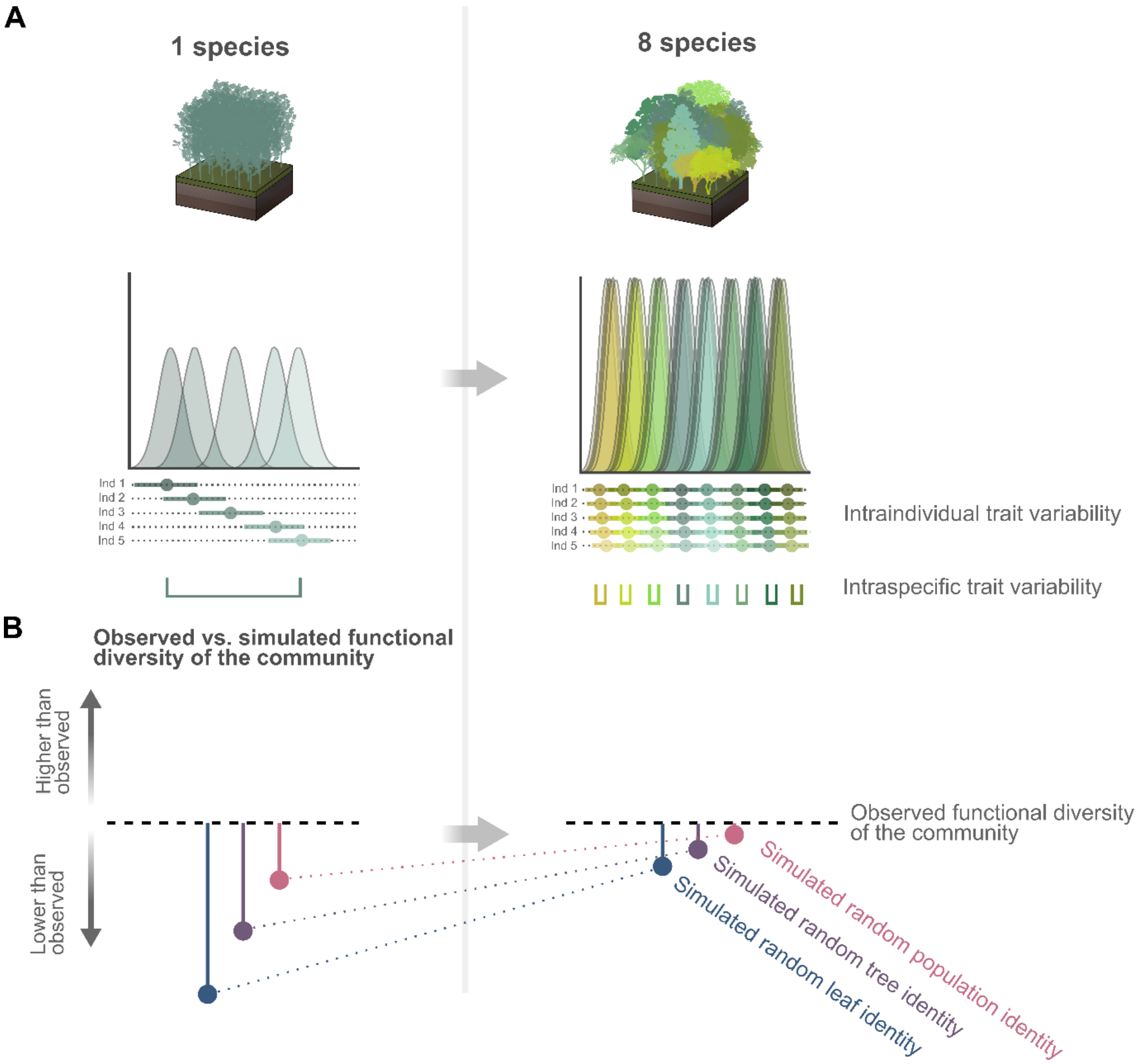

Fig. 1. Expected patterns along the experimental tree species richness gradient. Along the tree species richness gradient, we expect (**A**) a reduction of intraspecific trait variability (i.e. differences between the mean trait values of individual trees) and of intraindividual trait variability (i.e. differences between the trait values within a tree; represented as error bars around points of individual mean trait values), which would result in increasing intraspecific overlap (i.e. shared trait space between trees belonging to the same population). Curves represent the trait distributions of populations, with inner stacked curves belonging to the trait distribution of tree individuals. The structure of trait variation within species can influence community functional diversity and that is why (**B**) we expect functional diversity in observed communities (represented as a baseline with a grey dashed line) to be higher compared to the functional diversity of virtual assemblages (colored values) for which different sources of trait variation have been randomized. Specifically, we expect that the functional diversity of observed communities would be more similar to those assessed with models that randomize the identity of the populations (pink points) compared to those assessed with models that randomize the identity of the trees (purple points), and the total pool of leaves within a species (blue points), respectively. In addition, we expect these differences to be higher with low tree species richness due to the importance of intraspecific and intraindividual trait variability in the functional diversity of species-poor communities.

RESULTS

Responses of intraspecific and intraindividual variability to tree species richness

The first two axes of a principal component analysis (PCA) on the leaf-level values of seven functional traits (N = 4,568) explained 68% of the total variation in our dataset (Fig. 2A). PC1 was strongly associated with leaf dry matter content (LDMC), specific leaf area (SLA) and leaf carbon content (C) (with loadings -0.94, 0.89 and -0.73, respectively; Table S1) and reflected differences in the acquisition of resources from conservative leaves to acquisitive leaves. PC2 was related to leaf nitrogen content (N), leaf phosphorus (P) and stomatal density (SDens), followed by stomatal size (Ssize) (with loadings 0.75, 0.64, 0.63 and -0.42, respectively) and reflected differences in nutrition status, which seems to be closely linked to the stomatal morphology.

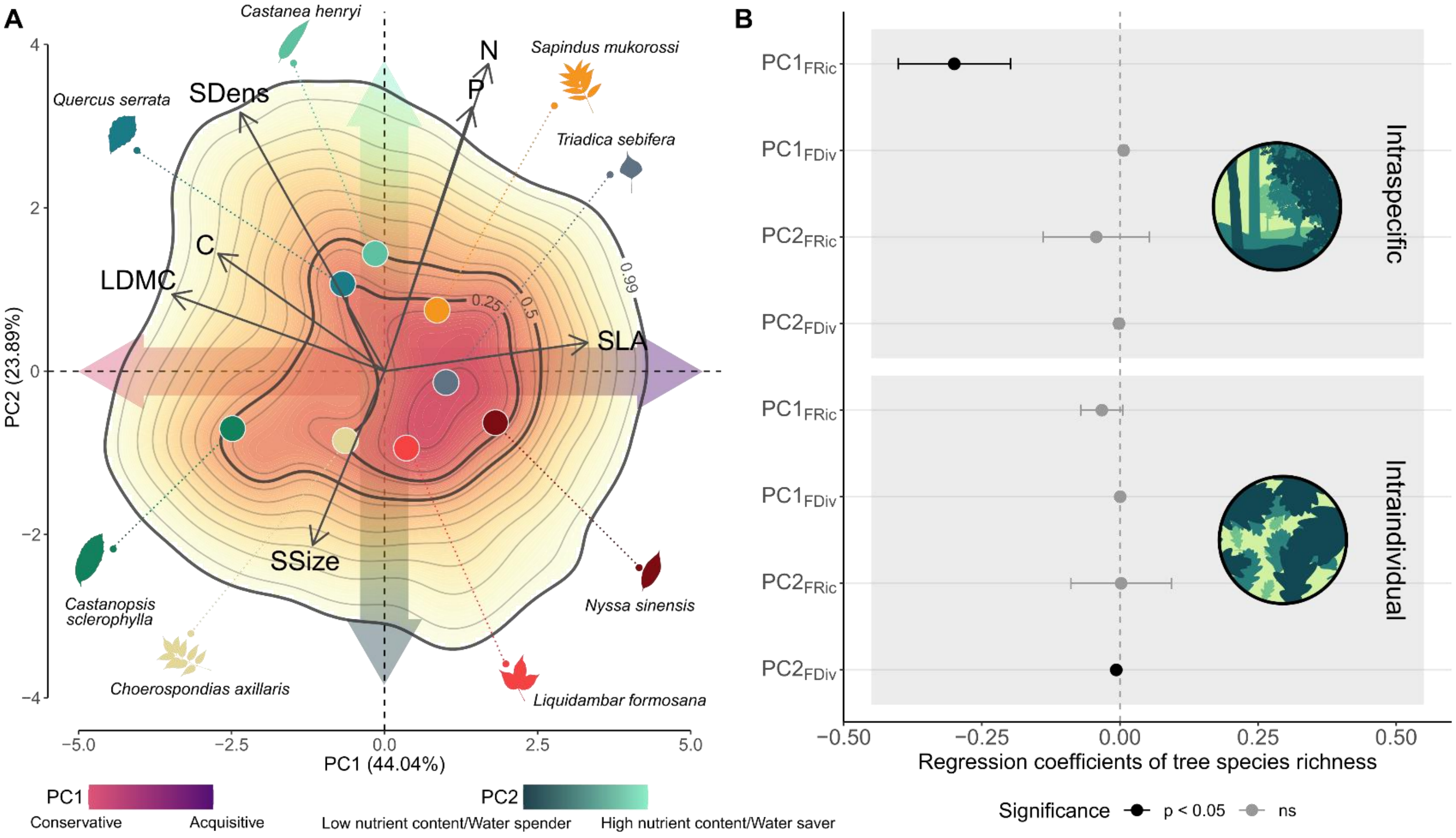

Fig. 2. Main axes of leaf trait variation and effect of tree species richness on intraspecific and intraindividual leaf trait variability. (**A**) Biplot for the first two axes of a principal component analysis (PCA) of seven functional traits predicted for leaves collected in eight tree species growing in a subtropical tree diversity experiment (N = 4568; colored points: mean species values). Data are based on spectroscopically predicted trait values of leaves collected from trees growing along an experimental species richness gradient with mixtures of 1, 2, 4 and 8 tree species. The color gradient visualizes different probability densities, with red colors corresponding to portions of the space displaying the highest densities of observations. The first component (PC1) reflects a gradient from “conservative” leaves (towards the left) that are expected to have a longer lifespan and higher survival probability in response to abiotic and biotic hazards than cheaply constructed “acquisitive” leaves (towards the right) which are expected to have higher photosynthetic rates (Fig. S2, S3). The second component (PC2) reflects a gradient in nutrition status and stomatal morphology ranging from “low nutrient content” and “water spender” leaves (towards the bottom) with low photosynthetic capacity and few large stomata to “high nutrient content” and “water saver” leaves that could show high photosynthetic rates and have many small stomata that could decrease stomatal conductance quickly in response to water shortage (*49*, *50*). Colors and leaf silhouettes correspond to the different tree species included in the study (Table S3). Regression estimates from linear mixed-effects models to study intraspecific and intraindividual variability of PC1 and PC2 (**B**) show a significant decrease in intraspecific functional richness of PC1 with increasing tree diversity (P = 0.01) and a significant decrease of intraindividual functional divergence of PC2 (P = 0.04). Regression coefficients are shown for the intraspecific level (top, N = 63) and intraindividual level (bottom, N = 381). Colors represent the significance as determined by a likelihood ratio test (black P < 0.05, grey P > 0.05).

Overall, intraspecific leaf trait variability within populations decreased with tree species richness. First, analyses with single axes of leaf trait variation, which aimed to detect changes associated with specific axis of leaf trait variation, revealed that intraspecific variability in PC1 decreased with tree species richness (P = 0.01 for functional richness (FRic; the extent of the functional volume of the population); Fig. 2B, Table S2). However, this effect was not significant for the intraspecific variability of PC2. Second, we estimated trait probability densities based on both PC1 and PC2 (multivariate FRic) to assess the main changes in the total trait space. This analysis revealed that tree species richness also had a significant negative effect on multivariate FRic (P = 0.047, N = 63; Fig. 3A). In contrast, we found no effect of tree species richness on multivariate functional divergence (FDiv; the degree to which the abundance in the trait space is distributed towards the extremes of the functional volume) (P = 0.36; Fig. 3C). The results for intraspecific variability contrast with the effects found for the intraindividual level, as only intraindividual FDiv of PC2 responded negatively to tree species richness, and this response was small (P = 0.04; Fig. 2B). This result is consistent with the effects of tree species richness on the intraindividual FRic of stomatal density (P = 0.03; Fig. S1). We found no effect of tree species richness on any of the multivariate functional indices used at the intraindividual level (FRic and FDiv with P = 0.51 and P = 0.69, respectively; N = 381; Fig. 3B, D).

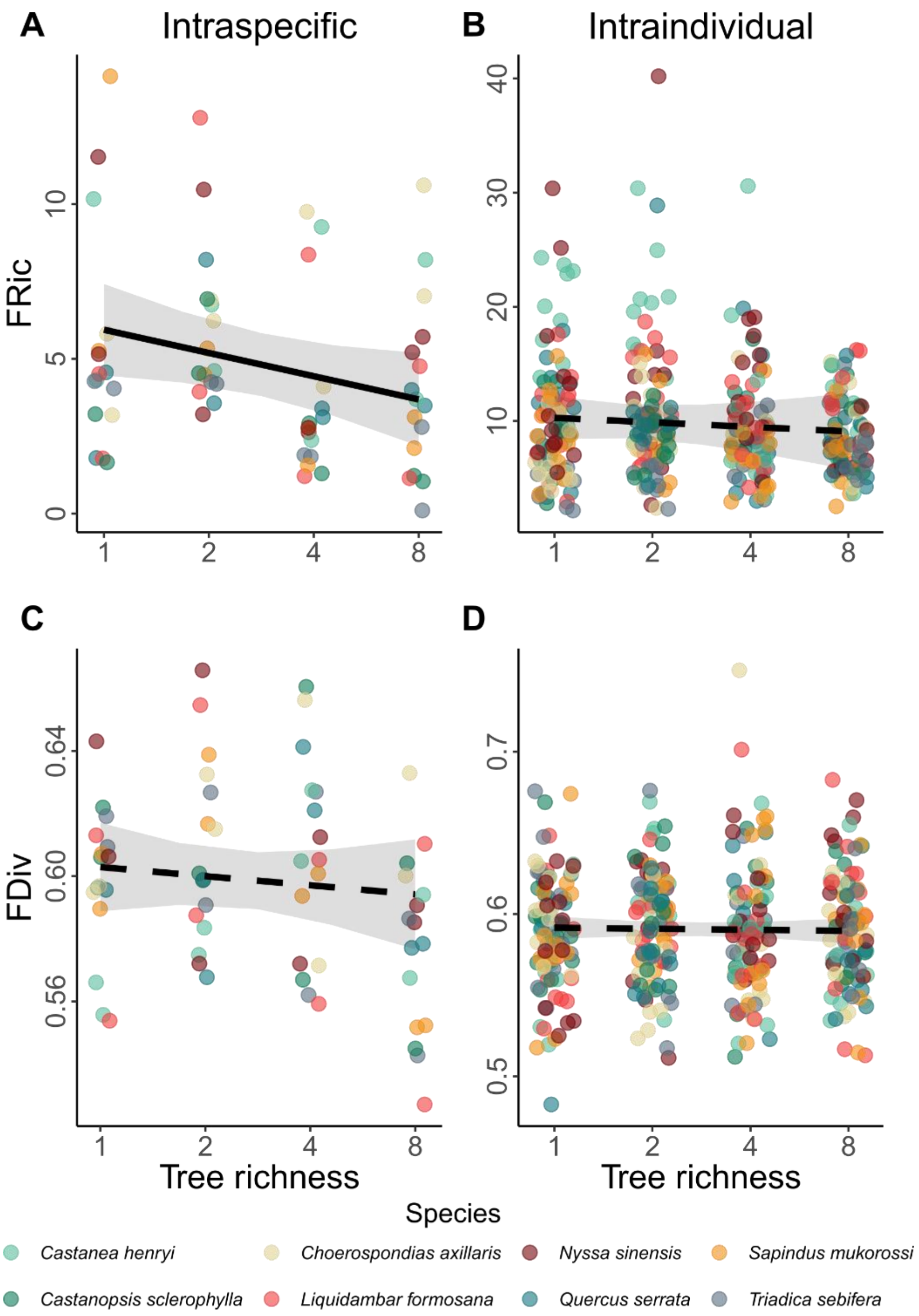

Fig. 3. Effect of tree species richness on leaf trait variability at the intraspecific and intraindividual levels, as reflected by two multivariate functional indices. Lines correspond to the results of linear mixed-effects models that show (**A**) a significant decrease of intraspecific functional richness (FRic) with increasing tree species richness (P = 0.047, N = 63) and non-significant effects of tree species richness on (**C**) intraspecific functional divergence (FDiv) and (**B**, **D**) intraindividual FRic and FDiv (N = 381). Data are based on measurements of seven morphological and chemical leaf traits in an experimental species richness gradient with monocultures and mixtures of 2, 4 and 8 tree species. Grey bands represent a 95% confidence interval. Colors correspond to the different tree species included in the study (Table S3), whose identity was included as a random effect in our models.

Effects of intraspecific and intraindividual trait variability on intraspecific overlap

We used a structural equation model (SEM) to understand the relationships leading conspecific trees to overlap in the functional trait space of their population (Fig. S4, S5). Our model fit the data well (Fisher's C = 0.68, df = 2, P = 0.76, N = 63). We found that changes in the intraspecific overlap (the mean overlap between the functional volumes of conspecific trees within a population; Fig. 4) are well explained by tree species richness and multivariate intraspecific and intraindividual FRic (marginal $R^2 = 0.57$, conditional $R^2 = 0.72$). Intraspecific FRic significantly decreased with tree species richness (P = 0.04) and had in turn a negative impact on intraspecific overlap (P < 0.001). However, we did not find any effect of tree species richness on intraindividual FRic (P = 0.49), and we found an increase of intraspecific overlap with intraindividual FRic (P < 0.001). Additionally, tree species richness also had a direct positive effect on intraspecific overlap (P = 0.01). These results remained qualitatively similar in SEMs with functional indices based on single axes of trait variation (PC1 or PC2 of Fig. 2A; Fig. S6).

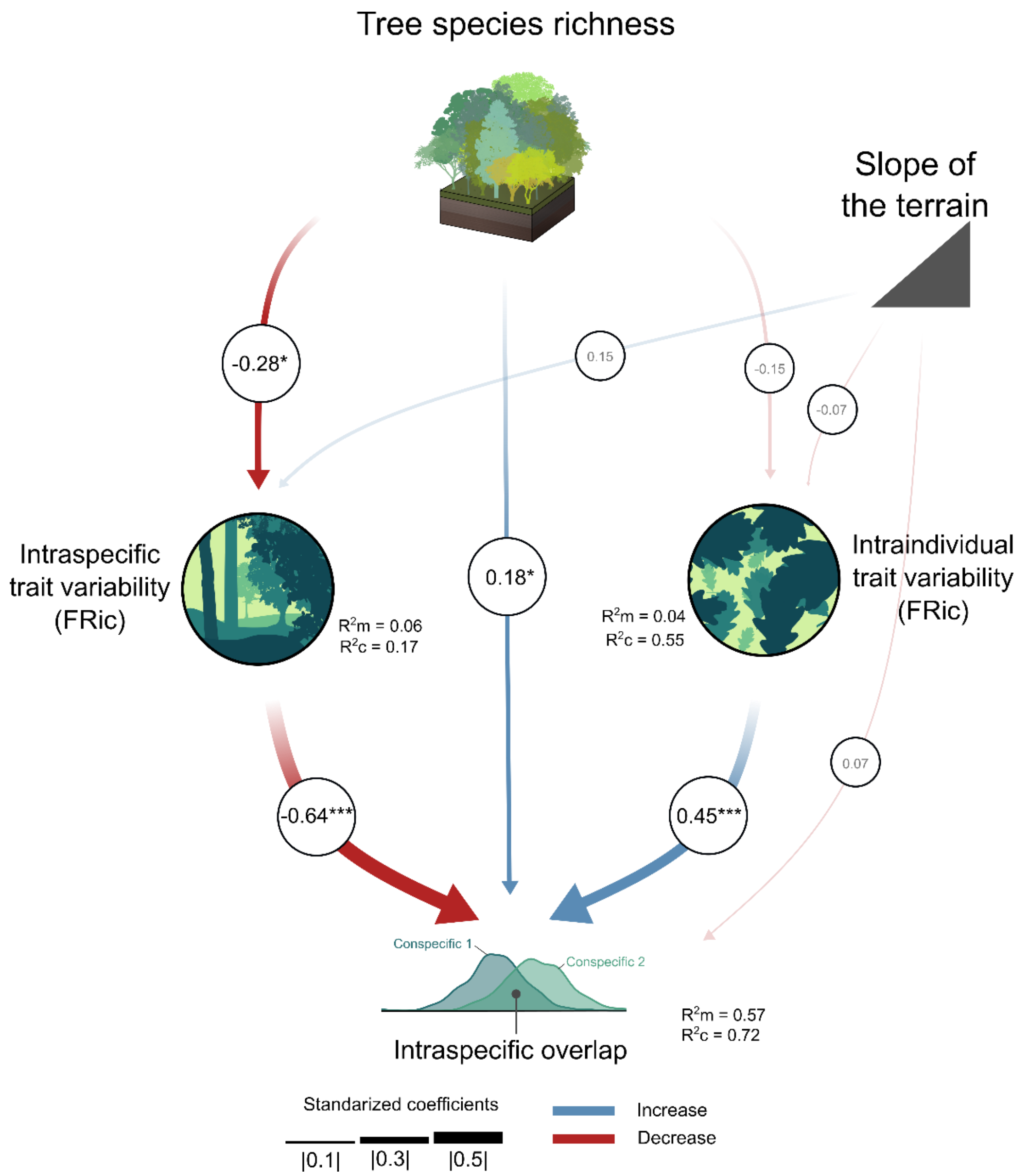

Fig. 4. Piecewise structural equation model (SEM) studying the mechanisms driving the intraspecific overlap in leaf functional traits. The SEM tests the direct effect of tree species richness on intraspecific overlap as well as its indirect effects mediated via multivariate intraspecific and intraindividual variability, which is expressed as functional richness (FRic) here, but see Fig. S6 for a non-simplified SEM in which intraspecific and intraindividual functional divergence (FDiv) were also included. Data are based on multivariate functional indices measured at the intraspecific and intraindividual level for eight tree species growing along an experimental species richness gradient with monocultures and mixtures of 2, 4 and 8 tree species. The width and color of the arrows indicate the strength and direction of the effect, with blue arrows showing positive effects and red arrows negative ones. Significant results are represented by solid lines while non-significant relationships are represented by semi-transparent lines. Asterisks indicate significant effects (*$p < 0.05$, ***$p < 0.001$). The marginal and conditional R^2 (R^2m and R^2c, respectively) are indicated for every model of the piecewise SEM.

Effects of trait variation on community functional diversity

In order to study the importance of intraspecific and intraindividual trait variability in the assessment of functional diversity of a community and its dependence on species richness, we built four null models that randomized trait probability densities at three different levels where trait variation arises (Fig. 5, S7, S8). This approach ensured that simulated assemblages had identical tree species composition as the observed communities, but differed in the trait variability within species. Specifically, the sources of random trait variation differed between the four null models: (1) random population model (assuming random trait distribution of the populations in an assemblage, but within the constraints of the species to which each population belongs), (2) random tree model (assuming random trait distribution among trees from the same population, but within the constraints of the species to which each tree belongs), (3) random leaf model (assuming random trait distribution among leaves from the same tree, but within the constraints of the species to which each tree belongs) and (4) population-restricted random leaf model (assuming random trait distribution among leaves from the same tree, but within the constraints of the population to which each tree belongs; see methods for details on the null models). Based on 500 simulations we calculated the standardized effect sizes (SESs) of FRic and FDiv for every type of null model and every sampled community to determine how much the observed functional diversity deviates from what would be expected under the null models. We then used linear mixed-effects models to study differences in SESs among null models and along a gradient of tree diversity (Fig. S8).

We found a significant interaction between tree species richness and the type of model on SESFRic ($P < 0.001$, $N = 128$; Table S4). Specifically, SESFRic did not differ from 0 in the random population model, suggesting no differences between the FRic of null models and observed communities. Still, SESFRic became significantly higher than 0 with increasing tree species richness in the random tree null model and the population-restricted random leaf model, suggesting that FRic in the diverse observed communities was higher compared to FRic from the null models. In the case of the random leaf model, SESFRic was lower than 0 with low tree species richness and similar to 0 in the highest levels of tree diversity, suggesting that in monocultures, observed SESFRic values were lower compared to the null model, and there were no differences between the null model and observed FRic in diverse communities. A significant interaction was also found for SESFDiv ($P < 0.001$, $N = 128$), but for this functional index the random population and the random tree models did not differ from 0 and only the random leaf and population-restricted random leaf models were significantly higher than 0 in more diverse communities, suggesting that only for these two last null models the observed values of FDiv were higher than the ones from the null models in diverse communities. All analyses remained qualitatively similar when studying functional indices on single axes of trait variation (PC1 or PC2 of Fig. 2A; Fig. S9).

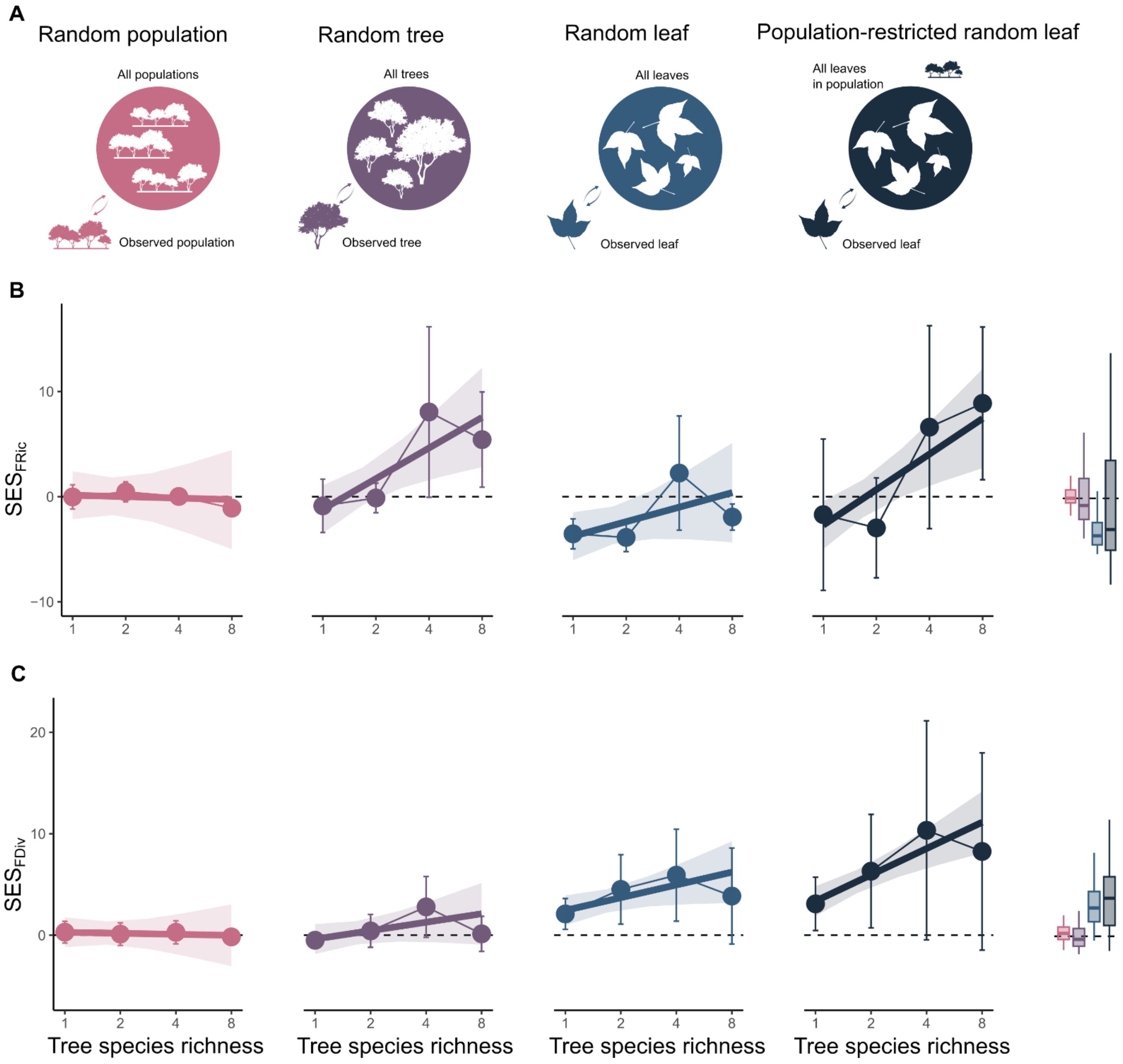

Fig. 5. Differences between observed and random values of community functional diversity along a species richness gradient for four null models that randomized different sources of trait variation. We built (**A**) null models that differ in the level of biological organization in which the randomization was performed (population level, tree level or leaf level; Figure S7) based on leaf-level data collected from plots with 1, 2, 4 or 8 tree species. Data are based on standardized effect sizes (SES) assessed for every null model and functional index (functional richness (FRic) and functional divergence (FDiv)). Linear mixed-effects models showed that the responses of (**B**) SES_{FRic} and of (**C**) SES_{FDiv} to tree species richness depended on the type of null model ($P < 0.001$ and $P = 0.01$, respectively; $N = 120$). SESs are lower than zero (below the dashed line) when the observed values of functional diversity are lower than the simulated ones, while SESs are higher than zero when the observed values are higher than the simulated ones. Semi-transparent bands represent a 95% confidence interval. Points correspond to the mean value of SESs and error bars represent their standard deviation. Boxplots for comparing the values for the SESs in different models are included in the right panels.

DISCUSSION

With our study, we show that intraspecific leaf trait variability correlated negatively with tree species richness and in turn, had a strong negative correlation with intraspecific trait overlap within a community. We interpret this to indicate that trees of a given species are on average functionally more similar in species-rich communities than when they are growing in monoculture. In contrast, intraindividual leaf trait variability was weakly correlated with tree species richness, but strongly and positively correlated with intraspecific trait overlap. We interpret that, as the leaves within each tree become functionally more similar, the trait expressions of individual trees become more dissimilar to each other. Our results also show that the organization of intraspecific and intraindividual variability influences community functional diversity, especially at higher levels of tree species diversity.

Leaf intraspecific and intraindividual trait variability decrease along tree species richness gradients

Our approach allowed us to study how trait variability responded negatively to tree species richness in terms of functional variation between and within individuals (H1). The negative association of tree species richness with functional variability between individuals of a species is consistent with the theoretical predictions suggesting that higher intraspecific variability would minimize intraspecific competitive interactions in monocultures, while intraspecific variability is of secondary importance for species coexistence in species-rich communities (10, 14, 51, 52). Indeed, responses of intraspecific variability in leaf traits were found to decrease with species richness in observational studies (18), and also in other BEF-experiments (15), supporting the idea that leaf variability between conspecifics is a mechanism for complementarity in trees. However, these results contrast with previous observational studies on trees that found an increase in intraspecific trait variability with tree species richness (19–21). These studies mentioned that higher structural complexity (i.e. the structural diversity in the occupancy of the aboveground space) could release competition, allowing species to occupy a larger niche space. In fact, most of the responses found in these studies involved architectural traits (e.g. crown projection area), for which increasing tree species richness often leads to higher complexity in canopy space-filling (53, 54). However, most of these studies were observational and included trees differing in age and distance from neighbors. Such heterogeneous settings would impede, for instance, separating the variability arising from neighborhood diversity from that associated to ontogeny (55, 56).

Our results also indicate that variability in the leaf economics spectrum (LES, 56), accounts for most of the changes at the intraspecific level (as indicated by the results of functional richness for PC1). Therefore, most of the variability occurs between conservative leaves which are expected to have a long lifespan and high resistance against abiotic and biotic hazards, and acquisitive leaves with short lifespans which are expected to be fast in the acquisition of resources and efficient in photosynthetic activity (58, 59). This pattern is consistent with the responses found in other studies for SLA (15), a trait widely used as a proxy for acquisitiveness. It suggests that conspecifics adjust their leaf design in terms of resource use (some individuals more conservative and some more acquisitive), resulting in intraspecific coexistence. Consistently, we found a trend towards a positive effect of tree species richness on intraspecific trait overlap (H2a). These results reinforce the idea that species richness leads to the convergence of conspecifics in their leaf phenotypic space, resulting in higher niche packing of species due to the higher similarity between conspecifics in the resource-use strategy (H2b).

Recent studies suggest that intraindividual variability, which primarily relies on epigenetics and phenotypic plasticity (23), has evolved in natural populations in order to adapt to changing environmental conditions (29, 30, 60). However, this source of variation has been widely disregarded and its role in the context of tree-tree interactions remains largely unclear. Proβ et al. (15) suggested that intraindividual variability in leaves could act in a similar way as intraspecific variability, meaning that higher intraindividual variability could minimize competitive interactions among conspecifics. Therefore, intraindividual trait variability should be higher in monocultures compared to mixtures. However, while a clear decrease in intraindividual variability in response to tree species richness previously reported, these studies did not explore how this could be related to intraspecific trait overlap (31, 61). In contrast, our results indicate that intraindividual leaf variability promotes trait overlap within a population, suggesting that the role of intraindividual variability in intraspecific complementarity could have been overestimated (H2b). Further, tree diversity seems to affect intraindividual variability in nutrition status and stomatal morphology, with more similar leaves in mixtures than monocultures (as indicated by the results of functional divergence for PC2 and functional richness for stomatal density; H1). Additionally, we have observed differences between leaves from the bottom and the top of the tree crown (Fig. S10), with lower stomatal density at the bottom compared to that at the top. Therefore, higher intraindividual differentiation in leaves may appear due to the higher photosynthetic activity of leaves in the upper part of the canopy, as higher photosynthetic rates require higher CO2 assimilation via stomata (62). Indeed, this may be consistent with the alignment between nutrient concentration and stomatal density we found. Thus, the lack of clear stratification of the canopy in monocultures (53) could result in a higher differentiation in the photosynthetic capacity between sun and shade leaves, which would be expected to affect stomatal morphology. As an additional explanation, as diversity can reduce water stress in mixtures (63) and provide microclimate buffering due to higher canopy density (32), stronger differentiation between "water spender" and "water saver" leaves within individuals could appear as a mechanism to cope with water stress in monocultures. In this context, it makes sense that leaves in the upper part of the crowns, which are exposed to higher evaporative demand, adopt a "water saver" strategy that allows them to respond more quickly to water stress (64). Indeed, intraindividual variability has already been suggested as a mechanism to prevent water stress in the case of perennial herbaceous species (29). In sum, our data suggest that intraindividual variability in tree-tree interactions is associated with intraspecific overlap and may arise as a response to varying light and microclimatic conditions within the canopy and the (associated) environmental stressors that trees face.

Intraspecific and intraindividual trait variability shapes the high functional diversity in ecological communities

Our results, interpreted in terms of the deviation of observed functional diversity from null models, showed that the structure of intraspecific and intraindividual trait variability contribute positively to communities' functional diversity (H3a). While differences among species are still the most prominent source of trait variation, intraspecific and intraindividual variability can represent almost half of the total leaf trait variability in our species, especially in the case of predicted leaf nitrogen and phosphorus contents (37; Fig. S11). Therefore, it becomes reasonable that the variability within species will also partly explain how functional diversity is distributed (65). Surprisingly, although we expected to find a higher contribution of trait variability in monocultures, the increase of the divergence from the null models suggests that intraspecific and intraindividual trait variability contributed more to functional diversity in species-rich communities (H3b). One possible explanation is that trees tend to differ in trait values from the other trees in the community (from the same or a different species). As a result, this would lead to an increase in the functional diversity of the community that would be more noticeable as the number of species increases (18).

Differences between the deviations in different null models revealed that intraspecific and intraindividual trait variability contribute to different facets of functional diversity (H3c). In the case of intraindividual variability, the negative departure of FRic from the random leaf null model indicate that, as different populations are exposed to different environmental conditions, the leaves belonging to the same population are highly similar among them compared to other leaves from the same species. Further, the coincidence in the discrepancies between the observed and expected FRic in the random tree and the population-restricted random leaf null models may mean that higher FRic in observed communities is only attributable to intraspecific trait variability. In contrast, the higher observed functional divergence (FDiv) in comparison to the expectations of the random leaf and the population-restricted random leaf null models suggests that communities have a more multimodal distributions, that is, there are several modes ("peaks") across the functional space, resulting from intraindividual trait variability. Indeed, while the role of intraspecific variability in producing multimodal trait distributions had already been studied (66), our results indicated that this effect could be amplified when considering intraindividual variability. This means that, even within one experimental plot, there is not a unique optimal trait value, but different optimal leaf designs are expressed. This is consistent with previous literature, as due to microenvironmental conditions along the tree crown, multiple leaf designs can be expressed in order to maximize fitness (67), affecting the distribution of traits in the community. Interestingly, while we found differences between the observed functional diversity and the expectations in the random tree, random leaf and population-restricted random leaf model, observed populations did not depart significantly from randomly chosen populations for either functional richness or functional divergence. This suggests that, despite the responses found for intraspecific trait overlap, population identity does not matter for functional diversity. However, we should be careful when interpreting this result because, while we did not find differences in the contributions of different populations from the experiment presented here, populations in natural systems with higher environmental heterogeneity could differ substantially in their contribution to the functional diversity of the community (44).

In summary, both intraspecific and intraindividual trait variability affected the distribution of functional diversity. This outcome provides a better understanding of how the variation within species influences functional diversity and supports the idea that intraspecific variability is an important component to be considered when studying the functional diversity of ecological communities at fine or local scales (40, 65). Additionally, we show that intraindividual variability does not only matter for the ecological processes occurring at the population level (68), but also shapes the trait distribution of ecological communities.

Outlook

Using a trait dataset that accounts for hierarchical sources of trait variation for eight tree species across a gradient of tree species richness, we showed that trait variability within and between individual trees is relevant for understanding patterns of intraspecific functional diversity. Traits are a response to pressures from the abiotic and biotic environment, but simultaneously affect ecosystem functioning (69). For instance, intraspecific variability in trees has also been shown to be an important factor increasing primary productivity (70), and similar effects are expected for intraindividual trait variability (61). Therefore, understanding the patterns of trait variation could reveal new facets of the mechanisms behind ecosystem functioning. Altogether, our study demonstrates the importance of considering biological units below the population or species level in trait-based ecology, thus highlighting the importance of moving from a species-based trait ecology to an individual-based trait ecology, that could enable better understanding of processes occurring at the local scales

MATERIALS AND METHODS

Study site and experimental design

This study was conducted in a biodiversity–ecosystem functioning (BEF) experiment, the BEF-China tree diversity experiment, located in Xingangshan, in Jiangxi Province, China (lat. 29°08′11″N, long. 117°90′93″E). While BEF-China was primarily created to investigate ecosystem functions in planted areas with different levels of tree-species diversity, thereby simulating the impact of species extinction, this experiment has also been used to address intraspecific changes of trees in response to their biotic context (71). The climate is subtropical with a mean annual temperature of 16.5°C (ranging from 0.4°C in January to 34.2°C in July) and mean annual precipitation of 1,821 mm (72). We worked on 'Site A', where trees were planted in 2009 and which extends over an area of 27 ha with an elevation ranging from 205 to 275 m a.s.l. and slopes from 8.5° to 40° (3; Fig. S12). In each plot, 400 saplings were planted in a uniform grid with 1.29-meter spacing, with species randomly allocated to planting positions. In the experiment, the trees are arranged according to the 'broken-stick' design outlined by Bruelheide et al. (47). This design involves dividing the species pool into two equal groups for each subordinate richness level. From the total pool of 24 species, we worked with eight tree species: Castanea henryi Rehder & E.H. Wilson, Castanopsis sclerophylla (Lindl. & Paxton) Schottky, Choerospondias axillaris (Roxb.) B.L.Burtt & A.W.Hill, Liquidambar formosana Hance, Nyssa sinensis Oliv., Quercus serrata Murray, Sapindus mukorossi Gaertn. and Triadica sebifera (L.) Small (see Table S3 for details on species family); in plots ranging from the monoculture to the 8-species mixture passing through 2- and 4-species mixtures. Hence, all species are equally represented at every species richness level.

Field sampling

Sampling took place from mid-August to mid-September 2023. In every plot, we randomly chose six individuals from every species, and every species was sampled in two plots at each diversity level. This results in a total of 384 trees from eight different species in 30 plots. However, the theoretical number of 384 trees was reduced to 381 due to the high mortality of Triadica sebifera in one of the plots, where we found only three individuals for that population. In order to capture the variability of the whole individual, from each tree we collected 12 fully developed leaves free from apparent mechanical or pathogen damage at three different heights and four different orientations of the crown, resulting in 4,572 leaves. Immediately after collection, leaves were stored in sealable plastic bags with moistened tissue. Samples were transported in an isothermal bag equipped with cooling bags to prevent dehydration. In the laboratory, samples were temporarily stored at 6–8 °C for a maximum of 12 hours before further processing.

In addition, we collected independent sets of leaf samples in order to predict the leaf economics spectrum (LES) and stomata trait values for the samples of the regular set based on the relationship between reflectance spectra and measured trait values of the calibration and stomata sets (see `leaf trait prediction` section for details). As the methods for measuring traits from the LES were incompatible with the methods used to measure stomata traits, we used two independent sets for these predictions: calibration LES set and calibration stomata set. For the calibration LES set, we included 20 leaf samples per species across all species richness levels, collected at different heights and orientations within the crown, in order to maximize the sampled scenarios (i.e., combinations of species considering closest neighbors, different positions of the leaf within the crown, and the tree's location within the experiment). Each of the 160 samples was composed of four leaves on average depending on the leaf size, to ensure sufficient material for laboratory analyses. For the calibration stomata set we collected 16 leaves per species (totaling 128 samples), also covering all species richness levels and four different heights within the tree crown.

Spectroscopy and laboratory analyses

Visible-near infrared spectrometry (Vis-NIRS) is emerging as a high-throughput phenotyping technique to manage large sample sizes and predict individual leaf trait values using calibration models (39, 73). For all leaves (regular, calibration LES and calibration stomata samples), we acquired reflectance spectra with a portable Vis-NIRS device (ASD “FieldSpec4” Wide-Res Field Spectroradiometer, Malvern Panalytical Ltd, Almelo, Netherlands). Reflectance was measured across the full range of the solar radiation spectrum (350-2500 nm) by taking three repeated measures on the adaxial side of each leaf while avoiding main veins. The equipment was optimized regularly with a calibration white panel (Spectralon, Labsphere, Durham, New Hampshire, USA). For each measurement, ten spectra were averaged internally to reduce noise. A splice correction was applied to the spectral data to minimize the disjunctions between the three sensors of the ASD FieldSpec (VNIR, SWIR1 and SWIR2, with ranges 350-1000 nm, 1001-1800 nm and 1801-2500 nm, respectively). Therefore, the splicing regions were configured according to the points between sensors (from 750 to 1000 and from 1800 to 1950; 74)). After splice correction, outlier detection was performed by using a similar procedure as in Li et al. (75). First, all spectra were visually inspected in the laboratory after acquisition. Additionally, for every species separately, we calculated the local outlier factor of every spectrum (76) and hence considered as outliers 25 spectra that had a value higher than 2 for the local outlier factor (Fig. S13).

For the samples of the calibration LES set, we determined five morphological and chemical leaf traits which are assumed to reflect a plant's strategy in terms of the investment of nutrients and dry mass in the leaves (59, 77) and are key components of the leaf economics spectrum (57, 58): specific leaf area (SLA; leaf area divided by leaf dry mass; mm^2/mg), leaf dry matter content (LDMC; leaf dry mass divided leaf fresh mass; mg/g), carbon content (C; %), nitrogen content (N; %), and phosphorus content (P; µg/g). Additionally, from the calibration stomata set, two traits involved in the regulation of the use of water regulation were assessed: stomatal density (SDens; mm^{-2}) and stomatal size (SSize; µm). In our experiment, these two traits have been shown to represent an orthogonal source of leaf variation relative to the traits from the leaf economics spectrum (78, 79). After collection, the saturated fresh leaves of the calibration LES samples were weighed (DeltaRange Precision Balance PB303-S; Mettler-Toledo GmbH, Gießen, Germany) and scanned at a resolution of 300 dpi to measure leaf area (WinFOLIA; Regent Instruments, Quebec, QC, Canada). Leaves were oven-dried at 80°C for 72 h and weighed to calculate SLA and LDMC. Dried leaves were ground (Mixer Mill 400; Retsch, Haan, Germany), and 200 mg of the resulting powder was used for a nitric acid digestion. After the digestion, P was measured through a molybdate spectrophotometric method (UV-VIS Spectrophotometer UV-1280; Shimadzu, Duisburg, Germany) (80). Additionally, we used an elemental analyzer (Vario El Cube; Elementar, Langenselbold, Germany) to gas-chromatographically determine C and N contents. Imprints for measuring SDens and SSize were taken from fresh leaves of the calibration stomata set by applying transparent nail polish to the abaxial leaf surface and peeling it off once it dried naturally (81). Afterward, stomata were counted at three different locations of the imprints by using an optical microscope (Zeiss Axiostar Plus) with a magnification of 400×. SDens of every leaf were estimated as the mean number of stomata divided by the area of the field of view. A magnification of 1000× was used later for assessing the length of the guard cells in three randomly selected stomata for every imprint. SSize of every leaf was calculated as the mean guard cell length.

Leaf trait prediction

The calibration LES and calibration stomata datasets (spectral data and corresponding trait measurements) were then divided into train and test sets, which account for a proportion of 75% and 25%, respectively. Finally, after excluding the spectral region between 350 and 399 nm for subsequent analyses due to the typical large amount of sensor noise in this region (82), we used a convolutional neural network (CNN) approach for leaf trait prediction based on the spectral data (31). First, input spectra from the train and test sets were augmented from 2,501 to 12,255 wavelength features by using transformations based on a combination of standard normal variates and Savitzky-Golay derivatives (83). Then, a CNN composed of one convolutional layer followed by three dense layers was fitted to train the samples. To avoid overfitting, batch normalization was applied after the convolutional layer (84). Hyperparameter tuning for every CNN was performed independently for every trait, by adjusting the number of filters, their size for the convolutional layers, and the number of nodes in the dense layers (Table S5). For model optimization, an Adam algorithm and a loss function based on the mean squared error was used (85). We tested the predictive ability of the CNNs by assessing the coefficient of determination (R^2) and the root mean squared error (RMSE) for the predicted and measured values in the test set and in the train set. The mean R^2 of the test set was 0.74 ± 0.15 (mean ± standard deviation), with a maximum R^2 for SLA and LDMC (both 0.91) and minimum for P (0.54). The mean R^2 of the train set was 0.80 ± 0.14, with a maximum for LDMC (0.96) and minimum for P (0.62; Fig. S14, S15). These trained CNNs were used for predicting trait values of leaves from the regular set of samples. After leaf trait prediction, we excluded, on average across all traits, 3.46% of the predicted trait values (1.35% for SLA, 1.78% for LDMC, 3.31% for C, 3.85% for N, 5.14% for P, 4.30% for SDens and

4.52% for SSize; Fig. S16, Table S6) as they lay outside the interval formed by the median, plus or minus 3 median absolute deviations (86). This threshold for excluding predicted data was chosen as these values seemed unrealistic and were negative in some cases (Fig. S17). Leaf trait predictions and consecutive statistical analyses were conducted in the R environment with R version 4.1.3 (87).

Metrics of intraindividual and intraspecific trait variability

We identified the main axes of functional trait variability by performing principal component analyses (PCA) on the scaled predicted functional traits of all our leaves. Then, by using a Horn's parallel analysis to choose axes of trait variability, as implemented in the `paran` package (88), we selected the first two axes, which accounted for 67.94% of the variability in our dataset (44.04% and 23.89% explained by the first and second axis, respectively; see Fig. 2, S2, S3, Table S1) and showed adjusted eigenvalues > 1 (3.03 and 1.64 for the first and the second axis, respectively). Due to the presence of missing values in our dataset as a consequence of the removal of extreme predicted trait data (see `Leaf trait prediction` section), missing values were imputed using a PCA-based method as implemented in the `missMDA` package (89) for every species independently prior to the PCA described above. This procedure, while avoiding unrealistic values, may also underestimate intraspecific and intraindividual variation.

The selected axes were used to measure the leaf intraindividual and intraspecific trait variability of a given individual or population, respectively, by estimating trait probability densities (37, 90) using the package `TPD` (48; Fig. S18). Therefore, we assessed trait variability by considering probabilistic multivariate trait distributions with two dimensions (PC1 and PC2). Further, trait variability for single axes (for PC1 and PC2 independently) was also assessed. First, by considering leaf-level values on PC1 and PC2, we compiled a trait probability density for every individual tree as an approach to intraindividual trait variability (Fig. S7). We estimated the bandwidth of the kernel functions by using an unconstrained bandwidth matrix as implemented in the `ks` package (91) and applied a 5% quantile threshold to the trait probability densities. In order to calculate intraspecific trait variability, we first assessed the mean PCA scores of every tree individual by using a bootstrap approach

(45) and used the individual-level data to assess trait probability densities for all populations (following the same procedure as described for the trait probability densities of individuals). In both cases (individual and species levels), from the trait probability densities, we calculated two functional indices that describe two components of trait variability: (1) functional richness (FRic) indicates the total extent of the trait probability density and aims to detect changes in the niche space of individual trees and of the populations (37, 92), respectively, and (2) functional divergence (FDiv) indicates the degree to which the abundance within the functional trait space is distributed toward the edge of the functional volume and, therefore, describes whether the distribution of leaves and tree individuals, respectively, in the trait space is clustered or dispersed (37, 48, 93). Last, the trait probability densities measured for individual trees based on leaf-level data were used to assess the intraspecific trait overlap as the mean overlap between all the trees belonging to the same population (37). Both functional indices and intraspecific trait overlap were estimated by using the `TPD` package (48).

Statistical analyses

To assess the effect of tree species richness on leaf intraindividual and intraspecific trait variability (for both multivariate functional indices and functional indices for PC1 and PC2), we used linear mixed-effects models (LMMs) with the functional indices as a response variable and tree species richness (log2-transformed) as a fixed factor. In addition, tree diameter at breast height (DBH) and slope of the terrain in the position of the tree were included as covariates in the model for intraindividual variability, while the mean slope of the terrain in the plot was included as a covariate in the model for intraspecific trait variability due to its variability across the study site (Fig. S12). We included species identity and plot identity nested in tree composition of the plot as crossed random effects in the model of intraindividual trait variability, and species identity and tree composition of the plot as random effects in the model of intraspecific trait variability. We used diagnostic plots of the residuals to study the assumptions of normality, homoscedasticity and linearity in our models: residuals vs fitted values plots, histograms of the residuals and Q-Q plots for the deviance of the residuals. Then, we tested the significance of fixed effects using likelihood ratio tests (94).

Finally, we assessed the quality of fit of our model by calculating the marginal and conditional R^2, which address the variance explained only by fixed effects and the variance explained by the entire model including the random effects, respectively.

In order to assess the effects of tree species richness on intraspecific leaf trait overlap and how this effect is mediated by the intraspecific and intraindividual leaf trait variability, we used a Piecewise Structural Equation Model (piecewise SEM) as implemented in the `piecewiseSEM` package (95). Here, species identity was included as a random effect. First, we defined the conceptual model as a set of regressions, representing the relationships between the variables and fit linear mixed models (LMMs) based on these relationships (Fig. S4). Correlated error terms were included between indices of intraindividual trait variability and between indices of intraspecific trait variability. The mean slope of the plot was included as a covariate in the models for intraspecific and intraindividual trait variability. Then, the model fit was evaluated based on d-separation test and Fisher's C statistics (95). Eventually, as intraindividual and intraspecific functional divergence did not show any significance and weak standard estimates, we reduced the SEM by excluding these two metrics and the correlated error terms. All of these results remained qualitatively similar when using the full and the reduced SEM (Fig. S6).

Null models for functional diversity

In order to assess the effects of intraspecific and intraindividual trait variability in the assessment of functional diversity of a community and its relationship with species richness, we used null models that randomized different sources of trait variation occurring within the species.

First, observed functional diversity in every plot was assessed by using sums of trait probability densities from the leaf-level to the community level, therefore, expanding to the individual level the trait probability density framework for functional diversity developed in Carmona et al. (38; Fig. S7). Thus, based on the leaf-level data (level 1), we estimated trait probability density for individuals (level 2) and, afterwards these trait probability densities were summed at the species level (considering the given species in a population; level 3).

Finally, by summing the trait probability densities of the different populations occurring in a community we obtained final trait probability densities at the community level (level 4). The community trait probability densities were then used to assess FRic and FDiv as metrics of functional diversity in a plot. For this last step, the contribution of the trait probability density of every population was weighted according to the sum of wood volume of every species in the central area of every plot (including the 36 trees in the centre of the plot). To assess the wood volume (WV) of the trees, basal area and height were measured in 2022 and the conversion factor calculated by Huang et al. (96) for our study species in our study site was used to estimate wood volume as:

$$WV\ = 0.5412\,m^3m^{-3} - 0.1985\,m^{-3} \times\ basal\,area\ \times\ height \tag{1}$$

Following this framework, we ran simulations randomizing hierarchically different sources of variation occurring within the species (Fig. S8). Therefore, these null models simulated communities with the same species composition and abundances, but they randomized data on different steps of the framework for measuring functional diversity (Fig. S12):

(1) Random population null model: The trait probability densities of every population were calculated based on observed data and, afterwards, these population trait probability densities were shuffled for every species. This model aims to test which is the effect of considering the functional identity of the population in the plot.
(2) Random tree null model: The trait probability densities of all trees were calculated based on the observed leaf values and the trees were shuffled for every species. This model aims to test the effect of intraspecific variability on community functional diversity.
(3) Random leaf null model: Leaf values were shuffled for every species before calculating functional diversity. This model aims to detect the whole effect of the variability occurring within species (intraspecifically and intraindividually) on functional diversity.

As the intraindividual variability tends to be clustered around the centroid of every tree and trees in the same population are more similar to each other compared to trees from other populations (Fig. S3), the random leaf null model could represent highly unrealistic scenarios. Therefore, we decided to build another null model for the assignment of random leaves in which the pool of leaves was more restricted than in the “random leaf null model” model:

(4) Population-restricted random leaf null model: Leaf values were shuffled for every population before calculating functional diversity. This model aims to detect the effect of the whole variability occurring within populations (intraspecific and intraindividual variability) in functional diversity.

We simulated 500 null assemblages for every plot and every type of null model. We visually inspected the changes and stabilization of the mean and variance of every null distribution with an additive number of simulations (Fig. S19). Finally, to assess the differences between the observed and the simulated values of the functional indices we used standardized effect sizes (SES) as in Gotelli and McCabe (97)

$$SES = \frac{FD_{observed} - mean(FD_{simulated})}{SD(FD_{simulated})} \qquad (2)$$

Where FD corresponds to any of the measured functional indices. SESs were calculated independently for every plot and type of null model. To test the effects of tree species richness and the type of null model on the SES for every functional index, we used LMMs and included the plot identity nested in tree species composition, as a random effect. In every model, we included the SES of every functional index as a response variable. Thus, we fitted two models with tree species richness (log2 transformed), type of the null model, and their interaction as response variables. Then, we tested the significance of fixed effects by using likelihood ratio tests, following the same procedure described previously for LMMs fitted for the intraspecific and intraindividual trait variability. All LMMs were fitted using the 'lmer' function in the 'lmerTest' package (98). We considered that the fitted null model coefficients were significantly different from the random scenario when the 95%-confidence intervals did not overlap with zero.

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Conflict of interest

The authors declare that they have no competing interests.

Data availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Data and R codes supporting the results presented in this manuscript are available at the Zenodo repository: https://doi.org/10.5281/zenodo.14190700.

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SUPPORTING INFORMATION

Fig. S1. Regression coefficients for the effects of tree species richness on the intraspecific and intraindividual variability on seven leaf functional traits and two main axes of leaf trait variability.

Fig. S2. Results of a principal component analysis (PCA) of seven leaf functional traits belonging to eight different tree species.

Fig. S3. Location of eight sampled species in a functional trait space assessed by a principal component analysis (PCA) for seven leaf functional traits.

Fig. S4. Conceptual model representing the relationships between variables that could affect intraspecific overlap in leaf functional traits.

Fig. S5. Effect of tree species richness on intraspecific overlap.

Fig. S6. Results of non-simplified piecewise structural equation models (SEM) studying the mechanisms driving the intraspecific overlap in leaf functional traits.

Fig. S7. Conceptual framework for measuring community functional diversity based on individual leaf trait values (following the approach of Carmona et al. 2016 (37)).

Fig. S8. Conceptual framework for the null model approach based on the randomization of different sources of variation.

Fig. S9. Results of linear mixed-effects models to test the joint effect of tree species richness and the type of null models on standardized effect sizes (SES) of two univariate functional indices (functional richness (FRic) and functional divergence (FDiv)) calculated from the two main axes of leaf variation (PC1 and PC2) and for four different sources of trait variation.

Fig. S10. Differences in stomatal density across different positions in the tree crown.

Fig. S11. Bar plots for the variance partitioning of leaf variation.

Fig. S12. Location and slope of the sampled trees within the experimental site.

Fig. S13. Leaf reflectance spectra for the eight study species.

Fig. S14. Scatter plot of predicted and measured trait values in the test and the train samples.

Fig. S15. Evolution of the error during the training of convolutional neural networks for trait prediction.

Fig. S16. Bar plot and heatmap of the distribution of missing trait data in the leaf-level dataset.

Fig. S17. Excluded values from predicted leaf-level data for seven leaf functional traits.

Fig. S18. Analytical framework used to assess the metrics of intraindividual variability, intraspecific variability and intraspecific overlap.

Fig. S19. Evolution of mean and variance of simulated values of FRic and FDiv from different null models with increasing number of randomizations.

Table. S1. Summary of a principal component analysis for seven leaf functional traits, including loadings, standard deviation, proportion of the variance explained by each component and the adjusted eigenvalue obtained in a Horn's parallel analysis.

Table. S2. Results for linear mixed-effects models studying the effects of tree species richness on multivariate functional indices used to estimate intraspecific variability, intraindividual variability and intraspecific overlap.

Table. S3. Species included in the study.

Table. S4. Results for linear mixed-effects models studying the effects of tree species richness and type of null model on standardized effect sizes of two functional indices.

Table. S5. Layers and hyperparameters used for building a convolutional neural network for every trait, and coefficient of determination (R^2) and root mean squared error (RMSE) for the test and the train samples.

Table. S6. Distribution of missing trait data in the leaf-level dataset across species and traits.

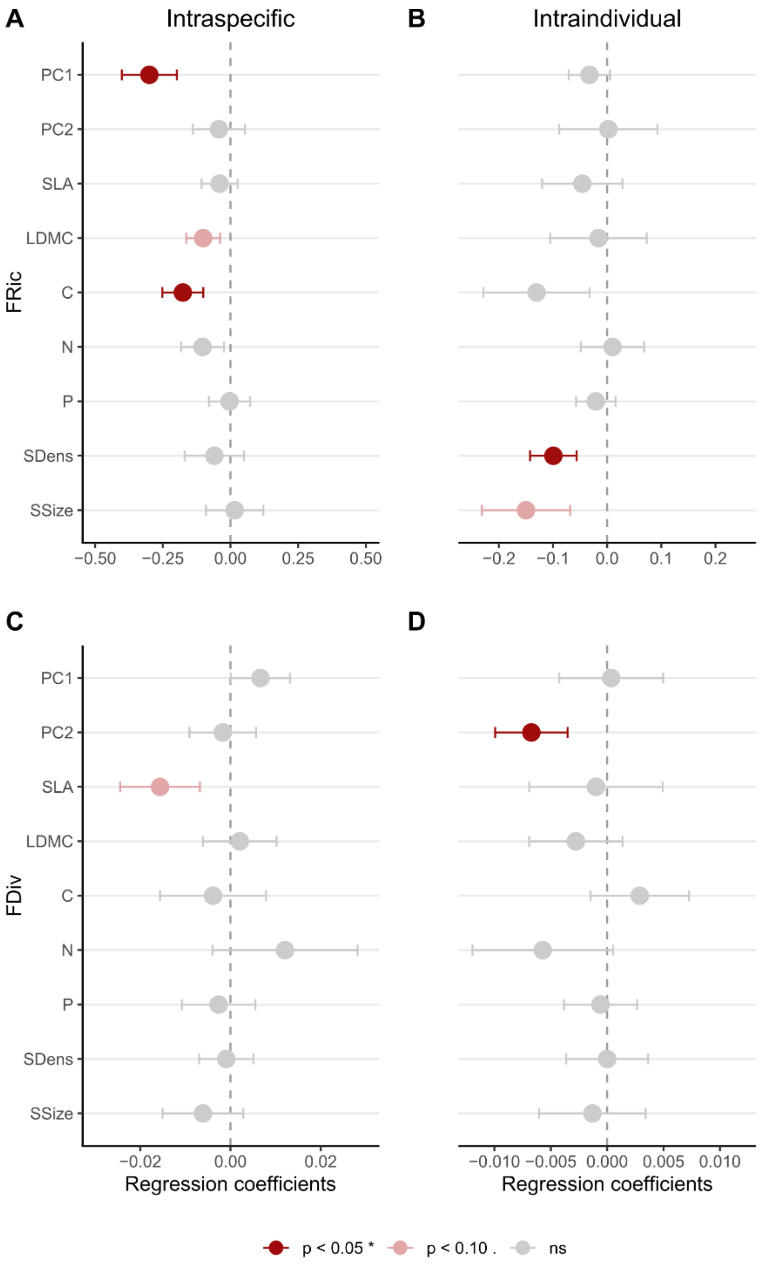


Fig. S1. Regression coefficients for the effects of tree species richness on the intraspecific and intraindividual variability on seven leaf functional traits and two main axes of leaf trait variability. Regression coefficients for the effects of tree species richness on the intraspecific and intraindividual variability on seven leaf functional traits and two main axes of leaf trait variability. The effects of tree species richness on intraspecific and intraindividual variability were studied for seven functional traits (specific leaf area (SLA), leaf dry matter content (LDMC), leaf carbon content (C), leaf nitrogen content (N), leaf phosphorus content (P), stomatal density (SDens) and stomatal size (SSize)) and for two main axes of leaf trait variability (PC1 and PC2). Colors represent the significance as determined by a likelihood ratio test (red $p < 0.05$, pink < 0.01, grey $p > 0.05$).

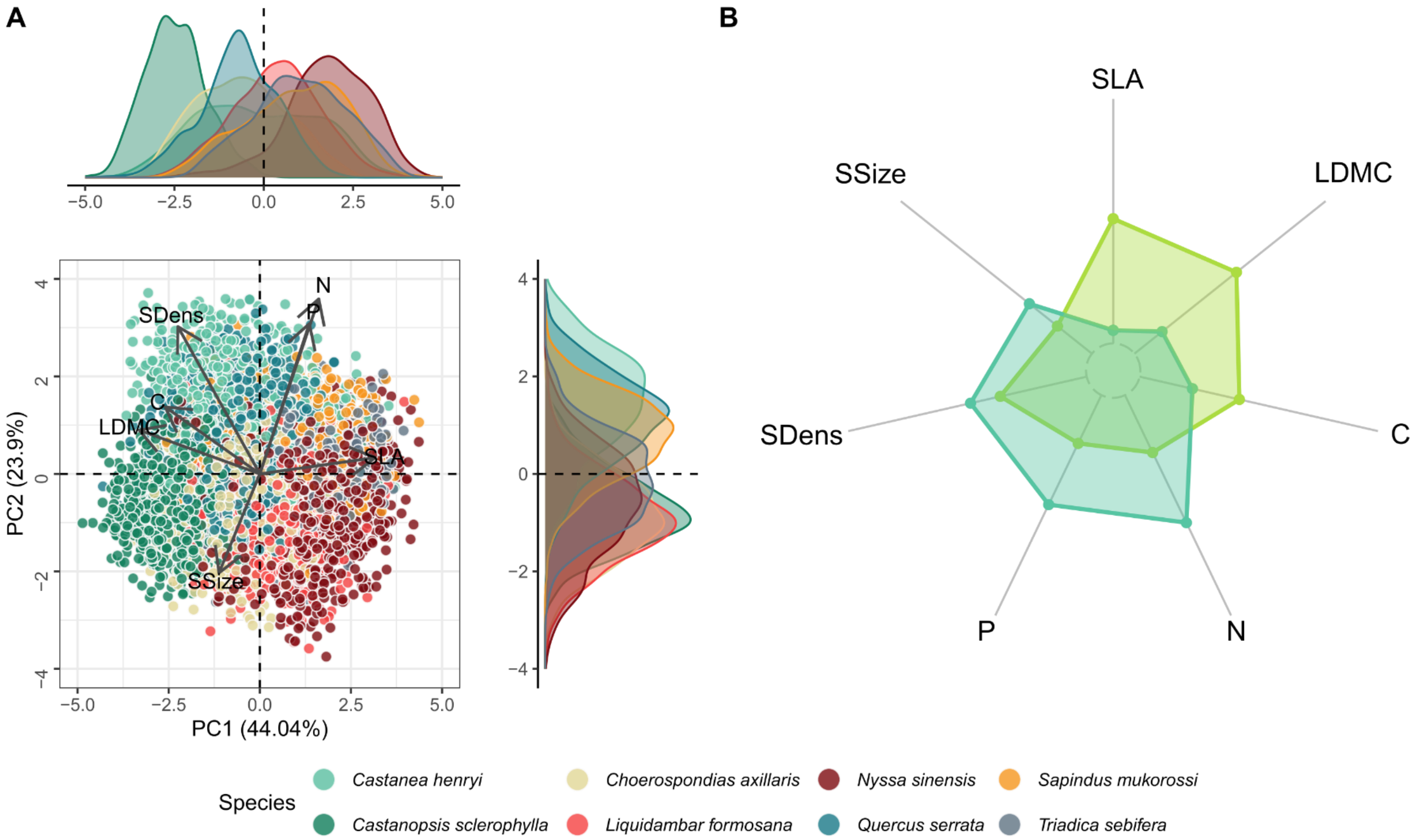


Fig. S2. Results of a principal component analysis (PCA) of seven leaf functional traits belonging to eight different tree species. (A) Main axes of a principal component analysis, including the location for every leaf and arrows representing the eigenvalues of every trait in the PCA axes, and (B) radar plot representing the eigenvalues of the traits in the two main axes. The first axis represents the variation in growth strategy, with lower values associated with a conservative strategy while higher values correspond to an acquisitive strategy. The second axis mainly associates with stomata density (SDens) and leaf P and N. This suggests that higher evapotranspiration rates linked to higher SDens might be associated with a higher content of P and N, which are key nutrients for photosynthetic activity (1).

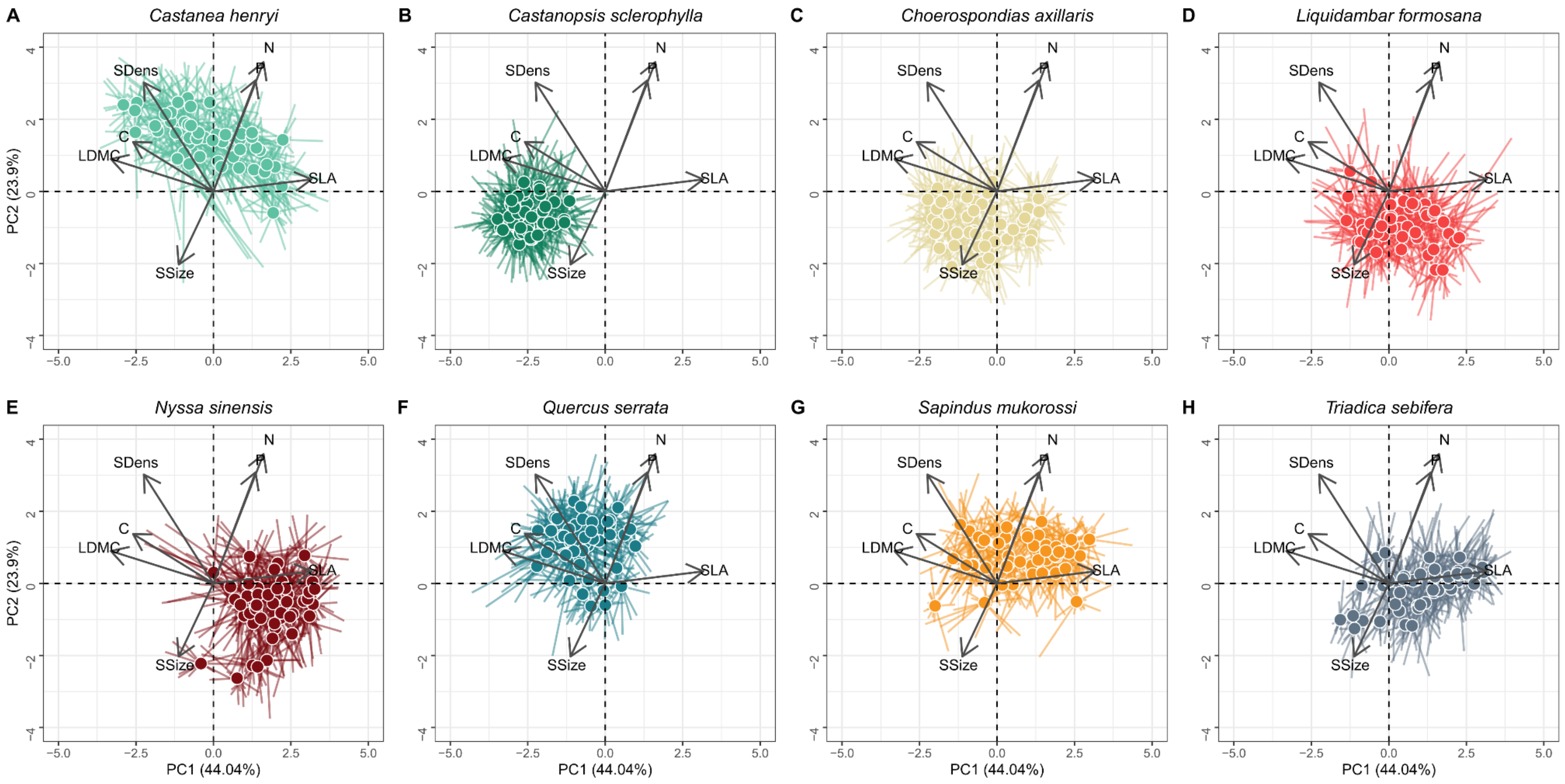


Fig. S3. Location of eight sampled species in a functional trait space assessed by a principal component analysis (PCA) for seven leaf functional traits. Each panel corresponds to a different species, dots represent the mean trait values of every tree (centroid) and lines connect the centroid to the individual values of the leaves belonging to the tree. The figure suggests that there are differences between species in terms of their leaf strategy (e.g. Castanopsis sclerophylla has a more conservative growth strategy compared to Triadica sebifera) and in terms of the extent of their intraspecific variation (e.g. Nyssa sinensis and Castanea henryi show high intraspecific variability compared to other species).

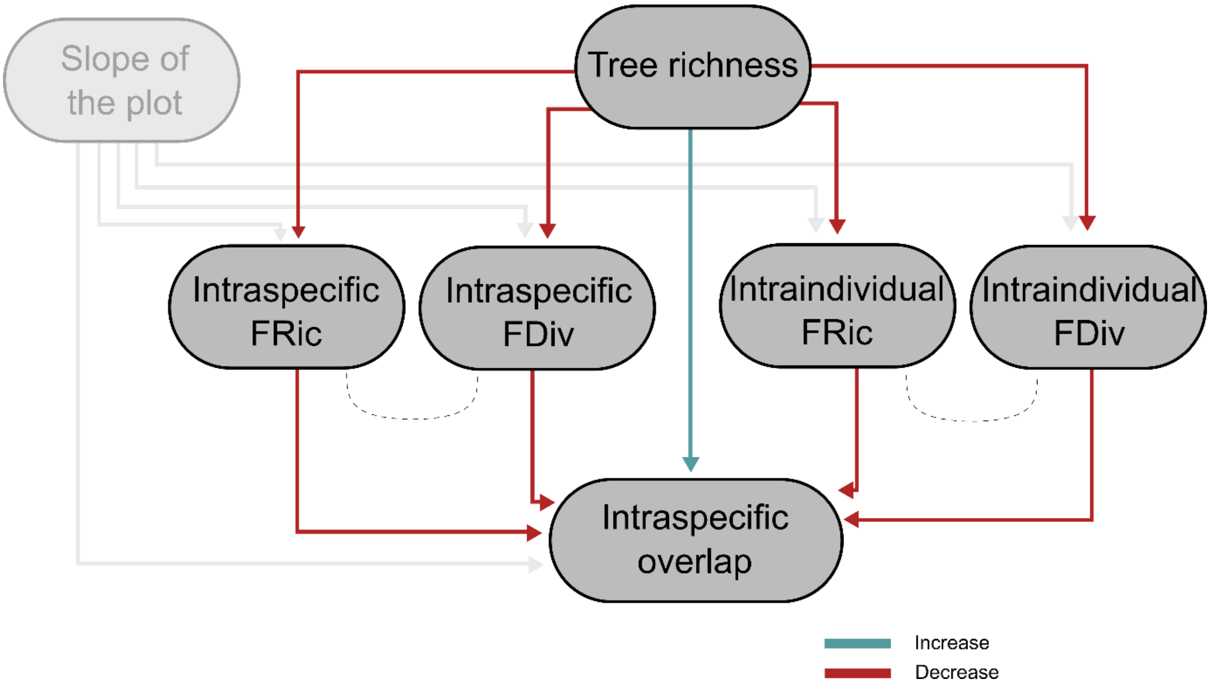


Fig. S4. Conceptual model representing the relationships between variables that could affect intraspecific overlap in leaf functional traits. Tree species richness is expected to affect negatively the intraspecific and intraindividual trait variability (for both indices), while these are expected to have a negative effect on the intraspecific overlap. This was expected as intraindividual and intraspecific trait variability were hypothesized to act as mechanisms to guarantee complementarity in intraspecific interactions. The slope of the plot was included as a covariate in the analyses in order to control for it, but hypotheses were not formulated for its effect on the other variables. Red lines represent negative relationships, blue lines represent positive relationships and dashed lines indicate correlated error terms.

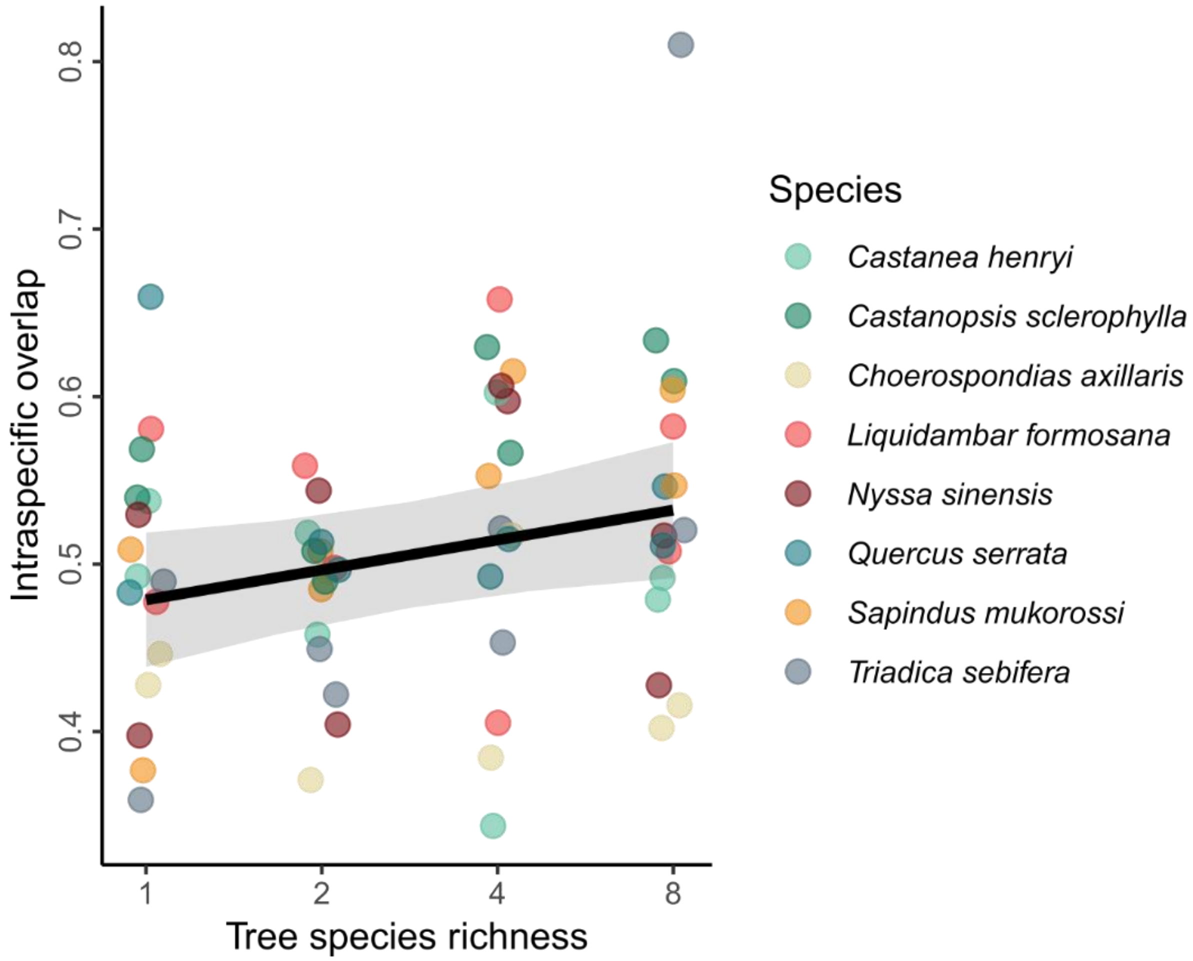


Fig. S5. Effect of tree species richness on intraspecific overlap. The line corresponds to the results of a linear mixed-effects model that shows a significant increase of intraspecific overlap with increasing tree species richness (P = 0.03, N = 63). Grey bands represent a 95% confidence interval. Colors correspond to the different tree species included in the study, whose identity was included as a random effect in our models. The slope of the terrain was included as a covariate in the model.

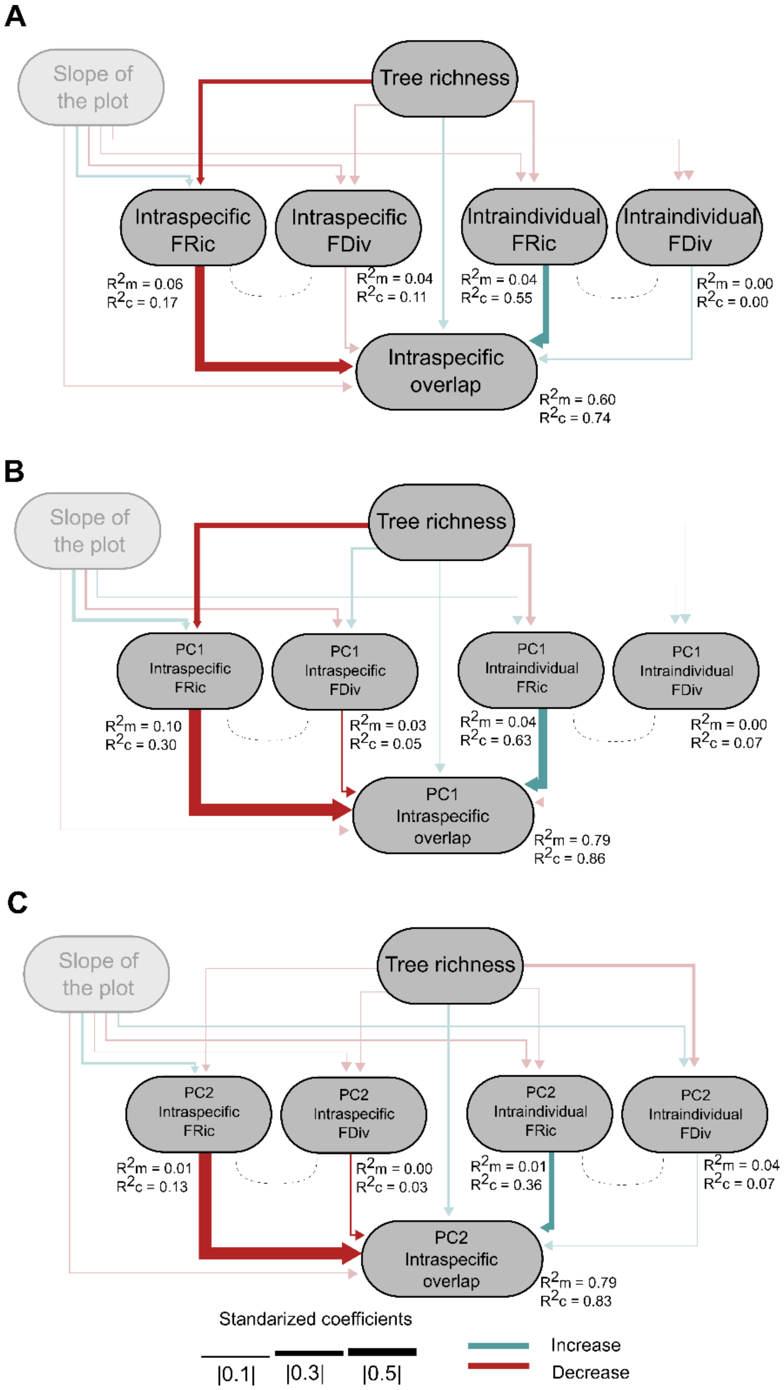


Fig. S6. Results of non-simplified piecewise structural equation models (SEM) studying the mechanisms driving the intraspecific overlap in leaf functional traits. Results are shown for (A) a complete SEM based on the conceptual model defined in Fig. S4, and for SEMs based on the variability on the two main axes of trait variation: (B) PC1 and (C) PC2. The width and color of the arrows indicate the strength and direction of the effects. Significant results are represented by solid lines while non-significant relationships are represented by semi-transparent lines. The marginal and conditional R^2 are indicated for every model of the piecewise SEM.

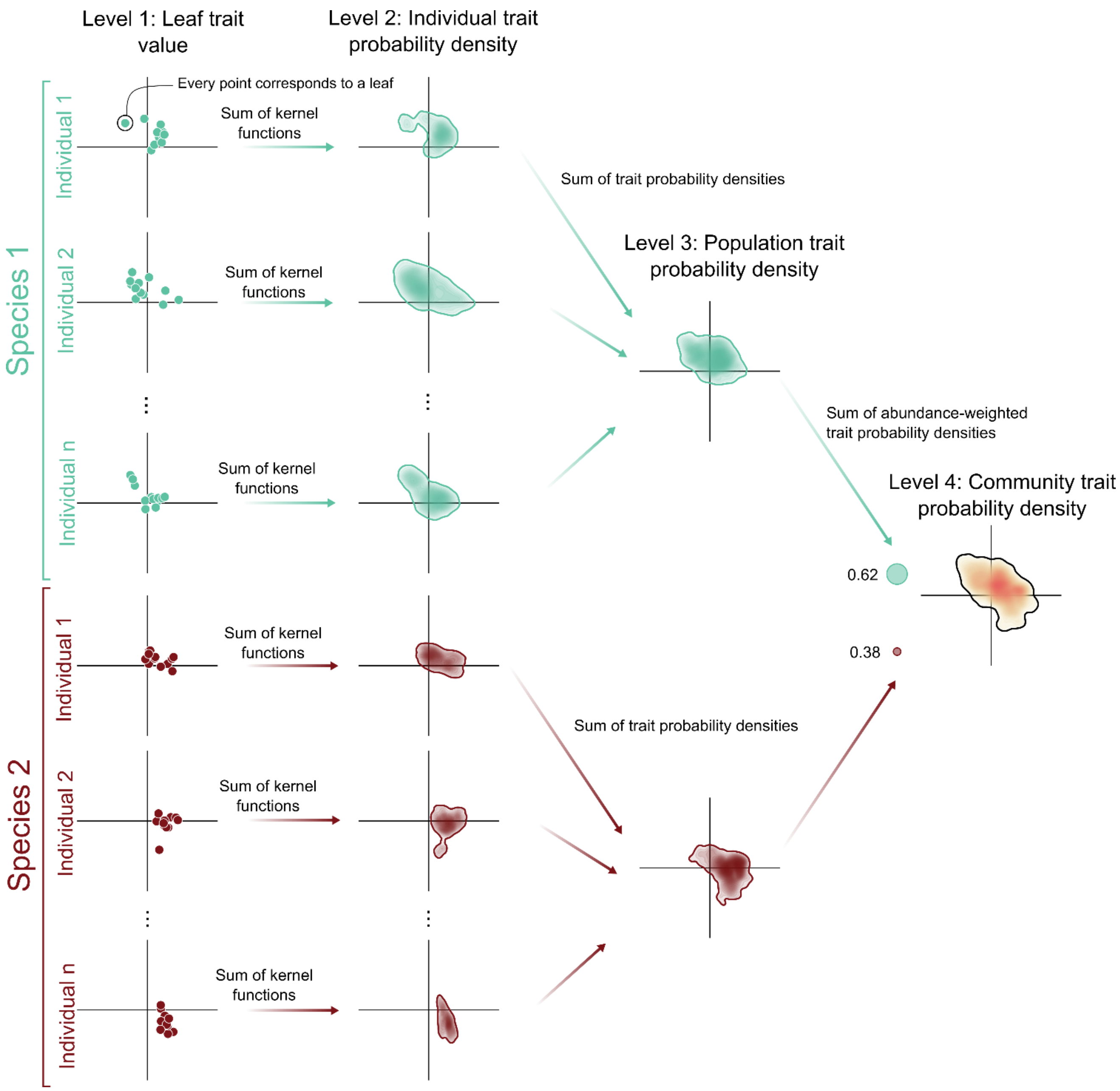
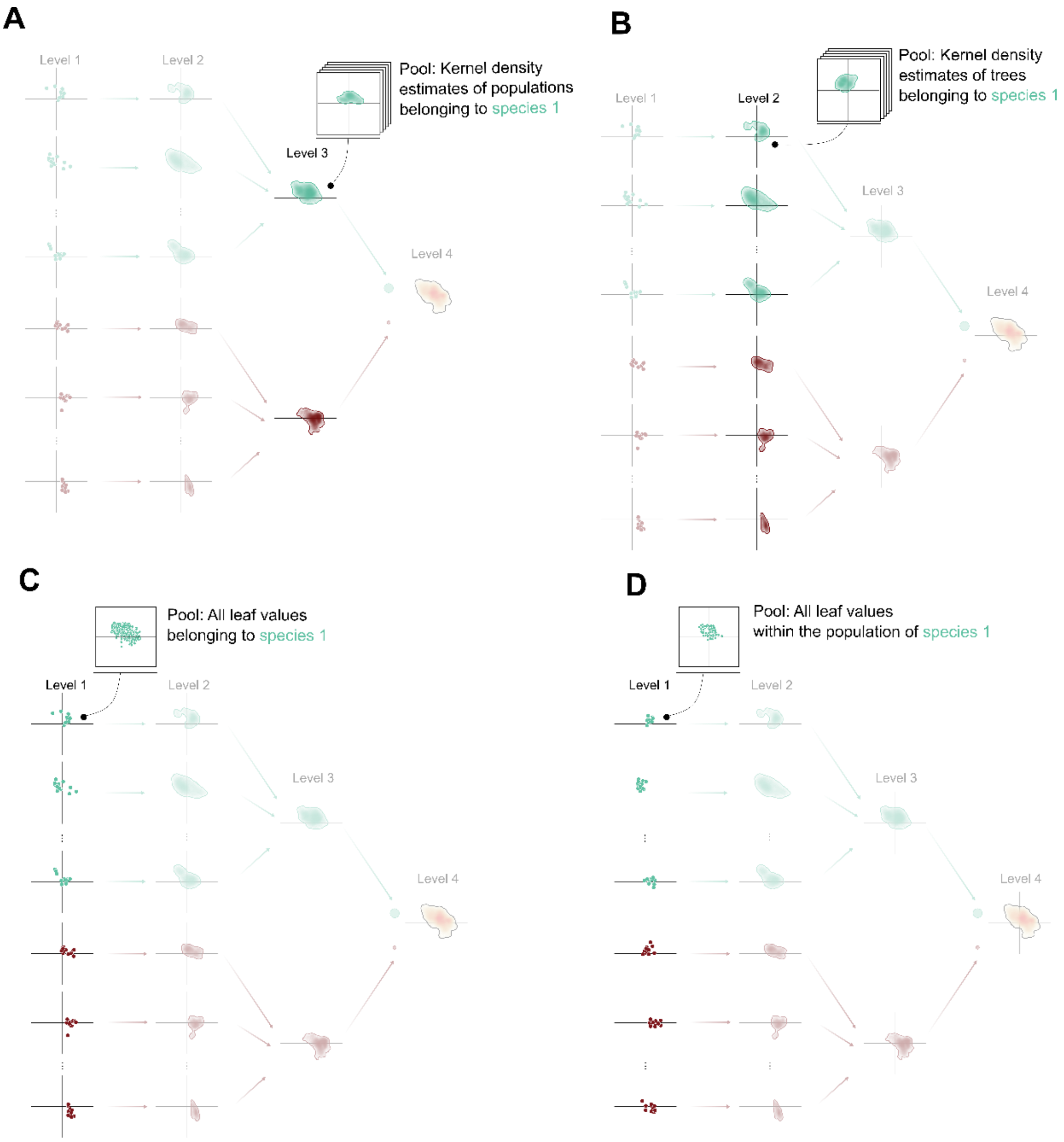


Fig. S7. Conceptual framework for measuring community functional diversity based on individual leaf trait values (following the approach of Carmona et al. 2016 (37)). As an example, we used a community with two species (species 1 in green and species 2 in red). First (Level 1), we applied kernel density functions to the leaf trait values (represented by the leaf's position on axis 1 and axis 2 of a PCA; Fig. 3) of every tree. Next, we summarized the kernel density functions to get one trait probability density for every tree (Level 2). After this, we calculated the sum of the trait probability densities of all trees belonging to the same population to get the population trait probability density (Level 3). Finally, we aggregated the population trait probability densities (Level 4). In this step, each trait probability density was rescaled according to the relative abundance of the species in the community.



Pool: Kernel density estimates of populations belonging to species 1

Pool: Kernel density estimates of trees belonging to species 1

Pool: All leaf values belonging to species 1

Pool: All leaf values within the population of species 1

Fig. S8. Conceptual framework for the null model approach based on the randomization of different sources of variation. Null models differ in the process for generating simulated communities by randomizing different steps of the framework for measuring functional diversity as shown in Fig. S7. The result in every case is an assemblage with the same species composition and abundances as the observed one, but different levels of the variability occurring within the species were randomized. (A) The random population null model is generated by randomizing the population trait probability densities generated in step 3 by using as a pool all the different population trait probability densities calculated for that species. (B) The random tree null model is generated by randomizing the tree trait probability densities generated in step 2 by using as a pool all the different tree trait probability densities calculated for that species in any community. (C) The random leaf null model is generated by randomizing the leaves that are used to estimate the trait probability densities of trees by using as a pool all the different leaves for that species across the whole experiment. Finally, (D) The population-restricted random leaf null model is generated by randomizing the leaves that are used to estimate the trait probability densities of trees by using as a pool all leaves for that species within the population.

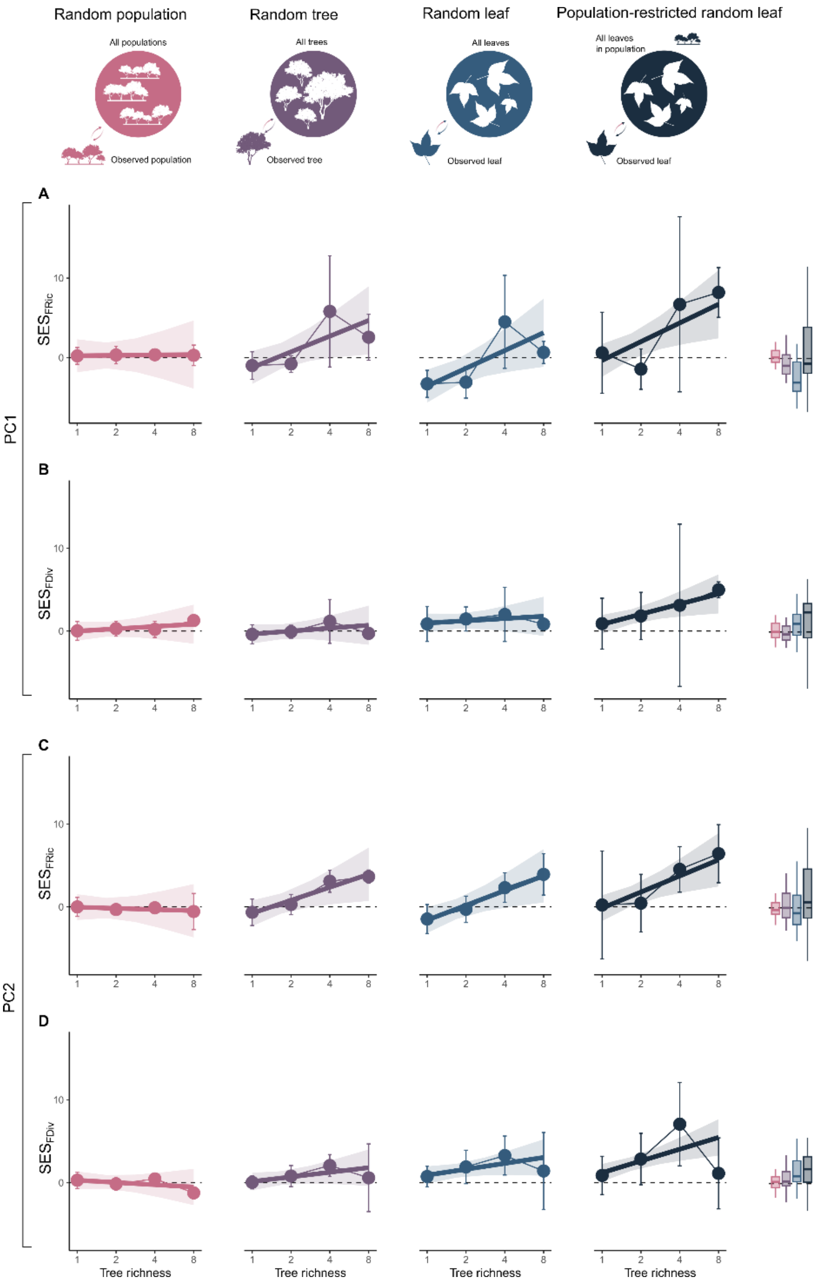


Fig. S9. Results of linear mixed-effects models to test the joint effect of tree species richness and the type of null models on standardized effect sizes (SES) of two univariate functional indices (functional richness (FRic) and functional divergence (FDiv)) calculated from the two main axes of leaf variation (PC1 and PC2) and for four different sources of trait variation. Linear mixed-effects models show a significant effect of the interaction of tree species richness and the type of model on SES(FRic) in the case of both axes ($P = 0.002$ for PC1 and $P < 0.001$ for PC1) and a significant effect of this interaction in the cases of SES(FDiv) in PC2 ($P < 0.001$), but this effect was not significant in the case of the SES(FDiv) of PC1 ($P = 0.17$). However, in the case of SES(FDiv) of PC1, the effects of tree species richness and the type of model were still significant ($P < 0.001$ in both cases).

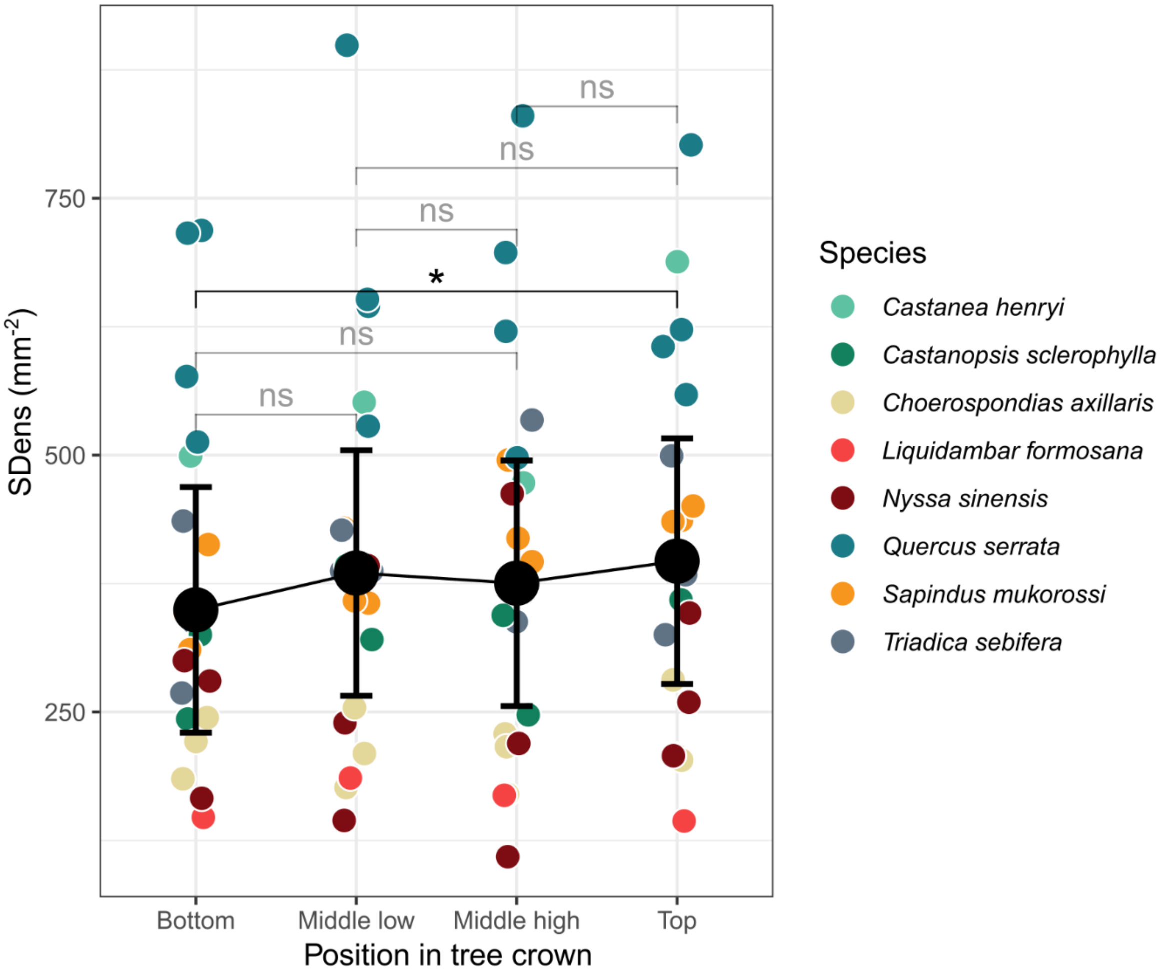


Fig. S10. Differences in stomatal density across different positions in the tree crown. Leaves of the calibration stomata set (see `Field sampling` section for description of the calibration stomata) were collected at different heights within the tree crown (bottom, middle low, middle high and top). A linear mixed-effects model to study differences in stomatal density (SDens) across different crown positions revealed significant differences between the top and the bottom (P = 0.03 as revealed by a Tukey post-hoc text). Black points and error bars represent estimate and confidence intervals, respectively. Every tree species is represented in a different color.

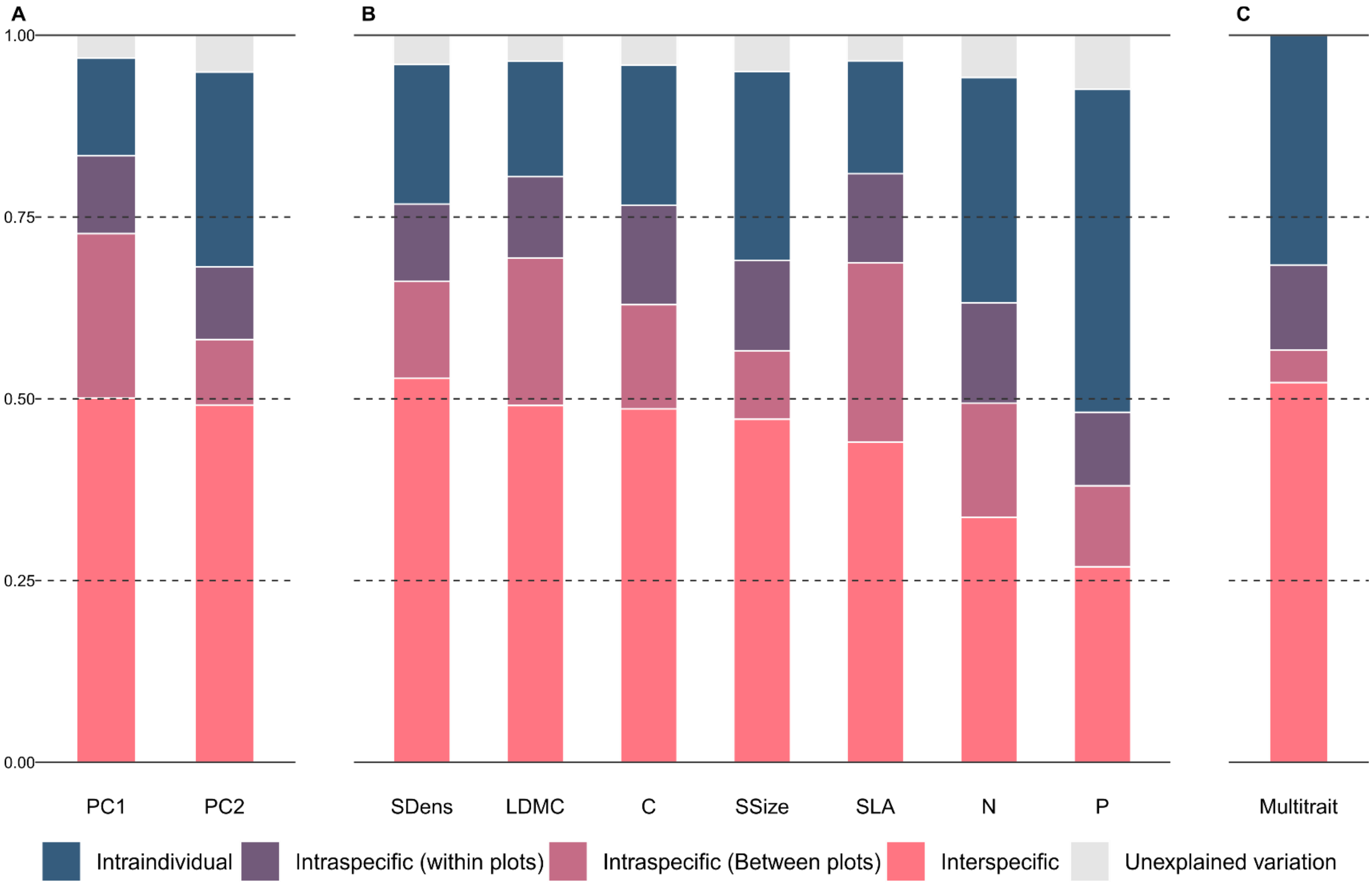


Fig. S11. Bar plots for the variance partitioning of leaf variation. Variance partitioning was studied for (**A**) the main axes of leaf variation found in a principal component analyses (PC1 and PC2; Fig. 2A), (**B**) independently for seven functional traits related to plant resource and water use (specific leaf area, SLA; leaf dry matter content, LDMC; leaf carbon content, C; leaf nitrogen content, N; leaf phosphorus content, P; stomatal density, SDens; stomatal size, SSize) and (**C**) jointly for the seven leaf traits mentioned. Variance partitioning in (**A**) and (**B**) was assessed by using an intercept only linear mixed-effects model with only random effects (leaf nested in tree, in turn nested in population, in turn nested in species identity), while we used a permutational multivariate analysis of variance (PERMANOVA) with the nested structure described as a predictor for the variance partitioning in (**C**).

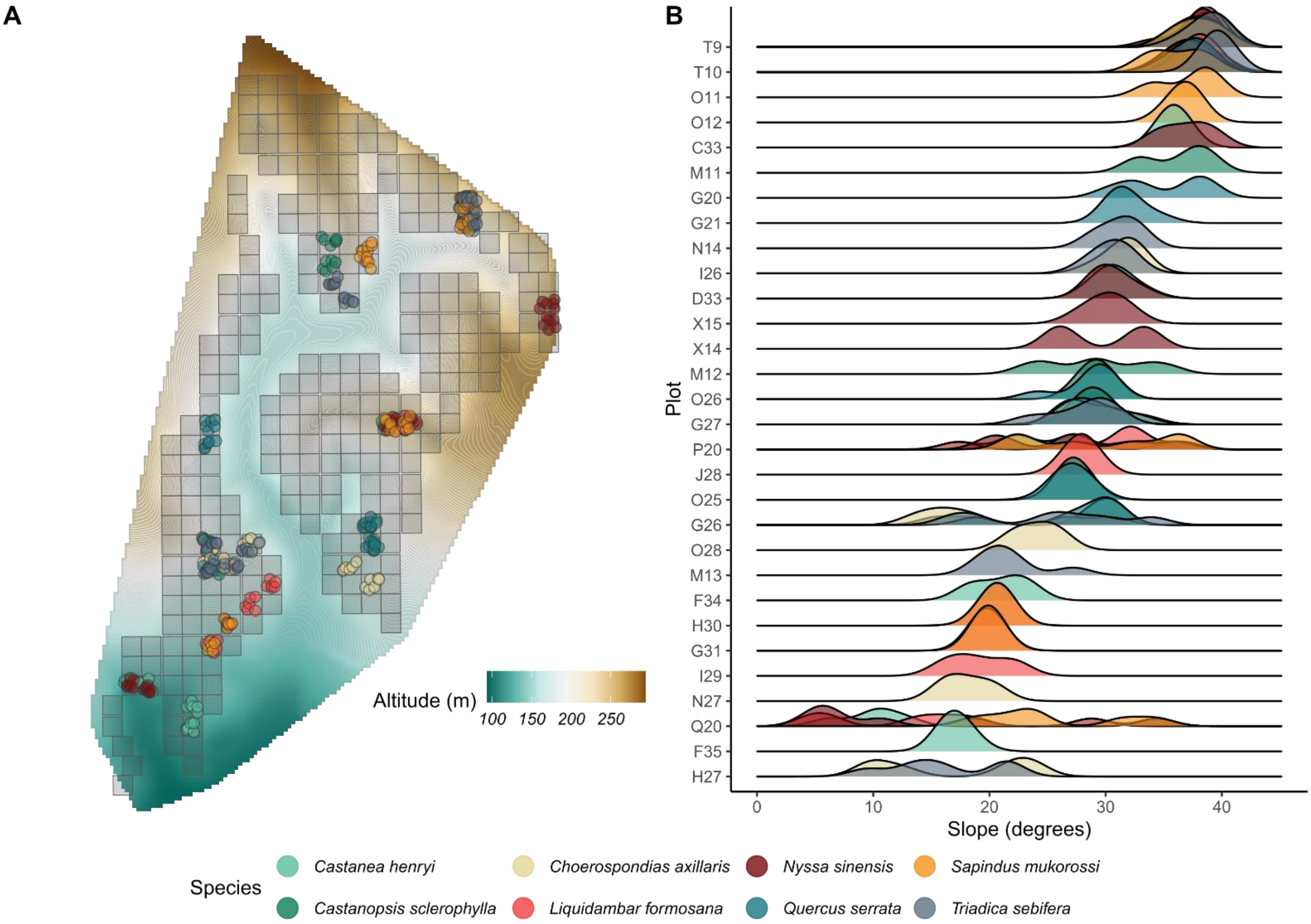


Fig. S12. Location and slope of the sampled trees within the experimental site. (**A**) Trees were sampled across 30 plots distributed in different parts of the experiment. (**B**) Density plots of the slope of every species in every plot (based on interpolated values of the slope of the terrain obtained from a 5 m resolution digital elevation model available at https://data.botanik.uni-halle.de/bef-china/datasets/53).

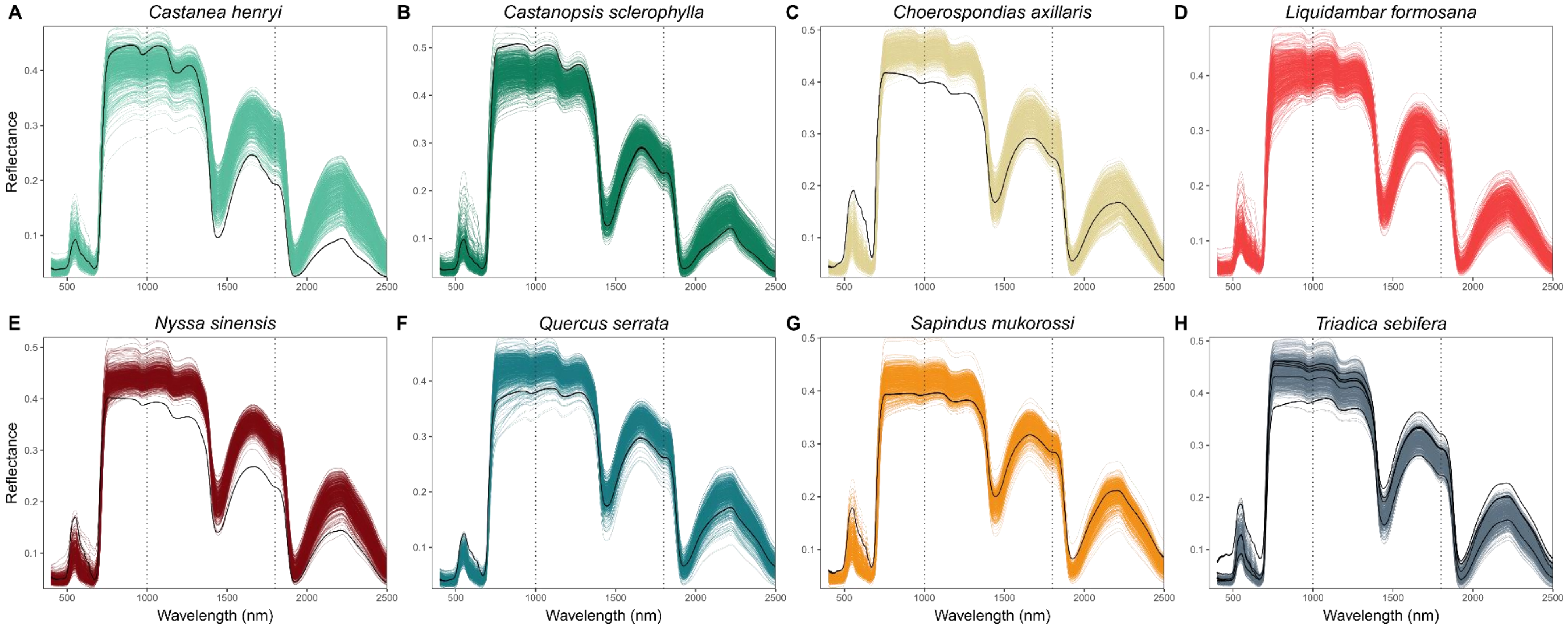


Fig. S13. Leaf reflectance spectra for the eight study species. Spectra of all leaves collected by species (represented in different panels and different colors). Lines in black represent those spectra, which were excluded for subsequent analyses as they had a local outlier factor higher than two. Dotted vertical lines represent the limits between the sensors of the spectroradiometer use.

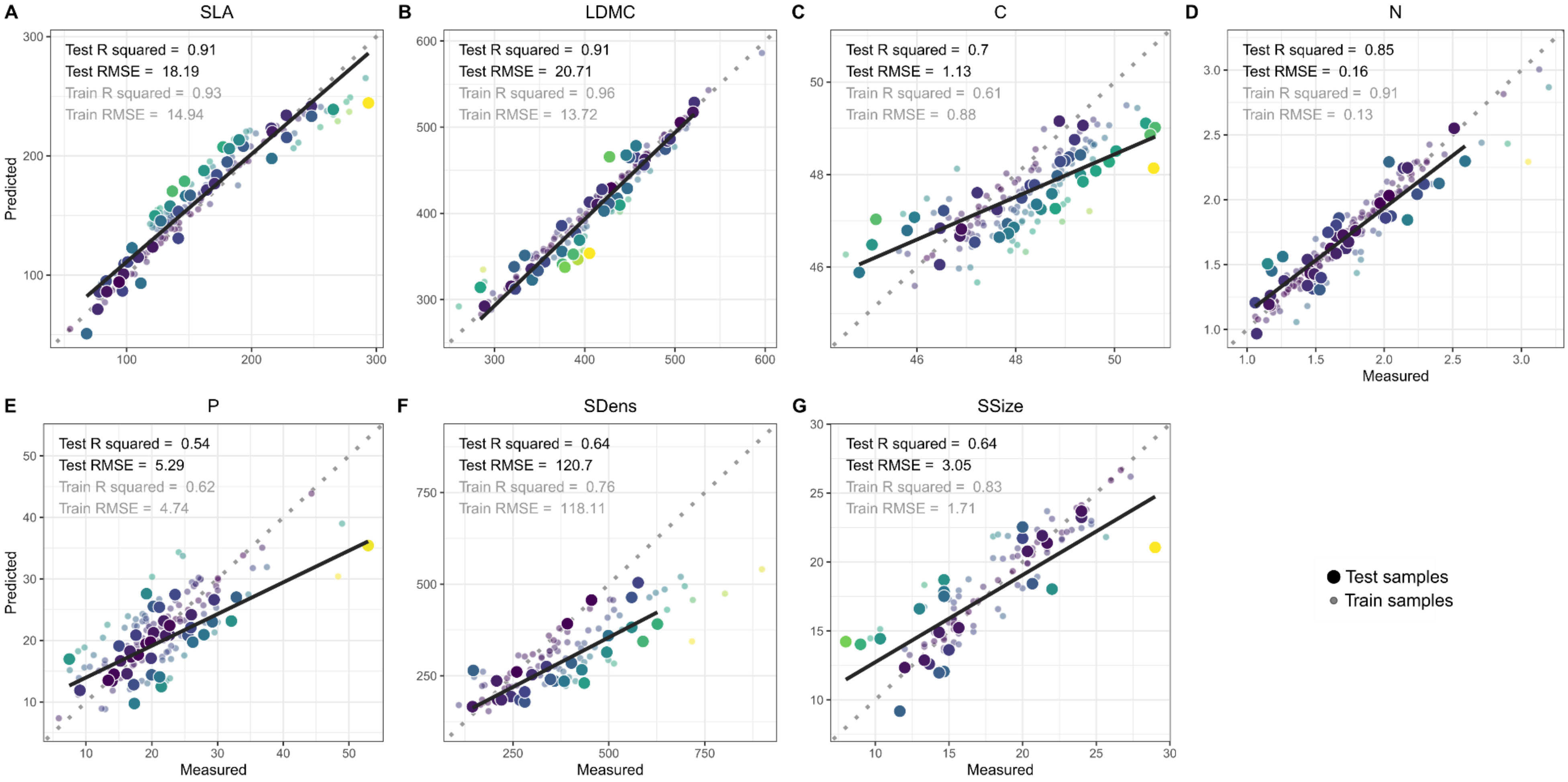


Fig. S14. Scatter plot of predicted and measured trait values in the test and the train samples. Correlation lines correspond only to the correlation between the predicted and measured values in the test samples. The dashed grey lines indicate the optimal fit in every case. The color of the points corresponds to the distance of the value from the optimal fit (dark blue colors indicate short distance while yellowish colors correspond to higher distances).

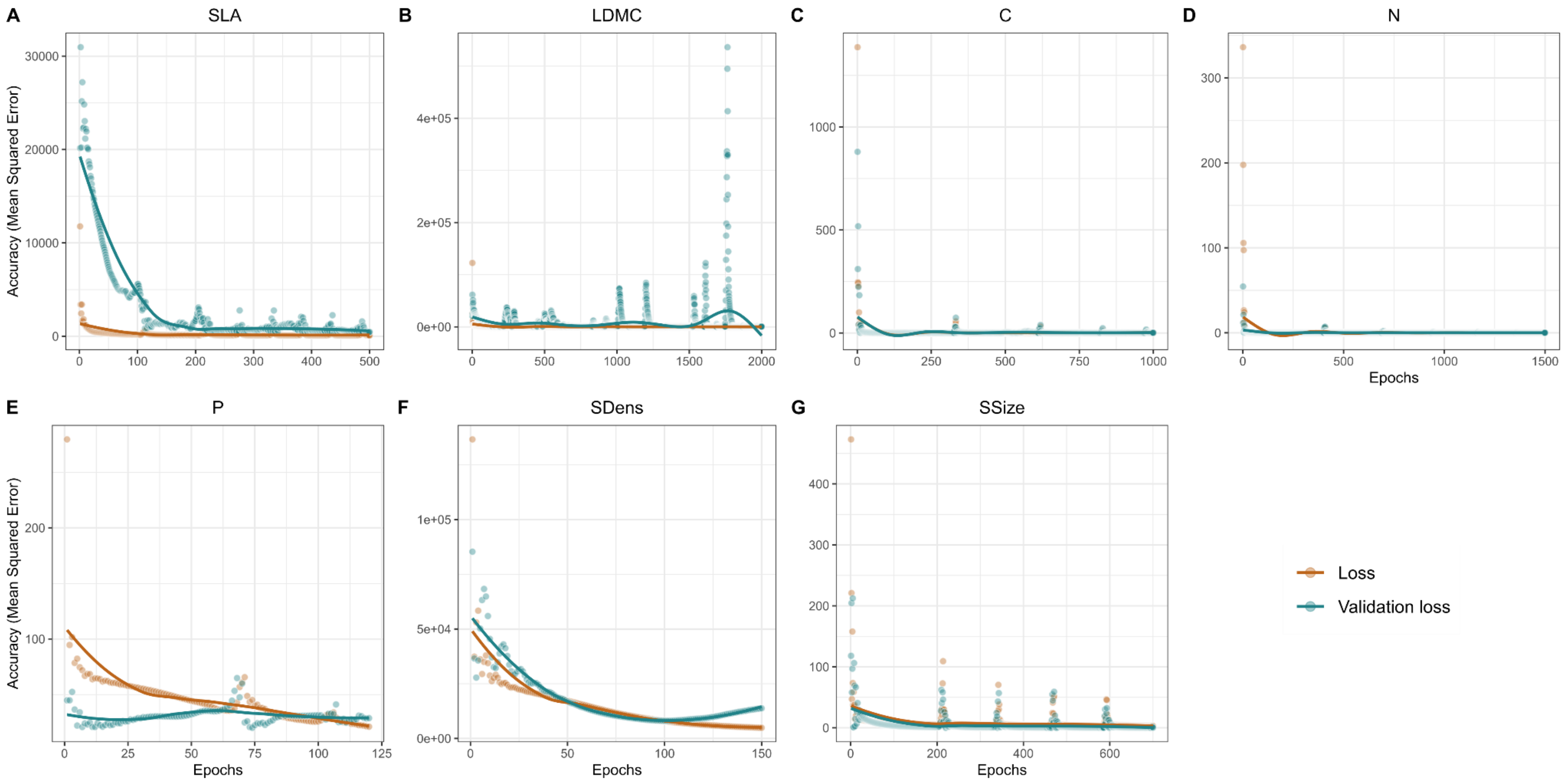


Fig. S15. Evolution of the error during the training of convolutional neural networks for trait prediction. Changes in the mean squared error in the loss function for all samples in the training set (in brown) and for a subset of samples used for validation during the training (in blue) were registered with an increasing number of epochs during the training process of the convolutional neural networks.

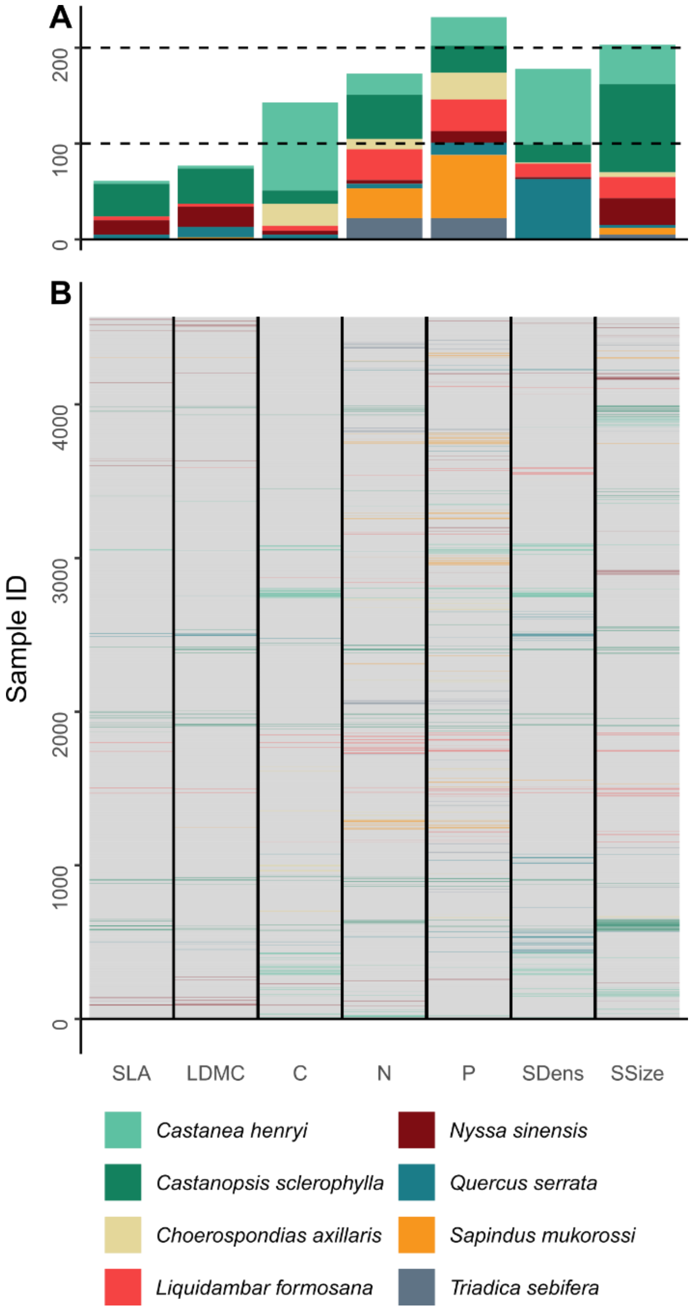


Fig. S16. Bar plot and heatmap of the distribution of missing trait data in the leaf-level dataset. (**A**) Represents a bar plot for the number of missing values for every trait (colored by species). In (**B**), vertical colored lines represent missing values and their distribution across the dataset (as ordered in the original dataset).

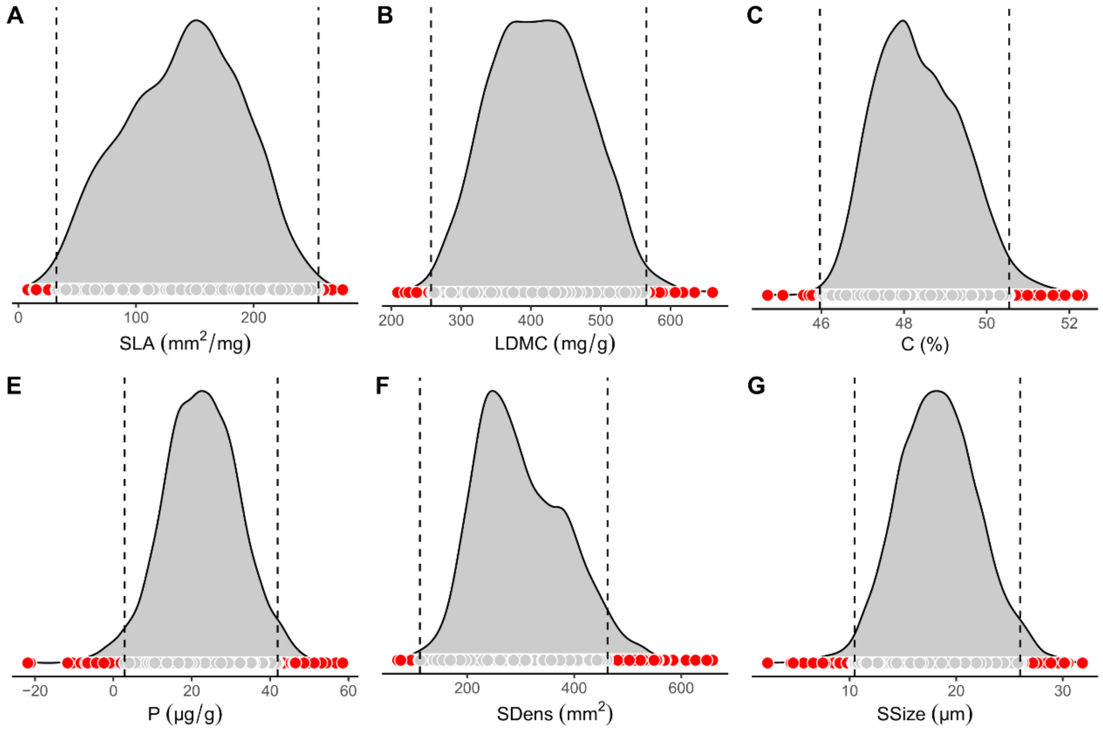


Fig. S17. Excluded values from predicted leaf-level data for seven leaf functional traits. Data excluded in trait predictions (showed in red) laid outside the interval formed by the median, plus or minus 3 median absolute deviations, as represented by the dashed vertical lines.

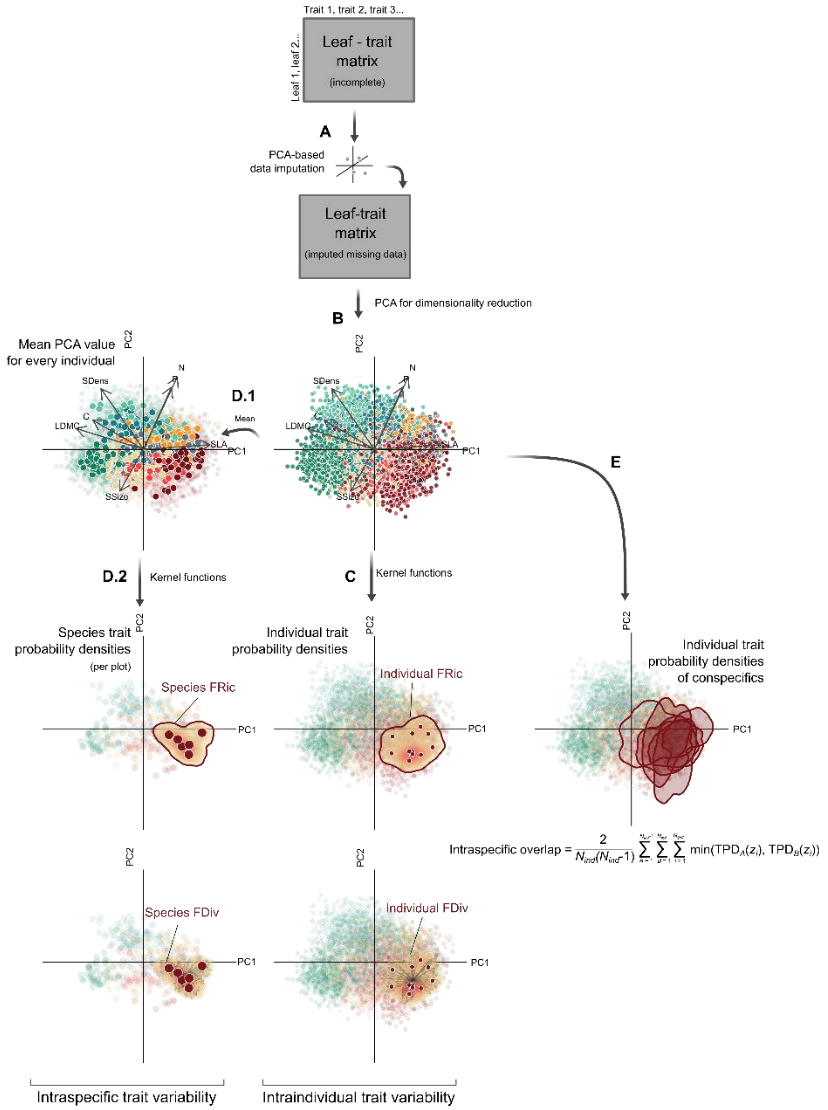


Fig. S18. Analytical framework used to assess the metrics of intraindividual variability, intraspecific variability and intraspecific overlap. All metrics were assessed by using (**A**) the leaf-level trait matrix. Due to missing values in the matrix, a principal component analyses (PCA)-based imputation approach was used to predict the missing data from the existing ones. With the completed dataset via imputation, (**B**) we performed a PCA to reduce the dimensionality of our data and used the first two principal components which together explained almost 70% of the variation. While, (**C**) trait probability densities were estimated for individual trees from this data, (**D.1**) mean values were obtained for individual trees in order to assess (**D.2**) trait probability densities for the intraspecific trait variability. From all these trait probability densities (the ones at the individual level and the population level) functional richness and functional divergence were used to estimate intraindividual and intraspecific trait variability. Last, (**E**) the trait probability densities estimated at the individual level for conspecifics (individuals from the same species occurring in the same plot) were used to estimate the mean intraspecific overlap of a species in a plot.

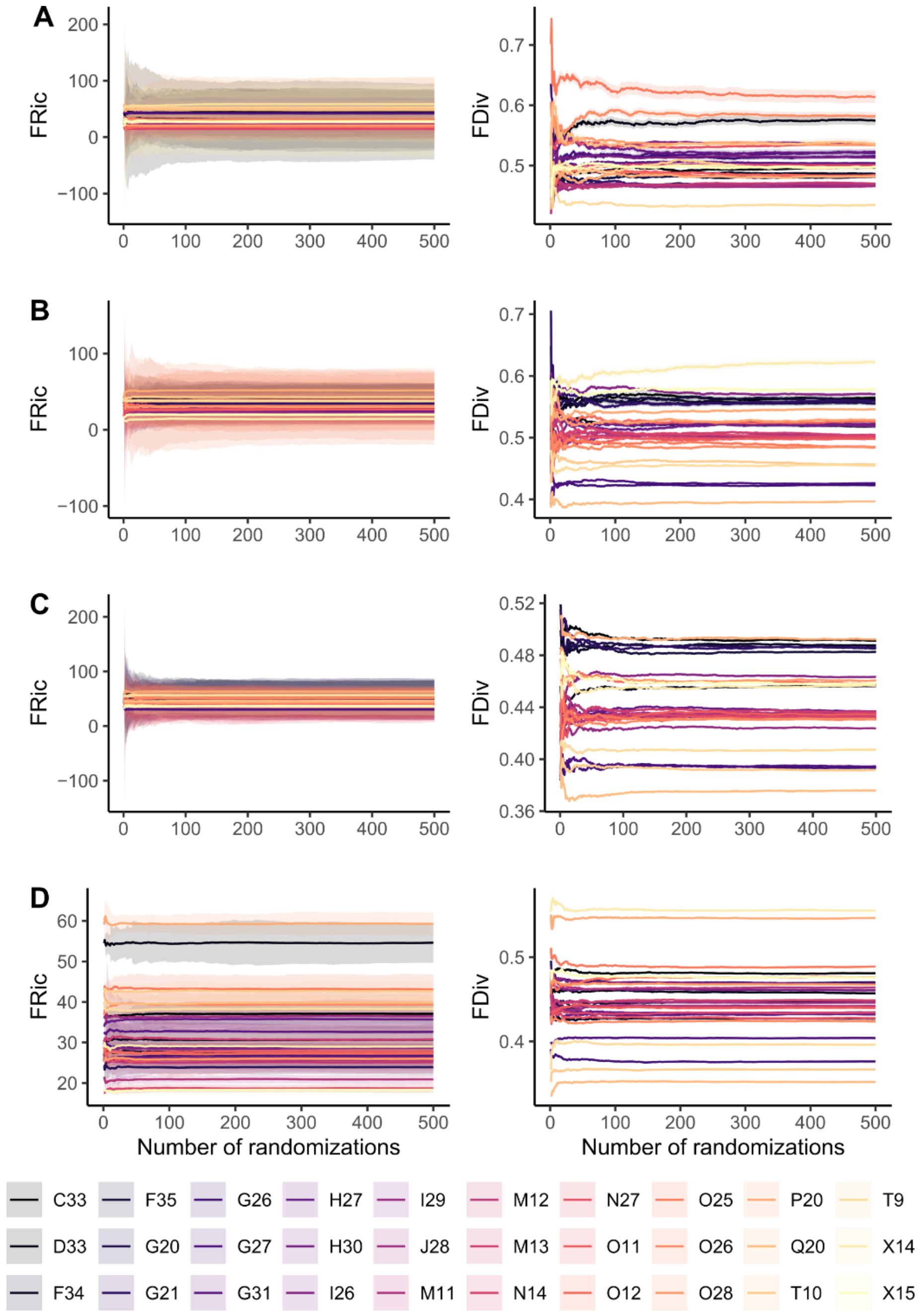


Fig. S19. Evolution of mean and variance of simulated values of FRic and FDiv from different null models with increasing number of randomizations. In order to assess the quality of using 500 randomizations, the changes in the mean (represented by lines) and the variance (represented as dashed areas) of functional diversity indices were studied in response to the number of randomizations for the null models of (**A**) random population, (**B**) random tree, (**C**) random leaf and (**D**) population-restricted random leaf null model. Colors correspond to the different plots included in our study.

Table. S1. Summary of a principal component analysis for seven leaf functional traits, including loadings, standard deviation, proportion of the variance explained by each component and the adjusted eigenvalue obtained in a Horn's parallel analysis.

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7
SLA	0.90	0.07	-0.03	-0.31	-0.01	0.28	-0.15
LDMC	-0.94	0.19	-0.07	0.16	0.08	-0.06	-0.22
C	-0.73	0.29	0.19	-0.44	-0.38	-0.03	0.02
N	0.46	0.75	0.16	-0.21	0.28	-0.29	-0.01
P	0.38	0.64	0.46	0.40	-0.25	0.09	-0.01
SDens	-0.64	0.63	-0.18	-0.03	0.24	0.32	0.09
SSize	-0.32	-0.42	0.81	-0.09	0.24	0.09	0.00
Standard deviation	1.75	1.29	0.98	0.72	0.64	0.53	0.28
Proportion of variance	0.44	0.23	0.14	0.08	0.06	0.04	0.01
Cumulative Proportion	0.44	0.68	0.82	0.89	0.95	0.99	1.00
Adjusted eigenvalue	3.03	1.64	0.94	0.53	0.42	0.31	0.13

Table. S2. Results for linear mixed-effects models studying the effects of tree species richness on multivariate functional indices used to estimate intraspecific variability, intraindividual variability and intraspecific overlap. Estimates (standard errors) and significance assessed with likelihood ratio tests are shown. The slope of the terrain (slope) was included as a covariate in the models for intraspecific variability and intraspecific overlap, while the models for intraindividual variability included slope and diameter at breast height (DBH) as covariates.

Level	Index	Tree species richness	Slope	DBH	R^2m	R^2c
Intraspecific	FRic	**-0.75 (0.36)***	0.07 (0.06)	-	0.06	0.17
Intraspecific	FDiv	-0.003 (0.004)	-0.0005 (0.001)	-	0.04	0.11
Intraindividual	FRic	-0.40 (0.68)	-0.03 (0.05)	**0.06 (0.02)***	0.03	0.29
Intraindividual	FDiv	-0.001 (0.002)	0.0001 (0.0003)	0.0001 (0.0001)	0.00	0.01
-	Intraspecific overlap	**0.02 (0.01)***	-0.002 (0.001)	-	0.06	0.29

Note: R^2m, marginal R^2; R^2c, conditional R^2;

*$p < 0.05$.

Table. S3. Species included in the study. Species names and families from World Flora Online (https://www.worldfloraonline.org/; accessed 19 June 2024).

Species	Family
Castanea henryi Rehder & E.H.Wilson	Fagaceae
Castanopsis sclerophylla (Lindl. & Paxton) Schottky	Fagaceae
Choerospondias axillaris (Roxb.) B.L.Burtt & A.W.Hill	Anacardiaceae
Liquidambar formosana Hance	Altingiaceae
Nyssa sinensis Oliv.	Nyssaceae
Quercus serrata Murray	Fagaceae
Sapindus mukorossi Gaertn.	Sapindaceae
Triadica sebifera (L.) Small	Euphorbiaceae

Table. S4. Results for linear mixed-effects models studying the effects of tree species richness and type of null model on standardized effect sizes of two functional indices. Significance assessed with likelihood ratio tests are shown. The interaction between the predictors is indicated by ":".

Response variable	Tree species richness	Type of null model	Tree species richness : Type of null model	R^2m	R^2c
SES_{FRic}	***	***	***	0.26	0.61
SES_{FDiv}	***	***	***	0.40	0.68

Note: R^2m, marginal R^2; R^2c, conditional R^2;

***p < 0.01.

Table. S5. Layers and hyperparameters used for building a convolutional neural network for every trait, and coefficient of determination (R^2) and root mean squared error (RMSE) for the test and the train samples.

Layer	Hyperparameter	SLA	LDMC	C	N	P	SDens	SSize
Spectral region	-	400-2500	400-2500	400-2500	1500-2400	1500-2400	400-2500	400-2500
1 dimension convolutional layer	Number of filters	2	2	1	2	2	2	2
1 dimension convolutional layer	Kernel size	50	2	35	77	77	2	1
Batch normalization layer	-	Yes	Yes	Yes	Yes	No	Yes	No
Max-pooling layer	Pool size	2	2	2	2	2	2	2
Layer flatten	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Layer dense	Number of nodes	128	64	128	64	256	128	64
Layer dense	Number of nodes	32	16	32	16	64	32	16
Layer dense	Number of nodes	8	4	4	4	16	8	4
-	Epochs	500	2000	1000	1500	120	150	700
-	Validation Split	0.2	0.2	0.2	0.2	0.2	0.2	0.01
R^2 test	-	0.91	0.91	0.7	0.85	0.54	0.64	0.64
RMSE test	-	18.19	20.71	1.13	0.16	5.29	120.7	3.05
R^2 train	-	0.93	0.96	0.61	0.91	0.62	0.76	0.83
RMSE train	-	14.94	13.72	0.88	0.13	4.74	118.11	1.71

Table. S6. Distribution of missing trait data in the leaf-level dataset across species and traits.

	SLA	LDMC	C	N	P	Sdens	Ssize	Mean
Castanea henryi	3 (0.07%)	3 (0.07%)	92 (2.01%)	22 (0.48%)	30 (0.66%)	79 (1.73%)	41 (0.9%)	38.57 (0.84%)
Castanopsis sclerophylla	34 (0.74%)	37 (0.81%)	14 (0.31%)	46 (1.01%)	28 (0.61%)	19 (0.42%)	92 (2.01%)	38.57 (0.84%)
Choerospondias axillaris	0 (0%)	0 (0%)	23 (0.5%)	11 (0.24%)	28 (0.61%)	1 (0.02%)	5 (0.11%)	9.71 (0.21%)
Liquidambar formosana	4 (0.09%)	3 (0.07%)	5 (0.11%)	32 (0.7%)	33 (0.72%)	14 (0.41%)	22 (0.48%)	16.14 (0.35%)
Nyssa sinensis	15 (0.33%)	21 (0.46%)	4 (0.09%)	4 (0.09%)	12 (0.26%)	2 (0.04)	28 (0.61%)	12.28 (0.27%)
Quercus serrata	4 (0.09%)	11 (0.24%)	5 (0.11%)	5 (0.11%)	13 (0.28%)	62 (1.36%)	3 (0.07%)	14.71 (0.32%)
Sapindus mukorossi	1 (0.02%)	2 (0.04%)	0 (0%)	31 (0.68%)	66 (1.44%)	1 (0.02%)	7 (0.15%)	15.43 (0.34%)
Triadica sebifera	0 (0%)	0 (0%)	0 (0%)	22 (0.48%)	22 (0.48%)	0 (0%)	5 (0.11%)	7 (0.15%)
Total	61 (1.34%)	77 (1.69%)	143 (3.13%)	173 (3.79%)	221 (4.84%)	178 (3.9%)	203 (4.44%)	150.86 (3.3%)

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CHAPTER 4

Within-individual leaf trait variation increases with phenotypic integration in a subtropical tree diversity experiment

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SUMMARY

Covariation of plant functional traits, that is, phenotypic integration, might constrain their variability. This was observed for inter- and intraspecific variation, but there is no evidence of a relationship between phenotypic integration and the functional variation within single plants (intraindividual trait variation; WTV), which could be key to understand the extent of WTV in contexts like plant–plant interactions.

We studied the relationship between WTV and phenotypic integration in c. 500 trees of 21 species in planted forest patches varying in species richness in subtropical China. Using visible and near-infrared spectroscopy (Vis-NIRS), we measured nine leaf morphological and chemical traits. For each tree, we assessed metrics of single and multitrait variation to assess WTV, and we used plant trait network properties based on trait correlations to quantify phenotypic integration.

Against expectations, strong phenotypic integration within a tree led to greater variation across leaves. Not only this was true for single traits, but also the dispersion in a tree's multitrait hypervolume was positively associated with tree's phenotypic integration. Surprisingly, we only detected weak influence of the surrounding tree-species diversity on these relationships.

Our study suggests that integrated phenotypes allow the variability of leaf phenotypes within the organism and supports that phenotypic integration prevents maladaptive variation.

Keywords: leaf functional traits, niche complementarity, phenotypic integration, plant–plant interactions, trait-based ecology, within-individual trait variation.

INTRODUCTION

Trait-based plant ecology assumes that plant attributes, that is, functional traits, mediate community assembly and ecosystem processes (Violle et al., 2007; Shipley et al., 2016). Traditionally, this discipline focused on mean differences between species' traits (i.e. interspecific trait variation) to address ecological questions related, for example, to plant coexistence or niche differentiation, ignoring that trait variation may also occur within species (i.e. intraspecific variation; Bolnick et al., 2011; Violle et al., 2012). However, by only considering interspecific trait variation we might fail to explain ecosystem processes at the spatial scales where individuals interact and, indeed, it is agreed that ignoring variability within species can lead to biased conclusions (Bolnick et al., 2011; de Bello et al., 2011; Chase, 2014). Recently, there has been a growing effort to understand the role of variation at lower levels of biological organization (Escudero & Valladares, 2016; Hart et al., 2016; Escudero et al., 2021). While variation among individuals within a species (i.e. intraspecific trait variation) has gained attention (Hart et al., 2016; Des Roches et al., 2018), only few studies addressed the ecological role of intraindividual trait variation (WTV; see Table 1). Intraindividual trait variation (WTV) refers to the plastic responses of plant individuals to express different trait values across different repeated architectural units of the plant body structure (De Kroon et al., 2005; Herrera et al., 2015; Herrera, 2017). Far from being ‘phenotypic noise’, plants show intraindividual responses in, for example, leaf (Winn, 1996; Valladares & Niinemets, 2008; Møller et al., 2022), fruit (Sobral et al., 2019) or flower traits (March-Salas et al., 2021). Furthermore, this variation may affect plant performance (Herrera, 2009, 2017) and can have evolutionary consequences (Herrera et al., 2022; Sobral & Sampedro, 2022).

Table 1. Definition of acronyms and abbreviations used.

Acronym/abbreviation	Definition
WTV	Intraindividual trait variation
PI_{ind}	Individual phenotypic integration
BEF	Biodiversity-ecosystem functioning
Vis-NIRS	Visible and near infrared-spectrometry
SLA	Specific leaf area
LDMC	Leaf dry matter content
C:N	Carbon to nitrogen ratio
C	Carbon leaf content
N	Nitrogen leaf content
Mg	Magnesium leaf content
K	Potasium leaf content
Ca	Calcium leaf content
P	Phosphorous leaf content
FRic	Functional richness
FDis	Functional Dispersion
SD	Standard deviation
LMM	Linear Mixed Model
AIC	Akaike Information criterion

The variation of one trait is not necessarily independent from the variation of other traits. Indeed, there are numerous and complex trait relationships resulting from genetic, developmental, and/or functional trade-offs and allometric constraints (Gould & Lewontin, 1979; Wright et al., 2004; Huneman, 2010; Armbruster et al., 2014; Nielsen & Papaj, 2022). For example, a reduction in specific leaf area (SLA) is usually coupled with an increase in leaf dry matter content (LDMC; Wright et al., 2004). Under this premise, traits vary in a coordinated way to optimize some functions at the cost of others (Messier et al., 2017; Vasseur et al., 2022). As a result, it is suggested that phenotypic integration (i.e. the pattern of coordination and covariation among traits reflected by the amount of significant correlations between traits, Schlichting & Pigliucci, 1998; Gianoli & Palacio-López, 2009; Armbruster et al., 2014) could play a role in constraining trait variation (Valladares et al., 2007; Matesanz et al., 2021). This assumption is also based on the impossibility of the evolution of organisms that can reach an optimal value for every trait simultaneously (Rees, 1993; Laughlin & Messier, 2015). That is why under scenarios of strong phenotypic integration, only a subset of possible trait combinations will exist due to the presence of strong constraints from functional trade-offs (Laughlin et al., 2017). Thus, different levels of phenotypic integration may be associated with the expression of plastic responses (He et al., 2021; Homeier et al., 2021; Silva et al., 2021; Li et al., 2022; Xie & Wang, 2022). However, while previous evidence describing the relationship between phenotypic integration and trait variation stems from studies on interspecific and intraspecific trait variation, the relationship between plant individuals' phenotypic integration (PI_{ind}; i.e. the number of significant correlations among traits between different repeated units of the same individual) and WTV is not known (but see Escribano-Rocafort et al., 2017). Therefore, understanding this relationship is crucial to assess the extent and intrinsic limits of WTV in modular organisms. To our knowledge, the only study focusing on PI_{ind} and WTV simultaneously suggested that for olive trees (Olea europaea) with higher leaf P_{lind}, leaf WTV tended to be lower (Escribano-Rocafort et al., 2017). According to these results, and similarly to what has been found at higher levels of biological organization (He et al., 2021), differences in PI_{ind} among individuals might explain differences in WTV. It could be therefore expected that individuals with higher PI_{ind} would be limited in their WTV. Therefore, phenotypic integration could affect the amount of WTV as a response to abiotic and biotic drivers.

Resource competition is considered a major component of plant–plant interactions. In theory, each individual inhabits a particular niche where it competes for resources with its local neighbors (Cabal et al., 2021). Accordingly, it is well established that there are stabilizing mechanisms that support species coexistence through functional differentiation and niche complementarity (Wright et al., 2014). Also, within species, niche complementarity between individuals of the same population can diminish the strength of intraspecific competition, since individuals from the same species need common resources and share similar uptake pathways (Grime, 1973; Tilman et al., 1982). Such niche complementarity through trait variation within species is particularly important in communities with low taxonomic diversity and, hence, high levels of intraspecific competition (Gross et al., 2008; Götzenberger et al., 2012). In this context, as WTV may improve efficiency in the use of resources (Møller et al., 2022), it has been suggested as a mechanism to foster niche complementarity and reduce intraspecific competition for resources among plants interacting directly (Davrinche et al., 2023). Indeed, WTV was reported to decrease with taxonomic diversity of the local neighborhood (Proß et al., 2021). However, as WTV is expected to be limited by phenotypic integration (Escribano-Rocafort et al., 2017), this could cause plants to fail to produce the optimal suites of traits for a given microenvironment (Pigliucci, 2005). Therefore, understanding the effect of neighborhood diversity on WTV and on the WTV-PI_{ind} relationship remains crucial to assess the role and intrinsic limits of trait variation in plant–plant interactions.

Leaves are repeated organs within plants with a crucial role in resource acquisition via photosynthesis. Furthermore, the light interception by leaves is a key factor in competition (Valladares et al., 2016). That is why plants express different leaf phenotypes within the crown in order to adjust to the light exposure (Sack et al., 2006; Escribano-Rocafort et al., 2016; Mediavilla et al., 2019). In addition, plastic responses in leaf traits are specifically noticeable in trees, which have great potential to express WTV as a consequence of their high modularity (Watkinson & White, 1986) and, therefore, could provide a suitable model to study the relationship between trait variation and phenotypic integration within an individual. As light heterogeneity is influenced, among others, by the canopy structure of the community, trees are expected to adjust their leaves to the different light exposures generated by different levels of taxonomic diversity. Consequently, a tree's leaf WTV may be strongly affected not only by the taxonomic identity of the closest tree neighbor but also by the taxonomic diversity of the surrounding tree neighborhood (Proß et al., 2021).

Here, we studied patterns of leaf trait variation and phenotypic integration within individual trees and how they were affected by local taxonomic diversity. As the closest adjacent tree is expected to have the strongest effect on intraspecific trait variation (Davrinche & Haider, 2021), we considered two scales of local taxonomic diversity: taxonomic diversity of the local neighborhood, that is, the trees surrounding a focal individual in a community, and identity of the closest neighbor. We used the currently largest tree diversity experiment, located in subtropical China, and measured nine morphological and chemical leaf traits in c. 500 individuals from 21 species across plots differing in species composition. We assessed different metrics of functional trait variation (WTV) and phenotypic integration for each individual tree. We expected that higher PI_{ind} constraints WTV (Fig. 1). As higher WTV is expected for scenarios of high intraspecific competition, we also expected that the WTV-PI_{ind} relationship depends on taxonomic diversity. Specifically, we hypothesized that the constraint should be more pronounced in scenarios of high taxonomic diversity (i.e. in diverse communities and, especially, when trees are directly interacting with a heterospecific adjacent neighbor) due to the lower ecological relevance of WTV in these environments (Fig. 1). Contrary, in scenarios of low taxonomic diversity, trees are expected to prioritize the display of alternative leaf designs, even if integration is high. We expected the constrain of PI_{ind} on WTV for the WTV of individual traits and also for metrics of multitrait functional diversity.

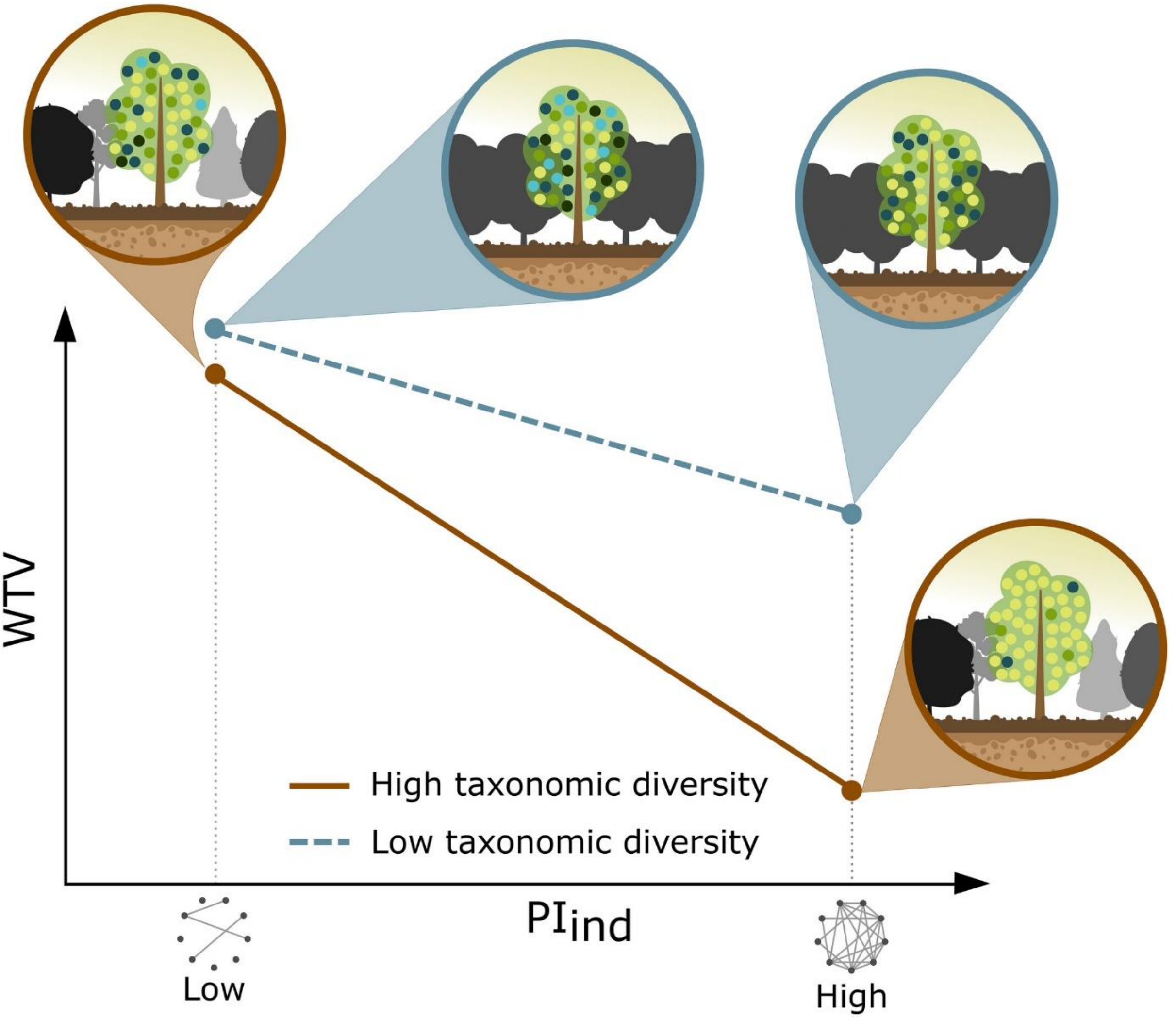

Fig. 1 Expected relationship between individual phenotypic integration (PI_{ind}) and intraindividual trait variation (WTV) at different levels of tree diversity. PI_{ind} is expected to constrain WTV at both levels of taxonomic diversity (Hypothesis 1), indicated in the figure through color variation in leaves. However, as WTV is an important mechanism to drive niche differentiation as response to intraspecific competition, we expect the WTV-PI_{ind} relationship to depend on the local taxonomic diversity, including both the identity of the adjacent neighbor and the diversity of species in the neighborhood (Hypothesis 2). Thus, the constraint of WTV by PI_{ind} should be less pronounced in scenarios of low taxonomic diversity. Networks on the bottom of the x-axis represent two scenarios of PI_{ind}, where lines ('edges') connecting points indicate coordination between two traits. Thus, low PI_{ind} (left) occurs when the number of coordinated pair of traits is low, while high PI_{ind} (right) indicates that the number of coordinated pair of traits is high. Zoom areas show the leaf WTV of a target tree (denoted by variable colored points within the crown) in different contexts of taxonomic diversity (represented by the shape and transparency of the tree silhouettes surrounding the target tree).

MATERIALS AND METHODS

Study site

This study was conducted in a biodiversity–ecosystem functioning (BEF) experiment, the BEF-China tree diversity experiment, located in Xingangshan, in Jiangxi Province (lat. 29°08’11”N, long. 117°90’93”E; Fig. 2a). BEF-China was designed to study ecosystem functions in planted patches of varying tree-species richness, hence simulating the effect of species extinction on the functioning of ecosystems. The climate is subtropical with a mean annual temperature of 16.5°C (ranging from 0.4°C in January to 34.2°C in July) and mean annual precipitation of 1821 mm (data from the adjacent Wuyuan County, Yang et al. (2013)). The natural vegetation in the region is dominated by mixed broadleaved forests with similar number of deciduous and evergreen species, but with evergreen species dominating in terms of abundance (Bruelheide et al., 2011; Su et al., 2020). The experiment consists of two sites, A and B, where trees were planted in 2009 and 2010, respectively (Bruelheide et al., 2014; Fig. 2b). Site A extends over an area of 27 ha with an elevation ranging from 205 to 275 m asl and slopes from 8.5° to 40°. Site B has a size of > 23 ha with an elevation ranging from 113 to 182 m asl and slopes ranging from 15° to 43°. In the experiment, trees are arranged following the ‘broken-stick’ design described in Bruelheide et al. (2014). This design is based on the partitioning of the pool of species in every site into two equal groups at each subordinate richness level. Thus, from the total pool of 40 species in both sites (see Bruelheide et al. (2014) for details on the plant species), plots range from the 24-species mixture to the monoculture passing through 16-, 8-, 4- and 2-species mixtures (Fig. 2c). Hence, at each site, all species are equally represented at every species richness level. In every plot, 400 saplings from local nurseries were planted in a regular grid with a distance of 1.29 m, with species randomly assigned to planting positions.

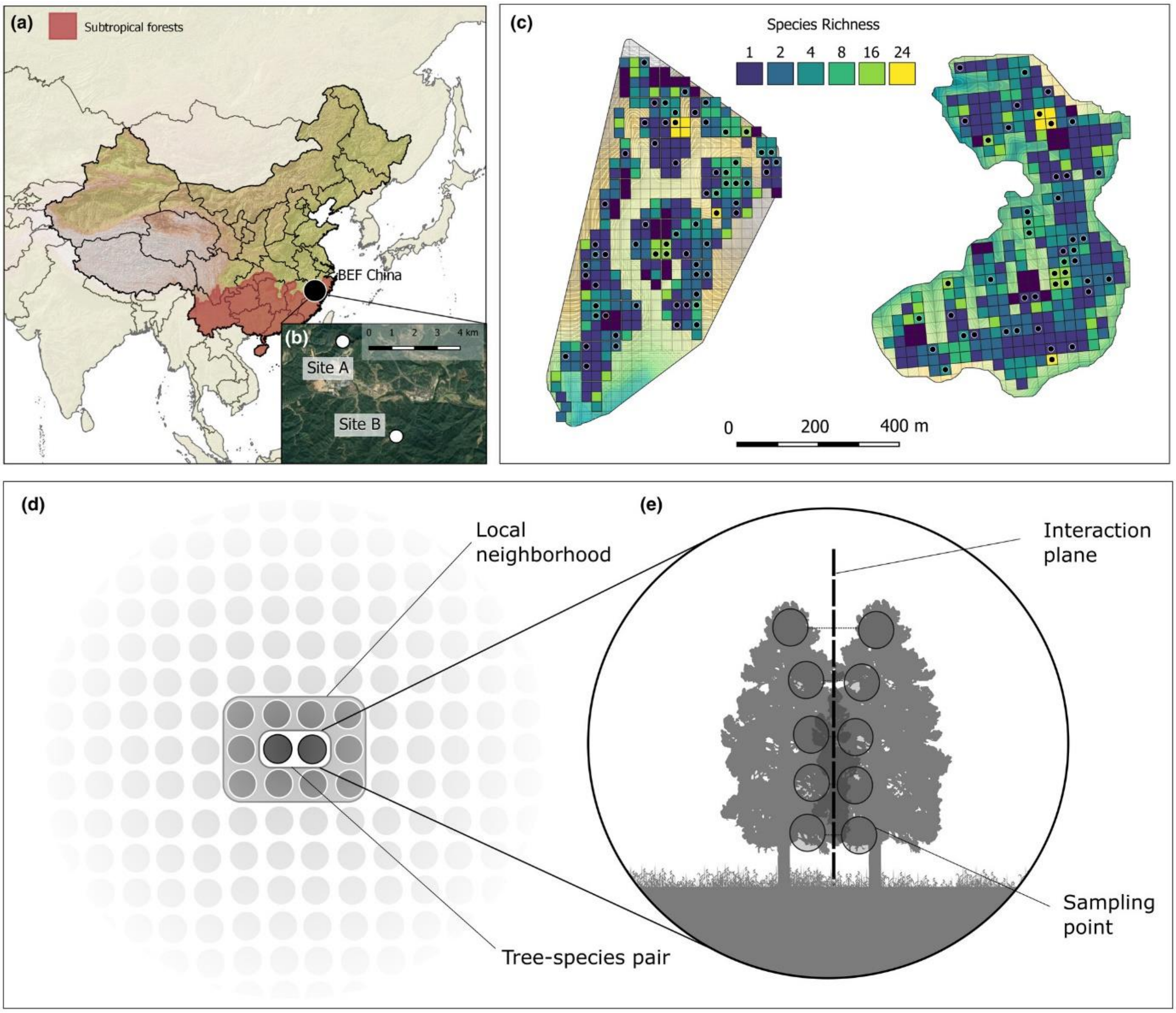

Fig. 2 Location of (a) the biodiversity–ecosystem functioning (BEF)-China experiment, (b) sites of the experiment and (c) plots within the sites, and (d, e) design of the tree-species pair sampling. The BEF-China experiment (a) is located in subtropical China (Xingangshan, Jiangxi Province). The map also shows in addition the distribution of subtropical forests in China (Olson et al., 2001). The experiment consists (b) of two sites (A and B) distanced by c. 5 km. Each site contains plots differing in the number of planted species (c). The black dots in (c) indicate the plots where sampling took place for this study. As shown in (d), within plots we selected at least one tree-species pair, consisting of two adjacent trees directly interacting, and defined the local neighborhood of a tree-species pair as the group of 10 trees that were directly surrounding the tree-species pair. For every tree-species pair, we sampled leaves at five different heights (sampling points) along the vertical plane between the trees (interaction plane), which is represented by the dashed line (e).

Field sampling

Sampling followed the tree-species pair design as described in Trogisch et al. (2021), which focuses on the interaction of a pair of directly adjacent neighbors (hereafter, referenced as tree-species pair) and the interaction of this tree-species pair with the surrounding local neighborhood (Fig. 2d). Hence, by sampling both trees in a tree-species pair, the design allows to study the interaction between WTV and individual phenotypic integration (PI_{ind}) at two different fine scales of local taxonomic diversity: the species identity of the tree-species pair partner, that is, the tree's closest neighbor (conspecific vs heterospecific); and the taxonomic diversity of the local neighborhood, that is, the 10 trees (or fewer in case of mortality) surrounding the tree-species pair. The diversity of the local neighborhood was assessed by calculating the Shannon index (Shannon, 1948), considering the frequency of the different tree species within the up to 10 neighbor trees of the tree-species pair.

Sampling took place from late August to early October 2018 and mid-August to mid-September 2019 for sites A and B, respectively. We sampled a total of 432 trees (216 tree-species pairs) in 69 plots at site A and 437 trees (219 tree-species pairs) in 57 plots at site B (hereafter, referenced as regular set). From each tree, we collected leaves along the interaction plane between the tree-species pair partners (the vertical plane where the two crowns of the tree-species pair partners meet, Fig. 2e). In order to encompass the variation of the whole individual, we sampled at five different heights along the interaction plane. At each height, we cut three fully developed leaves free from mechanical or pathogen damage. Immediately after collection, leaves were stored in sealable plastic bags with moistened tissue. Samples were transported in an isothermal bag equipped with cooling bags to prevent dehydration. In the laboratory, samples were temporarily stored at 6–8°C.

In addition, we collected an independent set of leaf samples for each site (hereafter, referenced as calibration sets). The aim of this was to predict the trait values for the samples of the regular set based on the relationship between reflectance spectra and measured trait values of the calibration set. For the calibration sets, we aimed to include 10 leaf samples per species per site across all plots of all species richness levels, collected at different heights and orientation within the crown, in order to maximize the sampled scenarios (i.e. combinations of species considering the closest neighbor and the local neighborhood, different position of the leaf within the crown and location of the tree within the experimental site). A total of 236 samples for site A and 252 for site B were collected for the calibration set, with each sample composed of 15 leaves on average depending on the leaf size, to ensure sufficient material for laboratory analyses.

Laboratory analyses

The number of leaf samples in the regular set was high, and the material from each leaf was too low to conduct all chemical analyses at the leaf level. For this reason, we used visible and near-infrared spectrometry (Vis-NIRS), a technique of massive phenotyping, to estimate trait values for each individual leaf based on calibration models (Foley et al., 1998; Escudero et al., 2021). For all leaves (regular and calibration sets), we acquired reflectance spectra with a portable Vis-NIRS device (ASD ‘FieldSpec4’ Wide-Res Field Spectroradiometer; Malvern Panalytical Ltd, Almelo, the Netherlands). Reflectance was measured across the full range of solar radiation spectrum (250–2500 nm), by taking three repeated measurements on the adaxial side of each leaf while avoiding main veins. For each of these repeated measurements, 10 spectra were averaged internally to reduce noise. The equipment was optimized regularly with a calibration white panel (Spectralon, Labsphere, Durham, NH, USA).

For the samples of the calibration set, we determined nine morphological and chemical leaf traits which are assumed to reflect a plant's strategy in terms of the investment of nutrients and dry mass in the leaves (Pérez-Harguindeguy et al., 2013; Díaz et al., 2016; see Supporting Information Table S1). Additionally, these traits are key components of the leaf economics spectrum and reflect the most important trade-offs along different leaf designs (Wright et al., 2004; Osnas et al., 2013): specific leaf area (SLA; leaf area divided by leaf dry mass), leaf dry matter content (LDMC; leaf dry mass divided leaf fresh mass), carbon-to-nitrogen ratio (C : N), carbon content (C), nitrogen content (N), magnesium content (Mg), potassium content (K), calcium content (Ca), and phosphorus content (P). After collection, the saturated fresh leaves of the calibration samples were weighed (DeltaRange Precision Balance PB303-S; Mettler-Toledo GmbH, Gießen, Germany) and scanned at a resolution of 300 dpi to calculate leaf area (WinFOLIA; Regent Instruments, Quebec, QC, Canada). Leaves were oven-dried at 80°C for 72 h and weighed to calculate SLA and LDMC. Dried leaves were ground

(Mixer Mill 400; Retsch, Haan, Germany), and 200 mg of the resulting powder was used for a nitric acid digestion. After the digestion, Mg, Ca, and K were analyzed with atomic absorption spectrometry (ContrAA 300 AAS; Analytik Jena, Jena, Germany), while P was measured through a molybdate spectrophotometric method. Additionally, we used an elemental analyzer (Vario El Cube; Elementar, Langenselbold, Germany) to gas-chromatographically determine C and N and, from these measurements, C:N.

Leaf spectra of the samples of the calibration set were analyzed with the Unscrambler X software (v.10.1; CAMO Analytics, Oslo, Norway) for all species together but separately for site A and site B. The use of multispecies calibrations aimed to cover the broadest trait space possible, in order to better reflect the possible variation in our samples (Burnett et al., 2021) and allowed for more data to build the calibration model. Spectral pretreatments were applied in order to optimize the prediction of traits (normalization, smoothing and 2nd derivate, orthogonal signal correction, standard normal variate, detrending according to Barnes et al. (1989), or baseline correction). Spectra were then used to fit partial least square regression models by using the NIPALS algorithm (Dayal & Macgregor, 1997; Burnett et al., 2021). Selection of the partial least square regression models was based on their quality (determined by a high R^2 value and a low root mean square error for a validation set), parsimony (indicated by a low number of factors), and predictive power (determined by a high R2 for predicted vs reference value). For site A, R^2 for predicted vs reference value of the best models for each trait was 66.94 ± 20.23 (mean ± standard deviation), with a maximum R^2 for SLA (88.90) and minimum for Ca (24.20), while for site B we obtained a mean R^2 of 74.60 ± 11.00, with maximum and minimum values for SLA (89.88) and Ca (58.84), respectively (see Davrinche & Haider (2021) and Davrinche et al. (2023) for methodical details). Finally, these models were used to predict the trait values from the reflectance spectra of the samples of the regular set. From the three predicted values for each leaf in the regular set (resulting from the three repeated measurements per scan), we excluded: negative ones; those with > 5% deviation from the range limits of the calibration data; and values outside of the 95% confidence interval of the model prediction. The remaining values per leaf were averaged. As a consequence of outlier detection, an average of 3.3 ± 1.1% (mean ± SD) of the leaves was removed depending on the trait (see Table S2).

Metrics of individual phenotypic integration and trait variation

To accurately estimate PI_{ind} and WTV, we only used data from those trees, which met several criteria in the regular dataset. Thus, as the study of phenotypic integration requires the lack of missing data (He et al., 2020) only leaves with trait data available for all nine traits were considered. Furthermore, in order to avoid low accuracy in the metrics for WTV, and especially, for PI_{ind}, only trees with data from a minimum of 10 leaves were considered (see Fig. S1 for details on data selection). Finally, as the underrepresentation of specific sampled species could make sample sizes across groups highly unbalanced and, thus, our models unstable (Grueber et al., 2011), we only worked with those species which after the filtering described before were represented by more than eight individuals. Thus, from the original number of 869 trees from 27 species, our study included 499 trees from 21 species located in 97 plots and represented across different levels of taxonomic diversity (see Tables S3, S4).

To measure PI_{ind}, we first computed for each tree all possible pairwise correlations between traits based on the trait values for individual leaves (Fig. S2a). Then, we evaluated the significance of the correlations by using permutation tests in which trait values were rearranged 20 000 times and pairwise correlations were calculated from each randomization. We calculated the P-values based on the permutation distribution of correlations obtained from randomizations. These tests were performed by using the function perm.cor.test in the jmuOutlier package (Garrenstjmuedu, 2019). Based on our correlation matrix, we built a plant trait network for each tree as described in He et al. (2020). To avoid spurious correlations among traits, we only considered connections ('edges') of significant correlation ($P < 0.05$) and with a Pearson coefficient $|\rho| \geq 0.6$ (Aggarwal & Ranganathan, 2016). Importantly, most trait–trait pairs considered for the network were highly significant ($P < 0.01$), indicating that the considered correlations are not likely statistical noise but, rather, a biological signal (see Figs S3, S4). We measured two properties that estimate the tightness of the networks: edge density, which measures the proportion of actual connections among traits out of all possible connections within a network and, thus, can be used to quantify the connectivity of all traits across the whole trait network (Benavides et al., 2021); and degree (Poorter et al., 2014; He et al., 2021), which represents a measure of coordination for each trait as it measures the number of connections of one focal trait ('node') to all others. These properties of the networks were assessed by using functions from the igraph package (Csardi, 2021).

We assessed multitrait WTV for each tree including all traits by using two indices related to different attributes of the functional hypervolume of all leaves in a tree: functional richness (FRic); and functional dispersion (FDis). Together, these two indices can reveal two different and complementary aspects of trait variation. Functional richness (FRic) measures the total functional hypervolume by using the minimum convex volume (Cornwell et al., 2006; Botta-Dukát & Czúcz, 2016). This index aims to detect reductions in the niche space occupied by individuals (Cornwell et al., 2006). Thus, a higher FRic indicates that an individual occupies a greater niche space. Functional dispersion (FDis) measures the distances of leaves to the centroid of the functional hypervolume, thus describing whether the distribution of leaves in a trait space is clustered or dispersed (Laliberte & Legendre, 2010). To calculate these indices, we first obtained a leaf-by-leaf trait distance matrix per tree by using Gower's distance. This was calculated with the gowdis function in the FD package (Laliberté et al., 2014). Then, for every tree we computed both indices through principal coordinate analysis (PCoA) by using the function dbFD from the same package. As these indices can be sensitive to the number of observations and in order to account for the different number of leaves per tree (Mason et al., 2013), we used standardized effect sizes (SES) as described in Gotelli & McCabe (2002). We used 500 randomizations in a null-model analysis to ensure accurate estimates of SES values. Additionally, we assessed single-trait WTV by using the standard deviation (SD) of each trait across all leaves within a tree, as it represents how trait values are spread around the mean value (Proß et al., 2021; Fig. S2b).

Statistical analyses

All statistical analyses were performed in R v.4.02 (R Core Team, 2021). To assess the joint effect of PI_{ind} and local taxonomic diversity on within-tree trait variation (WTV), we performed linear mixed models (LMMs) for the multitrait and single-trait metrics of WTV. To do so, we used the two functional indices (SES(FDis) and SES(FRic)) and the SD of each of the nine traits, respectively, as response variables, resulting in a total of 11 models. Explanatory variables were the two metrics for PI_{ind} (edge density in the case of multitrait WTV (SES(FDis) and SES(FRic)), and the degree in the case of the models for single-trait WTV (SD)), the diversity of the tree-species pair (conspecific vs heterospecific), the Shannon diversity of the local neighborhood of a tree-species pair, and all possible interactions of these variables (including the three-way interaction). We included tree-species pair identity nested in plot, in turn nested in site, and species identity as crossed random effects in order to account for the design of the experiment and differences among species, respectively (see Table S5). These analyses were conducted using the lmer function in the lmerTest package (Kuznetsova et al., 2017). We used diagnostic plots of the residuals to study the assumptions of normality, homoscedasticity and linearity in our models: residuals vs fitted values plots, histograms of the residuals and Q-Q plots for the deviance of the residuals. Thus, to meet the premises of homoscedasticity and normality of the residuals in the models, SD(SLA), SD(C), and SD(P) were log-transformed and SD(LDMC), SD(N), SD(K), and SD(Ca) were square-root transformed. We fitted 'beyond optimal' models, which included all of the fixed effects to fit the model. Then, by including only subsets of the predictors, the AIC was calculated for all possible models that varied in their fixed effects. We selected all models with ΔAIC lower than 2 as competing models holding similar information and followed the principle of parsimony to prioritize the simplest model with the smallest number of predictors among all competing models (Burnham & Anderson, 2004; Richards et al., 2011; Harrison et al., 2018). Finally, we assessed the quality of fit of our competing models by calculating the marginal and conditional R^2, which address the variance explained only by fixed effects and the variance explained by the entire model, respectively.

RESULTS

We found a positive relationship between multitrait WTV and PI_{ind}. However, of the two multitrait functional indices, only SES(FDis) responded to edge density, which was used to quantify PI_{ind} at the multitrait level (Table 2). In both competing models, SES(FDis) increased with edge density and this response was stronger for trees with a conspecific partner (significant interaction of edge density and the diversity of the tree-species pair; Figs 3, 4). Furthermore, one of the competing models, but not the simplest model, included a negative effect of local neighborhood Shannon diversity, indicating that higher local neighborhood diversity was associated with lower SES(FDis; see Fig. S5). The effect of edge density on SES(FRic) was included in three of the competing models, but the most parsimonious model neither included edge density nor any of the other predictor variables. In the three competing models including edge density among the predictors, two suggested that SES(FRic) increases with edge density while the third indicated that the response depends on the identity of the closest neighbor, increasing in the case of a conspecific partner and decreasing in the case of a heterospecific one (see Fig. S6). This suggests that the effect of edge density on SES(FRic) was rather weak and could be attributable to correlations between metrics (see Fig. S4). For the most parsimonious model of SES(FDis), marginal R^2 accounted for 13% of the variance, and 17% of the variance was explained when also considering the random effects as well. In the case of SES(FRic), for the simplest model, conditional R^2 accounted for 8% of the variance.

Table 2. Competing models to identify the drivers of intraindividual trait variation (WTV).

Response	Int	PI_{ind}	S	TSP_{Div}	PI_{ind}:S	PI_{ind}:TSP_{Div}	S:TSP_{Div}	PI_{ind}:S:TSP_{Div}	Df	delta	R^2m	R^2c
SES(FDis)	**-3.085**	**1.992**		**-0.824**		**×**			**9**	**0**	**0.133**	**0.174**
	-3.021	2.002	-0.075	-0.845		×			10	1.471	0.135	0.177
SES(FRic)	-0.493	0.236							7	0	0.005	0.093
	-0.374								**6**	**0.585**	**0**	**0.084**
	-0.339	-0.08		-0.244		×			9	1.48	0.01	0.109
	-0.503	0.24	0.011						8	1.942	0.005	0.1
log(SLA_{SD})	**2.385**	**0.089**							**7**	**0**	**0.062**	**0.709**
	2.374	0.088		0.023					8	1.557	0.062	0.71
	2.401	0.088	-0.019						8	1.671	0.062	0.71
	2.292	0.106		0.15		×			9	1.853	0.063	0.712
sqrt($LDMC_{SD}$)	**3.889**	**0.242**							**7**	**0**	**0.163**	**0.267**
	3.691	0.238	0.21	0.295			×		10	1.115	0.169	0.272
	3.513	0.278	0.21	0.585		×	×		11	1.401	0.173	0.273
	3.907	0.242	-0.025						8	1.94	0.163	0.268
$(C:N)_{SD}$	**2.069**	**0.299**							**7**	**0**	**0.123**	**0.344**
	2.177	0.295	-0.127						8	0.1	0.126	0.345
	1.987	0.337	0.137		×				9	1.017	0.127	0.348
	2.044	0.297		0.052					8	1.773	0.123	0.343
log(C_{SD})	-0.805	0.08	-0.037						8	0	0.093	0.745
	-0.831	**0.08**							**7**	**0.632**	**0.092**	**0.743**
	-0.838	0.089	0.009		×				9	0.989	0.093	0.744
	-0.848	0.079		0.03					8	1.067	0.093	0.744
	-0.82	0.08	-0.03	0.019					9	1.543	0.093	0.745
sqrt(N_{SD})	**0.308**	**0.02**							**7**	**0**	**0.145**	**0.416**
	0.304	0.02		0.007					8	0.55	0.147	0.415
	0.313	0.019	-0.005						8	0.924	0.146	0.414
	0.312	0.018		-0.007		×			9	1.947	0.148	0.418
Mg_{SD}	0.469	0.033	-0.059	-0.023	×				10	0	0.048	0.789
	0.44	0.042	-0.018	-0.023					9	1.055	0.046	0.79
	0.424	0.042		-0.017					8	1.255	0.046	0.788
	0.415	**0.041**							**7**	**1.294**	**0.045**	**0.787**
	0.451	0.033	-0.051		×				9	1.57	0.046	0.788
	0.474	0.033	-0.064	-0.031	×		×		11	1.935	0.048	0.789
sqrt(K_{SD})	0.544	0.09	0.152	0.299	×	×	×	×	13	0	0.124	0.549
	0.699	0.057		0.098		×			9	0.75	0.116	0.533
	0.717	0.057	-0.021	0.092		×			10	0.758	0.118	0.537
	0.761	**0.044**							**7**	**1.65**	**0.11**	**0.531**
	0.7	0.056	0.00	0.116		×	×		11	1.755	0.118	0.538
	0.776	0.043	-0.019						8	1.777	0.111	0.535
sqrt(Ca_{SD})	**1.003**	**0.049**	**-0.029**						**8**	**0**	**0.155**	**0.427**
	1.013	0.049	-0.033	-0.012					9	1.352	0.156	0.428
log(P_{SD})	**-2.468**	**0.094**							**7**	**0**	**0.055**	**0.853**
	-2.486	0.094		0.032					8	0.252	0.056	0.852
	-2.455	0.094	-0.015						8	1.588	0.056	0.852

The simplest model for each trait according to the parsimony principle is highlighted with a grey background. For each model, information about the estimates of all the included explanatory variables, degrees of freedom, delta of Akaike information criterion (AIC), and marginal and conditional R^2 are provided. '×' indicates that the interaction term was included in the model. See Fig. S5 for more details in the effect sizes of the variables included in the competing models. Int, intercept; PI_{ind}, individual phenotypic integration; S, Shannon diversity of the local neighborhood; TSP_{Div}, tree-species pair diversity; Df, degrees of freedom for the model; R^2m, marginal R^2; R^2c, conditional R^2.

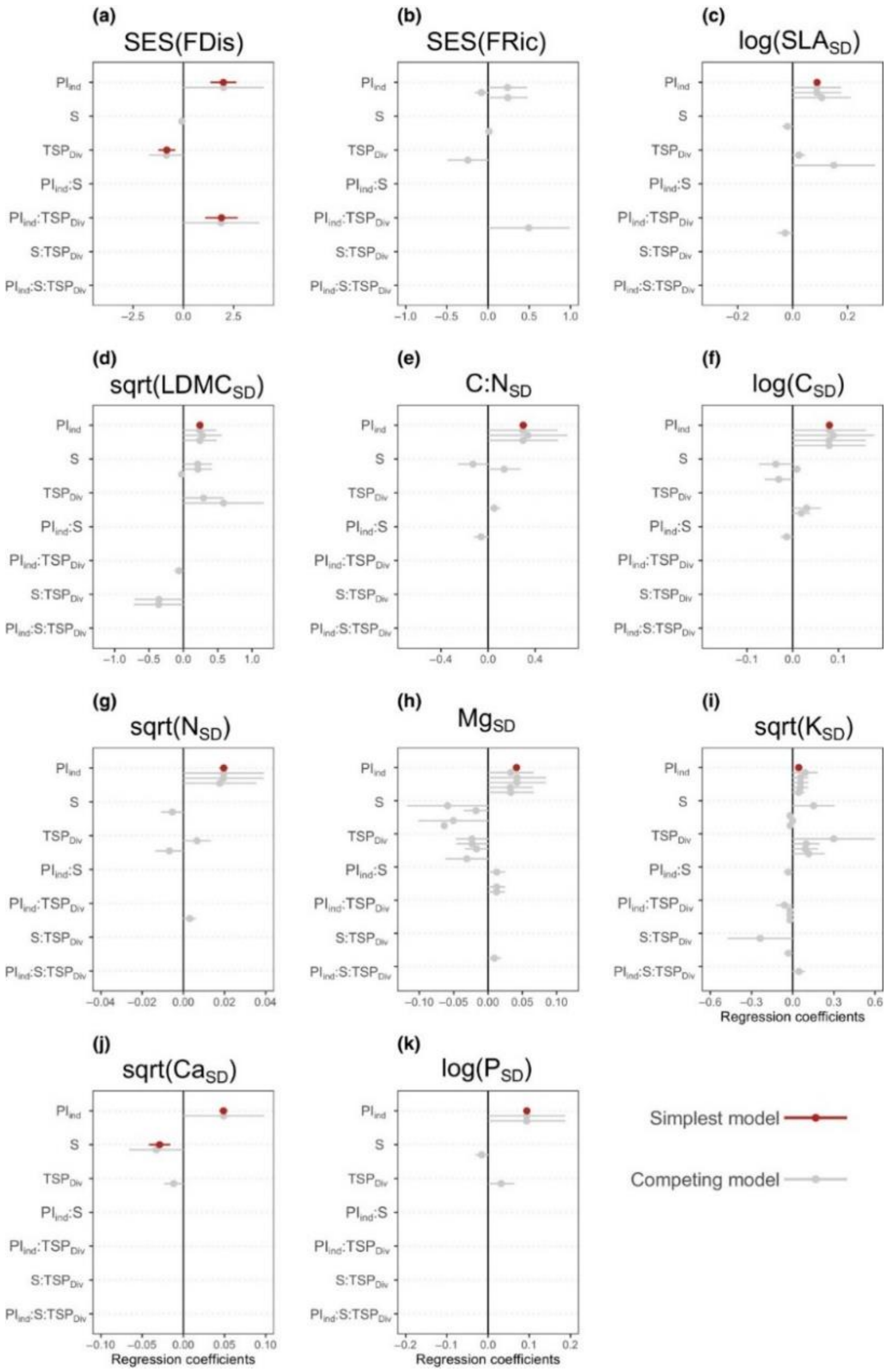

Fig. 3 Effects of the predictors in the simplest (red) and competing models (gray; $\Delta AICc < 2$) for (a) the standarized effect size of functional dispersion (SES(FDis)) and (b) functional richness (SES(FRic)), and the standard deviation (SD) of (c) specific leaf area (SLA), (d) leaf dry matter content (LDMC), (e) carbon-to-nitrogen content (C : N), (f) leaf carbon content (c), (g) leaf nitrogen content (n), (h) leaf magnesium content (Mg), (i) leaf potassium content (K), (j) leaf calcium content (Ca), and (k) leaf phosphorous content (P), with 95% confidence intervals. Log and square-root transformations of the variables were indicated for every trait by log and sqrt, respectively. The acronyms correspond to the different predictors (PI_{ind}, individual phenotypic integration; S, Shannon diversity of the local neighborhood; TSPDiv, tree-species pair diversity), and interactions between predictors are indicated by ':'. For tree-species pairs, positive and negative coefficients indicate higher and lower values for conspecific tree-specific pairs compared with heterospecific tree-specific pairs, respectively. The lack of red error bars in (b) indicates that the simplest model for FRic did not include any predictor.

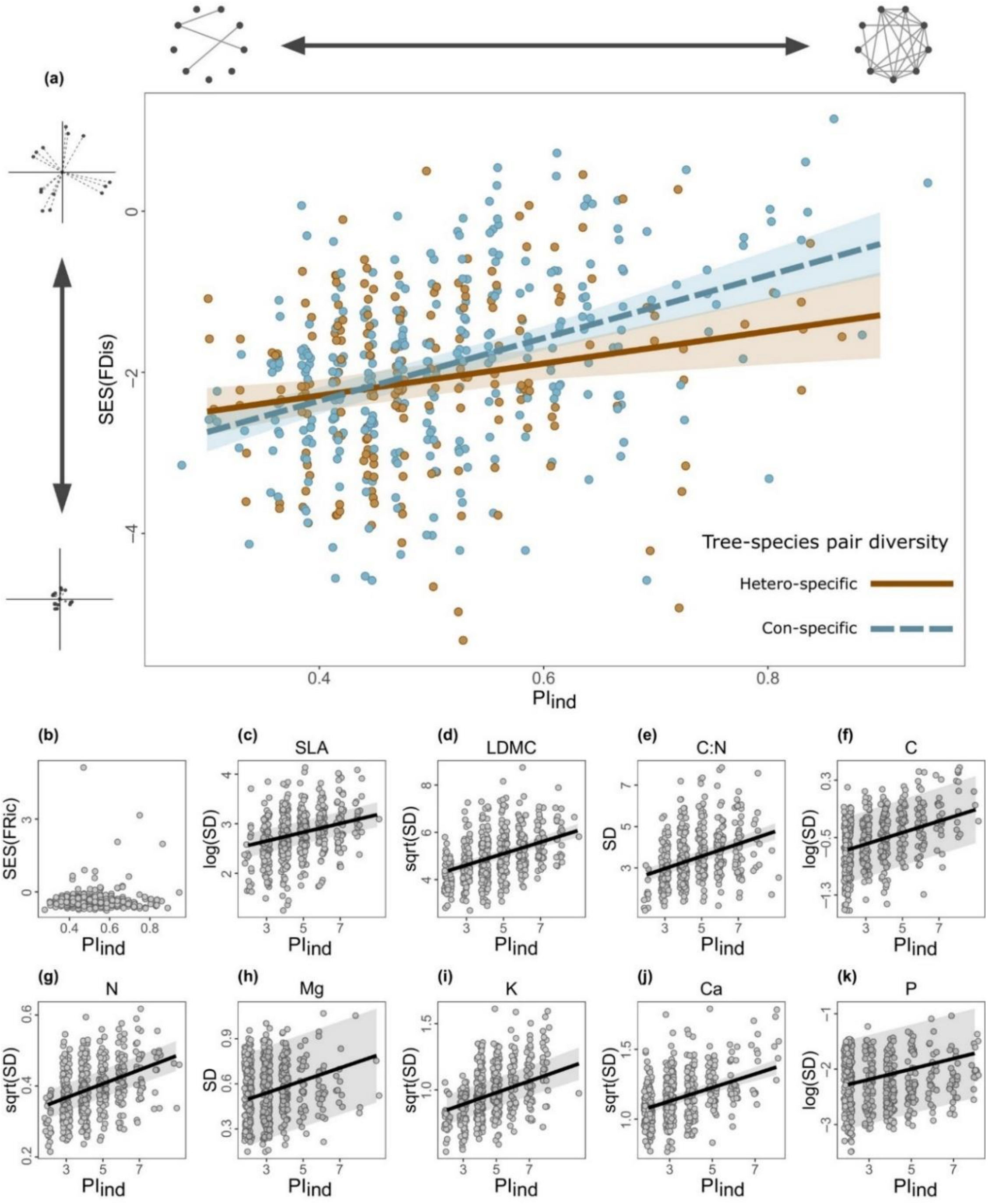

Fig. 4 Effects of PI_{ind} obtained in the simplest model according to the parsimony principle for (a) standardized effect size of functional dispersion (SES(FDis)), (b) standardized effect size of functional richness (SES(FRic)), (c) log-transformed standard deviation (SD) of specific leaf area ($\log(SLA_{SD})$), (d) square-root-transformed SD of leaf dry matter content ($\mathrm{sqrt}(LDMC_{SD})$), (e) SD of carbon-to-nitrogen ratio ($(C:N)_{SD}$), (f) log-transformed SD of carbon leaf content ($\log(C_{SD})$), (g) square-root-transformed SD of leaf nitrogen content ($\mathrm{sqrt}(N_{SD})$), (h) SD of leaf magnesium content (Mg_{SD}), (i) square-root-transformed SD of leaf potassium content ($\mathrm{sqrt}(K_{SD})$), (j) square-root-transformed SD of leaf calcium content ($\mathrm{sqrt}(Ca_{SD})$), and (k) log-transformed SD of leaf phosphorous content ($\log(P_{SD})$). Colored areas represent the confidence intervals at 95%. No effect was found for SES(FRic) in the simplest model (see Fig. 3; Table 2). Network diagrams on the upper panel illustrate the gradient from low to high P_{Iind}.

In the single-trait analyses, WTV (quantified through trait SD) increased with increasing PI_{ind} (quantified through degree, i.e. the number of significant associations of that trait with others) in all competing models (Table 2; Fig. 4c–k). In contrast, the effect of taxonomic diversity and its role in mediating the response of single-trait WTV to PI_{ind} was not included in all competing models. Local neighborhood Shannon diversity appeared as a predictor in at least one of the competing models for each trait (see Figs 3, S7–S15), suggesting that Shannon diversity of the local neighborhood may cause a decrease in WTV. However, only in the case of Ca this effect was included in the simplest model (Fig. 5). Similarly, tree-species pair diversity (conspecific vs heterospecific) was maintained as a predictor in at least one competing model for all traits, but never in the simplest model. For all traits, except for Mg and Ca, there was slightly more WTV in trees with conspecific partners. Interactions between PI_{ind} and local neighborhood Shannon diversity were found among some of the competing models for C : N, C, K and Ca, suggesting in most cases that the response of WTV to PI_{ind} could be slightly stronger when local neighborhood diversity decreases. In the case of the interaction between degree and tree-species pair diversity, this effect was present among some of the competing models for SLA, LDMC, N, and K, but the effect of the interaction was inconsistent across traits (see Figs S7, S8, S11, S13). In most cases, marginal R^2 accounted for a small portion of the total variance in the simplest models (varying between c. 5% in the case of Mg and P and c. 15% for the models of SLA, LDMC, N, and Ca), but conditional R^2 accounted for a greater portion of the variance (varying from c. 27% in P to c. 85% in P; see Table 2). This suggests that even though we found a correlation between PI_{ind} and WTV, the predictive ability of these models is rather low.

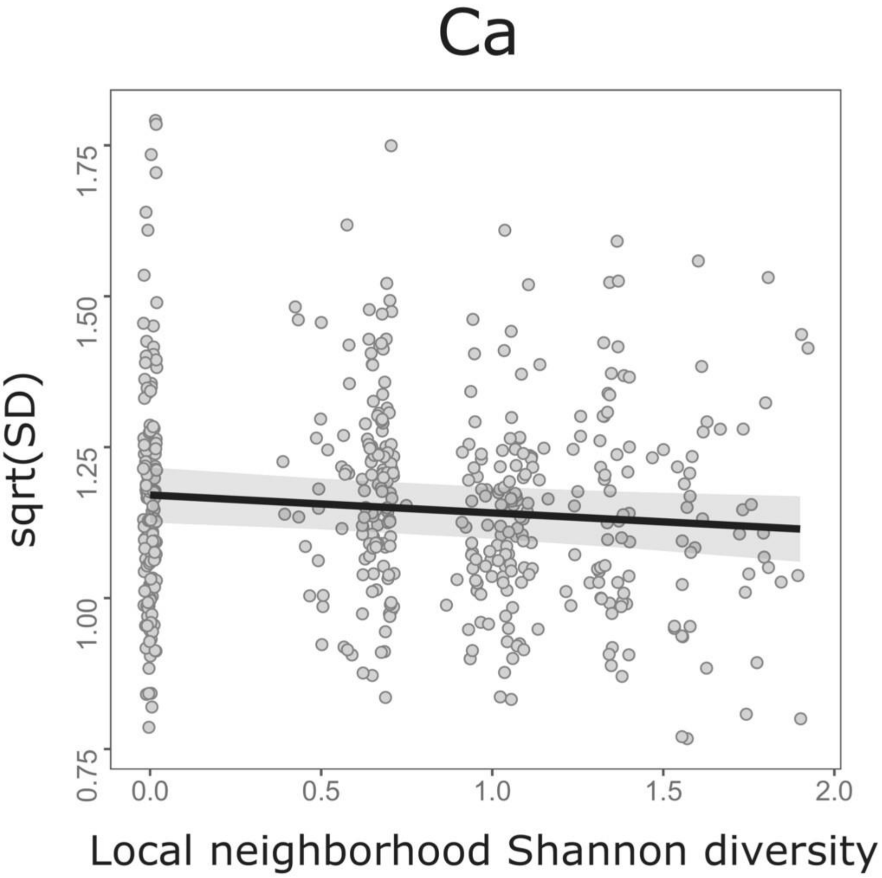

Fig. 5 Relationship between intraindividual variation of leaf calcium (square-root transformed standard deviation (SD)) and Shannon diversity of the local neighborhood (see Table 2). Gray areas represent the confidence intervals at 95%.

DISCUSSION

By using multiple leaves from each of 499 tree individuals from 21 species in a tree diversity experiment in subtropical China, we assessed whether WTV is influenced by PI_{ind}, and how tree-species diversity affects this relationship. Contrary to our expectation, our results showed that high individual WTV was associated with higher PI_{ind}. We found this response for FDis, a metric including all traits measured and reflecting the mean distance of each leaf to the centroid in a multidimensional trait space (i.e. how much a tree's leaves differ from the average trait values of all leaves of this tree), and for single traits' SD (i.e. leaves' deviation from the tree's mean considering single traits). To our knowledge, this is the first study showing such a consistent positive relationship between the integration of the phenotype and WTV. In contrast to the consistent response of FDis, the total trait hypervolume estimated by FRic did not show a clear pattern. Referring to the best and simplest model, the positive associations between WTV and PIind were mediated by taxonomic diversity only in the case of FDis, for which the response was stronger in conspecific tree-species pairs. Furthermore, WTV of leaf Ca showed a decrease in response to local neighborhood Shannon diversity.

In disagreement with our first hypothesis, our results suggest that PI_{ind} and WTV did not follow the same pattern described for higher levels of biological organization (inter- and intraspecific trait variation). The trade-off between trait variation and phenotypic integration is a widely spread statement in ecology (Valladares et al., 2007) and was observed for interspecific trait variation (Dwyer & Laughlin, 2017; He et al., 2021; Silva et al., 2021) and intraspecific trait variation (Carvalho et al., 2020; He et al., 2021). However, at the intraindividual level of biological organization used here, most metrics of trait variation showed an increase with PI_{ind}. Therefore, far from representing a constraint, PI_{ind} seems to be coupled with variation within an individual. Our results suggest that there is a link between the need of individuals to express alternative leaf designs and the maximization of the trait–trait coordination. As stated by Armbruster et al. (2014) and Zimmermann et al. (2016), phenotypic integration could act as a facilitator of adaptation by reducing maladaptive uncoordinated variation. Indeed, this could be a strategy that would allow individuals to maximize their fitness while adjusting to heterogenous microenvironmental conditions within the canopy. However, even though the general patterns observed point out an increase in WTV with increasing PI_{ind}, the use of two complementary functional indices revealed that there are still intrinsic limits to trait variation (Valladares et al., 2007; Auld et al., 2010). While FDis was positively related to PI_{ind}, the total functional space filled by the individual (represented by FRic; Cornwell et al., 2006) tended to remain unaffected by PI_{ind}. Thus, our results suggest that instead of occupying a larger niche volume, individual trees with higher PI_{ind} fill a similar trait space, but there are differences in the density and distribution of individual leaves within this trait space (Fig. 6). Leaves tend to be located in the inner part of the trait hypervolume when PI_{ind} is low and move toward the extremes as PI_{ind} increases. As a result, it seems that even though the studied trees increase leaf diversity with PI_{ind}, trait variation is not unlimited (Valladares et al., 2007).

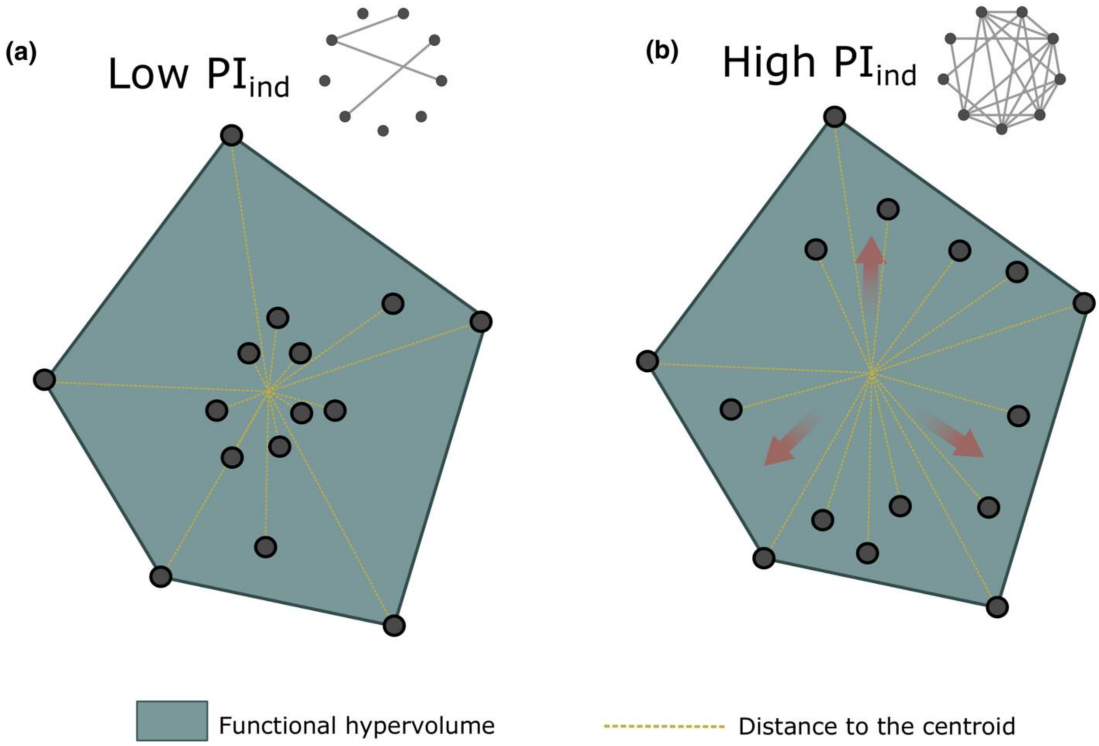

Fig. 6 Conceptual representation of the occupancy of the functional trait space under scenarios of (a) low and (b) high individual phenotypic integration (PI_{ind}), as suggested by our results. While overall trait space does not change, leaves (black points) tend to increase their distance to the centroid with higher PI_{ind}. Red arrows in (b) indicate the direction of the change detected by functional dispersion. Network diagrams illustrate scenarios of low and high PI_{ind}.

Overall, we found weak effect of taxonomic diversity on WTV, suggesting that WTV could facilitate intraspecific plant–plant interactions by promoting slight niche complementarity. First, in the case of single traits, local neighborhood Shannon diversity reduced WTV of leaf Ca, and similar trends were found in at least one competing model for all other traits. Therefore, our results, even though are rather weak, suggest that trees may display higher WTV in monospecific communities. Indeed, this is consistent with similar patterns found for trees in a similar experiment in the tropics (Proß et al., 2021). Second, the identity of the closest neighbor mediated the WTV-PI_{ind} relationship, as shown by the results for FDis, supporting that the positive effect of phenotypic integration on trait variation is stronger for trees with a conspecific closest neighbor. This result evidences that the role of phenotypic integration to prevent uncoordinated variation is even more important in the context of intraspecific competition. Thus, as higher WTV could facilitate intraspecific interactions by, for example, improving the efficiency of light capture (Møller et al., 2022) and providing niche complementarity (Proß et al., 2021), the steeper relationship with PI_{ind} in the presence of a conspecific may prevent maladjustment of leaf designs (Armbruster et al., 2014). Considering these responses to taxonomic diversity and taking in account that WTV represents a great portion of the total trait diversity occurring within a species (Herrera et al., 2015), we suggest that coexistence of individuals is not only driven by inter- and intraspecific trait variation. Rather, WTV could also constitute a mechanism that fosters niche complementarity.

Furthermore, the lack of strong responses of most single traits to taxonomic diversity (either heterospecificity of the closest neighbor or Shannon diversity of the local neighborhood) could have two complementary explanations: as drivers of trait variation act on multiple traits simultaneously, multitrait approaches reflect variation patterns better than single-trait analyses (Albert et al., 2010); and the relationship between WTV and taxonomic diversity could be hampered by other important drivers of WTV such as plant–animal interactions, and environmental factors like resource availability and climate predictability. Regarding plant–animal interactions, there is a growing literature showing the effect of pollination and seed dispersal on WTV of reproductive traits (Sobral et al., 2010, 2019) and, specifically for the case of leaves, antagonistic interactions such as leaf herbivory have been suggested to select for higher WTV (Herrera, 2017). Concerning resource availability, Davrinche et al. (2023) showed that the relationship between WTV in leaves and diversity was dependent on the availability of nutrients in the soil. Last, higher WTV has been proposed to be an adaptive strategy to cope with unpredictability in rain regimes (March-Salas et al., 2021).

While the variance explained by the predictors was not large, a large proportion of the variance in our data was attributed to random effects, including species identity and the location of the tree within the experiment. Species identity explains differences in trait variation (Mudrák et al., 2019), supporting that species differing in their evolutionary history and adaptations exhibit differences in their plastic responses (Schlichting, 1986; Davidson et al., 2011). Furthermore, because of its large spatial extent, the BEF-China experiment comprises environmental heterogeneity concerning, for example, slope, soil nutrients, and erosion (Scholten et al., 2017), and it already has been observed that these differences influence intraspecific variation in the trees' crown shape (Perles-Garcia et al., 2022). Indeed, as WTV also changes in response to small differences in abiotic conditions within the same habitat (Sobral et al., 2019), it seems that differences in WTV among individuals could also be explained by their location within the experiment.

Concluding remarks

We aimed to provide new insights into the functional constraints of WTV, which, even though it is still widely understudied, seems to play a role in ecological processes (Sobral & Sampedro, 2022) and could be key for plants to adaptively respond to future scenarios of global change (March-Salas et al., 2021). Although WTV is not unlimited, our study supports that integrated phenotypes maintain dissimilar leaf designs within the organism. This means that high PI_{ind} is needed to express large WTV and, as shown by our results, this is particularly important in the case of intraspecific interactions, where WTV could act as a stabilizing mechanism. Furthermore, if we aim to better understand WTV and its adaptive role for plants/trees in the response to future environmental conditions (Nicotra et al., 2010), it should acknowledge that PI_{ind} also responds to abiotic factors (García-Verdugo et al., 2009) and, therefore, following research on WTV and its limits should consider not only the WTV-PI_{ind} relationship but also its changes across environmental conditions.

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Conflict of interest

None declared.

Data availability

Code and data for the data analysis of this study are available at the Zenodo repository: doi: 10.5281/zenodo.8243092.

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SUPPORTING INFORMATION

Fig. S1 Selection of suitable data for the study of trait variation and phenotypic integration on individual trees

Fig. S2 Analytical framework used to obtain the metrics of phenotypic integration and trait variation for every tree.

Fig. S3 P values for trait-trait correlations.

Fig. S4 Testing independency between metrics of trait variation and phenotypic integration.

Fig. S5 Effect plots for all competing models for SES(FDis).

Fig. S6 Effect plots for all competing models for SES(FRic).

Fig. S7 Effect plots for all competing models for SLA.

Fig. S8 Effect plots for all competing models for LDMC.

Fig. S9 Effect plots for all competing models for C:N.

Fig. S10 Effect plots for all competing models for C.

Fig. S11 Effect plots for all competing models for N.

Fig. S12 Effect plots for all competing models for Mg.

Fig. S13 Effect plots for all competing models for K.

Fig. S14 Effect plots for all competing models for Ca.

Fig. S15 Effect plots for all competing models for P.

Table S1 Leaf traits included in our study, their ecological function and literature describing them.

Table S2 Number and percentage of scans and leaves excluded in the process of outlier removal for every trait in every site of the experiment.

Table S3 Species included in our study and number of individuals in both study sites included in the analyses.

Table S4 Number of plots, TSPs and trees sampled across all richness levels in the BEF-China experiment included in the analyses.

Table S5 Structure of the linear mixed models to study the relationship between individual phenotypic integration and trait variation under different scenarios of local taxonomic diversity.

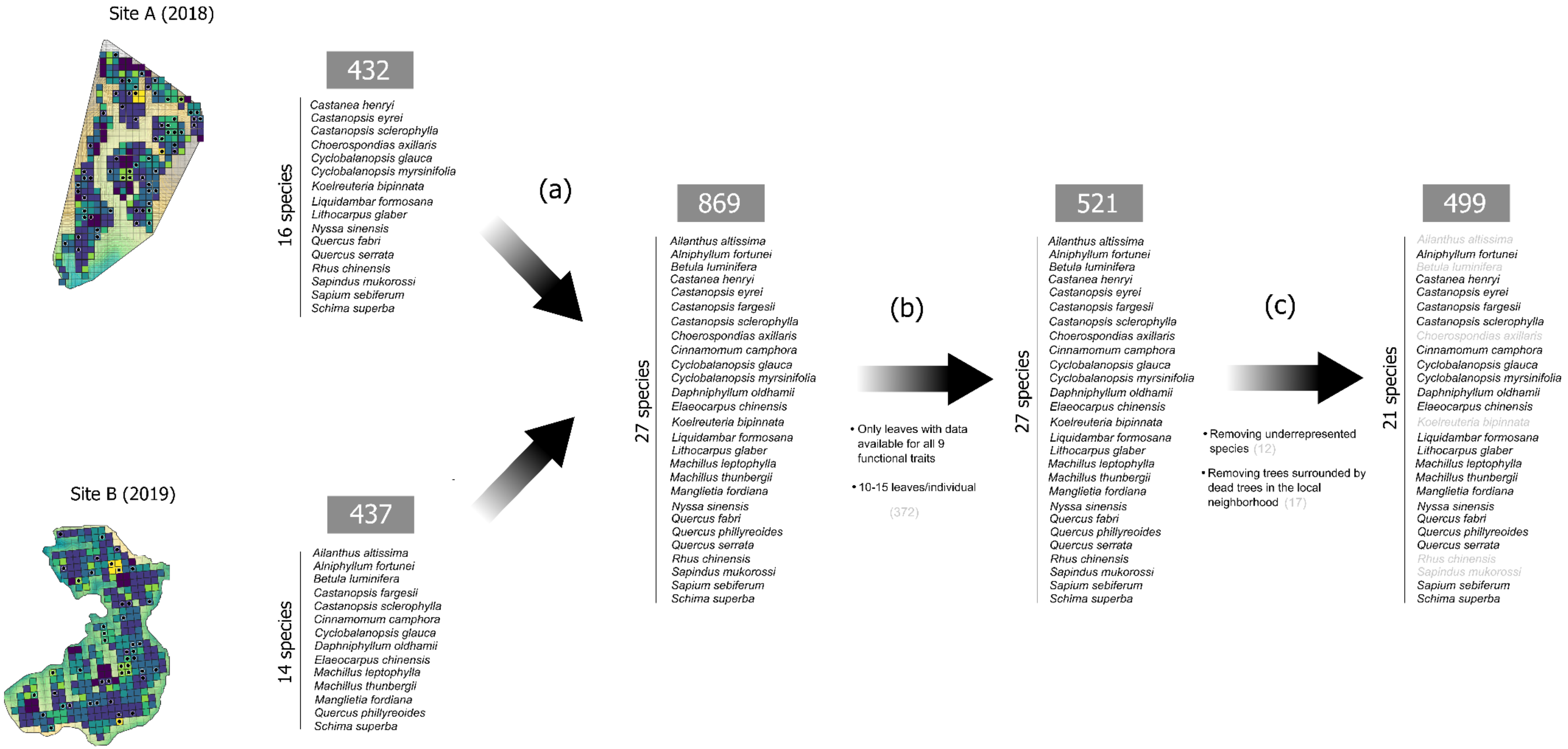

Fig. S1 Data selection process before statistical analyses. (a) First, leaf-level trait data collected for 16 species in site A and data collected for 14 species in site B in 2019 were merged together, resulting in a dataset of leaf-level trait data for 869 trees belonging to 27 species. (b) Afterwards, we filtered the data in order to select the suitable information to perform further analyses. For this selection only leaves with trait data available for all nine traits (SLA, LDMC, C:N, C, N, Ca, K, Mg and P) were considered, as the study of phenotypic integration requires the lack of missing data. Further, in order to avoid the low accuracy in the metrics for intraindividual trait variation (WTV), and especially, for phenotypic integration ($\mathrm{PI}_{\mathrm{ind}}$), only trees with data for a minimum of 10 leaves were considered. In this process data for 372 trees were excluded. (c) Finally, after the filtering processes, six species out of the 27 were underrepresented and, thus, 12 trees belonging to these species were discarded for further analyses. Excluded species are marked in light grey. In addition, 17 trees completely surrounded by dead trees (i.e. lack of surrounding neighborhood) were excluded. The result was a dataset with leaf-level data for 499 trees from 21 species represented across the different levels of taxonomic diversity considered in the sampling design (see Table S3).

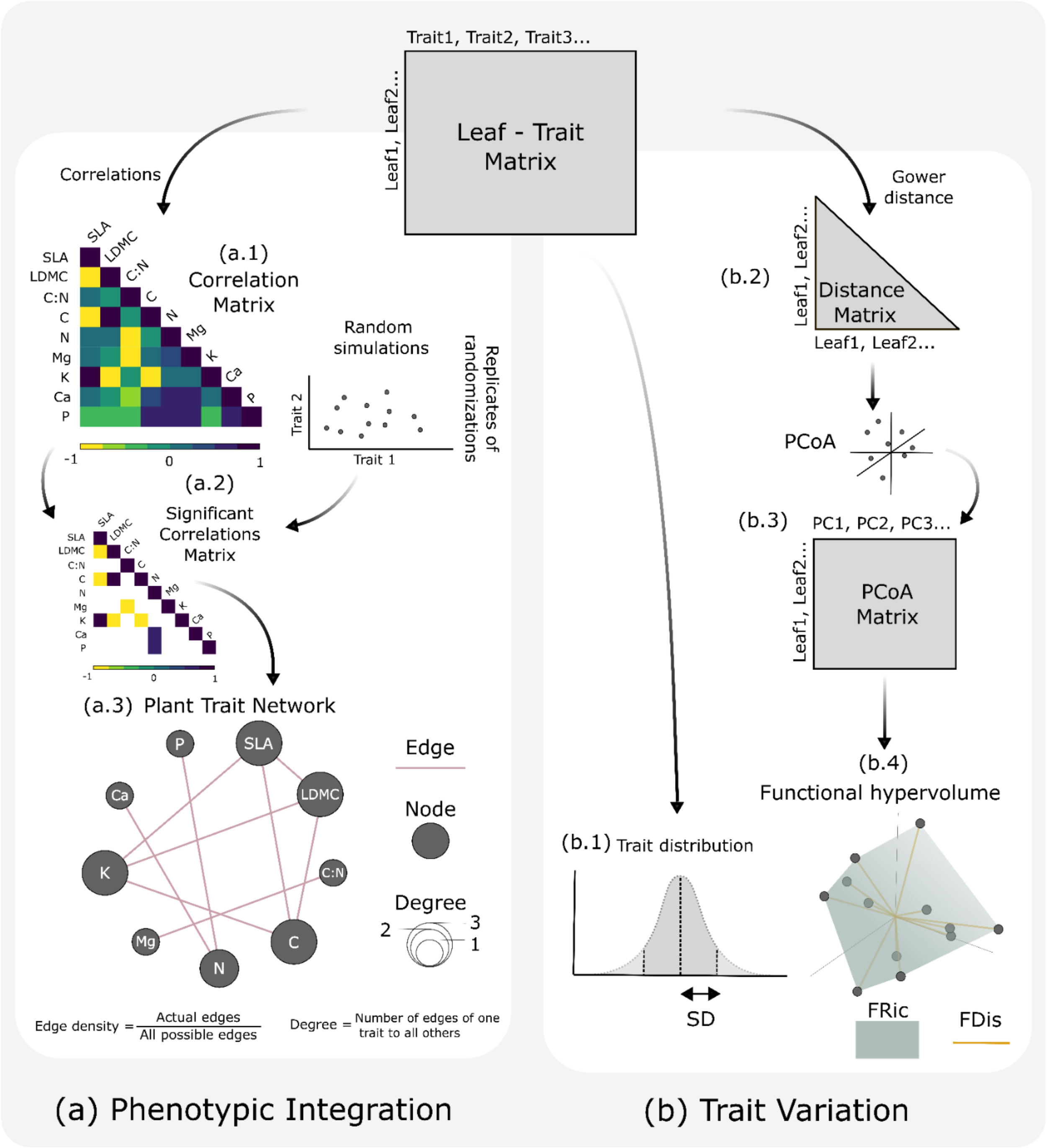

Fig. S2 Analytical framework used to obtain the metrics of (a) phenotypic integration and (b) trait variation for every tree. The metrics of individual phenotypic integration (edge density and degree) were addressed by using (a.1) correlation matrices and (a.2) permutation tests to evaluate which correlations were significant. Then, the significant correlations were used to build the (a.3) plant trait network, which describes the degree of every trait and the edge density of the network. The metrics of intraindividual trait variation for single traits were addressed by using (b.1) the standard deviation (SD) of every trait. Multi-trait functional indices were addressed by (b.2) constructing a distance matrix considering the Gower distance between individual leaves. Based on the distance matrix (b.3) we built a Principal Coordinates Analysis (PCoA). Finally, we assessed (b.4) Functional Dispersion (FDis) and Functional Richness (FRic) from Euclidean distances based on leaf scores obtained from PCoA axes.

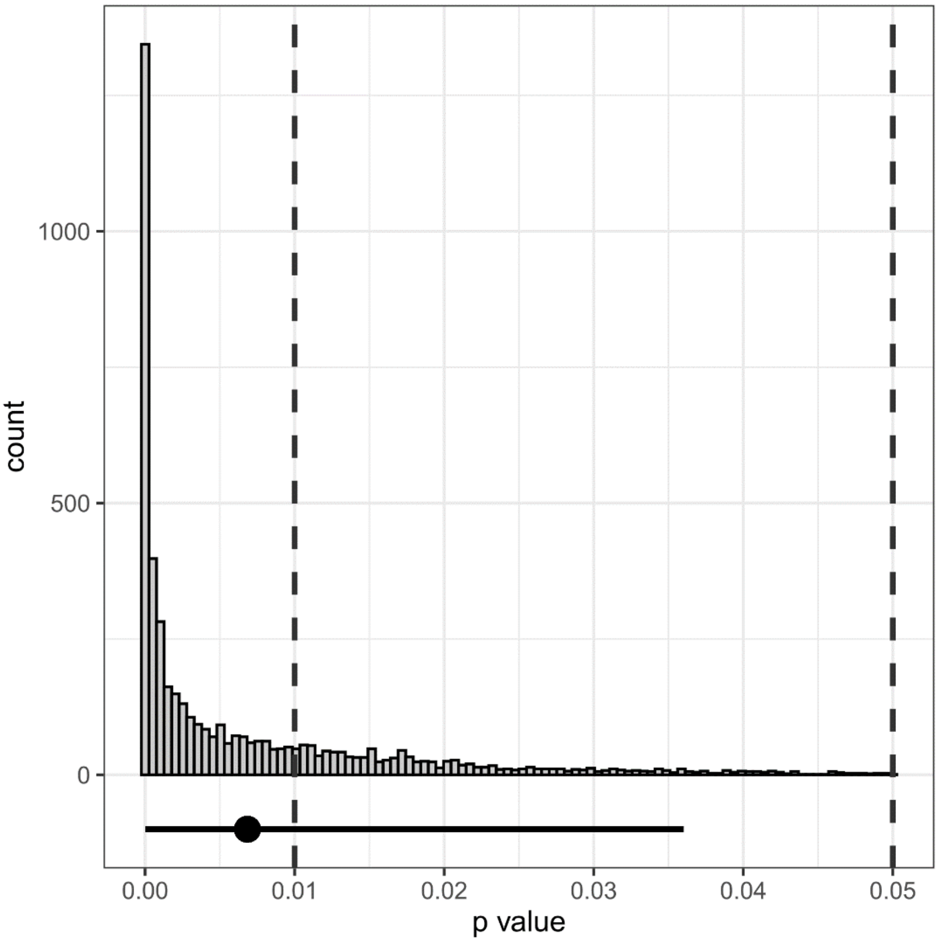

Fig. S3 Histogram of all p values of the trait-trait correlations considered significant. correlations of a trait with itself were not included here as the p value is always 0 in those cases. The dot and error bars below indicate the mean (0.007) and the 95% confidence interval of the p values, As indicated by the dashed lines, most of the p values were lower than 0.01, while the presence of p values near the threshold of 0.05 was minimal.

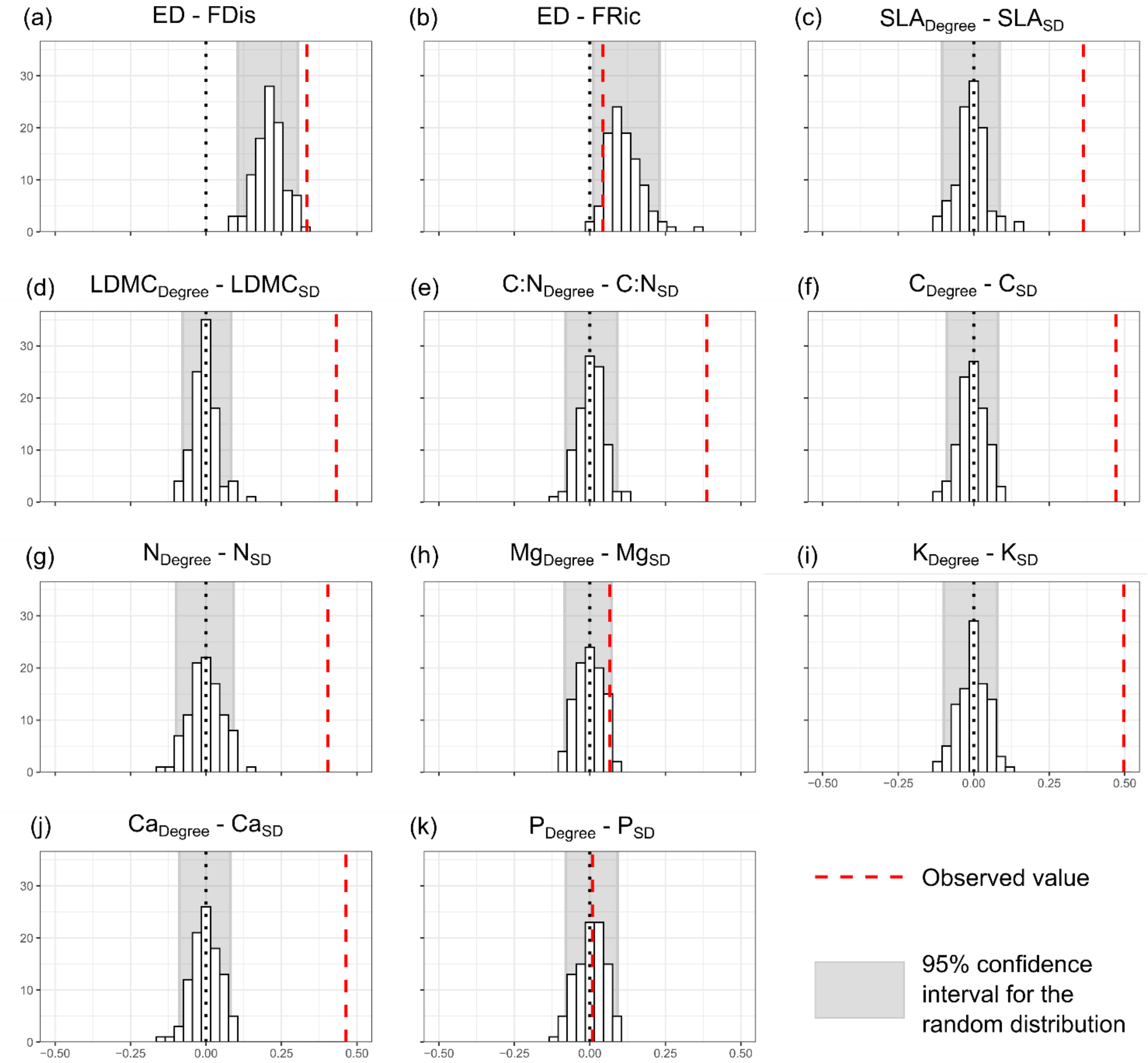

Fig. S4 Testing independency between metrics of trait variation and phenotypic integration. We shuffled the values of every trait 100 times and assessed functional dispersion (FDis), functional richness (FRic), the standard deviation of every trait (SD), edge density (ED) and the degree of every trait for every randomization. Then, for every randomization we calculated the Pearson correlation coefficient between the metrics of trait variation and the metrics of phenotypic integration (Manly, 2018). We considered that the randomizations were significantly different from zero when their 95% confidence intervals did not overlap with zero. For the correlations between degree and SD (c-k), we found in every case that the random correlations were distributed around zero, suggesting that these metrics were not correlated. For the correlations between multi-trait functional indices and edge density (a and b) we found that random correlations tended to be slightly over 0. However, in the case of FDis (a), the observed correlation was significantly higher than those occurring by chance, which suggest that the observed pattern in the linear mixed-effect models does not occur due to correlated metrics.

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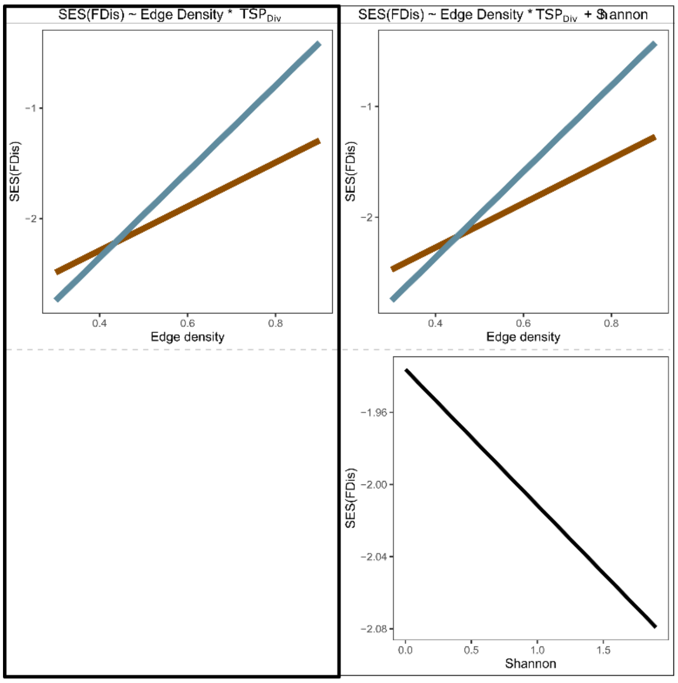

Fig. S5 Effect plots for all competing models for SES(FDis). Interactions with TSP diversity (TSP_{Div}) are represented in different colors (blue for conspecific and brown for heterospecific). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

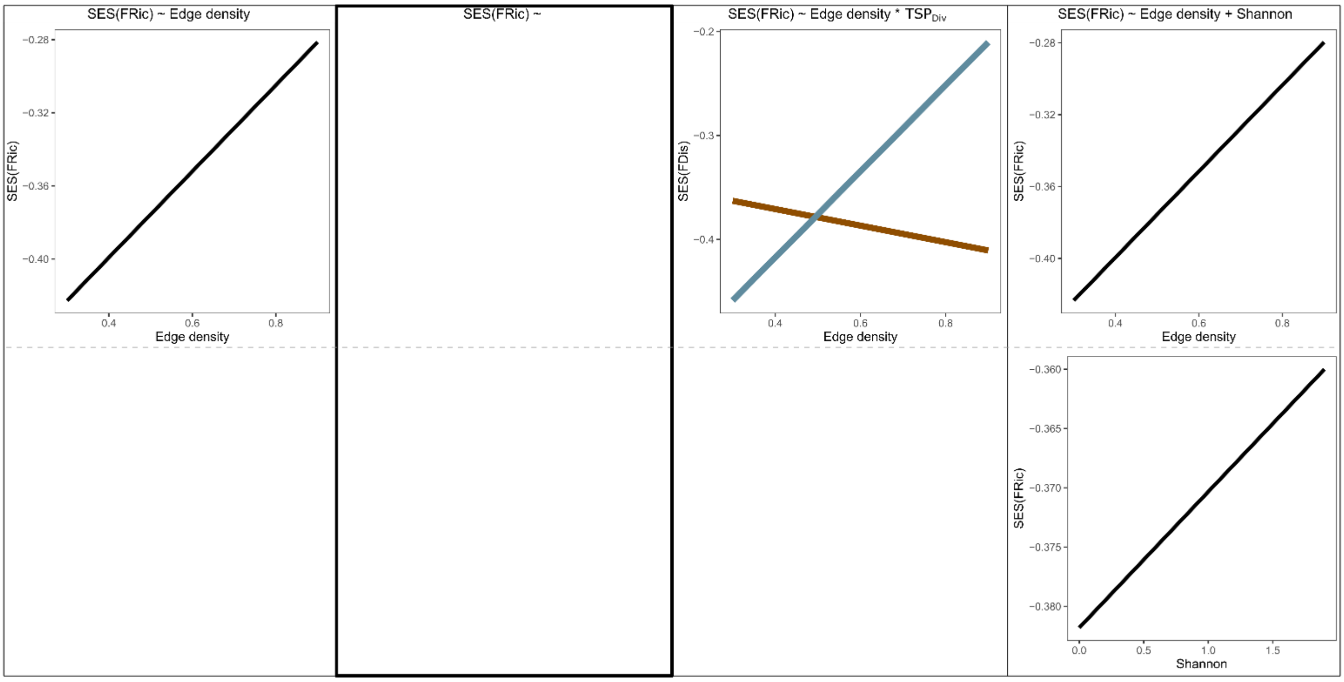

Fig. S6 Effect plots for all competing models for SES(FRic). Interactions with TSP diversity (TSP_{Div}) are represented in different colors (blue for conspecific and brown for heterospecific). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

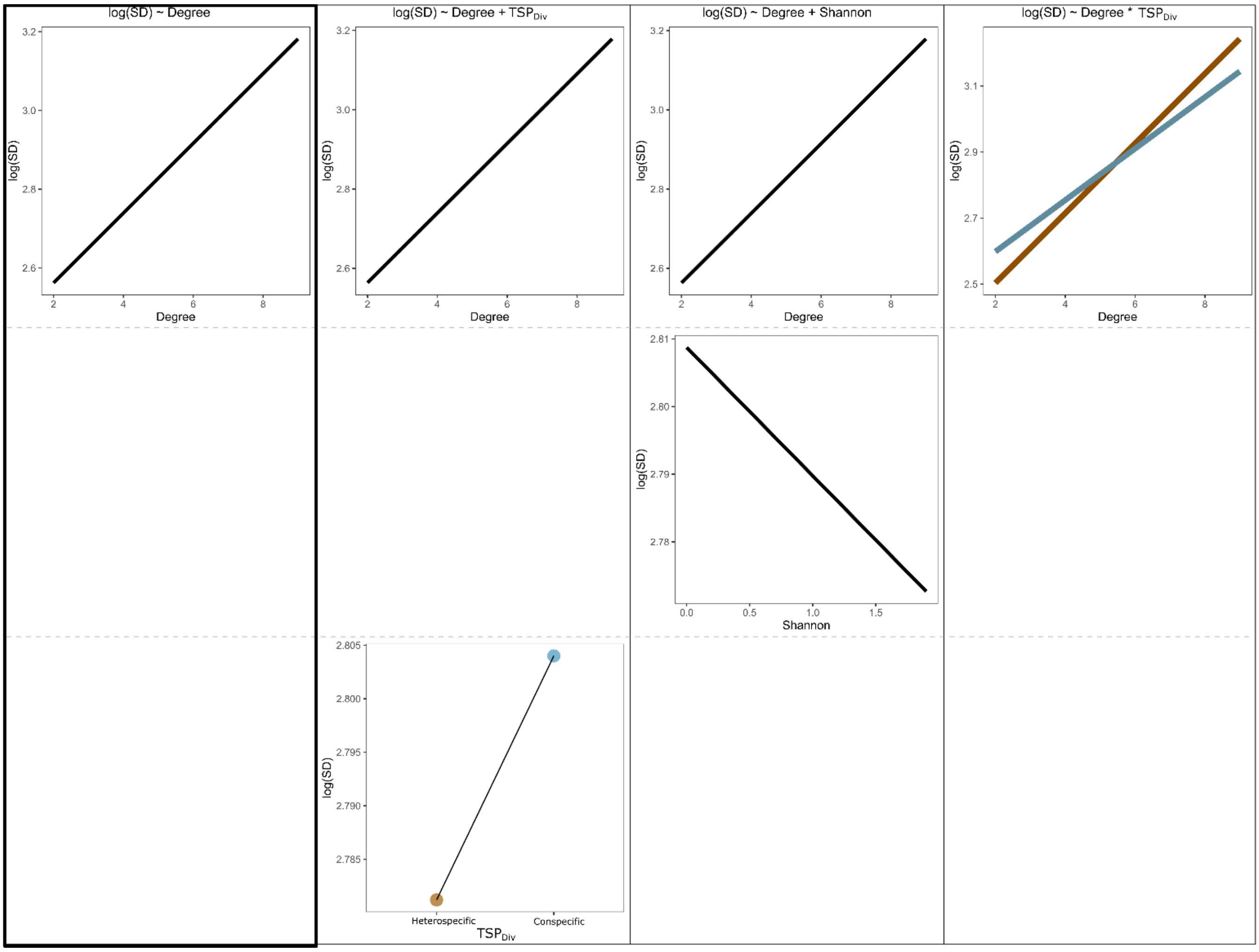

Fig. S7 Effect plots for all competing models for SLA. Interactions with TSP diversity ($\mathrm{TSP}_{\mathrm{Div}}$) are represented in different colors (blue for conspecific and brown for heterospecific). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

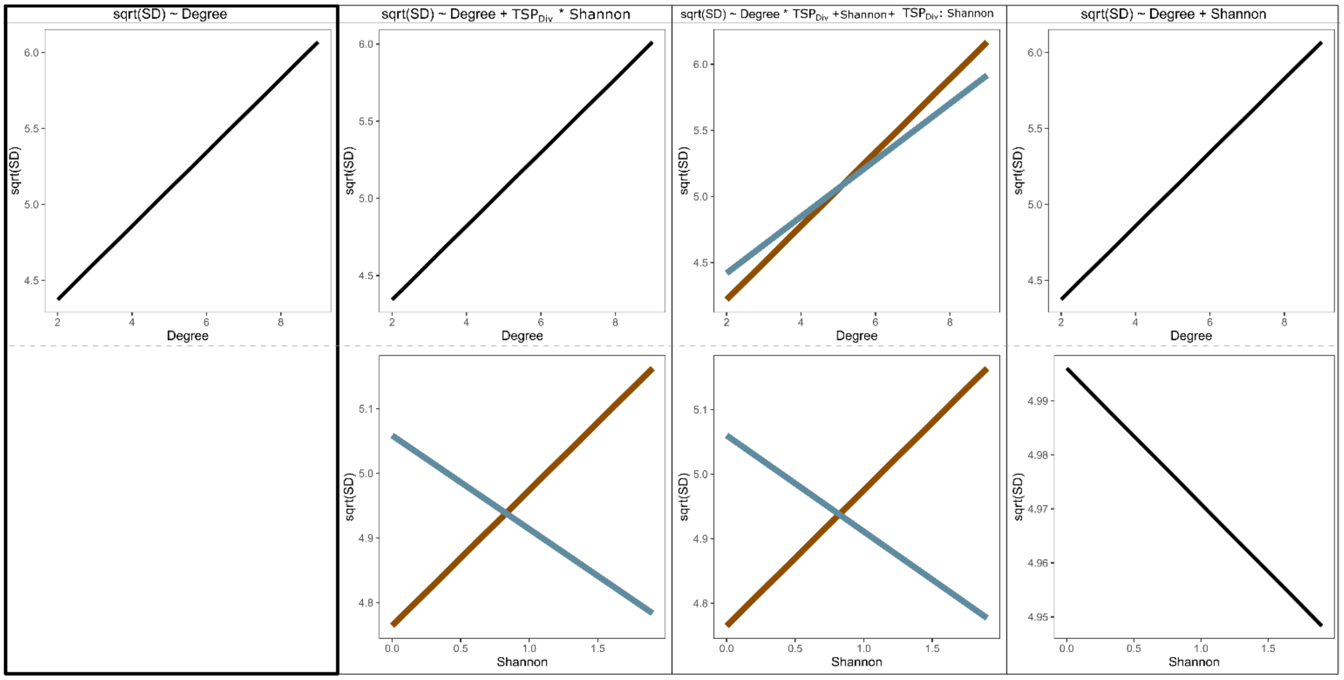

Fig. S8 Effect plots for all competing models for LDMC. Interactions with TSP diversity (TSP_{Div}) are represented in different colors (blue for conspecific and brown for heterospecific). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

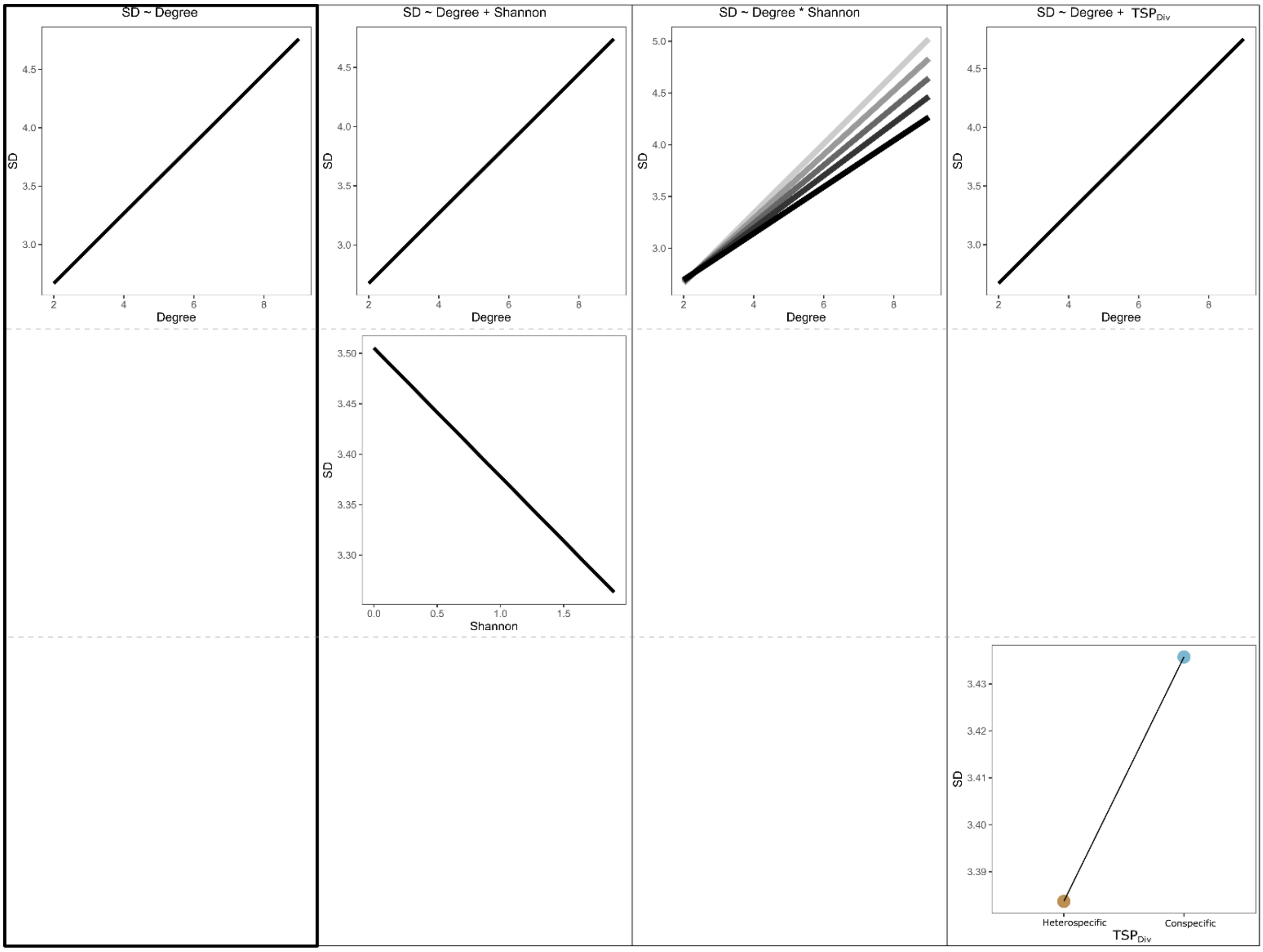

Fig. S9 Effect plots for all competing models for C:N. Interactions with local neighborhood Shannon diversity (Shannon) are represented with different color intensity (darker colors for higher values of Shannon diversity). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

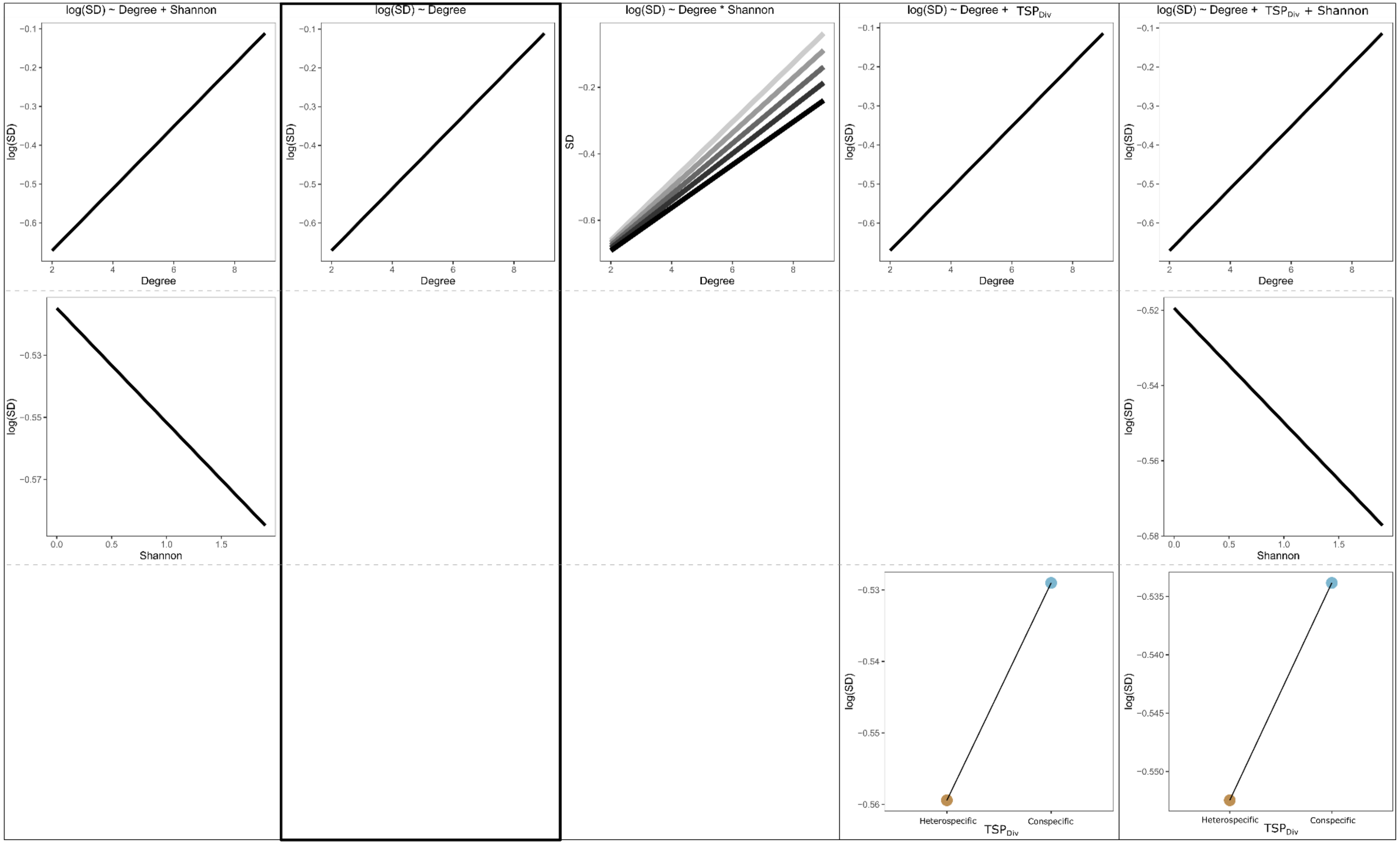

Fig. S10 Effect plots for all competing models for C. Interactions with local neighborhood Shannon diversity (Shannon) are represented with different color intensity (darker colors for higher values of Shannon diversity). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

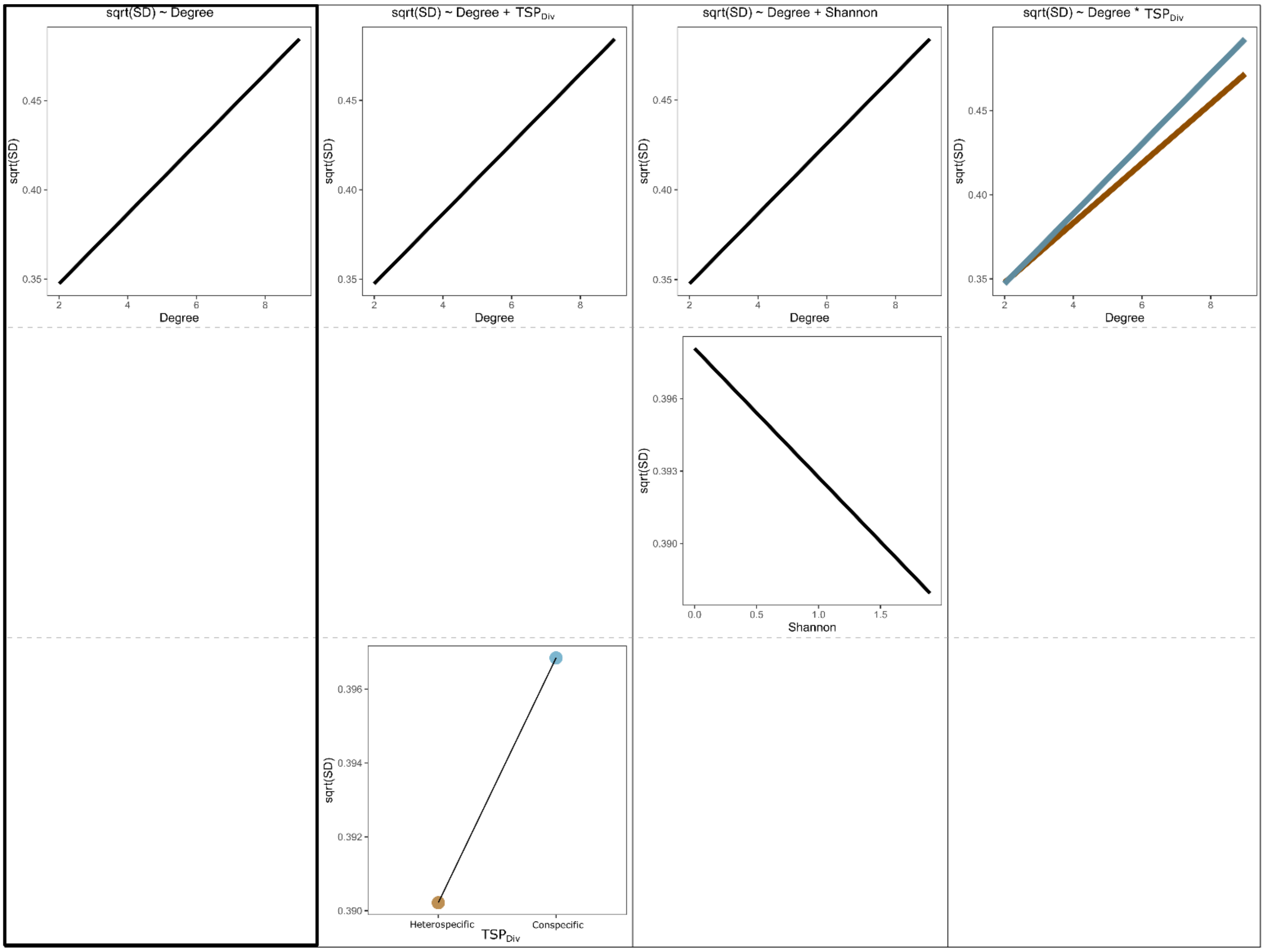

Fig. S11 Effect plots for all competing models for N. Interactions with TSP diversity (TSP$_{Div}$) are represented in different colors (blue for conspecific and brown for heterospecific). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

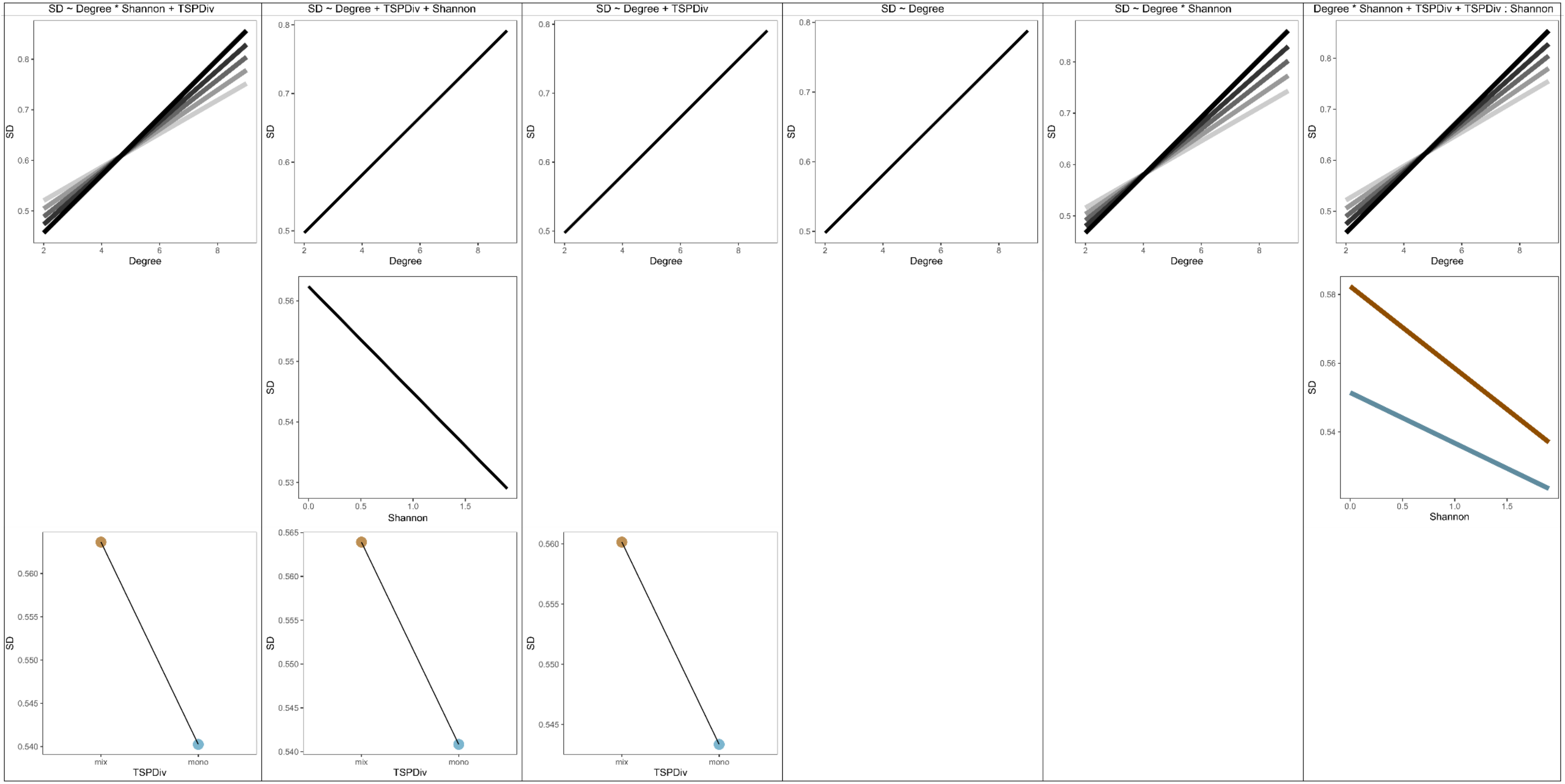

Fig. S12 Effect plots for all competing models for Mg. Interactions with TSP diversity (TSP$_{Div}$) are represented in different colors (blue for conspecific and brown for heterospecific). Interactions with local neighborhood Shannon diversity (Shannon) are represented with different color intensity (darker colors for higher values of Shannon diversity). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

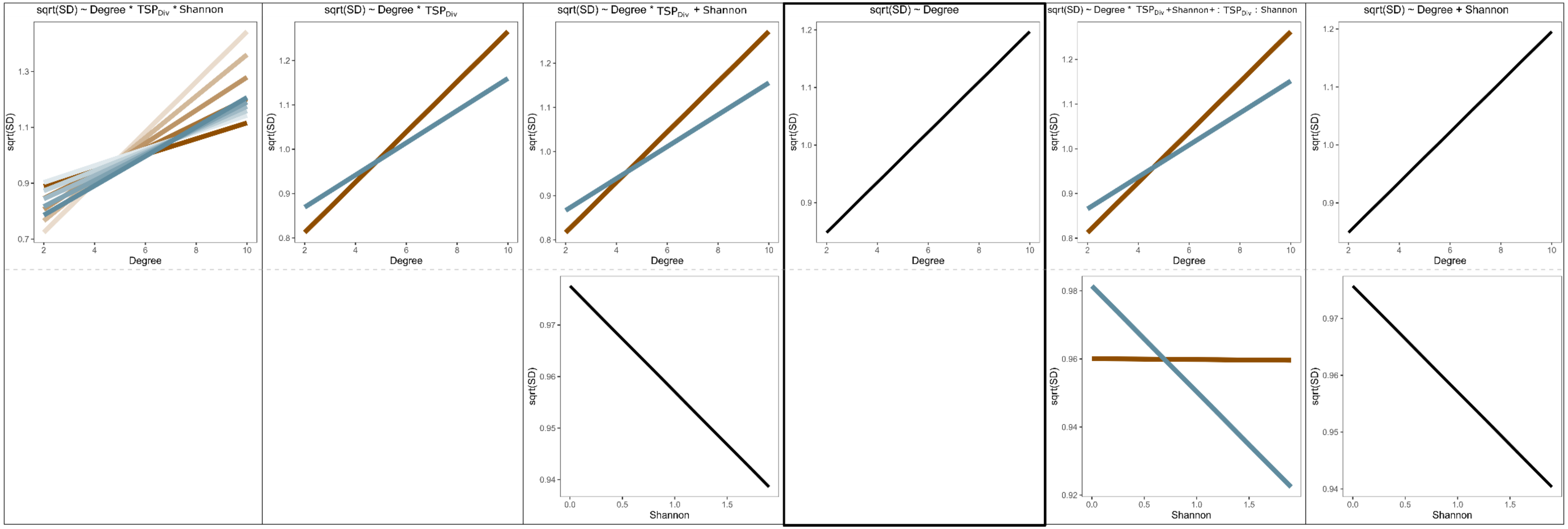

Fig. S13 Effect plots for all competing models for K. Interactions with TSP diversity (TSP$_{Div}$) are represented in different colors (blue for conspecific and brown for heterospecific). Interactions with local neighborhood Shannon diversity (Shannon) are represented with different color intensity (darker colors for higher values of Shannon diversity). The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

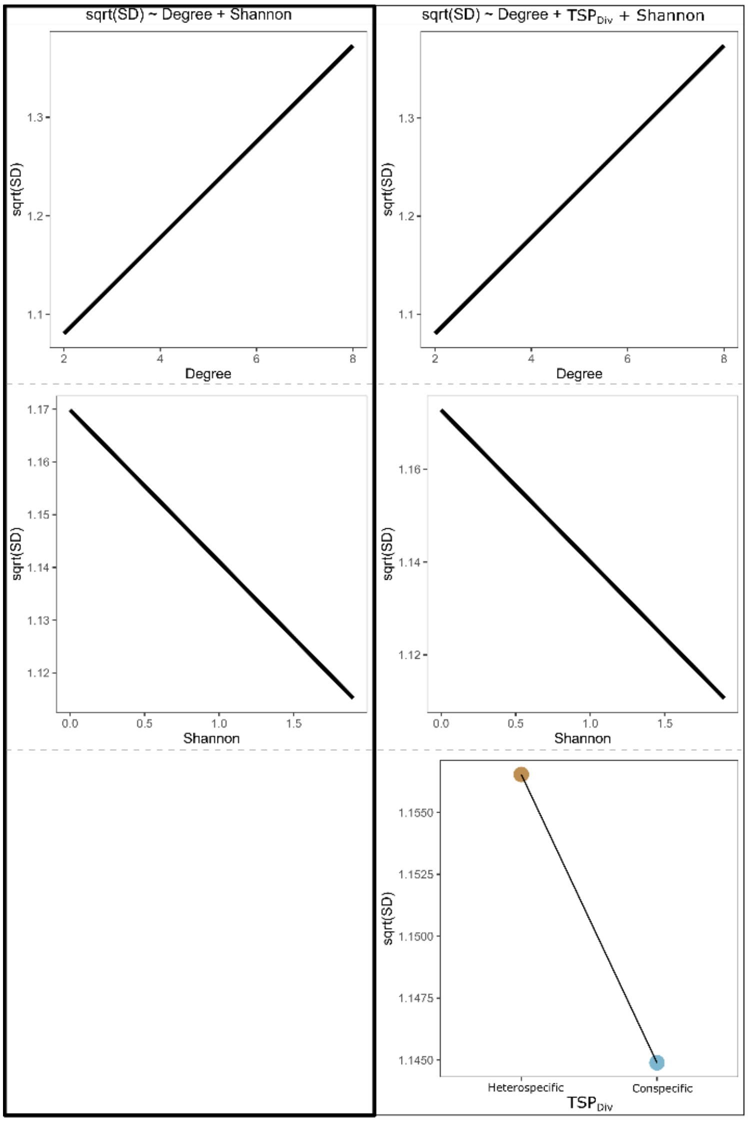

Fig. S14 Effect plots for all competing models for Ca. The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

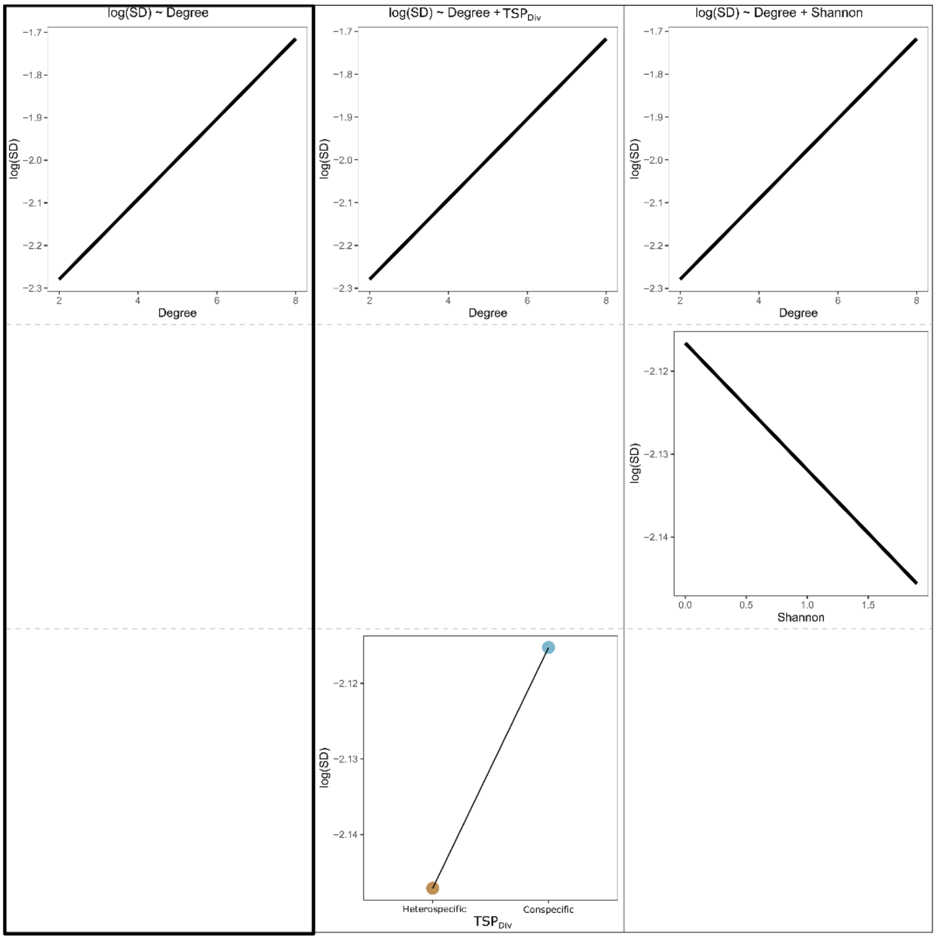

Fig. S15 Effect plots for all competing models for P. The results for the simplest model that we prioritized to interpret the results are highlighted in the box marked in bold.

Table S1 Leaf traits included in our study, their ecological function and literature describing them.

Trait	Abbreviation	Units	Ecological function	References
Specific leaf area	SLA	mm²/mg	Resource acquisition, photosynthetic rate, relative growth rate, shade-tolerance.	Reich et al. (1992); Niinemets and Kull (1995); Reich et al. (1997); Reich et al. (1999); Kazakou et al. (2006); Legner et al. (2014)
Leaf dry matter content	LDMC	mg/g	Structural support of the leaf, herbivory resistance, leaf tissue density, leaf life-span, relative growth rate.	Poorter & Bergkotte (1992); Niinemets & Kull (1995); Niinemets (1999); Niinemets (2001); Westoby et al. (2002); Kazakou et al. (2006); Poorter et al. (2009); Pérez-Harguindeguy et al. (2013)
Leaf carbon to nitrogen ratio	C:N	g/g	Structural support of the leaf, leaf tissue density, leaf life-span.	Poorter & Bergkotte (1992); Niinemets et al. (2007)
Leaf carbon content	C	%	Leaf palatability, leaf lignin content, leaf density, relative growth rate and structural leaf support, water use efficiency.	Poorter & Bergkotte (1992); Niinemets et al. (2007); Kazakou et al. (2006); Pérez-Harguindeguy et al. (2003)
Leaf nitrogen content	N	%	Net photosynthetic capacity, relative growth rate and N availability in the soil, leaf life-span and leaf decomposability.	Reich et al. (1992); Enríquez et al. (1993); Cornelissen et al. (1997); Reich et al. (1997); Reich et al. (1999); Niinemets et al. (2002); Kazakou et al. (2006)
Leaf magnesium content	Mg	mg/g	Leaf senescence, Mg availability in the soil.	Rao et al. (1987); Kobayashi (2015); Pérez-Harguindeguy et al. (2003)
Leaf potassium content	K	mg/g	Nitrogen use efficiency, K availability in the soil, relative growth rate.	Pérez-Harguindeguy et al. (2003); Xu et al. (2020)
Leaf calcium content	Ca	mg/g	Ca availability in the soil, relative growth rate, leaf senescence.	Poovaiah, & Leopold (1973), Pérez-Harguindeguy et al. (2003)
Leaf Phosphorus content	P	mg/g	Net photosynthetic capacity, relative growth rate, P availability in the soil, leaf life-span.	Tuohy et al. (1991); Raaimakers et al. (1995); Hevia et al. (1999); Pérez-Harguindeguy et al. (2003)

Table S2 Number and percentage of scans and leaves excluded in the process of outlier removal for every trait in every site of the experiment.

Site A

Trait	SLA	LDMC	CN	C	N	MG	CA	K	P
original number of scans	15257	15257	15257	15257	15257	15257	15257	15257	15257
final number of scans	14492	14684	14500	14644	14399	14676	14280	14381	13862
original number of leaves	5067	5067	5067	5067	5067	5067	5067	5067	5067
final number of leaves	4816	4944	4845	4895	4822	4906	4937	4800	5006
% of excluded scans	5.01	3.76	4.96	4.02	5.62	3.81	6.40	5.74	9.14
% of excluded leaves	4.95	2.43	4.38	3.39	4.84	3.18	2.57	5.27	1.20

Site B

Trait	SLA	LDMC	CN	C	N	MG	CA	K	P
original number of scans	17874	17874	17874	17874	17874	17874	17874	17874	17874
final number of scans	16867	17245	16920	16608	16878	15679	16674	17041	16777
original number of leaves	5958	5958	5958	5958	5958	5958	5958	5958	5958
final number of leaves	5650	5755	5767	5808	5748	5895	5844	5725	5752
% of excluded scans	5.63	3.52	5.34	7.08	5.57	12.28	6.71	4.66	6.14
% of excluded leaves	5.17	3.41	3.21	2.52	3.52	1.06	1.91	3.91	3.46

Table S3 Species included in our study and number of individuals with con-specific and hetero-specific neighbors in both study sites included in the analyses. Species names and families are taken from Tropicos (http://www.tropicos.org/; accessed 22 August 2022).

Species	Family	Leaf habit	Site A		Site B	
			Con-sp	Hetero-sp	Con-sp	Hetero-sp
Liquidambar formosana Hance	Altingiaceae	Deciduous	14	7	0	0
Daphniphyllum oldhamii Hayata	Daphniphyllaceae	Evergreen	0	0	15	11
Elaeocarpus chinensis Hook.f. ex Benth.	Elaeocarpaceae	Evergreen	0	0	19	12
Sapium sebiferum (L.) Roxb.	Euphorbiaceae	Deciduous	5	3	0	0
Castanea henryi Rehder & E.H.Wilson	Fagaceae	Deciduous	7	6	0	0
Castanopsis eyrei Tutch.	Fagaceae	Evergreen	4	5	0	0
Castanopsis fargesii Franch.	Fagaceae	Evergreen	0	0	26	16
Castanopsis sclerophylla (Lindl. & Paxton) Schottky	Fagaceae	Evergreen	15	10	13	8
Cyclobalanopsis glauca Oerst.	Fagaceae	Evergreen	15	11	16	9
Cyclobalanopsis myrsinifolia (Blume) Oerst.	Fagaceae	Evergreen	16	4	0	0
Lithocarpus glaber Nakai	Fagaceae	Evergreen	17	9	0	0
Quercus fabri Hance	Fagaceae	Deciduous	8	1	0	0
Quercus phillyreoides A.Gray	Fagaceae	Evergreen	0	0	7	6
Quercus serrata Thunb.	Fagaceae	Deciduous	8	4	0	0
Cinnamomum camphora (L.) J.Presl	Lauraceae	Evergreen	0	0	11	7
Machilus leptophylla Hand.-Mazz.	Lauraceae	Evergreen	0	0	10	4
Machilus thunbergii Siebold & Zucc.	Lauraceae	Evergreen	0	0	12	11
Manglietia fordiana Hu	Magnoliaceae	Evergreen	0	0	13	10
Nyssa sinensis Oliv.	Nyssaceae	Deciduous	4	7	0	0
Alniphyllum fortunei Makino	Styracaceae	Deciduous	0	0	15	14
Schima superba Gardner & Champ.	Theaceae	Evergreen	18	8	16	12

Table S4 Number of plots, tree-species pairs and trees sampled across all richness levels in the BEF-China experiment included in the analyses. Tree-species pairs (both conspecific and hetrospecific) were selected across a large number of plots differing in richness in order to represent the different situations of conspecifity and diversity in the local neighborhood across all species.

Site	Richness	Number of plots	consp TSP	Heterosp TSP	Trees in consp TSPs	Trees in hetosp TSPs
A	1	18	29	0	49	0
	2	11	22	11	39	14
	4	10	9	17	15	23
	8	6	7	16	13	19
	16	0	0	0	0	0
	24	6	10	14	15	19
B	1	15	29	0	53	0
	2	12	35	20	63	31
	4	7	14	25	25	42
	8	3	6	12	11	17
	16	4	7	12	12	19
	24	5	5	6	9	11
		Total: 97	Total: 173	Total: 133	Total: 304	Total: 195
			Total: 306		Total: 499	

TSP, tree-species pair; Consp, conspecific; heterosp, heterospecific

Table S5 Structure of the linear mixed models to study the relationship between individual phenotypic integration and trait variation under different scenarios of local taxonomic diversity.

Response variable	Fixed factors	Random effects
SES(FDis)	Edge density * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
SES(FRic)	Edge density * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$\log(SLA_{SD})$	SLA_{Degree} * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$\mathrm{sqrt}(LDMC_{SD})$	$LDMC_{Degree}$ * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$(C{:}N)_{SD}$	$C{:}N_{Degree}$ * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$\log(C_{SD})$	C_{Degree} * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$\mathrm{sqrt}(N_{SD})$	N_{Degree} * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
Mg_{SD}	Mg_{Degree} * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$\mathrm{sqrt}(K_{SD})$	K_{Degree} * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$\mathrm{sqrt}(Ca_{SD})$	Ca_{Degree} * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity
$\log(P_{SD})$	P_{Degree} * TSP Diversity * Local Diversity	Species + Site/Plot/TSP Identity

TSP, Tree-Species pair

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CHAPTER 5

SYNTHESIS

Despite the importance of functional traits in ecological processes, trait variation still holds many unresolved aspects, especially regarding the variation within species and individuals. In particular, the responses of this variation to diversity, which could reveal key facets of species interactions and biodiversity-ecosystem functioning (BEF), have been often disregarded. Since BEF experiments provide a useful setting for studying experimental gradients of species diversity and trees express high plasticity in functional traits, I looked into the patterns of intraspecific and intraindividual trait variation along tree and mycorrhizal fungi species gradients in tree diversity experiments. In this chapter, I summarize my findings and expand on their meaning and implications in a broader context, to contribute to a better understanding of processes underlying trait variation and discuss the potential implications that this could have for ecosystem functioning.

Summary of the results

In **Chapter 2**, I studied changes in the individual mean trait values and intraindividual trait variation in a tree diversity experiment that relies on the combination of gradients of tree richness and mycorrhizal associations. I found that both tree diversity and mycorrhizal fungal diversity drive changes in functional traits. Specifically, specific leaf area (SLA) increased with increasing tree species richness and the carbon to nitrogen ratio (C:N) decreased with increasing arbuscular mycorrhizal fungal richness. This suggests that both aboveground and belowground diversity contribute to trait variation. In addition, I found a decrease of intraindividual variation of traits from the leaf economics spectrum (LES) with increasing tree species richness, highlighting a potential role of intraindividual trait variation in tree-tree interactions. Last, I could not find differences between two groups of trees associated preferably with arbuscular mycorrhizal fungi and ectomycorrhiza fungi, respectively, regarding their acquisitive versus conservative strategy.

In **Chapter 3**, I studied changes in intraspecific trait variability within populations and intraindividual trait variation along an experimental gradient of tree species richness. I found that intraspecific trait variability decreased with increasing tree species richness and intraindividual variation of traits associated with the stomatal morphology decreased with increasing tree species richness. Further, I found that intraspecific trait overlap of conspecifics is small, when intraspecific variability is high, but high when intraindividual variation is high. This suggests that intraindividual trait variation may not be related to complementarity, but could instead appear as a mechanism to improve water use efficiency or as a result of higher differentiation between sun and shade leaves in the canopy. Last, by using a framework that allows including hierarchical sources of trait variation for calculating functional diversity of communities, I found that the organization of the trait variation within individuals and within populations is important for functional diversity, especially in mixed stands.

In **Chapter 4**, by using a large leaf-level dataset for ca. 500 trees, I explored the relationship between intraindividual trait variation and trait covariation. I found that intraindividual variation in leaf traits increases with trait covariation, meaning that trees with more coordinated traits exhibit greater variability in leaf traits. This relationship was influenced by the identity of the closest neighbour, suggesting that the positive effect of trait covariation on intraindividual trait variation is stronger for trees with a conspecific closest neighbor. These results indicate that intraindividual leaf trait variation requires strong coordination of traits and functions in order to avoid the expression of maladaptive phenotypic syndromes. In addition, I found that intraindividual variation of leaf calcium was negatively associated with the Shannon diversity of the neighborhood.

Intraindividual variation: patterns and role in tree-tree interactions

In **Chapter 2**, **Chapter 3** and **Chapter 4** I showed that intraindividual trait variation is not a neglectable part of trait-based ecology, but functional traits can vary subindividually in response to drivers that also have an effect at higher levels of biological organization (Herrera, 2017). In **Chapter 3** I discussed that intraindividual trait variation could represent up to one quarter of total trait variation in leaves and it can even overcome intraspecific trait variation in traits, such as leaf phosphorous (P) or leaf nitrogen content (N). It is also important mentioning that intraindividual trait variation is not “phenotypic noise” that occurs randomly, but results from patterns of phenotypic integration conserved across scales. For instance, I found that trade-offs between conservative and acquisitive traits also emerge when comparing different leaves within the same tree (as seen in **Chapter 4**) or when using leaf-level data to study the main axes of trait variation. Specifically, I found trade-offs between SLA or N and leaf dry matter content (LDMC) as we expect from the leaf economics spectrum framework (Wright et al., 2004). Further, in **Chapter 3** I found that patterns of leaf trait covariation, such as the orthogonal variation of the leaf economics spectrum and the stomatal morphology, which describes a trade-off between the density and size of the stomata in the leaf, aligned with studies that used species-level data to demonstrate the existence of different syndromes shaping leaf form and function (Kröber & Bruelheide, 2014; Schnabel et al., 2021). However, in this case I also found that the content of nutrients seems to be aligned with leaf stomatal traits, indicating that, leaf photosynthetic capacity, which is strongly related to N and P, may be tightly related to the gas exchange of the leaves (Tanaka et al., 2013).

Only recently intraindividual trait variation became of interest in ecological studies and, since then, researchers have been trying to understand its responses to different drivers to better understand the ecological meaning of this source of phenotypic variation. For instance, March-Salas et al. (2021) studied the responses of reproductive traits to variability in precipitation regime and suggested that intraindividual variation could emerge as a mechanism to cope with environmental uncertainty. Further, Møller et al. (2022, 2024) studied the patterns of intraindividual variation of *Galium odoratum* in response to light and water availability and suggested that intraindividual variation, especially in leaf traits, could explain adaptative mechanism related to efficiency in the use of resources. Regarding species interactions, in this thesis I aimed to explore the role of intraindividual trait variation in tree-tree interactions. In **Chapter 1** I hypothesized that intraindividual trait variation was highest in monocultures and decreased with increasing tree species richness. While my finding overall confirmed this trend, there were differences regarding the statistical significance of the results depending on the experiment. In **Chapter 2** I showed that, for the MyDiv experiment (Germany), the intraindividual variation of traits from the leaf economics spectrum significantly decreased with tree species richness. These results are aligned with those of Proß et al. (2023) in the Kreinitz experiment, located at ca. 100 km from our experimental site. Nevertheless, the corresponding patterns of intraindividual trait variation in BEF-China were much weaker. In **Chapter 4**, I showed that leaf calcium content was the only trait responding to Shannon diversity of the neighborhood, and no significant responses for LES traits were found in **Chapter 3**. Indeed, responses of intraindividual variation in LES traits in species from BEF-China have been shown to be complex and dependent on other environmental variables such as soil microbiome or soil nutrients (Davrinche & Haider, 2024). The differences between experiments could rely on (1) climate, (2) species pool, (3) experimental design, (4) environmental heterogeneity within the experiment or (5) age of the experiment. As for the climate, the regions present differences regarding their temperature and precipitation (Figure 1a, b), with MyDiv being colder and drier compared to BEF-China. Interestingly, our results contrast with previous evidence suggesting that phenotypic plasticity of species tends to be lower in colder and drier climates (Stotz et al., 2021). However, as MyDiv is located in a temperate region, which usually shows higher climatic variability compared to subtropical regions where BEF-China is located, the ability of plants to exhibit plasticity in intraindividual variability could be prominent in this region as a mechanism to cope with climate variability (March-Salas et al., 2021). Regarding the pool of species, even though some of the MyDiv species were more related, as many were members of the same family (this is the case for the Betulaceae or Rosaceae family), there was not a clear segregation in terms of phylogenetic relatedness between species belonging to different experiments (Figure 1c). However, it seems that MyDiv species have higher values for traits related to an acquisitive strategy compared to the species from BEF-China (Figure 1d). This is probably because the pool of species in the MyDiv experiment only include deciduous species, which are typically more acquisitive (Pringle et al., 2010). As phenotypic plasticity of conservative species is often lower because the production of alternative phenotypes is likely to be unsustainable (Valladares et al., 2007; Stotz et al., 2022), this could explain why the response of intraindividual variation only shows a trend but it is rarely significant for BEF-China (but see below a discussion about intraspecific trait variation in both tree diversity experiments in the section `*Intraspecific responses: From conservative heterogenous populations to acquisitive homogenous populations*`). Further, regarding the experimental design, experiments differ in the extent of the plots and the planting distance within them, with BEF-China having larger plots and higher planting distance compared to MyDiv. For instance, even though the tree species richness gradient was smaller in MyDiv compared to BEF-China, the higher planting density in MyDiv results in high canopy density and may lead to larger differences in intraindividual trait variability between monocultures, where crowns of the trees overlap substantially, and mixtures, where there is a stratification of the canopy (Ray et al., 2023). Last, there is a lot of microenvironmental variation in BEF-China that could blurr responses (e.g. slope, altitude, orientation). Indeed, Davrinche et al. (2023) showed that the responses of intraindividual trait variation were dependent on the changes of soil nutrients in the BEF-China experiment.

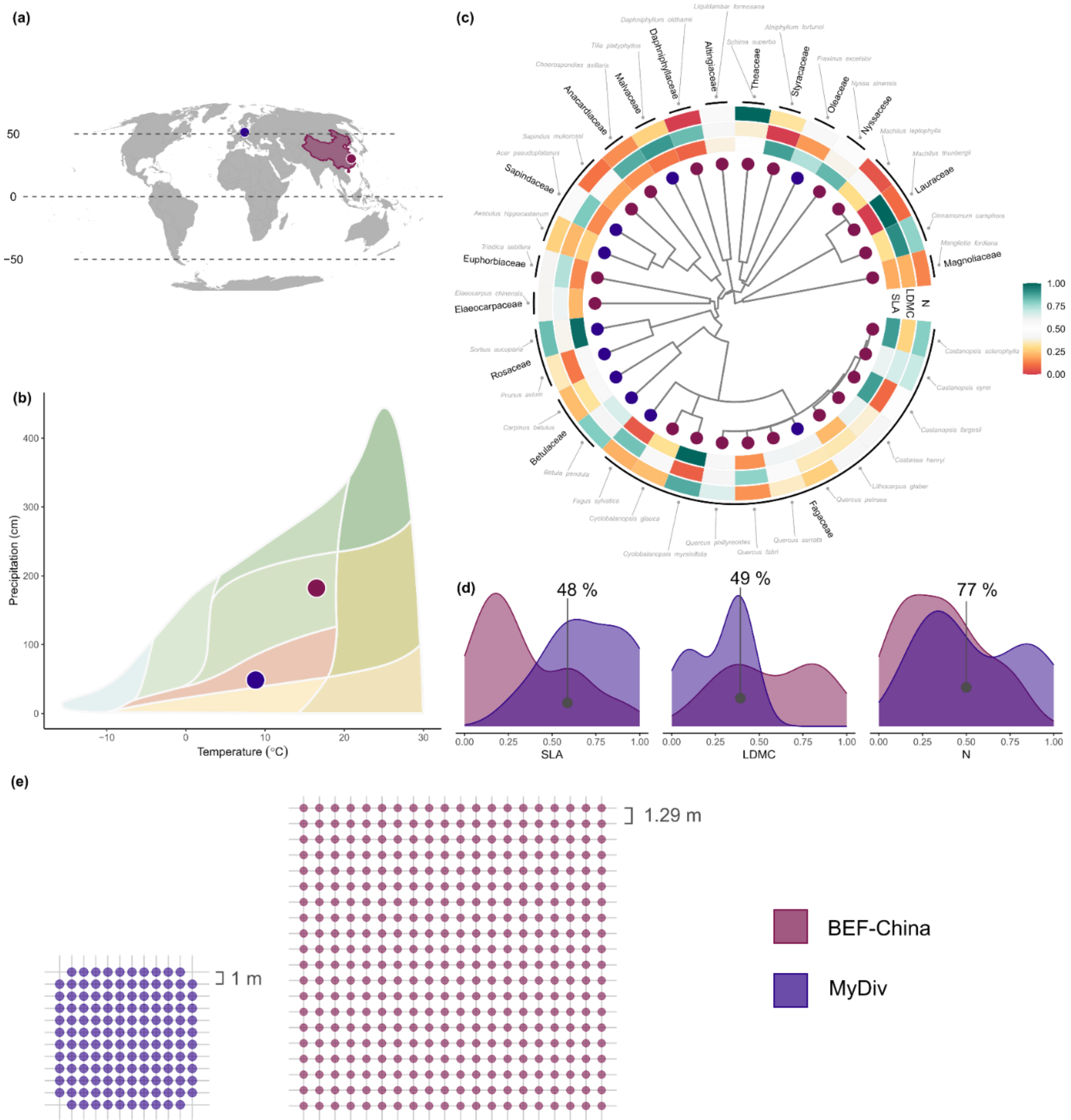

FIGURE 1 Comparison of the (a) location, (b) climate and (c, d) sampled species between BEF-China (red violet) and MyDiv (dark purple). (b) The different tree diversity experiments show differences regarding their mean annual temperature (16.5°C in BEF-China and 8.8°C in MyDiv) and precipitation (1821 mm in BEF-China and 484 mm in MyDiv) and, therefore, they are located in different biomes according to the Whittaker classification (Whittaker, 1975). Background colours in (b) correspond to the different biomes as defined by Whittaker. The phylogenetic tree of sampled species in both experiments is shown in (c), with the outer circles representing the rescaled mean values of three functional traits that are key components of the leaf economics spectrum (specific leaf area (SLA); leaf dry matter content (LDMC); leaf nitrogen content (N)) for every species. Red colours in (c) are associated with low trait values and blue values indicate high trait values. The phylogenetic tree was generated by using the ´V.PhyloMaker´ R package (Jin & Qian, 2019). Further, the density plots for the mean trait values of the species in each experiment are shown in (d). The percentage in every of the density plots corresponds to the shared area between both experiments. The schemes in (e) show the extent of a plot and the distribution of the trees within this in both experiments. Every point in (e) represents the planting position of a tree.

The decrease of intraindividual trait variation with increasing tree species richness was expected as intraindividual variation could facilitate intraspecific interactions. Specifically, it was suggested that higher leaf variability in the canopy would allow conspecific trees to be more phenotypically dissimilar and therefore reduce intraspecific competition (Proß et al., 2021). I tested for this mechanism on **Chapter 3**, and contrary to our expectations higher intraindividual variation caused conspecifics to share more trait features. Therefore, our results suggest that intraindividual variation was not related to species niche partitioning as I hypothesized from the results in **Chapter 2**. However, in **Chapter 3** I suggest that the responses of intraindividual trait variation to tree species diversity could be related to the microenvironmental conditions in monocultures. As the microclimate buffer is smaller in monocultures or stands with low species richness, increased intraindividual variation could emerge in these stands due to their role for coping with environmental variability mentioned before (Møller et al., 2022, 2024). As an alternative explanation, I suggest that higher leaf variability may emerge as greater differentiation between sun and shade leaves as a result of the canopy structure. Therefore, an interesting topic for future research would be how intraindividual variability is related to canopy structure and how diversity and canopy structure affect the epigenetics of the plant individuals.

Intraspecific responses to species diversity: from conservative heterogenous populations to acquisitive homogenous populations

Scaling-up in the levels of biological organization, our results show that differences between trees within the same species are also related to tree-tree interactions. Intraspecific trait variation has attracted ecologists in recent years, as studying its extent and patterns could reveal new facets of species interactions (Hart et al., 2016). Indeed, intraspecific interactions have been shown to be involved, among others, in plant-animal interactions (Gorné et al., 2020; Westerband et al., 2021; Arroyo-Correa et al., 2024), predator-prey interactions (Bolnick et al., 2011; Allesina et al., 2021), and, as we saw in previous chapters, tree-tree interactions. In **Chapter 2** I showed that the phenotypes of trees are respond to species diversity. Specifically, our results suggested that increase in SLA was related to species diversity, probably as a result of higher resource-use complementarity in mixed stands (Barry et al., 2019). In fact, similar results were already shown by Davrinche & Haider (2021) for BEF-China, suggesting that the shift from conservative to acquisitive with increasing tree species richness occurs in both of our experimental settings. In addition, in **Chapter 3** I showed that intraspecific diversity of populations was decreasing with tree species richness. All this information together suggests that, in forests, tree species diversity may drive intraspecific variation from conservative heterogeneous populations, where trees adopt a more conservative trait strategy and maximize phenotypic dissimilarities, to acquisitive homogenous populations where trees from the same species converge towards an optimal trait value. Additionally, these results may point out strong differences between the mechanisms involved in intraspecific and interspecific competitive interactions. Indeed, if trait similarity is the main driver of the intensity of competitive interactions, plants with similar traits should compete more strongly (MacArthur & Levins, 1967; Mason et al., 2011). In contrast, individuals could also converge towards optimal trait values if competitive interactions were driven by trait hierarchy instead of trait differences. Therefore, for our context of tree diversity experiments, the results from **Chapter 4** suggest that intraspecific interactions are driven by trait dissimilarities, while the shift to a more acquisitive strategy with increasing tree diversity found in **Chapter 2** suggests that interspecific competitive interactions depend on the optimal values of traits.

Species diversity as drivers of leaf functional traits

The overall goal of BEF research is to understand how diversity can influence ecosystem functioning. In this framework, we have seen how traits, which may be the link between biodiversity and ecosystem functioning, respond to the diversity of different organisms. First, as I have discussed in the previous sections of this chapter, the diversity of tree species drives trait variation (shifts towards a more acquisitive trait strategy, decrease of intraspecific and intraindividual trait variability). These results could be explained by resource partitioning in diverse forest stands (Barry et al., 2019). In fact, resource partitioning, that is related the acquisition of resources (light, water, nutrients) and how different species have different requirements and manners for the uptake of those (Mckane et al., 2002; Kahmen et al., 2006), could explain the use of a more acquisitive strategy in mixed stands. Further, in **Chapter 2**, I further analysed diversity effects in forest by considering, not only the most visible part of forest (i.e. trees), but also the diversity of mycorrhizal fungi associated with the roots of the trees. Mycorrhizal fungal communities have been shown to be important components of forests and are drivers of ecosystem functioning (Ferlian et al., 2018; Deng et al., 2023). Our results suggest that the diversity of mycorrhizal fungal communities (specifically, arbuscular mycorrhizal fungal communities) can enhance nutrient uptake and, therefore, affect leaf traits in the tree. This indicates that the drivers of trait variation in forests (and potentially, ecosystem functioning) go beyond considering the effects of tree-tree interactions, but the interactions with other organisms and trophic guilds could also have an effect on forest functioning. Thus, our results contribute to numerous studies suggesting that ecosystem functioning in forests may be also driven by other biotic interactions, including tree-soil organisms' interaction (Luo et al., 2023). Thus, in view of these results, I suggest that future research should address how the diversity of different trophic guilds can jointly affect functional traits in trees.

Conclusions and future directions: towards novel frameworks for community assembly and ecosystem functioning

As described above, functional traits are useful tools to describe the phenotypic variability of life and relate to the main axes of phenotypic variation (Wright et al., 2004; Díaz et al., 2016). In addition, functional traits are essential tools to understand the ecological processes shaping ecological communities (McGill et al., 2006; Pavoine & Bonsall, 2011; Götzenberger et al., 2012; see Figure 2a). As trait variation is important for coexistence, in this thesis I studied the patterns of intraspecific and intraindividual variation in response to species diversity (of trees and mycorrhizal fungi). Thus, my results may contribute to a better understanding of how trait variation changes in order to reduce competitive tree-tree interactions in forests. Further, the results highlight the need of going beyond in the use of functional traits to study ecological processes in local assemblages (both populations and communities). Violle et al. (2012) took an important step by suggesting a novel framework for including intraspecific trait variation in community ecology (Figure 2b). Thus, this new perspective started to consider that the filters shaping ecological communities affected not only species selection but also the selection of specific phenotypes of the species. In addition, considering this variation within species allows to understand why certain species are able to coexist in ecological communities. However, as plants are the result of the repetition of the same structure or organ along the body plan (branches, leaves, flowers, etc.), Herrera (2024) suggested that individuals can be studied as a collection of phenotypes. As our results suggest that intraindividual variation changes with species diversity, and contribute to a growing body of literature studying the responses of intraindividual variation to different abiotic and biotic drivers (Sobral et al., 2019; March-Salas et al., 2021; Møller et al., 2022, 2024), I suggest that there is a need of going beyond established approaches in trait-based ecology to study of the assembly of plant communities (see Figure 2c). Specially in forests, where we have already seen that intraindividual variation can be enormous (Escribano-Rocafort et al., 2016), this would allow to better understand different ecological processes. Indeed, this novel framework would allow to study new facets of the trait space (e.g. how much variation within individuals is selected, how much do plants from the same or different species overlap in the trait space) that, especially at local scales where trees interact, could help to understand ecological processes such as environmental filtering or limiting similarity. However, there are still many limitations in order to consider intraspecific and intraindividual variation. Apart from the technical challenge, which could be overcome thanks to the use of new technologies such as spectroscopy, it is still unknown how abiotic and biotic drivers would jointly shape intraindividual trait variation. That is why, future research should focus on understanding the contributions of intraspecific and intraindividual responses in ecological communities, not only by experimentally manipulating the species composition in the community as in the experiments I used here, but also by understanding these patterns along environmental gradients (for example, temperature or water availability gradients). This would help to elucidate, for instance, if the assembly of ecological communities depends on traits that can vary intraindividually (e.g. leaf traits) or if, in contrast, is mediated by other traits.

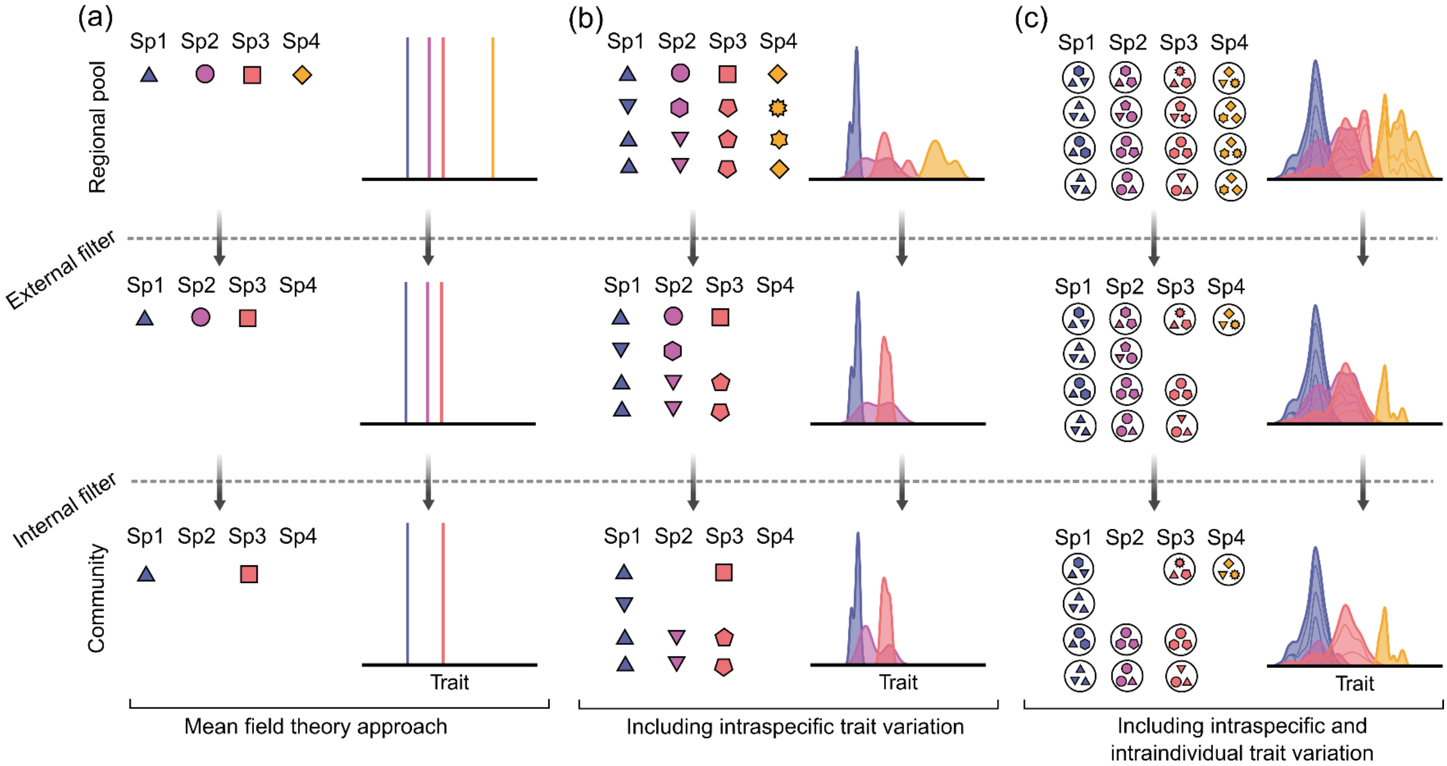

FIGURE 2 Framework for the assembly of ecological communities considering (a) one single value per species (mean field approach), (b) individual level data and (c) intraindividual level data by representing plants as trait distributions, based on figure 2 by Violle et al. (2012). In every case, a regional pool of four species (represented in different colours) is filtered by external filters, i.e. assembly processes operating on the regional pool, and internal filters, i.e. assembly processes internal to the community (represented by dashed lines), giving as a result the observed community. In (a) the filters act on species, while in (b) and (c) these filters select for specific phenotypes. In addition, in (c) the selection occurs on traits, but also on the distribution of traits within an individual.

So far in this section, we have seen that functional traits are the result of abiotic and biotic factors. Nevertheless, at the same time, functional traits drive ecosystem functioning (Díaz & Cabido, 2001; Violle et al., 2007). For example, trait differences between species have been able to explain productivity in BEF-China (Bongers et al., 2021) and have been proposed to be the Holy Grail to link diversity to ecosystem functioning (Díaz & Cabido, 2001). However, the role of functional traits as predictors of ecosystem functioning has also been often reported to be weak or non-existing in the context of BEF research (van der Plas et al., 2020; Chacón-Labella et al., 2023). In this thesis I made use of biodiversity-ecosystem functioning (BEF) experiments to understand the patterns of trait variation to species diversity, but I did not study how intraspecific and intraindividual variation jointly drive different facets of ecosystem functioning. For instance, intraspecific trait variation has been shown to affect arthropod herbivory (Bu et al., 2017) and productivity (Raffard et al., 2019), among others. Less is known about the role of intraindividual variation, but it has been suggested to influence plant-animal interactions (Herrera, 2017) or carbon fluxes (Sobral, 2023). Interestingly, Proß et al. (2023) demonstrated in tree diversity experiments that intraindividual leaf variation was positively related to tree growth (that could be related to stand productivity). That is why, I hypothesize that these sources of trait variation could contribute substantially to ecosystem functioning and help to clarify the role of functional traits in the relationship between diversity and ecosystem functioning. Therefore, future directions in the study of trait variation should attempt to link trait variation across scales to ecosystem functioning.

Overall, my results point out the complexity of the mechanisms underpinning diversity effects and the role of trait variation to mediate coexistence. Tree species show plasticity in their phenotype in order to adapt to the species composition of the stand where they grow (including the tree composition of the stand and the fungal community interacting with the tree) at different levels of biological organization. This could present new challenges, but also possibilities, for the understanding of plant responses, diversity patterns and for improving our scaling from traits to ecosystem functioning.

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APPENDICES

Author contributions

Chapter 2

PCS-B and SH conceived the idea and designed methodology; TM collected the leaf trait data with assistance from PCS-B and SH; KG and OF collected the mycorrhizal diversity data; PCS-B analysed the data with assistance from SH and HB; PCS-B led the writing of the manuscript. All authors contributed critically to the draft and gave final approval for publication. My overall contribution was 70 %.

	PCS-B [%]	TM [%]	KG [%]	OF [%]	NE [%]	HB [%]	ZM [%]	SH [%]
Entwurf (Design)	90	0	0	0	0	0	0	10
Umsetzung (Implementation)	15	50	10	10	0	0	0	15
Auswertung (Analysis)	90	0	0	0	0	5	0	5
Schreiben (Writing)	72.5	7.5	1.5	1	1.5	5	1	10
Insgesamt (Overall)	70	10	2.5	1	1.5	4	1	10

Chapter 3

PCS-B and SH conceived the idea of the study with support from CPC and MCS. SL and XL collected the tree basal area and height data. PCS-B collected the leaf trait data with support from LS and SH. PCS-B analyzed the data, created the figures and interpreted the data with support from RB, CPC, MCS and SH. PCS-B. wrote the first draft of the manuscript with support from SH. PCS-B, LS, RB, CPC, XL, MCS and SH contributed to the discussion of the results and revisions. My overall contribution was 80 %.

	PCS-B [%]	CPC [%]	MCS [%]	RB [%]	LS [%]	SL [%]	XL [%]	SH [%]
Entwurf (Design)	80	5	5	0	0	0	0	10
Umsetzung (Implementation)	80	0	0	0	5	5	5	5
Auswertung (Analysis)	85	5	5	0	0	0	0	5
Schreiben (Writing)	67.5	5	5	5	5	1	1.5	10
Insgesamt (Overall)	80	4.5	3.5	2.5	2.5	1	1	5

Chapter 4

PCS-B, SH, SM and WSH conceived the idea and developed the methodology; under the supervision of SH, AD was responsible for leaf sample collection, laboratory analyses and trait predictions with Vis-NIRS, and PCS-B performed the statistical data analysis; PCS-B led the writing of the manuscript, with support from SH and SM. All the authors contributed to the reviewing and editing of the manuscript. My overall contribution was 70 %.

	PCS-B [%]	AD [%]	SM [%]	WSH [%]	SH [%]
Entwurf (Design)	90	0	2.5	2.5	5
Umsetzung (Implementation)	5	85	0	0	10
Auswertung (Analysis)	95	0	0	0	5
Schreiben (Writing)	81.5	2.5	5	1	10
Insgesamt (Overall)	70	10	7.5	2.5	10

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After more than 50,000 words and 13,000 leaves, this journey full of adventures is coming to an end. But before I run out of pages, I would like to write a few lines to thank all the special people who have crossed my path. Do not get me wrong, during this journey, I have also encountered people capable of destroying the dreams and motivation of PhD students, as well as some behaviors that are more than questionable. However, I do not want to give room to those in the following paragraphs. Instead, I prefer to highlight the role of those who made a difference.

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Curriculum Vitae

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Education

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List of publications

Published

Castro Sánchez-Bermejo, P., Monjau, T., Goldmann, K., Ferlian, O., Eisenhauer, N., Bruelheide, H., Ma, Z., & Haider, S. (2024). Tree and mycorrhizal fungal diversity drive intraspecific and intraindividual trait variation in temperate forests: Evidence from a tree diversity experiment. Functional Ecology, 38, 1089-1103. https://doi.org/10.1111/1365-2435.14549

Castro Sánchez-Bermejo, P., Davrinche, A., Matesanz, S., Harpole, W. S., & Haider, S. (2023). Within-individual leaf trait variation increases with phenotypic integration in a subtropical tree diversity experiment. New Phytologist, 240, 1390-1404. https://doi.org/10.1111/nph.19250

Castro Sánchez-Bermejo, P., deCastro-Arrazola, I., Cuesta, E., Davis, A. L. V., Moreno, C. E., Sánchez-Piñero, F., & Hortal, J. (2022). Aridity drives the loss of dung beetle taxonomic and functional diversity in three contrasting deserts. Journal of Biogeography, 49, 2243-2255. https://doi.org/10.1111/jbi.14506

Submitted

Castro Sánchez-Bermejo, P., Carmona, C.P., Schuman, M.C., Benavides, R., Sachsenmaier, L., Li, S., Liu, X., Haider, S. (submitted). Intraspecific and intraindividual trait variability decrease with tree species richness in a subtropical tree biodiversity experiment. Submitted to Science Advances on 26.11.2024.

Conference contributions

Pablo Castro Sánchez-Bermejo, Lena Sachsenmaier, Raquel Benavides, Carlos Pérez Carmona, Shan Li, Xiaojuan Liu, Meredith Christine Schuman, Sylvia Haider (2024). Assessing the role of intraspecific and intraindividual variation in tree-tree interactions. Oral presentation in the 109th Annual Meeting of the ESA (Long Beach, United States).

Pablo Castro Sánchez-Bermejo, Tilo Monjau, Kezia Goldmann, Olga Ferlian, Nico Eisenhaue, Helge Bruelheide, Zeqing Ma, Sylvia Haider (2023). Tree and mycorrhizal fungal diversity drive intraspecific and intraindividual trait variation in temperate forests: Evidence from a tree diversity experiment. Oral presentation in the XIV Congress of the Asociación Española de Ecología Terrestre (Almería, Spain).

Pablo Castro-Sánchez-Bermejo, Andréa Davrinche, Stanley Harpole, Sylvia Haider (2022). Patterns of tree phenotypic integration and intra-individual trait variation. Oral presentation in the World Biodiversity Forum 2022 (Davos, Switzerland).

Pablo Castro-Sánchez-Bermejo, Andréa Davrinche, Stanley Harpole, Sylvia Haider (2022). Tree phenotypic integration promotes intra-individual trait variation in a subtropical forest. Oral presentation at the Annual iDiv Conference (Leipzig, Germany).

Pablo Castro Sánchez-Bermejo, Rut Sánchez de Dios, Felipe Domínguez Lozano. Retos en la aplicación de los criterios IUCN a las comunidades vegetales españolas (2017). Poster presented in the VIII Congress of Biología de la Conservación de las Plantas (Madrid, Spain).

Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit mit dem Titel „Patterns of intraspecific and intraindividual trait variation in trees along experimental diversity gradients" eigenständig und ohne fremde Hilfe verfasst sowie keine anderen als die im Text angegebenen Quellen und Hilfsmittel verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommen wurden, wurden von mir als solche kenntlich gemacht. Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe. Die vorliegende Doktorarbeit wurde bis zu diesem Zeitpunkt weder bei der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt.

Pablo Castro Sánchez-Bermejo, Halle (Saale) 27.11.2024