The role of economic, venation and morphological leaf traits in plant and ecosystem function along forest-savanna gradients in the tropics

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Abstract

Predictions about the impacts of current and projected anthropogenic climate change on tropical forest ecosystems require a better understanding of sensitivity and variability of plant attributes to biotic and abiotic factors. Leaf functional traits are particularly informative because they determine carbon and water fluxes between plants and atmosphere. However, due to the lack of data in the tropics, especially, in Africa, many uncertainties surround the interpretations of trait-environment relationships as well as trait-trait trade-offs and their importance on plant and ecosystem functioning. Therefore, the principal aim of the thesis was to better understand, and test widely held assumptions surrounding the functional significance of key leaf economic, morphological and venation traits in the tropical forest ecosystems. The data was collected for 90 angiosperm adult tree species along a strong environmental gradient (rainfall, fertility, midday temperature and vegetation) in Ghana, West Africa and presented in four data chapters.

The first data chapter of the thesis (**Chapter 4**) focuses on the links between leaf photosynthetic capacity and key nutrients along a vegetation gradient from forest to savanna. This is important because these relationships are employed in the modelling of global primary productivity, with great uncertainties evident across the tropical forest-savanna boundaries. To allow for better

interpretation of the results, this chapter includes an additional data set of neotropical forest-savanna boundary in Cerrado, Brazil. Special attention is paid towards patterns of nitrogen allocation between leaf structure and photosynthetic apparatus. In contrary to the expectations, the key findings of this chapter suggest that studied species do not maximise photosynthetic capacity per available foliar nitrogen, phosphorus or potassium due to adaptation to intermittent water availability in these strongly seasonal environments.

The two following data chapters explore morphological adaptations of leaves to the extremes of the studied environmental gradient. **Chapter 5** examines the drivers and functions of leaf morphology, specifically, leaf shape. Based on the leaf energy budget theory, the findings of this chapter demonstrate the importance of narrow and elongated leaves in thermoregulation and maintenance of photosynthetic rates in the savanna study plot, which is characteristic of high irradiance, midday temperatures and seasonal water deficits. Additionally, this chapter suggests that narrow and elongated leaf blades are also important for optimising canopy light capture in shaded canopies of wet evergreen forests. Including leaf shape metrics in plant performance modelling can improve understanding of ecosystem response to projected increases in and extreme temperatures.

The penultimate data chapter of the thesis (**Chapter 6**) explores the role and diversity of functional traits that describe leaf venation network. The data presented in this chapter test and support the hypothesis that minor vein traits play an important hydraulic function that underlies plants adaptation/acclimation to intermittent water availability. This is seen in denser and more reticulated networks present at the driest end of the gradient and strong links between minor veins and potassium (important nutrient for osmoregulation), leaf size, leaf deciduousness and wood density. These relationships were shown to be affected more significantly by environmental filtering than by phylogenetic relatedness among species.

The final data chapter (**Chapter 7**) joins the recurring themes of the thesis by assessing the relationships between studied leaf economic, morphological and minor venation trait dimensions and plant and community ecological strategies. Leaf functional traits clustered along three significant ecophysiological axes of variability related to gas exchange, structure and water transport. Additionally, the findings highlight the role that leaf functional traits play in the ecosystem functioning as leaf trait dimensions relate to aboveground net primary productivity and biomass. Finally, this chapter emphasises the need for more large-scale studies linking functional traits with plant and ecosystem function at different scales of tropical forests. Such studies would enable the development of a significant mechanistic understanding of what creates and drives functional trait variability across tropical forests and in modelling their effects on water and carbon fluxes in tropical forest ecosystems.

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everything is possible

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Chapter 1

Introduction

1.1. Understanding tropical forest functioning in a changing climate

Increases in atmospheric greenhouse gas concentrations over the last century are causing changes in climate which are particularly evident across the tropical latitudes (Stocker *et al.*, 2013; Fischer & Knutti, 2015). Here, temperatures have risen by ~0.05°C decade^{-1} since the start of 20th century (Harris *et al.*, 2014) and are expected to continue increasing even under different climate mitigation scenarios (Stocker *et al.*, 2013). Additionally, climate models are predicting overall increase in the global and tropical rainfall levels, as well as changes in precipitation extremes (longer dry seasons, and more droughts and floods) (Harris *et al.*, 2014). However, some regions, such as West Africa, have experienced consistent reductions in rainfall over the last few decades with further projections in an increase in dry season water deficits (James *et al.*, 2013). These environmental changes across the tropical regions are and will continue having a significant effect on the global climate transformations as tropical forests are one of the key "engines" behind the global atmospheric circulation (Malhi *et al.*, 2014; Wang *et al.*, 2014).

Tropical forests are also major influencers of the global carbon cycle as they store 60% of terrestrial live biomass, exchange more carbon with the atmosphere than any other terrestrial biome (Beer *et al.*, 2010) and account for one-third of net primary productivity (NPP) (Aragão *et*

al., 2014). However, during the last decade tropical forests have experienced greater carbon and, consequently, NPP losses than gains which can be attributed to deforestation and to reductions in carbon density (Baccini *et al.*, 2017). There has been a widespread concern that these changes could weaken the current tropical forest carbon sink as observed in Amazonia (Corlett, 2016). A few studies have shown that recent drought events are having a significant effect on the aboveground biomass storage in the Amazon (Lewis *et al.*, 2011) due to the vulnerability to increasing moisture stress (Phillips *et al.*, 2009). Drought was suggested to be one of the key climatic drivers behind tree mortality events which have been observed worldwide (Pillet *et al.*, 2018). Additionally, tropical forests are extremely sensitive to rising temperatures as they may already function close to photosynthetic temperature threshold (Doughty & Goulden, 2009). Therefore, increases in respiration and reduction in photosynthesis can influence the ability of tropical forests to act as carbon sinks (Wang *et al.*, 2013a).

Ecosystems that are particularly sensitive to changes in climate are the widespread transition zones between tropical forests and savannas. The stability of the boundaries between the two biomes is usually weak, reflecting both local and regional variation in abiotic conditions (Oliveras & Malhi, 2016). Changes in precipitation and strongly linked fire feedbacks are thought to be major drivers of short and long-term shifts of boundaries between tropical forest and savannas (Hirota *et al.*, 2011; Staver *et al.*, 2011a). Specifically, resilience of savanna boundaries to becoming treeless increases with reducing precipitation. Reduced tree cover promotes growth of flammable grasses resulting in greater fire occurrence and intensity (Cardoso *et al.*, 2018). Salazar *et al.* (2007) predicted that climate change together with anthropogenic pressures would increase "savannisation" of tropical forests and aridisation of savannas in South America. Conversely, increases in precipitation and/or length of rainy season can exclude fire and encourage greater sapling recruitment. Climate change effects on the stability of biome boundaries are further exacerbated by anthropogenic pressures such as deforestation (Wuyts *et al.*, 2018). This is suggested by paleoecological studies that found contemporary savannas established in areas that are now wet enough to support forest (Desjardins *et al.*, 1996). Furthermore, changes in vegetation

type from forest into savanna are expected to have significant effects on climate directly via changes in solar irradiance reflection and transpiration or indirectly through changes in carbon cycling (Anadon *et al.*, 2014).

Considering projected climatic change and significance of tropical forest ecosystems, understanding the sensitivity of trees and forests to changing environmental conditions is, therefore of particular importance. Greatest uncertainties in the models predicting the response of undisturbed tropical forests to climate change are the physiological responses rather than differences amongst climate model projections (Huntingford *et al.*, 2013). Specifically, there are research gaps in the understanding of tropical forest ecosystem responses to nutrient limitations (in the soils and canopies), responses to elevated temperatures and changes rainfall regimes.

1.2. Functional trait dimensions

Studying certain morphological, anatomical, and biochemical plant traits (functional traits) can bring us one step closer towards understanding the ecosystem's response to environmental change. Traits are known to collectively and individually affect the functioning of a plant (Violle *et al.*, 2007) and, while the links between functional traits and ecosystem function are complex, functional trait diversity can be indicative of resilience of tropical forest ecosystems to climate change. This is according to the complementarity effect hypothesis (Tilman *et al.*, 1997), which states that greater functional trait diversity facilitated by a higher biodiversity can better utilize a pool of limiting resources, thereby increasing total ecosystem performance. Indeed, use of plant functional traits is becoming increasingly popular in modelling of tropical forests response to climate change (Lavorel *et al.*, 2007). Such modelling approaches are exceptionally well suited for application to diverse tropical ecosystems. They are able to account for the continuous variation and high variability of functional traits within such ecosystems, and thereby provide improved predictions of their biogeochemical and physical dynamics (Verheijen *et al.*, 2013).

Some of the most informative functional traits are leaf properties that determine carbon and water fluxes between plant and atmosphere (Lambers *et al.*, 2006). For example, photosynthetic capacity (V_{cmax}) is widely used as a parameter to help simulate global gross primary production in terrestrial biosphere models (Kattge *et al.*, 2009; Walker *et al.*, 2017). This functional trait is widely regarded to be limited by leaf nutrient content in the tropics, particularly by nitrogen (*N*) (Bahar *et al.*, 2016; Norby *et al.*, 2017), phosphorus (*P*) (Bloomfield *et al.*, 2014; Domingues *et al.*, 2015) and/or potassium (*K*) (Lloyd *et al.*, 2015), the latter being particularly important in the more seasonal tropical forest-savanna boundaries due to the osmotic role it plays in plant functioning (Sardans & Peñuelas, 2015). The concentration of nutrients allocated towards photosynthetic machinery also depends on leaf mass per area (LMA). The trade-offs among these traits optimise plant carbon acquisition and are summarized by the leaf economic spectrum (LES) (Wright *et al.*, 2004). The LES predicts that greater gas exchange rates of the leaves are enabled by large foliar *N* investments. The trade-off of this is shorter leaf life span, due to leaves being less robust, as a result of their low LMA, and being highly palatable, and therefore readily consumed by herbivores (Coley, 1983). Conversely, long-lived leaves are predicted to have low carbon assimilation rates and low *N*. Understanding these trade-offs is important for predicting the effects of climate change on tropical vegetation structure. Leaf economic spectrum is known to be correlated with plant's response to drought, indicated by leaf turgor loss point, (Zhu *et al.*, 2018), water transport via venation networks (Blonder, 2017) and with thermal regulation and energy budgets (Michaletz *et al.*, 2015).

Leaf morphology, in addition to governing the LES, plays an important role in plant and ecosystem ability to cope with environmental change (Davis *et al.*, 2005). This has been suggested in numerous studies observing strong links between climatic gradients and traits, such as leaf shape, width, size and LMA (e.g. Poorter *et al.*, 2009; Leigh *et al.*, 2017; Wright *et al.*, 2017; Fauset *et al.*, 2018). Variability of these morphological traits is particularly important adaptation in sites of high evaporative demand, where stomatal conductance (g_s) is not able to regulate the temperature via transpiration (Slot & Winter, 2017). These conditions can result in reduction of carbon gain,

disruption of enzyme function or even critical damage to photosynthetic machinery (Mathur *et al.*, 2014). Larger and wider leaves are more susceptible to damage from extremely high temperatures (~48° C (Slot *et al.*, 2018)) when soil water is limiting as predicted by daytime leaf energy budgets (Penman, 1948; Wright *et al.*, 2017; Fauset *et al.*, 2018). Meanwhile, LMA has been proposed as a correlate of drought tolerance in, partly because it is associated with modulus of elasticity (Poorter *et al.*, 2009; Markesteijn *et al.*, 2011). Indirectly, in sites of limited water supply, high LMA is often a feature of drought adapted species with deciduous leaf habit (Markesteijn *et al.*, 2011).

Leaf venation architecture is an important aspect of leaf morphology and it poses significant constraints on water and gas exchange (Sack & Scoffoni, 2013). This was demonstrated empirically and theoretically by connecting the LES with venation network (Blonder *et al.*, 2011; Sack *et al.*, 2013; Blonder, 2017). While the mechanisms linking vein and economic traits are still debated, there is consensus that minor vein density (*VD*) controls leaf photosynthetic traits via leaf hydraulic conductance and/or leaf mass per area adjustments (Brodribb *et al.*, 2007; Blonder *et al.*, 2013). Together with *VD*, higher reticulation of veins (greater hierarchical branching and looping (Roth-Nebelsick *et al.*, 2001)) enables more efficient transport of water and photoassimilates within the leaf (Katifori *et al.*, 2010). This is of particular importance in environments with a high evaporative demand (Boer *et al.*, 2016), as denser and more reticulated vein networks provide more pathways for rerouting the flow in order to minimise the effects of drought stress induced xylem cavitation on gas exchange rates (Sack *et al.*, 2008; Price & Weitz, 2014; Brodribb *et al.*, 2016). On the other hand, higher biomass investments in veins present plant functioning trade-offs; Veins are rich in lignin and therefore have higher carbon costs when compared to other leaf tissues (Lambers & Poorter, 1992). Additionally, high *VD* can also displace photosynthetic tissue, further reducing available carbon and thus potentially reducing plant fitness (Feild & Brodribb, 2013).

Evidently, the aforementioned leaf trait dimensions (economic, morphological and venation) are closely interlinked and, therefore, have been used to define, describe and explain species ecological strategies and ecosystem functioning (Fyllas *et al.*, 2012, 2017a). Specifically, grouping of functional traits according to the rates of resource acquisition and processing (Reich, 2014) reflects growth and survival of trees (Wright *et al.*, 2010a; Fyllas *et al.*, 2017b). For example, greater constructions costs of evergreen leaves (Eamus 1999) enable them to maintain photosynthesis during dry seasons in the tropics, while deciduous species maximise carbon gain during the wet season (Tomlinson et al. 2013), which in turn affects their growth rates and survival (Tomlinson *et al.*, 2014; Santiago *et al.*, 2016). To a degree, these trait dimensions have also been used to explain species and ecosystem distributions along geographical gradients (Westoby & Wright, 2006; Kraft *et al.*, 2008).

1.3. Research gaps

It is now widely accepted that leaf functional traits are imperative to understanding the response of tropical forest ecosystems to fast-paced and continuously intensifying climate change. However, numerous challenges remain in advancing the use these traits in models capable of predicting the sensitivity of forests to change.

With an increasing number of studies empirically testing the relationships between traits and environmental conditions a lack of consensus is evident. Some of the examples might include venation architecture being linked with water availability in some studies (Sack & Scoffoni, 2013) but not in others (Blonder *et al.*, 2018), soil *P* and/or *K* has limiting functioning of the tropical ecosystem rather than *N* (Haridasan, 2008; Bond, 2010). Some of these uncertainties are particularly evident in sites of intermittent water availability; Here the findings are often substantially different from those seen in temperate forests or laboratory and greenhouse experiments (Xiang et al., 2013). The lack of agreement among these studies might arise because the study plots are often situated along complex and extensive rainfall, temperature, altitude as

well as fertility gradients, which makes them difficult to compare and interpret. Additionally, trait variability in response to environmental conditions might be different depending on the scale they are assessed at: tree, species, or community (Anderegg *et al.*, 2018a). These uncertainties are particularly important to consider in the complex and rich tropical forest ecosystems.

Great uncertainties are also evident when trait-trait relationships and trade-offs are assessed. This is, for example, seen in an increasing number of studies questioning widely accepted relationship between photosynthetic capacity and leaf *N* (Dusenge *et al.*, 2015; Varhammar *et al.*, 2015). Likewise, contrary to current understanding, some research has suggested that leaf water transport/use and economic trait dimensions are decoupled (Li et al., 2015; Males & Griffiths, 2017), especially in tropical regions when large number of species is considered (Li et al., 2015). Understanding the links between photosynthetic and water transport traits is of particular importance, as they play an important role in plant survival under environmental stress (Choat *et al.*, 2012).

In contrast to the LES traits, some plant features and their functional role in changing climate are rarely discussed. Leaf shape, for example, is known to be important for plant thermoregulation, however, most of the previous work linking leaf shape, heat transfer and boundary layers has been based on steady-state conditions and metal leaf replicas which are not indicative of a realistic fluctuating environment (Vogel 2009). Additionally, to the best of our knowledge, there are no empirical studies that investigate links between leaf shape and photosynthesis, apart from one study assessing the effect of leaf dissection on gas exchange in African *Pelargonium* species (Nicotra *et al.*, 2008). The way leaf shape is measured in the tropical studies is also lacking improvements. Considering the importance of leaf thermoregulation under increasing global temperatures, seasonality and aridity (Coumou & Robinson, 2013; Harris *et al.*, 2014), understanding the role that leaves play in adapting to changing environment is of particular importance.

Knowledge of species ecological and evolutionary background can provide explanations to observed syndromes (consistent associations between traits (Kattge *et al.*, 2011)), however, are seldom used in current research of functional traits. Specifically, many trait-trait and trait-environment relationships are significantly affected by species phylogenetic relatedness (Cavender-Bares *et al.*, 2009) and not accounting for it may result in statistical non-independence of data in cross-species comparative analysis (Stone *et al.*, 2011). In addition to phylogenetic clustering of traits, species ecological strategies, such as deciduousness or light requirements for germination and establishment, can also affect the relationships between traits and environmental conditions, however, numerous studies produce conflicting results (e.g. Cianciaruso *et al.*, 2013; Tomlinson *et al.*, 2013) and require further investigation.

Finally, studies exploring bivariate relationships between specific functional traits and environmental variables in the context of plant function can be mechanistically informative, however, it is a combination of ecologically relevant traits (trait space (Díaz *et al.*, 2015)) that allow plants to grow and survive (Lebrija-Trejos *et al.*, 2010). Studies that consider links between trait space and ecosystem function in a defined set of environments are very few (Messier *et al.*, 2017; van der Sande *et al.*, 2018).

Many of the aforementioned challenges arise from the lack of functional trait data, especially in certain tropical regions. While a lot of research has been focused on the Amazonia, the tropical forests of Africa have been rather overlooked. This has been for several reasons, including political instability and logistical obstacles (Malhi *et al.*, 2013). Additionally, widespread forest-savanna ecotones (boundaries between forest and savanna biomes (Oliveras & Malhi, 2016)) have received little attention from a functional traits perspective, despite being particularly sensitive to ongoing environmental change.

1.4. Research questions

Given the above context, the principal aim of the thesis is to better understand and test widely held assumptions surrounding the functional significance of leaf economic, morphological and venation traits in the tropical forest ecosystems. Because contrasting study environments provide perfect setting to achieve this aim, the data collection was conducted along a forest-savanna vegetation gradient in the tropical forests of Ghana, West Africa. This region was chosen because of the predicted reductions of rainfall, intensifying seasonality, rising temperatures and overall lack of data. To allow for phylogenetic and functional group comparisons, the data spanned ~90 angiosperm tree species from 27 families with different ecological strategies. The principal aim of the thesis will be addressed by four key questions, which are answered using research objectives:

Question 1: *Are photosynthetic capacity, key leaf and soil nutrients linked along forest-savanna boundaries?*

Objective 1: Test the relationships between soil and leaf chemistry in study plots along the vegetation transitions.

Objective 2: Investigate if photosynthetic capacity varies along the forest savanna boundary and if it is related to leaf nutrient concentrations.

Objective 3: Explore the role that ecological strategies (deciduousness, light guilds, nitrogen fixation, phylogeny) play in the variability in photosynthetic capacity and its relationships with nutrients.

Question 2: *How and why does leaf shape vary along the studied environmental gradient?*

Objective 4: Identify the most variable features of leaf shape and measure their dynamics along the gradient.

Objective 5: Evaluate abiotic and biotic drivers of leaf shape**.**

Objective 6: Quantify the effect that leaf shape has on leaf thermoregulations and gas exchange.

Question 3: *Does venation architecture play an important role in plant's adaptation to intermittent water availability?*

Objective 7: Explore venation trait dynamics along the rainfall gradient.

Objective 8: Test the relationships between vein metrics and traits considered to be indicative of plant drought response.

Objective 9: Investigate the role that phylogeny and species ecological strategies play in regulating the relationships between traits.

Question 4: *How does multivariate leaf trait space affect plant and ecosystem performance?*

Objective 10: Identify leaf traits that respond the most to to environmental filtering.

Objective 11: Asses the links between trait space and plant ecological strategies (deciduousness, light requirements and *WD*).

Objective 12: Test if trait space is correlated with NPP and aboveground biomass.

1.5. Thesis outline

The background, methodology and findings required to answer these questions were outlined in eight chapters of the thesis. This chapter (**Chapter 1**) has presented the rationale aspects to the research carried out in this thesis, highlighting the remaining knowledge gaps. **Chapter 2** gives a concise background literature review on each of functional trait dimension (economic, morphological and venation) and the role they play in plant and ecosystem functioning. Descriptions of study sites, data collection campaigns and general methods are outlined in **Chapter 3**. Where necessary, more specific methods and field site descriptions are detailed within individual chapters. The first data chapter of the thesis (**Chapter 4**) investigates the dynamics of photosynthetic capacity in relation to soil and leaf nutrients in forest-savanna boundaries (Question 1). **Chapter 5** assesses the functional significance of leaf shape along the gradient in Ghana (Question 2). **Chapter 6** investigates the functional role of minor venation traits with specific

attention paid to water availability (Question 3). **Chapter 7** combines the different dimensions of functional traits, assesses the relevance of these traits in plant and ecosystem function (Question 4). Finally, **Chapter 8** discusses the three previous data chapters simultaneously, placing the findings in a global context and summarises the main conclusions of the investigation, with recommendations for further work.

Chapter 2

Literature review

2.1. General introduction

Studying traits that reflect species fitness directly or indirectly can help us to understand and predict patterns of species abundance and diversity, as well as community and ecosystem functioning (Díaz *et al.*, 2007). This is particularly relevant when traits are studied along environmental gradients as not all ecosystems respond to environmental changes in the same way. For example, wet tropical forests may be more sensitive to climatic change than drier forest-savanna ecotones due to functional composition of the ecosystem (Aguirre-Gutiérrez *et al.*, 2019). Nevertheless, interpreting responses of the forest to environmental change can be extremely challenging as trait-trait and trait-environment interactions are complex and often unique to individual species. Additionally, high diversity of species of tropical forests that often is dynamic to given past and current environmental conditions further complicates our current understanding of the role that functional traits play in ecosystem functioning.

While numerous leaf functional traits have an effect on plant functioning, some suites of traits such as economic (Wright *et al.*, 2004) and morphological (size and density (Diaz *et al.*, 2004)) are most commonly discussed in the modern literature. More recently, leaf venation traits have been gaining recognition because of their influence on plant functioning as well as on economic

and morphological traits (Blonder *et al.*, 2011; Sack & Scoffoni, 2013; Sack *et al.*, 2013). For this reason, this thesis focuses on leaf economic, morphological, and venation suites of plant functional traits.

This chapter will discuss the existing literature and research gaps surrounding the three main leaf functional trait dimensions (economic, morphological and venation). Firstly, I will review biotic and abiotic drivers of gas exchange between leaves and atmosphere and functional adaptations to unfavourable environmental conditions (**section 2.2.**). Secondly, I will introduce literature assessing the importance of venation traits (**section 2.3.**). Thirdly, I will review recent research that addressed the relationship between leaf water transport and gas exchange (**section 2.4.**). Fourthly, I will discuss how the aforementioned traits position themselves in leaf multidimensional trait space (**section 2.5.**). Specifically, I will discuss studies that previously considered sets of traits in a multivariate context and the environmental variables behind the trait filtering. In addition to environmental factors, the following section (**2.6.**) will highlight the role that the phylogenetic history of species plays in filtering of functional traits. Finally, I will discuss the potential links between the studied leaf functional traits and ecosystem function, focussing on forest biomass and biomass gains (**section 2.7**).

2.2. Biotic and abiotic factors controlling leaf gas exchange

Photosynthetic gas exchange is one of the most important ecophysiological traits; it affects individual plant survival and functioning, forest stand growth and ecosystem carbon gain (Flexas *et al.*, 2012; Fatichi *et al.*, 2014b). Additionally, gas exchange between leaves and the atmosphere controls carbon sink-source balance (Fatichi *et al.*, 2014b) and is, therefore, the main natural tool to counteract anthropogenic climate change. Gas exchange traits are very dynamic; they vary in response to many biotic and abiotic factors, such as climate, growth, soil fertility, nutrient and light availability, among others (Lawson *et al.*, 2012). These interactions are often well studied in

controlled environments, however, in complex tropical forest ecosystems, this variability is less studied and understood (Dietze, 2014).

2.2.1. Light

Light is a fundamental aspect of photosynthesis because it onsets electron transfer reactions and activates Calvin cycle enzymes (Flexas *et al.*, 2012). Leaves have adapted in many ways to efficiently harvest light because light availability is extremely variable depending on the time of the day, season and forest stand structure. Leaf morphological and anatomical features are particularly important for optimum internal processing of light. For example, traits such as waxiness of the leaves, leaf thickness, LMA or density of mesophyll can have an effect on light distribution within a leaf (Evans & Poorter, 2001). These modifications are often seen in studies comparing leaf morphology and anatomy between sunlit and shaded leaves (Keenan & Niinemets, 2016).

Leaf anatomy, morphology and architecture are known to acclimatize and adapt to changes in the light environment over time to optimize leaf optical features, such as absorptance, reflectance and transmittance (Fig. 2.1; Flexas *et al.* (2012)). These changes can vary from short term changes in stomatal movements to coordinated modification of leaf morphology, such as leaf size, shape and inclination angles. Longer term increases or decreases in light availability can result in advancement of fast-growing species that require light for germination and establishment of juveniles (pioneers) or slow-growing shade-bearers that function well in the dark understories of tropical forests and these changes of community dynamics can affect plant and ecosystem functioning. For example, Dusenge et al. (2015) suggested that pioneer and shade-bearing species allocate different amounts of leaf *N* to photosynthetic machinery, which can in turn affect ecosystem carbon gain and, potentially, NPP (Cai *et al.*, 2016). Links between leaf morphology, species ecological strategies, community structure and light availability are, however, seldom studied across large numbers of species in tropical forests.

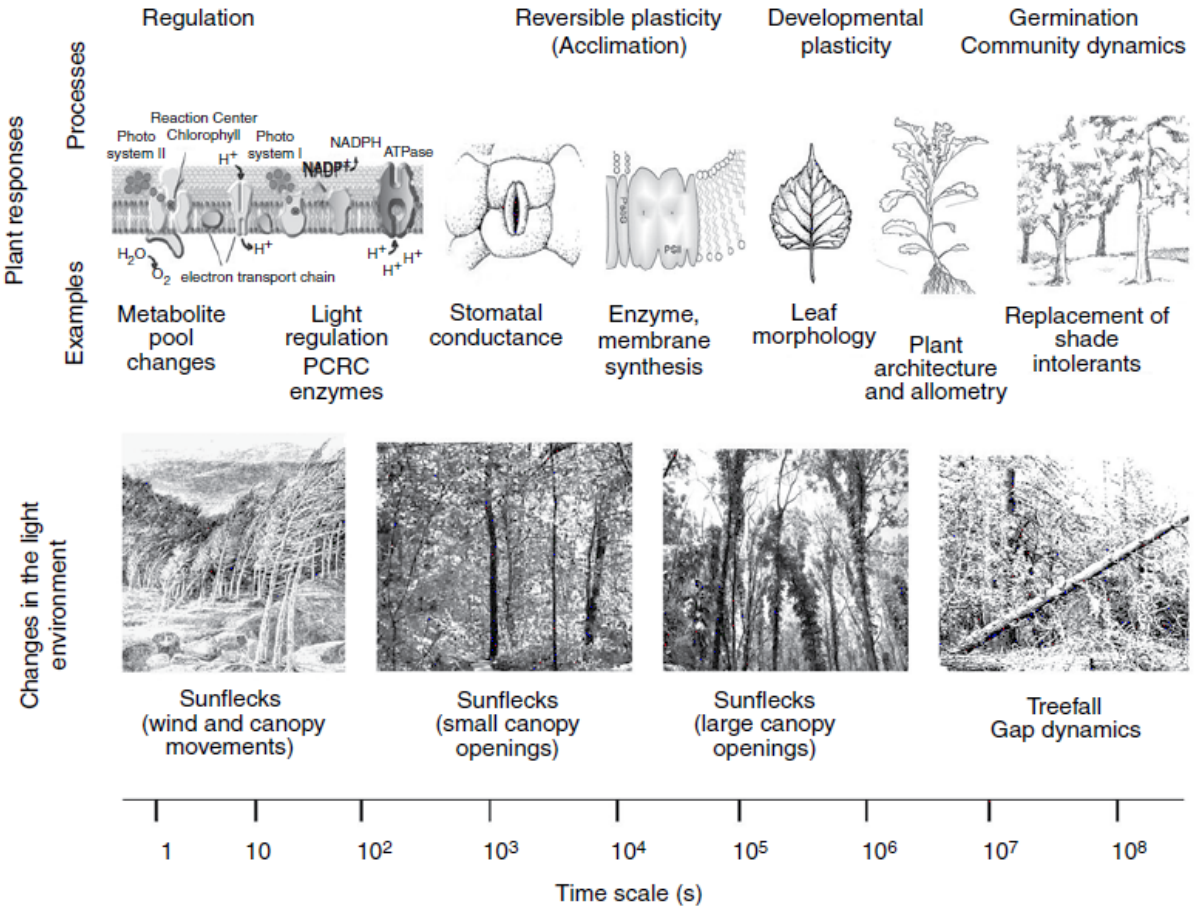


Fig. 2.1 Leaf, plant and community responses to increasing light availability over time. Figure taken from Flexas *et al.* (2012).

While light is essential for photosynthetic processes, plants that are not acclimatized to high intensity irradiance or are not able to adapt due to the fast rate of changes in their light environment can experience photoinhibition due to production of harmful reactive oxygen species (ROS) or even permanent photosynthetic machinery damage (Foyer, 2018). These negative effects can be exacerbated by other environmental factors, such as drought or extremely high temperatures as they can reduce the rates of carbon assimilation and increase the generation of ROS (Demmig-Adams & Adams, 2006). Additionally, absorbed and not utilized light can be dissipated as heat across the leaves, making leaf adaptations to high temperatures of particular importance.

Anthropogenic climate change, together with deforestation and exploitation of land are initiating changes of light environment experienced by forests globally. Changes in cloud density, global dimming due to areosols (Wild, 2009), increases in forest gaps and edge effects are either increasing or decreasing light intensity in many tropical regions, which in turn may affect ecosystem carbon balance. Therefore, it is of vital importance to better understand species adaptations and requirements for light at leaf, tree and community levels. These themes are further investigated in **Chapter 4, Chapter 5** and **Chapter 7**.

2.2.2. Temperature

Optimal photosynthetic temperature is considered to be 25 ˚C at which gas exchange peaks and is followed by declines at either side. Generally, photosynthesis is lower in tropical trees (Kattge *et al.*, 2009) as the high air temperatures in the tropics exceed the optimum carbon fixation temperature (Helliker & Richter, 2008). Nevertheless, species of some tropical biomes exhibit considerably higher photosynthetic optimum temperatures, for example gas exchange peaks at 31.4 ˚C in three lowland tropical forest species (Fig. 2.2; Slot *et al.* (2018)). Light availability and species adaptations to it are also important as optimum photosynthetic temperatures differ between sunlit and shaded leaves. This might be a result of modifications in leaf morphology which can significantly reduce the negative effects of high temperature on the functioning of the leaf (Curtis *et al.*, 2012). For example, small leaves enable smaller leaf-to-air temperature differences (Wright *et al.*, 2017) due to a reduced boundary layer and are likely to be adaptive in hot and dry environments (Leigh *et al.*, 2017). In the context of climate change, specifically, increases in average temperatures as well as extremes, more species may experience reductions in carbon assimilation rates and catastrophic damages to photosynthetic apparatus. Implications and adaptations of temperature on species morphology (specifically, leaf shape) and gas exchange in different light environments are further investigated and discussed in **Chapter 5**.

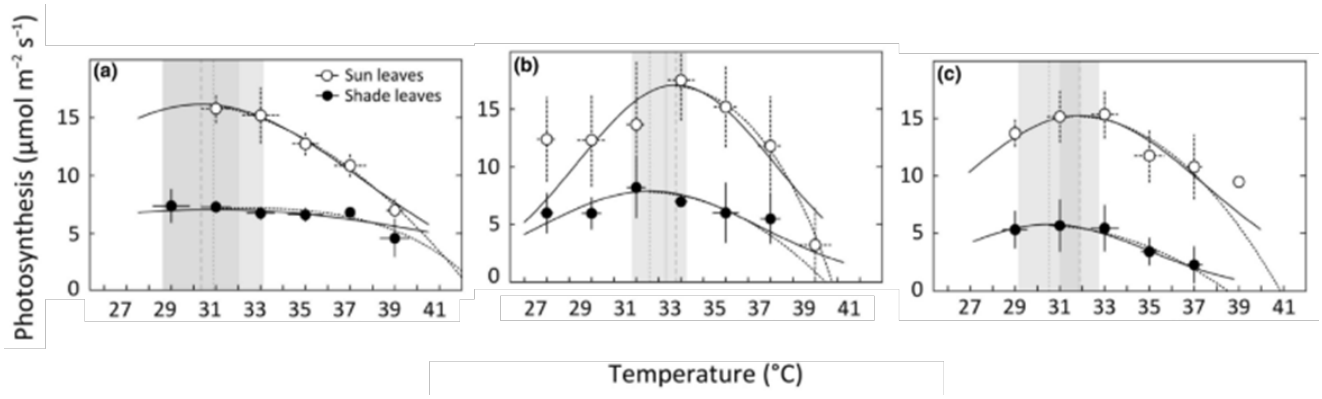


Fig. 2.2 Relationships between photosynthesis and temperature in sunlit and shaded leaves of a) *Calophyllum inophyllum*, b) *Inga spectabilis*, and c) *Ormosia macrocalyx*. Figure taken from Slot *et al.* (2018).

2.2.3. Water stress

Short term water stress, for example, seasonal droughts, is known to have a significant effect on leaf gas exchange (Zhou *et al.*, 2014). Directly, this is largely due to reduced stomatal conductance or complete stomata closure which is caused by abscisic acid signals sent to the leaves from roots that detect a lack of available water in the soil (Saradadevi *et al.*, 2017). Stomatal depression can also be triggered by factors such as stem embolism, higher xylem-sap pH and other signalling hormones such as ethylene (Saradadevi *et al.*, 2017). Indirectly, water stress can also affect hydraulic conductance, which is linked with photosynthetic capacity (Skelton *et al.*, 2017).

Plants often recover quickly and without lasting damage from short term water stress, however, during severe, longer lasting droughts, plants that are not well adapted to these conditions can incur metabolic impairments which sometimes results in permanent damage of photosynthetic apparatus (Gallé & Feller, 2007). This may be because water stress accelerates leaf senescence which is characteristic of declining gas exchange rates. Permanent metabolic impairment is often exacerbated by other unfavourable environmental conditions, such as high light intensity and temperature (Zhou *et al.*, 2007).

In contrast to light and temperature, water availability may not be a strong predictor of photosynthetic capacity, which varies significantly in response to short term changes in rainfall (Flexas *et al.*, 2006), but adapts to long term water stress (Gouveia & Freitas, 2008; Rowland *et al.*, 2015). This is because plants have evolved many strategies to escape, avoid or tolerate lack of water. Water use efficiency, increased rooting depth, foliage deciduousness and leaf morphological strategies are examples of adaptations that maintain photosynthesis through intermittent water availability (Eamus, 1999). Furthermore, dense venation networks have previously been suggested to be important in water stressed environments (Sack & Scoffoni, 2013) and are discussed in detail in **Chapter 6** and further in this chapter (**section 2.3.**).

2.2.4. Growth

While aforementioned environmental conditions can have a direct impact on gas exchange, it is sometimes suggested that long term changes in gas exchange are driven by plant growth reductions induced by adverse climatic conditions (Körner, 2013). This is indicated by more immediate changes in growth of a plant in conditions of limited water availability which, in-turn downregulates photosynthesis (Fig. 2.3; Fatichi *et al.* (2014b)).

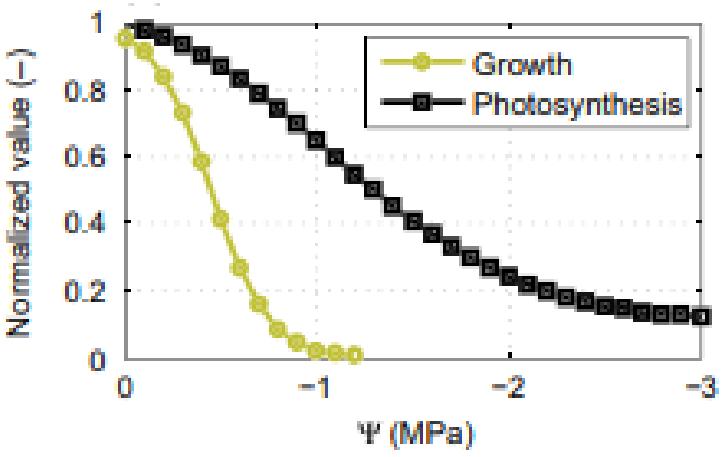


Fig. 2.3 Difference between normalised photosynthesis values (square symbols) and growth (circle symbols) as functions of soil water deficit. The figure was taken from Fatichi *et al.* (2014a).

2.2.5. Nutrients

Biochemically, photosynthetic capacity depends on the amount, kinetics and activity of the foliar *N*-demanding enzyme Rubisco (Dong *et al.*, 2016). Therefore, *N* availability is often the key limiting factor for carbon acquisition at the leaf and ecosystem level (Wright *et al.*, 2004; Kattge *et al.*, 2009). While a strong positive relationship between photosynthesis and *N* is observed in several large cross-biome datasets (e.g. Wright *et al.*, 2004), it becomes weak to non-existent when investigated at smaller scales, specifically, when the range of *N* values decreases (Coste *et al.*, 2005; Domingues *et al.*, 2015). There are numerous reasons for this, however, they are rarely discussed. Some species may allocate higher fractions of *N* towards leaf structure to increase leaf turgidity, which is particularly important in water-stressed environments (Niinemets & Tenhunen, 1997). Some species demonstrate strong coordination between plant growth and new leaf generation meaning that they would seldom produce leaves that do not have sufficient amounts of nitrogen for photosynthesis/leaf structure (Flexas *et al.*, 2012). However, this has not been investigated in the generally infertile tropical forests. The relationship between *N* and gas exchange is further discussed and investigated in **Chapter 4**.

Leaf phosphorus is an important element as it limits ecosystem functioning in tropical forests, often more so than *N* (Domingues *et al.*, 2010; Bloomfield *et al.*, 2014). However, the role of *P* in the regulation of photosynthesis is less understood than that for *N*, but it is thought that *P* is essential for the activation and acceleration of rubisco. Additionally, lack of *P* is often reflected in reduced stomatal conductance, as it is necessary for adenosine triphosphate synthesis. Indirectly, as is the case with other nutrients, *P* can limit growth of the plant which in turn downregulates photosynthesis (Flexas *et al.*, 2012). Nevertheless, the amount and fraction of *P* involved in the primary processes of photosynthesis are variable and often very small which adds to the uncertainty over why there are strong relationships between *P* and photosynthesis.

In addition to *N* and *P*, potassium can also be an excellent predictor of photosynthesis in seasonally water stressed environments (Lloyd *et al.*, 2015) where it is an important element for drought survival (Wang *et al.*, 2013b; Anschütz *et al.*, 2014). Soil potassium can limit plant growth, particularly in sandy soils due to the high mobility of the element in soil. It is also highly mobile in plant tissues where it is required for growth, particularly in stomatal movements (Tränkner *et al.*, 2018). Further roles of *K* in plant and forest functioning are discussed in **Chapter 4** and **Chapter 6**.

Morphological adaptations of leaves are particularly important in plants that lack key nutrients. Even when nutrient deficiency is suppressing normal gas exchange rates, leaves continue to collect light which can result in permanent damage to photosynthetic machinery (Demmig-Adams & Adams, 2006). In such cases to avoid damage some species via leaf morphology (petioles, shape) are able change leaf inclination angles to avoid high intensity light when they are not able to maintain gas exchange (Niinemets & Fleck, 2002).

2.3. Venation traits

Since the 19th century, botanists have been using leaf venation for systematics in modern and fossil plants (e.g. Ettingshausen 1861; Melville 1969). More recently, studies have shown that intraspecific variability in venation patterns are affected by the environment due to their functional adaptations (Nelson & Dengler, 1997; Uhl & Mosbrugger, 1999). A seminal review by Roth-Nebelsick et al. (2001) emphasised the importance of the functions of leaf venation architecture, such as structural support, water and carbohydrate transport. Since then, venation has been considered an important plant functional trait that can affect ecosystem dynamics and functioning (Brodribb *et al.*, 2007; Niinemets *et al.*, 2007; Sack *et al.*, 2008).

Key vein traits, their definition and suggested functional role are listen in the Table 2.1 (Blonder *et al.*, 2018). Some traits are more studied than others. Vein density has received a lot of attention.

Table 2.1 Summary of venation trait definitions. Table taken from Blonder *et al.* (2018).

Venation trait name	Definition	Higher values indicate
Vein density	Length of veins per unit leaf area	More veins
Areole loopiness	Number of areoles per unit leaf area Number	More looping
Areole circularity	Median of the distribution across areoles of 4π times the area divided by squared perimeter	Areoles with less infolding
Minimum spanning tree ratio	Summed length of the minimum spanning tree connecting all vein segments divided by the summed length of all vein segments. The minimum spanning tree is defined as a subset of vein segments that maintains connectivity between all vein intersections but has less or no more total length than any other subset of vein segments. Scale-	More tree-like pathways
Freely ending veinlet ratio	Number of vein segments that are only connected to other vein segments at one end divided by number of all vein segments	More non-redundant pathways
Meshedness ratio	Number of loops in the network divided by the maximum possible number of loops given the number of vein intersections	Less tree-like pathways

High *VD* is particularly beneficial in environments of high evaporative demand as it enables higher water transport capacity and consequently more effective cooling of the leaves (Sack & Scoffoni, 2013). Additionally, leaves with greater vein reticulation are less vulnerable to drought induced leaf xylem embolism as they provide more alternative routes for flow (Scoffoni *et al.*, 2011a). In one way, higher vein density provides redundant pathways for water transport when other major veins are blocked by drought-induced xylem embolism (Sack *et al.*, 2008; Scoffoni *et al.*, 2011a). In addition to water and photosynthate transport, vein networks play an important role in leaf structural support and defense against herbivory (Sack *et al.*, 2008; Blonder *et al.*, 2011; Price & Weitz, 2014), which are not further discussed in the thesis.

2.4. Relationships between water transport and carbon acquisition

Water transport efficiency within leaves is considered to be a major bottleneck to plant carbon acquisition (Sack *et al.*, 2003), with theoretical work incorporating venation traits into leaf economic spectrum (LES (Wright *et al.*, 2004)) showing links between the two (Fig. 2.4; Blonder *et al.*, 2011, 2014; Sack *et al.*, 2013). Higher *VD* increases photosynthetic rates (Sack *et al.*, 2013) by increasing leaf hydraulic conductance via reduction in water flow distances within the leaf, greater water permeability from xylem to cells, and greater evaporative surface (Sack & Holbrook, 2006). The link between leaf hydraulic capacity and photosynthetic rates is evident in many environments and among numerous clades (Scoffoni *et al.*, 2016b).

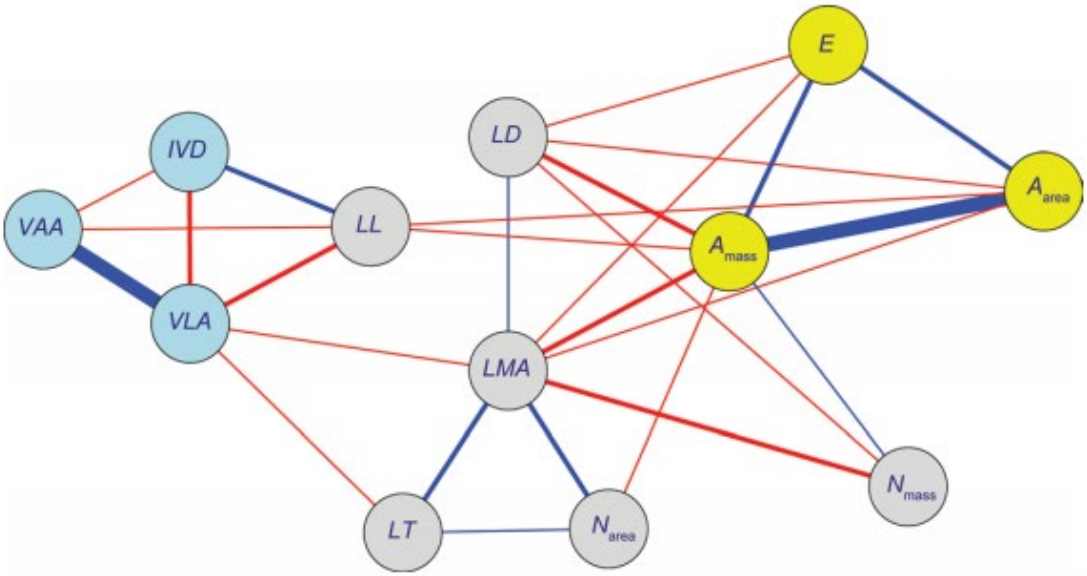


Fig. 2.4 Diagram illustrating links between venation traits and leaf economic spectrum. The figure is based on study by Blonder *et al.* (2011) and taken from Sack *et al.* (2013). Abbreviations: *VAA* - vein areole loopiness, *VLA*– vein density, *IVD*- interveinal distance, *LL*- leaf lifespan, *LMA*– leaf mass per area, *LT*– leaf thickness, N_{area}– area-based leaf nitrogen concentration, N_{mass}- mass-based leaf nitrogen concentration, A_{mass}- mass-based net photosynthesis rate, A_{area}- area-based net photosynthesis rate and *E*- leaf transpiration rate.

Despite these links, an increasing number of studies are providing evidence for independent variation of leaf hydraulic and economic traits among and within species (Li *et al.*, 2015;

Marechaux *et al.*, 2015; Blackman *et al.*, 2016). Li *et al.* (2015) shows photosynthetic and water transport related processes to be decoupled because they take place in two different parts of the leaf mesophyll; upper palisade and lower spongy mesophyll. This is supported by differences in the evolutionary history of these traits (Beerling, 2005). Such proposed independent variation of photosynthetic and hydraulic traits may allow for better adaptation to environmental conditions, which is particularly important in sites with strong environmental pressures, such as strong seasonality, poor soil fertility or limited light conditions (Boer *et al.*, 2016). More empirical studies are required to better untangle the links between economic and hydraulic traits which is addressed in **Chapter 6**.

2.5. Multidimensional trait space

Traits have been used to explain functioning of individual species in many studies, however, predicting power of community performance and assembly from these relationships is limited. This is partly due to the majority of studies linking individual link traits with plant function and/or single environmental variables. Quantifying multidimensional functional trait space (Hutchinson, 1957) can tie together ecological and evolutionary links between traits, environment and community functioning (Lamanna *et al.*, 2014; Li *et al.*, 2018). Trait space can be defined as fitting a combination of functional traits for specific biotic and abiotic conditions that lead to trait convergence (Díaz *et al.*, 2015).

The creation of trait space is often facilitated by habitat filtering, as over time, it can exclude species with unsuitable trait combinations for a specific environment. Generally, it segregates trait spaces along the axes of plant functional strategies. For example, Lebrija-Trejos *et al.* (2010) previously found that functional traits of species growing along the subtropical-dry forest transition in Mexico were filtered along two main axes of variability; the first dimension indicated trait space that deals with heat load reduction and water status control, while the second dimension indicated traits that are important for drought-coping strategies.

These axes often characterize species functional strategies, with one of the most important being growth-longevity trade-off axis, where leaves with shorter life-spans have lower photosynthetic rates but higher LMA than leaves with shorter life-spans (Reich, 2014). This trade-off axis is often studied using growth rate and wood density (Fyllas *et al.*, 2017b). Fast growing species tend to have lower wood densities and greater mortality rates than slow growing species (Martínez-Vilalta *et al.*, 2010; Adler *et al.*, 2014; Fyllas *et al.*, 2017b) due to reduced structural strength and pest resistance of a tree (Chave *et al.*, 2009). Few studies have investigated the relationship between plant functional traits and growth/survival strategies, often with inconclusive or opposing results (Santiago & Wright, 2007; Wright *et al.*, 2010a; Paine *et al.*, 2015). The discrepancy between theoretical expectation, empirical results and interpretation can be attributed to a lack of functional trait and environmental data, poor choice in trait selection and low power of statistical analyses. Finally, the growth-longevity trade-off often enables species coexistence due to niche specialization (Kitajima & Poorter, 2008).

2.6. Phylogenetic control of functional trait dynamics

Increasing development in computational tools and knowledge of species evolutionary history, has resulted in an increasing research effort to incorporate the evolutionary history of species in explaining community assembly, species distributions, plant and ecosystem function (e.g. Blomberg *et al.*, 2003; Chave *et al.*, 2006; Cavender-Bares *et al.*, 2009). This is useful because in addition to environmental factors, the evolutionary history of species can also have an effect on trait-trait relationships (Reich, 2014). Species that are closely related tend to have more similar suites of traits than would occur randomly (Fig. 2.5; Cavender-Bares *et al.* (2009). A study along the Andes-Amazonia elevation gradient found that more than 80% of turnover in LMA and leaf nitrogen along the transect was determined phylogenetically (Asner *et al.*, 2014). Conversely, when considering trait variability, phylogenetic distance does not necessarily have a significant effect on functional trait differences among species (Losos, 2008; Gravel *et al.*, 2012). This can

be attributed to convergence in trait values among evolutionary distant taxa as a result of strong environmental filtering (Cavender-Bares *et al.*, 2004)

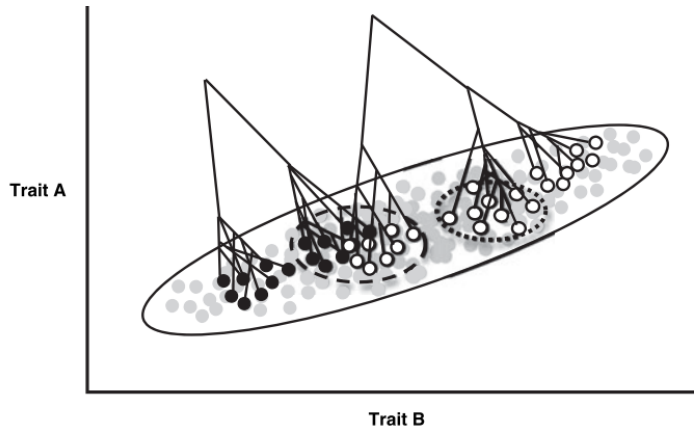


Fig. 2.5 Hypothesised relationship between trait A and B in the context of phylogenetic history (from different species from different clades). Arrangement of trait relationships in trait space (solid oval line) can be random within a clade (non-circled black dots), limited to the specific clade (dotted circle) or labile (dashed circle), latter often resulting from selective pressures, such as environmental filtering. Figure taken from Cavender-Bares *et al.* (2009).

Understanding which functional traits are phylogenetically conserved or labile can provide more power to predictive models investigating the responses of forest ecosystems to global change. This is because information about species phylogenetic history can sometimes be more informative in predicting community assembly and functioning than species diversity metrics (Cadotte *et al.*, 2008)

2.7. Linking functional traits and ecosystem function

Net primary productivity (NPP), the rate of biomass (wood, leaf and root tissues) formation, is one of the key parameters that can describe ecosystem functioning (Malhi *et al.*, 2011). NPP is not only indicative of the amount of organic material available locally to higher trophic levels, it has significant global influences on the balance between terrestrially and atmospherically stored

carbon. Specifically, it determines the magnitude and turnover of the carbon and nutrient cycles of ecosystems and potential response times to disturbance (Malhi *et al.*, 2011). For example, Espírito-Santo *et al.* (2013) found that in the Amazon alone, natural disturbances release an estimated 1.3 Pg C a^{-1}, which is more than compensated for by CO_2 sequestration through tree growth. Hence, CO_2 exchanges between the tropical forest biome and the atmosphere clearly influence atmospheric CO_2.

Tropical forest ecosystem productivity, defined by GPP and NPP, can be controlled by biotic and abiotic factors (Fig. 2.6; Fyllas *et al.* (2017a). Abiotic, or environmental conditions, such as temperature, rainfall, solar radiation and soil fertility (*N* and *P* availability) have been shown to affect tree growth and, therefore, forest productivity (e.g. Finegan *et al.*, 2015b; Taylor *et al.*, 2017). Additionally, functional traits and species ecological strategies can also have an effect on the ecosystem productivity (Xia *et al.*, 2015; Fyllas *et al.*, 2017a). For example, Bakker *et al.* (2011) has demonstrated that leaf morphological traits associated with LES can predict the litter decomposition rates and hence nutrient and carbon cycling in lowland tropical forests in Bolivia.

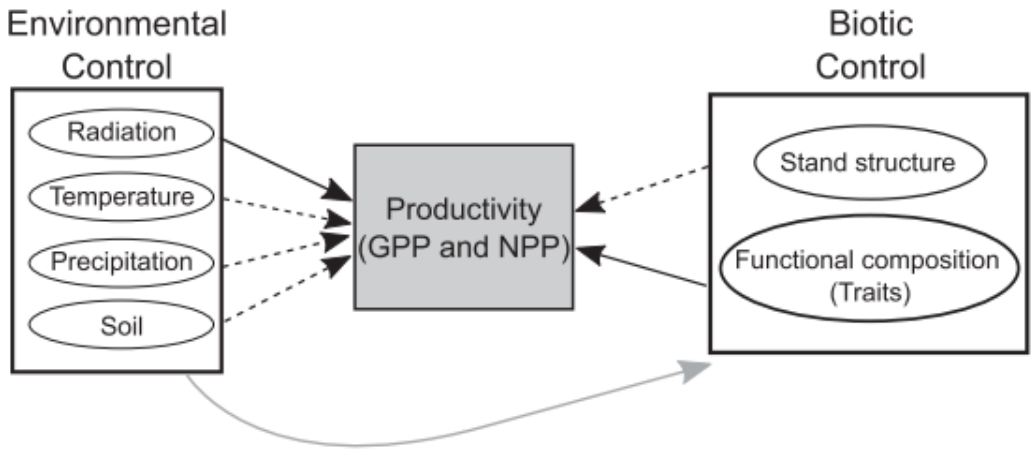


Fig. 2.6 Environmental and biotic factors that directly and indirectly affect ecosystem productivity, specifically gross primary productivity (GPP) and net primary productivity (NPP). Figure taken from Fyllas *et al.* (2017a).

Despite the importance of NPP it remains one of the least quantified components of the global carbon cycle due to the laborious nature of the data collection. Additionally, this data is particularly scarce in Africa, the second largest tropical region after Amazonia. A recent study by Moore *et al.* (2017) was the first to fully quantify all major components of NPP, including aboveground and belowground components, in West Africa. This study discussed the abiotic drivers of NPP dynamics, however, no links between functional traits and carbon cycling were investigated. In other regions, only a few studies have explored relationships between functional trait space and ecosystem productivity (e.g. van der Sande *et al.*, 2015). Further investigation of relationship between ecosystem productivity and leaf functional traits is presented in **Chapter 7**.

Chapter 3

Methodology

3.1. GEM Network

The data collection for the thesis was facilitated by Global Ecosystems Monitoring Network (GEM; http://gem.tropicalforests.ox.ac.uk/). This network of study sites was first established in order to monitor all key ecosystem carbon stocks and fluxes in tropical forests. This project is continuously expanding across all tropical regions (Fig. 3.1) with additional sites being added every year.



Fig. 3.1 Distribution of GEM plots across around the world.

Following the establishment of GEM long term carbon monitoring plots, GEM-Traits projects began to take place in these sites worldwide to measure functional diversity of these sites. In 2013,

the first GEM-Traits campaign ("CHAMBASA") took place ten 1-ha size plots situated along the Andes elevation gradient in the departments of Cusco and Madre de Dios in South East of Peru. In 2014, this campaign was replicated at a smaller scale in a forest-savanna ecotone in Mato Grosso, Brazil ("BACABA") in 2014. A few months later, a large GEM-Traits campaign began in Ghana, West Africa ("Kwaeema") encompassing seven 1-ha plots situated along a strong environmental gradient. Part of the data collected in the two campaigns in Ghana and Brazil was used for the data chapters of the thesis.

GEM-Traits projects aim to collect data for leaf and wood functional traits using the same methodologies for approximately 25000 individual trees and 2000 species. The traits collected so far include leaf and wood anatomy, chemical properties, gas exchange, crown dimensions, branch architecture. These trait are contributing towards the development of the individual-based forest models (Fyllas *et al.*, 2014).

3.2. Study sites

The data collection for the thesis was conducted in eleven 1 ha plots in Ghana (seven plots) and Brazil (four plots). Figure 3.2. summarises which plots and associated vegetation types were used as study sites for each of the data chapters. Chapter 5, 6 and 7 included all seven plots from Ghana, while Chapter 4 was based on data collected in the Brazil plots and dry semideciduous plots from Ghana (KOG01, KOG03 and KOG04). The main reason for this distinction was the focus of Chapter 4 – forest-savanna ecotone, a unique mosaic of tall seasonally dry woodland and savanna patches that fall under the same climatic regime. Forest-savanna ecotone in Chapter 4 should not be confused with the wider vegetation transition considered in Chapter 5, 6 and 7.

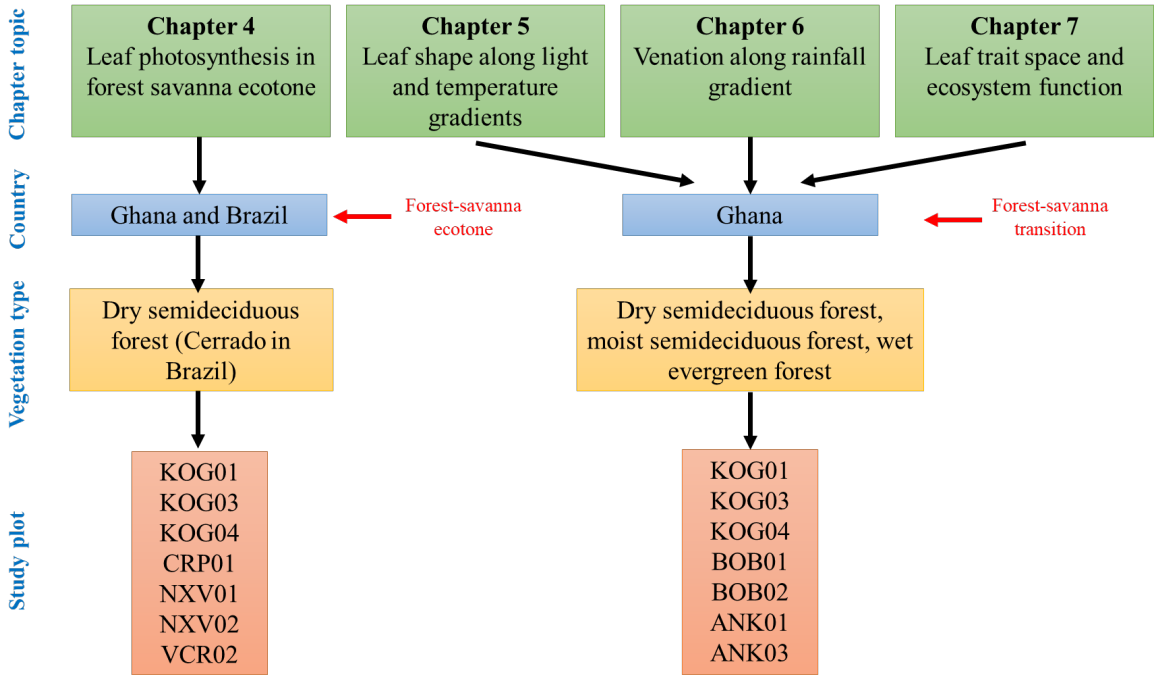


Fig. 3.2 Scheme summarising study sites used in each data chapter.

3.2.1. Ghana

Data were collected in seven 1 ha study plots situated along a precipitation gradient in Ghana (1,030 mm -2,200 mm a^{-1}). The plots are situated along a forest-savanna vegetation gradient of three tropical forest types, each associated with distinct environmental conditions (Fig. 3.1). Two are located in wet evergreen forest (MAP of 2,200 mm a^{-1} and MNT of 30.7° C), two in moist semideciduous forest (MAP of 1,500 mm a^{-1} and MNT of 34.0° C) and three in dry semideciduous forest (MAP of 1,030 mm a^{-1} and MNT of 36.0° C), . The latter tropical vegetation type, dry semideciduous forest, also represents a strong vegetation transition- forest-savanna ecotone. Within the different forest zones, plots are situated in close proximity to one another, usually, between a few hundred metres to a few kilometres apart, however, they differ in their vegetation composition.

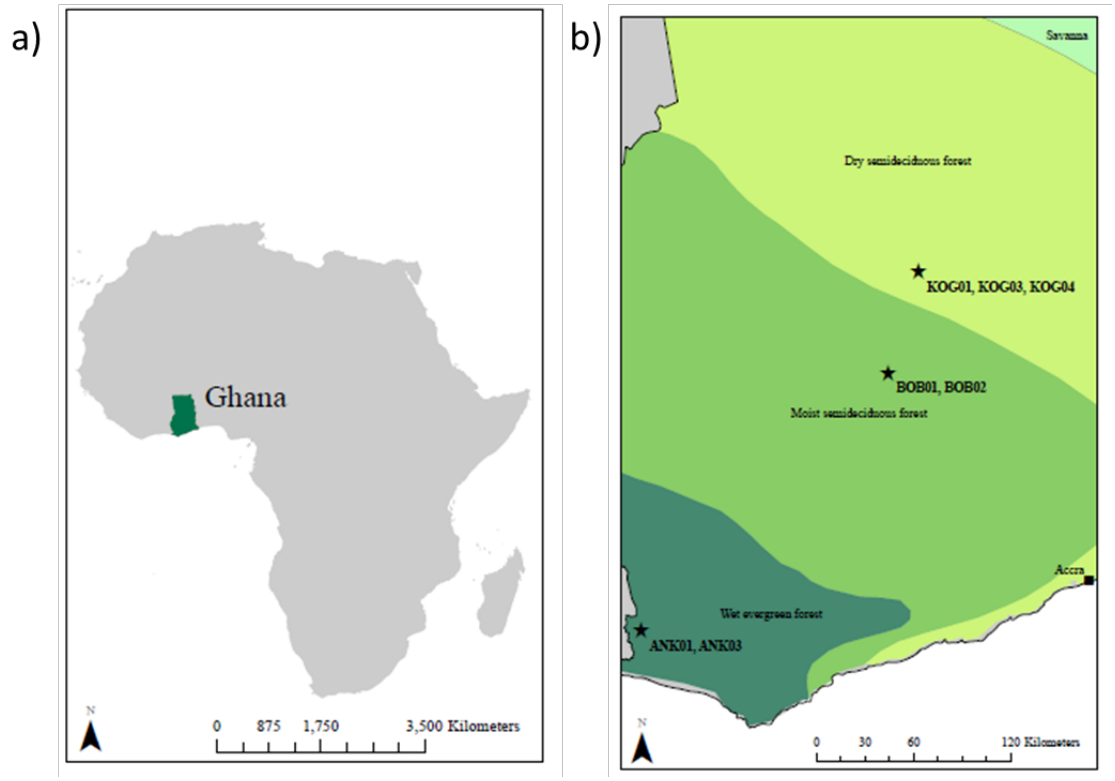


Figure 3.2 Map of the location of a) Ghana within the African continent b) and the study plots and forest types in Ghana (Hall & Swaine, 1981). The colours represent different vegetation types.

Plots within the driest and hottest site (KOG01, KOG03, KOG04) are situated within the Kogyae Strict Nature Reserve along a dry forest-savanna ecotone meaning that the plots are of distinct composition even though under the same climatic conditions (Fig. 3.3). The savanna woodland (KOG04) has the lowest species diversity, low basal area (8.79 m^2 ha^{-1}) but similar stem density when compared to other plots in the ecotone (Table 5.1). This results in a relatively open canopy which allows enough light to reach the ground to support a largely continuous herbaceous layer consisting primarily of C4 grasses. Extensive flammable grass coverage and hunting and farming practices mean that this plot experiences yearly fires. and The fires in the nearby situated transitional woodland (KOG03) and dry forest (KOG01) plots are also recorded but usually they are less intense and frequent. This is likely due to reduced flammable grass cover, greater basal area and higher species diversity and cooler and moister air in the understorey. Some species are shared amongst the three plots, however, the savanna woodland plot is dominated by deciduous pioneers while other plots have a mix of light demanding and some evergreen species. Here, the

climate is classified as tropical savanna (Aw) according to the Köppen classification. The sites exhibit strongly seasonal distribution of rainfall with two wet seasons and two dry seasons per year. The main wet season lasts from April through June with a secondary wet season from September to November. .

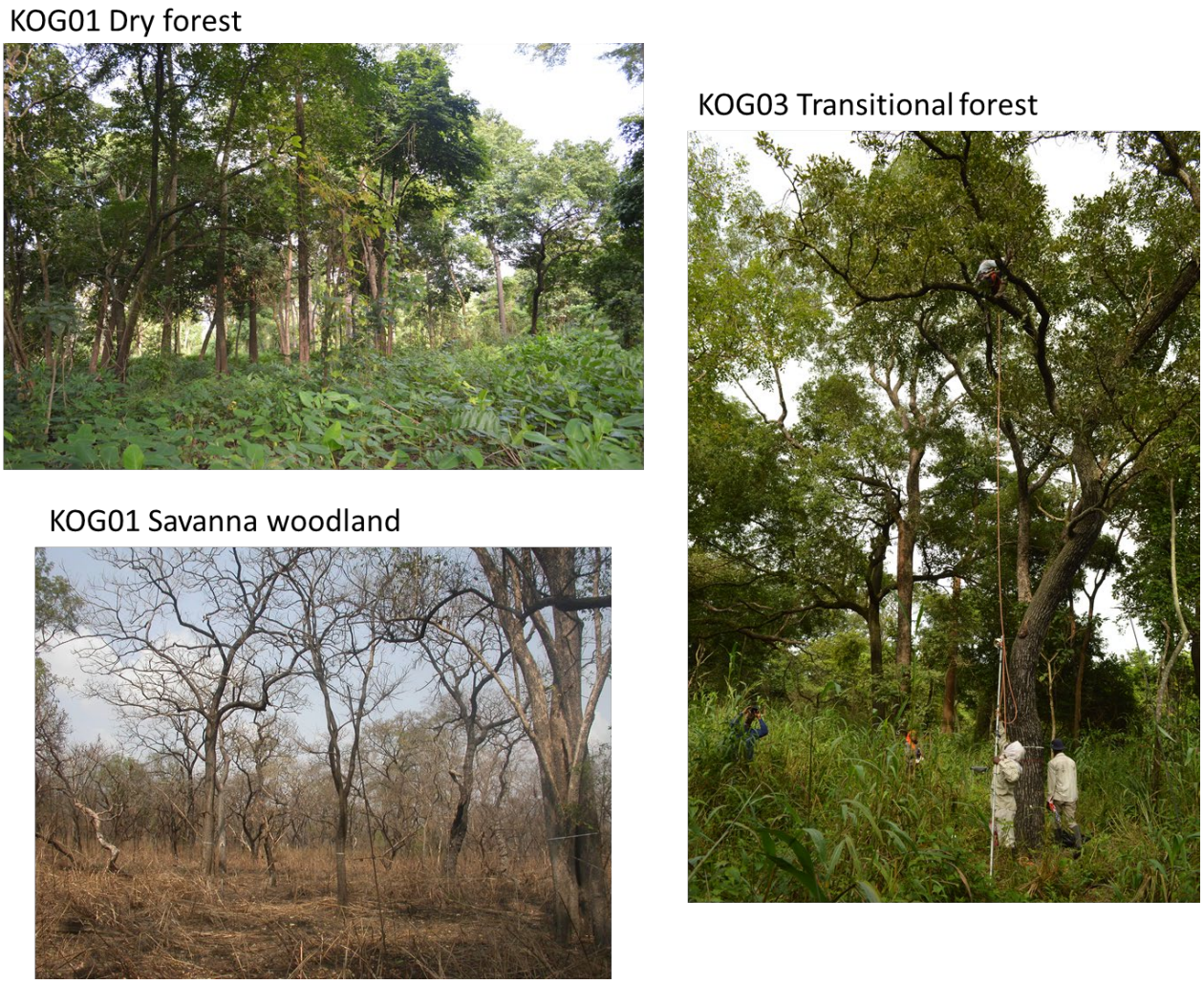


Fig. 3.3 Pictures of study plots in dry semideciduous forest (forest-savanna ecotone) in Ghana.

The moist semideciduous forest plots (BOB01 and BOB02) are situated the Bobiri Forest Reserve (BFR) close to the second largest city in Ghana, Kumasi (Fig. 3.4). One of the plots in the moist semideciduous zone (BOB01) exhibits more gaps in the canopy than the other study site, potentially due to disturbance as it is situated on the edge of the forest reserve. The disturbance is also indicated by a large amount of lianas covering tree trunks and canopies.

BOB01 Moist semideciduous forest (with edge effect)





BOB02 Moist semideciduous forest

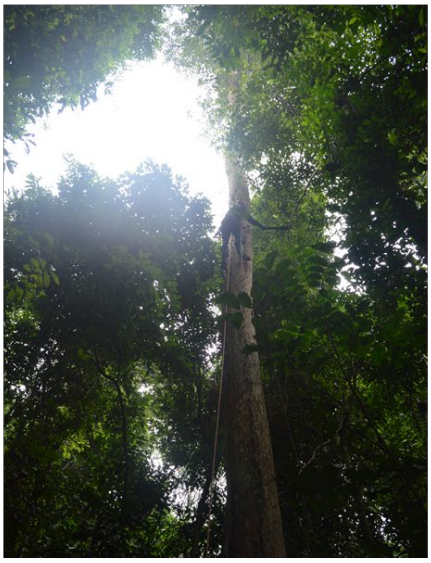




Fig. 3.4 Pictures of study plots in moist semideciduous forest in Ghana.

The two wet evergreen forest plots (ANK01 and ANK03) are situated in Ankasa Conservation Area (ACA) in the extreme southwest of Ghana, close to the Cote d'Ivoire border (Fig. 3.5). One of the plots in the wet evergreen forest zone is classified as a swamp due to seasonal flooding (ANK03), whereas the other plot is an upland forest on a ridge top (ANK01).

ANK01 Upland evergreen forest



ANK03 Seasonal swamp forest



Fig. 3.5 Pictures of study plots in wet evergreen forest in Ghana. Orange arrow indicates a slope and blue arrow indicates a small stream.

3.2.2. Brazil

The forest-savanna gradient plots in Brazil consisted of four 1 ha plots in or near the Bacaba Municipality Reserve, Nova Xavantina, (14°41'S; 52°20'W). The climate of the area is Aw (tropical savanna) with a MAP of 1635 mm a^{-1} and mean annual temperature of 24.9 °C (Farias *et al.*, 2016). The region exhibits strong seasonality, with the rainy season between October and

March and a very strong dry season between May and September. The soils of the study area are ferralsols, plinthosols and leptosols.

The predominant vegetation in the area is *cerrado sensu stricto,* which is described as continuous grassy layer intercepted by trees with up to 60% canopy cover (Ribeiro & Walter, 1998). The study plots were situated in patches of gallery and transitional (*cerradão*) forests and short savanna vegetation on shallow, rocky soils (*cerrado rupestre*). The study plots were classified into three different vegetation types within the ecotone: forest (VCR02), transition (NXV02) and savanna (CRP01 and NXV01) plots (Fig. 3.6).



Fig. 3.6 Photographs of the four study plots in the forest-savanna ecotone in Brazil.

The non-forest plots were situated within 4 km of each other and the forest plot was 40 km from the others. One of the savanna plots (NXV01) and transition plot (NXV02) were situated very closely to each other but exhibit significantly different vegetation structures (Fig. 3.7)



Fig. 3.7 Savanna and transition plots situated in very close proximity of each other.

3.3. Fieldwork campaigns

Within each study plot we aimed to sample the minimum number of species that contributed to 80% of basal area. We sampled the most common species because rare species are likely transients that are performing poorly at population level and are sink populations from metacommunity propagule pressure (Orrock & Watling, 2010). The species were chosen based on the data from the most recent census (2013). Within each sampled species in each plot, three largest trees were chosen for sampling. We sampled more than one tree per species as some traits are known to vary more within species than between species. Due to high species diversity within each study site and low stem density in dry semideciduous forest, three was an optimal number of trees to sample for each species to allow for sampling consistency.

The data collected during the field campaigns covered many aspects of plant function (Fig. 3.8), specifically, the depth and size of crowns, light availability throughout the canopy, bark and wood properties, wood and leaf hydraulics, leaf chemistry and gas exchange. Two large teams in both

countries (~25 people in each team) worked tirelessly for months (two in Brazil and almost seven months in Ghana) to collect such a large amount of data.



Fig. 3.8 Trait dimensions sampled in Brazil and Ghana campaigns by different teams.

With an assistance of tree climber, we sampled one fully sunlit canopy branch (positioned on the most outer edge of the central part of the canopy) and a fully shaded branch (that is shaded throughout the day), each at least 1 cm diameter, from each tree. From each branch, we measured three leaves for trait measurements (Fig. 3.9). In addition, we collected two extra branches for hydraulics. Leaf gas exchange traits were measured using an open gas exchange system LI-6400XT (Li-Cor Inc., Lincoln, NE, USA) in the field. For detailed description of procedures for gas exchange measurements please refer to **Chapter 4**.

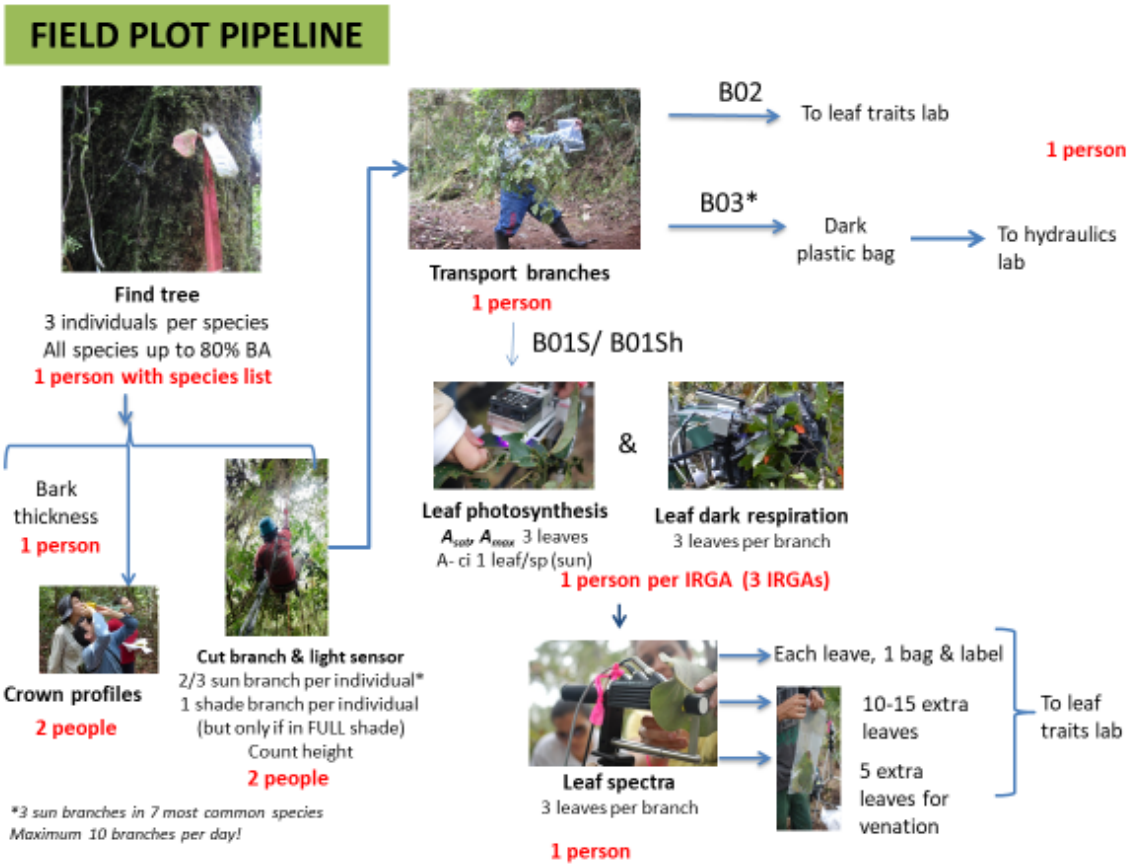


Fig. 3.9 Data collection in the field. B01, B02 and B03 indicate different cut branches.

Leaves were collected immediately following the gas exchange measurements, placed in moist sealed bags in a cool shaded environment until being transported to the lab in a few hours. There leaves were scanned with a digital flatbed scanner (Canon LiDE 110) and weighed (Fig. 3.10). Thickness was measured using a micrometer (Tresna, 211- 101F). Leaves were then saturated with water overnight, weighed again and then placed in an oven at 70°C and dried until the mass reached constant weight. Extra plant material was collected for further venation analysis. Detailed descriptions of methodology for nutrient analysis, leaf shape and venation are described in detail in **Chapter 4**, **Chapter 5** and **Chapter 6**, respectively.

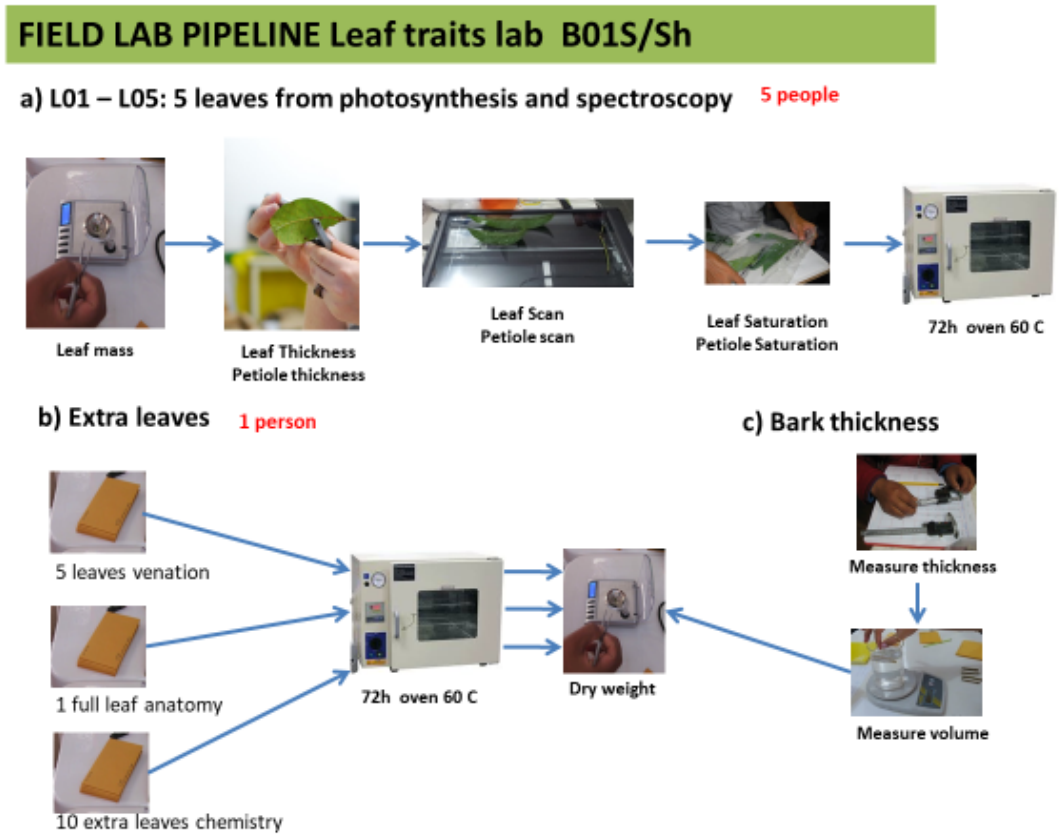


Fig. 3.10 Data collection in the field laboratories.

Chapter 4

The first data chapter addresses **Question 1** of the thesis: *Are photosynthetic capacity, key leaf and soil nutrients linked along forest-savanna boundaries?* The data used to answer this question comes from 89 woody angiosperm species growing in seven study plots situated in the Ghana (three plots) and Brazil (four plots) in the forest-savanna ecotones. Specifically, I used gas exchange values to derive photosynthetic capacity (V_{cmax}) and linked it with soil and leaf nitrogen, phosphorus and potassium. To aid the interpretation of the results, I used information about species ecological strategies, such as leaf deciduousness, light requirements for germination and establishment, ability to fix nitrogen, and evolutionary history. The findings of the study are presented in a paper entitled "Leaf-level photosynthetic capacity dynamics in relation to soil and foliar nutrients along forest-savanna boundaries in Ghana and Brazil" which has been accepted for publication in *Tree Physiology* on 12th September 2018.

I was the main author and wrote the paper, conducted the fieldwork in both countries and carried out all of the data analysis with guidance from my supervisors Imma Oliveras and Yadvinder Malhi. The field campaigns were planned by Yadvinder Malhi and Imma Oliveras and coordinated by Imma Oliveras and collaborators in Ghana and Brazil: Theresa Peprah, Stephen Adu-Bredu, Beatriz Schwantes Marimon and Ben Hur Marimon Junior. In addition to data collection assistants (Mickey Boakye, Lydia Afriyie, Karine Peixoto da Silva, Josenilton de Farias, Edmar Almeida de Oliveira, Cassia Cristina Almeida Farias, Nayane Cristina Candida dos Santos Prestes and Margot Neyret), Tomas Ferreira Domingues taught me the processing of gas exchange data and Sam Moore provided soil chemistry data as well as set up and coordination of the plots in Ghana.

Chapter 4:

Leaf-level photosynthetic capacity dynamics in relation to soil and foliar nutrients along forest-savanna boundaries in Ghana and Brazil

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4.1. Abstract

Forest-savanna boundaries extend across large parts of the tropics but the variability of photosynthetic capacity in relation to soil and foliar nutrients across these transition zones is poorly understood. For this reason, we compared photosynthetic capacity (maximum rate of carboxylation of Rubisco at 25 C° (V_{cmax}^{25}), leaf mass, nitrogen (N), phosphorus (P) and potassium (K) per unit leaf area (LMA, N_{area}, P_{area} and K_{area}, respectively), in relation to respective soil nutrients from 89 species at seven sites along forest-savanna ecotones in Ghana and Brazil. Contrary to our expectations, edaphic conditions were not reflected in foliar nutrient concentrations but LMA was slightly higher in lower fertility soils. Overall, each vegetation type within the ecotones demonstrated idiosyncratic and generally weak relationships between V_{cmax}^{25} and N_{area}, P_{area} and K_{area}. Species varied significantly in their $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship due to reduced investment of total N_{area} in photosynthetic machinery with increasing LMA. We suggest that studied species in the forest-savanna ecotones do not maximise V_{cmax}^{25} per given total N_{area} due to adaptation to intermittent water availability. Our findings have implications for global modelling of V_{cmax}^{25} and forest-savanna ecotone productivity.

Keywords: photosynthesis; carboxylation capacity; tropical vegetation transitions; leaf traits; nitrogen.

4.2. Introduction

Forest-savanna boundaries represent the ecotone between tropical forest and savanna biomes, and extend across large parts of South America, Africa, Australia and Asia (Torello-Raventos *et al.*, 2013; Veenendaal *et al.*, 2014; Lloyd *et al.*, 2015). These ecotones are particularly sensitive to ongoing environmental change as they are border regions between contrasting biomes, reflecting both local and regional variation in abiotic conditions (Oliveras & Malhi, 2016). On one hand, increasing CO_2 may favour the expansion of forests into the savanna vegetation (Bond & Midgley, 2012), and fire exclusion policies may reinforce this advance (Staver *et al.*, 2011b). On the other hand, an intensification of the duration and intensity of dry season and of general climate variability, and an increase in maximum temperatures may favour the expansion of drought-tolerant savanna vegetation into the forest (Hely *et al.*, 2006). Changes in floristic composition can in turn have important implications on the biodiversity, ecosystem functioning and carbon balance of these ecotones.

To date, forest-savanna boundaries have received little attention from a functional traits perspective, and comprehensive characterization of leaf economic, chemical and structural trait distributions along the transitions is still lacking. Trait-based analyses are critical in explaining plant strategies, ecosystem functioning and species assembly mechanisms (Kraft *et al.*, 2008). Functional traits associated with leaf economic spectrum (leaf chemistry, structure and photosynthesis) (Wright *et al.*, 2004) have proved to be valuable in modelling nutrient fluxes, changes in ecosystem productivity and shifts in vegetation boundaries with land-use and climate change (Yang *et al.*, 2016; Fyllas *et al.*, 2017b,a). Therefore, understanding the diversity and variability of functional traits along the forest-savanna ecotones could provide vital insights into mechanism governing the ecology of these transitions.

Photosynthetic capacity, expressed as maximum rate of carboxylation per area (V_{cmax}), is one of the key functional traits that determine individual plant fitness (Smith *et al.*, 2004). This parameter has been successfully modelled at the biochemical level (Farquhar *et al.*, 1980) and is widely used

to simulate photosynthesis in terrestrial biosphere models (Kattge *et al.*, 2009). Photosynthetic capacity is widely regarded to be limited by leaf nutrient content in the tropics, particularly by *N* (Bahar *et al.*, 2016; Norby *et al.*, 2017). The $V_{cmax} \leftrightarrow N$ relationship is considered to be linear and invariant in most terrestrial biosphere models (Luo *et al.*, 2004; Prentice *et al.*, 2015). However, an increasing number of studies in tropical regions are observing either a variable $V_{cmax} \leftrightarrow N$ relationship (Domingues *et al.*, 2015) or do not find any relationship (Coste *et al.*, 2005; Dusenge *et al.*, 2015). Lloyd et al. (2013) have suggested that, because of many roles that foliar *N* can play (*e.g.* anti-herbivory and apoplastic immune defence, cell wall proteins (Burns *et al.*, 2002; Wang & Dong, 2011; John *et al.*, 2017)), it is possible that $V_{cmax} \leftrightarrow N$ relationships appear to be insignificant or weak, especially when numerous species across biomes are considered. In addition to leaf *N*, foliar *P* and/or *K* can have a significant effect on photosynthesis along forest-savanna ecotones (Bloomfield *et al.*, 2014; Domingues *et al.*, 2015; Lloyd *et al.*, 2015) Potassium is thought to be a particularly important element in these drought prone environments as it is essential for plant osmotic processes and plays a vital role in long-distance water transport (Wang *et al.*, 2013b; Anschütz *et al.*, 2014)

In addition to direct leaf-level nutrient limitation, V_{cmax} may be indirectly influenced by some aspects of soil fertility (Ali *et al.*, 2015). Correlation between soil nutrients relevant for photosynthesis and corresponding foliar nutrient concentrations have been observed across Amazonia (Fyllas *et al.*, 2009; Quesada & Lloyd, 2016). However, Bloomfield et al. (2014) observed a lower slope between leaf and soil phosphorus ([P]) along forest-savanna transition in Australia than in the Amazon. This finding was speculated to be because of the inability of studied Australian forests to utilise *P* efficiently, as evolutionary they are adapted to *P*-limited soils. Indeed, exploring the link between soil fertility and canopy nutrient stocks can provide us with additional insights into floristic adaptations to abiotic factors.

Many forest-savanna ecotones are situated on old, nutrient-depleted soils, where soil nitrogen ([N]), potassium ([K]) and/or [P] can limit functioning of the ecosystem (Haridasan, 2008; Bond,

2010). Hence, exploring relationships between soil and leaf nutrient concentrations is particularly important when studying photosynthesis dynamics. These links have been addressed along the forest-savanna ecotones in the Amazon (Lloyd *et al.*, 2015), however, the findings were difficult to interpret as sites in this study were situated along extensive rainfall as well as fertility gradients. To our knowledge, only one study by Domingues et al. (2015) investigated leaf gas exchange dynamics within forest-savanna ecotone in West Africa without the complexity of rainfall variation.

Species' ecological strategies can provide additional insights into variability of photosynthesis (Niinemets *et al.*, 1998; Eamus, 1999). For example, greater constructions costs of evergreen leaves (Eamus, 1999) enable them to maintain photosynthesis even during dry season, while deciduous species maximise carbon gain during the wet season (Tomlinson *et al.*, 2013). Nevertheless, this generally accepted variability of photosynthetic capacity among the species of different leaf habit, was shown not to be the case in Brazilian Cerrado, where deciduous and evergreen species did not differ in leaf trait values (Cianciaruso et al. 2013). Authors suggested that species of forest-savanna ecotones employ different ecological strategies not to maximise carbon gain, but to cope with strongly pronounced environmental pressures. In addition to deciduousness, potential ability of certain Fabaceae species to fix *N* (Batterman *et al.*, 2013) can also be advantageous in sites with strong seasonality; during drought additional *N* can enable greater water use efficiency (Adams *et al.*, 2016). It is particularly important to investigate the effects of these ecological strategies on gas exchange in forest-savanna ecotones as these zones are often dominated by deciduous legumes (Lock, 1989).

In this study, we addressed the above-discussed research gaps using a novel and large dataset comprising of V_{cmax} values and key nutrient concentrations for 89 tree species distributed along two forest-savanna ecotones, one in the Palaeotropics (Ghana) and another in the Neotropics (Brazil). We asked the following questions: are there significant differences in V_{cmax} along the

forest-savanna transitions? Are these differences linked with soil and, consequently, foliar nutrient concentrations? What role do taxonomy and ecological strategies play in regulating V_{cmax}?

Specifically, we hypothesised that:

H1: edaphic conditions vary in a consistent direction along the forest-savanna transitions. Specifically, savanna soils exhibit lower [N], [P] and [K] values than nearby forests (Lloyd *et al.*, 2015).

H2: differences in soil fertility along the transitions are reflected in foliar nutrient concentrations and LMA (Lloyd *et al.*, 2015).

H3: foliar nutrient concentrations have an effect on V_{cmax}. Specifically, *P* has a significant positive effect on V_{cmax}, especially in savanna plots (Domingues *et al.*, 2015); *K* shows a negative relationship with V_{cmax}, especially in savanna plots (Lloyd *et al.*, 2015); and leaf *N* is positively linked with V_{cmax}. in all study plots types.

H4: differences in V_{cmax} are reflected in plant ecological strategies. Specifically, 4a) evergreen species have lower V_{cmax} than deciduous species (Eamus, 1999); 4b) pioneer species have higher V_{cmax} than shade-bearers (Silvestrini *et al.*, 2007); and 4c) N_2-fixing species have higher foliar *N* (Adams *et al.*, 2016) and, consequently, higher V_{cmax}.

4.3. Materials and Methods

Study sites

The forest-savanna gradient in the Palaeotropics consisted of three 1 ha plots situated in close proximity (< 10 km separation) in Kogyae Strict Wildlife Reserve (7º15' N, 1º04' W), central Ghana. The vegetation in the area is classified as dry semideciduous forest (Hall & Swaine, 1981).

The climate is classified as equatorial savanna with dry winter (Aw) (Kottek *et al.*, 2006) with a mean annual precipitation (MAP) of 1030 mm a^{-1} and mean annual temperature of 26.4 °C and maximum midday temperature of 36 °C. The main wet season lasts from April through June with a secondary wet season from September to November. Soils in the plots are classified as haplic nitisols and arenosols (Table 4.1).

The forest-savanna gradient in the Neotropics consisted of four 1 ha plots in or near the Bacaba Municipality Reserve, Nova Xavantina, (14°41'S; 52°20'W), Brazil. The predominant vegetation in the area is *cerrado sensu stricto,* which is described as continuous grassy layer intercepted by trees with up to 60% canopy cover (Ribeiro & Walter, 1998). The study plots were situated in patches of gallery and transitional (*cerradão*) forests and short savanna vegetation on shallow, rocky soils (*cerrado rupestre*). The non-forest plots (Table 4.1) were situated within 4 km of each other and the forest plot was 40 km from the others. The climate of the area is Aw with a MAP of 1635 mm a^{-1} and mean annual temperature of 24.9 °C (Farias *et al.*, 2016). The region exhibits strong seasonality, with the rainy season between October and March and a very strong dry season between May and September. The soils of the study area are ferralsols, plinthosols and leptosols (Table 4.1).

Table 4.1 Key study site characteristics. Abbreviations: mean annual precipitation (MAP); basal area of trees (BA; >10 cm DBH); total soil phosphorus ([P]); nitrogen ([N]); carbon ([C]); calcium ([Ca]); potassium ([K]); magnesium ([Mg]). Soil nutrient concentrations are for the soil depth of 0-0.3m except for CRP01 plot where data was only available for 0-0.2m (*). Soil chemistry data were provided by ForestPlots database Lopez-Gonzalez et al. (2011) and Moore et al. (2017).

Region	External plot code	Study vegetation classification	Forest type	MAP (mm)	BA (m^2 ha^{-1})	Soil type	[P] (mg kg^{-1})	[N] (%)	[C] (%)	[Ca] (mg kg^{-1})	[K] (mg kg^{-1})	[Mg] (mg kg^{-1})	CEC ($mmol^+$ kg^{-1})	Sand (%)	Clay (%)	Silt (%)
Ghana																
	KOG01	Forest	Dry forest	1030	17.5	Haplic Nitisol	67.2	0.05	0.71	378.8	42.5	75.5	26.3	82.4	2.3	15.3
	KOG03	Transition	Transitional forest	1030	13.5	Haplic Arenosol	74.6	0.05	0.67	307.9	35.6	78.6	22.8	79.7	3.3	17.0
	KOG04	Savanna	Woody savanna	1030	12.4	Haplic Arenosol	81.9	0.04	0.62	237.1	28.6	81.8	19.4	76.9	4.3	18.7
Brazil																
	VCR02	Forest	Tall forest	1635	12.8	Plinthosol	160.5	0.09	1.15	96.3	40.6	30.9	11.8	66.4	23.6	10.0
	NXV02	Transition	Tall closed woodland	1635	16.1	Ferralsols	129.5	0.08	1.19	50	46.5	27.5	18.1	75.3	17.8	6.9
	NXV01	Savanna 1	Savanna woodland	1635	7.5	Ferralsols	107.5	0.05	0.78	19.5	39.9	7.6	12.0	85.5	11.1	3.4
	CRP01	Savanna 2	Savanna woodland	1635	14	Lithic Leptosol	171.4*	0.12*	2.7*	303.7*	73.9*	79.7*	9.9*	-	-	-

The study plots were classified into three different vegetation types within both forest-savanna ecotones: forest, transition and savanna (two in Brazil) plots. This classification was based on differences in species diversity, grass cover and fire occurrence and consistent with classification used by Oliveras & Malhi (2016) and Torello-Raventos et al. (2013).

Data collection

Data collection was conducted in March 2014 in Brazil and in October 2015 in Ghana. These periods were representative of the usual climate for the areas. In total we sampled 89 angiosperm tree species from 36 families (Table S4.1) that constituted approximately 80% of the plot basal area (tree diameter at breast height > 10cm). For each species within a plot, we sampled three mature and canopy emergent trees (total of 298 trees); and within each tree we randomly selected three mature (but not senescing) leaves from one fully sunlit branch, yielding a total of 831 leaf samples for LMA, N_{area}, P_{area}, and K_{area} and 713 for gas exchange traits (no data was available for *Buchenavia capitata, Aspidosperma macrocarpon* and *Syagrus flexuosa*). The cut branch was immediately placed and recut under water.

Leaf gas exchange sampling

Leaf gas exchange traits were measured using an open flow gas exchange system LI-6400XT (Li-Cor Inc., Lincoln, NE, USA). Three leaves from each branch were sampled for light-saturated rate of net CO_2 assimilation at ambient (400 ppm) CO_2 (A_{sat}). One individual tree per species within each study plot was sampled for photosynthetic response to variation in substomatal CO_2 concentration, C_i, by generating A-C_i curves. These were measured by changing CO_2 concentration to the following sequence: 400, 300, 200, 100, 50, 400, 600, 800, 1200, 1500 and 2000 μmol m^{-2} s^{-1}. The photosynthetic photon flux density was set to 2000 μmol m^{-2} s^{-1} and block temperature was kept closest to ambient and constant throughout the sampling period at 30º C. Measurements of other photosynthetic traits were beyond the scope of this paper.

The A-C_i curve fitting method and extraction of V_{cmax} values followed the procedure described in detail by Domingues et al.(2010). Using this method, 85 measurements (one for each sampled species) were derived for V_{cmax} and R_{day}, respiration in the light. To increase the number of V_{cmax} measurements we further estimated additional photosynthetic capacity values based on the V_{cmax} estimation from the one-point method described by De Kauwe et al. (2016):

$$V_{\mathrm{cmax,\,a}} = (A_{\mathrm{sat}} + R_{\mathrm{day}}) \frac{(C_i + K_m)}{(C_i - \Gamma^*)} \quad (1)$$

where Γ* is the CO_2 compensation point in the absence of day respiration (μmol mol^{-1}). R_{day} values were calculated following Domingues et al. (2010) and available at the species level. K_m is the Michaelis–Menten constant, given by:

$$K_{\mathrm{m}} = K_c \left(1 + \frac{O_i}{K_0}\right) \quad (2)$$

where K_c and K_o are the Michaelis constants for CO_2 and O_2, respectively (μmol mol^{-1}), and O_i is the intercellular concentration of O_2 assumed to be 210 mmol mol^{-1}. K_c, K_o and Γ* values were calculated according to equations listed in De Kauwe et al. (2016) and (Bernacchi *et al.*, 2001).

Estimated V_{cmax} values were in good agreement with V_{cmax} calculated from A-C_i curves (r^2=0.96, Fig. S4.1) with an overall positive bias of 3.4 μmol m^{-2} s^{-1} and root mean square error of 5.26 μmol m^{-2} s^{-1}.

Finally, to make our data and findings comparable to the wider literature of photosynthetic capacity variability we scaled the measured and estimated values of V_{cmax} to a reference temperature of 25°C following Sharkey et al. (2007) and further refer to it in text as V_{cmax}^{25}.

Leaf structure and chemistry determination

Leaves, or all leaflets if compound, were collected immediately following the gas exchange measurements, had petioles and rachis removed, scanned at 300 pixels per inch resolution with a digital flatbed scanner (Canon LiDE 110) and weighed. Leaves were then placed in an oven at 70°C and dried until the mass reached constant mass. Total leaf lamina area (cm^2) was calculated from scanned images using NIH ImageJ (http://rsbweb.nih.gov/ij/) with a custom MATLAB script (https://github.com/bblonder/leafarea). Dried leaf mass was divided by leaf area to calculate LMA. Leaf mass per area values for leaves from Brazil were analysed and presented in Neyret et al. (2016).

Dried leaf samples were transferred to the laboratory for further nutrient analysis. Plant material were pulverised and digested in a sulfuric-salicylic acid mixture and selenium (Novozamsky *et al.*, 1983) before colorimetric determination of *N* and *P* concentrations using a continuous flow analyser (SKALAR SAN plus system, The Netherlands). Potassium levels were measured using flame atomic emission spectroscopy (Walinga *et al.*, 1989). Foliar nutrients were expressed per area basis (divided by LMA).

Nitrogen investments in the major photosynthetic compartments

We estimated the proportion of total leaf *N* invested in Rubisco, P_r, based on work by Niinemets & Tenhunen (1997) from V_{cmax}^{25} values (Equation 3). The proportion of foliar *N* in Rubisco, P_r, is expressed as:

$$Pr = {V_{cmax}^{25}}/{6.25\, N_{area}\, V_{cr}} \qquad (3)$$

where V_{cr} is the specific activity of Rubisco (20.5 qmol CO_2 [g Rubisco]$^{-1}$ s^{-1} at 25 ºC, Jordan & Ogren, 1984), N_{area} is the total leaf nitrogen and the value of 6.25 converts nitrogen content to protein content.

Area versus mass based analysis

We analyse and present trait data on a per area (*cf.* per mass) basis. This was done because photosynthetic function is proportional to area, as only a few cell layers of mesophyll are responsible for the majority of light interception and photosynthesis rather than all of the leaf volume (Evans & Poorter, 2001; Poorter *et al.*, 2009). There are also significant mathematical issues related to interpreting relationships among traits on a per mass basis, i.e. when they have been divided by LMA (Lloyd *et al.*, 2013; Osnas *et al.*, 2013). Most relevantly, this includes photosynthesis being much more variable on a mass than on an area basis due to additional resource investments in non-photosynthetic functions. This results in greater regression covariance and in 'spurious correlations' among traits. Lloyd et al. (2013) also noted that when comparing different sites and/or functional groups, mass based photosynthesis and *N* relationships are potentially confounded by many factors not directly related to photosynthetic metabolism. Finally, area based photosynthetic capacity is useful for the parameterization of terrestrial biosphere models, which utilise area-based values only (e.g. Fyllas et al. (2014)). Mass correction of photosynthesis related traits are as appropriate as area based, however, might be more suitable when considering carbon economy, functioning and/or growth of an entire plant or when defensive and structural functions of *N* and LMA are discussed.

Species grouping

All tree species in Ghana were classified into three ecological guilds according to their light requirements for germination and seedling establishment: pioneers, non-pioneer light demanders (N.P.L.D.), and shade bearers (Hawthorne, 1993; 1995) (Table S4.1). Pioneers have the greatest light requirements for germination and establishment, non-pioneer light demanders require light to

develop beyond sapling stage and shade bearers can germinate and survive in the shaded parts of the forest. Due to lack of ecological data this classification was not available for Brazil. Additionally, the study considered 57 species with deciduous leaf habit (semideciduous and brevideciduous included) (17 in Ghana and 40 in Brazil) and 26 evergreen species (five in Ghana and 21 in Brazil) (Table S4.1). Finally, seven species of Fabaceae family in Brazil and three in Ghana were identified as potentially capable of biological nitrogen fixation via symbiotic association with rhizobia according to Sprent (2009).

Statistical analyses

All statistical analyses were performed in RStudio version 1.1.453 (RStudio Team, 2018). Differences in soil nutrients between the countries were tested using Student's t-test, and among the study plots using one-way ANOVA followed by post hoc Tukey's HSD test in the "agricolae" package (de Mendiburu, 2017) (H1). Pearson's Product-Moment Correlation (*r*) was used to test the relationship between soil and community-weighted (Appendix 4.1) foliar nutrients (H2).

Hypotheses 3 and 4 were tested using linear mixed-effect models (LMMs) (package "lme4" (Bates *et al.*, 2015)) as they allowed to account for a hierarchical nested structure of our dataset. Additionally, LMMs can effectively resolve the phylogenetic non-independence of data that, in our case, stems from sampling leaves from the same taxonomic units (families, species and trees).

Model assumptions were checked using protocols outlined in Zuur *et al.* (2009). Generally, studied traits did not follow a normal distribution due to positive or negative skewness. In most cases, squared-root transformation applied to a response variable in the LMMs, resulted in model residuals conforming to normal distribution. In a small number of cases, we transformed the variables with a Box-Cox method using "bcPower" function in "car" package (Fox & Weisberg, 2011).

We tested the effect that foliar nutrient had on the V_{cmax}^{25} values using LMMs with taxonomic units as random effects (Family/Species/Tree) (H3). The relationships were investigated at three levels: throughout the entire dataset, within each ecotone and within each study plot. We report *t*, *P* values and marginalised r^2 for fixed effects (derived using "MuMin" package (Barton, 2018)), and Akaike information criterion (AIC). To asses if a combination of foliar nutrients were more likely to explain V_{cmax}^{25} variability than individual nutrients, we compared the model structures using a goodness of fit test (function "anova") and made the decision for the best fit model using AIC and chi-squared test (χ^2) values (Tang *et al.*, 2014). This model structure was also used to test the effect that LMA and N_{area} have on P_r.

We tested the differences in LMA, foliar nutrient concentrations and V_{cmax}^{25} among species of different leaf deciduousness, guilds and ability to fix *N* (H4) with a similar model structure as for H3. In this case, we added study plot as a separate random effect to account for the uneven number of species of different ecological strategies in each site. Model significance was derived using "anova" function with Satterthwaite's method implemented in "lmerTest" package (Kuznetsova *et al.*, 2017). Post-hoc Tukey's comparisons were derived using "lsmeans" package (Lenth, 2016).

Finally, we performed a variance partitioning analysis to examine the factors that best explain the variance of each studied trait (Fyllas *et al.*, 2009). The LMM for this analysis did not include any fixed effects but incorporated study sites and taxonomic scales as random effects. The study plot was nested separately from taxonomic units to allow families and species to occur in multiple plots.

4.4. Results

Variations in leaf and soil nutrients

The soils (0-30 cm) in the Ghana transect were slightly poorer in [N] ($t_{24.86}$ =3.69, P<0.01) and in [P] ($t_{17.08}$=6.59, P<0.001) and not significantly different in [K] ($t_{24.37}$=1.57, P=0.13) (Table 4.1). Savanna

1 plot in Brazil was found on soils of significantly lower [P] ($F_{2,15}$=121.5, P<0.001) and [N] ($F_{2,15}$=8.76, P<0.01) than closely situated transition and forest plots. There was no difference in soil fertility among the plots within the Ghana transect (P=0.19, P=0.75, P=0.09 for [N], [P] and [K] respectively).

Overall, mean foliar nutrient concentrations were significantly different among the different study plots types (P<0.01, Table 4.2). On average, N_{area} values were higher in Brazil, while P_{area} and K_{area} were significantly greater in the Ghana plots. Within the Brazil transect, savanna 1 plot exhibited leaves with the highest nutrient concentrations and forest plot had particularly low P_{area} values (0.07 g m^{-2}). Average LMA value across the study sites was 124.93 g m^{-2} (±3.12) with the lowest and the highest values present in Brazilian Forest and savanna 1 plots, respectively. Leaf mass-area ratio increased moving from forest to savanna in both transects, but foliar nutrients showed no consistent trends.

Table 4.2 Mean leaf mass per area, LMA (g m^{-2}), and area based foliar nutrient concentrations (g m^{-2}) across the study plots in Ghana and Brazil. S.E. represent standard errors (±1). The denominator degrees of freedom (*Den. d.f.*) and *F* values are for mixed effect models comparing trait averages among the plots. Letters indicate results of post-hoc tests. Significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. = $P > 0.05$.

		LMA (g m^{-2})			N_{area} (g m^{-2})			P_{area} (g m^{-2})			K_{area} (g m^{-2})		
Country	Plot	Mean	S.E.	Post-hoc	Mean	S.E.	Post-hoc	Mean	S.E.	Post-hoc	Mean	S.E.	Post-hoc
Ghana	Forest	119.64	3.29	ab	2.21	0.06	ab	0.19	0.01	bc	0.82	0.03	c
	Transition	130.53	4.00	ab	2.33	0.06	bc	0.17	0.01	bc	0.73	0.03	c
	Savanna	132.07	4.62	ab	1.66	0.04	a	0.21	0.01	c	0.80	0.04	abc
Brazil	Forest	103.33	2.31	a	2.57	0.06	bcd	0.07	0.00	a	0.48	0.03	a
	Transition	122.96	2.71	a	2.63	0.05	bc	0.16	0.00	bc	0.54	0.02	ab
	Savanna 1	141.02	2.40	b	3.03	0.05	e	0.18	0.00	c	0.70	0.02	bc
	Savanna 2	124.98	2.52	ab	2.57	0.05	cd	0.12	0.00	b	0.45	0.01	a
	F		3.28			10.61			11.36			6.60	
	Den. d.f.		187.66			178.00			175.45			170.56	
	P		**			***			***			***	

Correlation between [P] and P_{area} was strong and negative (r=-0.84, P<0.05) and driven by the Brazilian forest and savanna 2 plots (Fig. 4.1). There was no relationship between [N] and [K] and foliar nutrients when expressed on area basis. Foliar nutrient concentrations expressed on mass basis and plotted against soil nutrient concentration demonstrated very similar patters (Fig. S4.2) with phosphorus being the only nutrient showing significant correlation with soil nutrients.

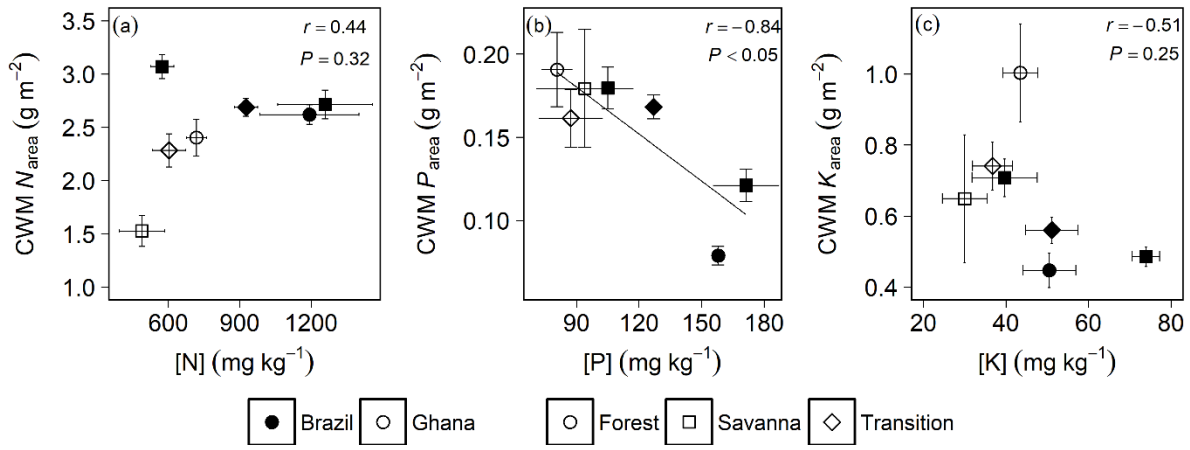


Fig. 4.1 The relationship between community weighted (CWM; Appendix 4.1) foliar and soil nutrient concentrations (top 20 cm; mg kg^{-1}) with standard errors: (a) nitrogen (*N*); (b) phosphorus (*P*); and (c) potassium (*K*). Symbols indicate vegetation types in Ghana and Brazil. *P* and *r* values are for Pearson's correlations.

Variations in photosynthetic capacity

Photosynthetic capacity (V_{cmax}^{25}) values ranged from 4.06 to 88.72 µmol m^{-2} s^{-1}, with the mean value across all study sites being 34.45 µmol m^{-2} s^{-1}. Mean V_{cmax}^{25} was significantly different among the study plots ($F_{156.52}$ =3.88, P<0.01; Fig. 4.2) with the lowest values in the Brazilian forest plot (23.91±1.1 µmol m^{-2} s^{-1}) and the highest in the Brazilian Savanna 1 plot (38.83±1.2 µmol m^{-2} s^{-1}). When the Brazilian forest plot was excluded from the analysis, there was no significant difference in V_{cmax}^{25} among the study plots ($F_{151.61}$=1.98, P=0.08). Remarkably, the mean V_{cmax}^{25} was not

significantly different between the two transects in Ghana and Brazil ($F_{78.39}$=0.79, P=0.37) despite the differences in soil and leaf nutrient status.

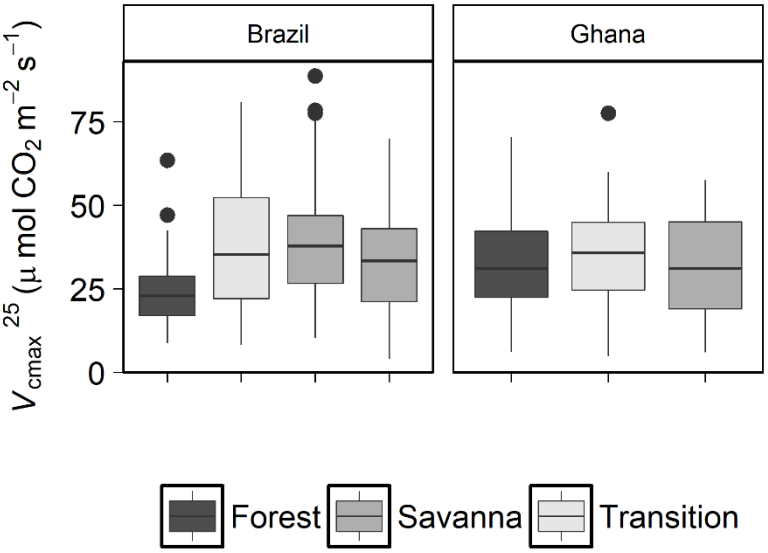


Fig. 4.2 Area-based photosynthetic capacity (V_{cmax}^{25}) among the study plots. The box boundaries represent 25th and 75th percentiles; the median (solid line); whiskers: 10th and 90th percentiles; outliers (dots).

Relationships between photosynthetic capacity and foliar nutrients

We observed a large spread of $V_{cmax,a}^{25}$ values in relation to the foliar nutrient concentrations which resulted in often weak and idiosyncratic to scale associations between the traits (Fig. 4.3). Photosynthetic capacity was significantly affected by both N_{area} and P_{area} but not by K_{area} when the entire dataset was used for the analysis (Table 4.3). In the Ghana transect, N_{area} was the only foliar nutrient that had a significant (and positive) relationship with V_{cmax}^{25} (r^2=0.10, t=3.99, P<0.001). In Brazil, on the other hand, all nutrients showed significant positive relationships with the V_{cmax}^{25} (P<0.05). Within transects, the transition plot in Ghana was the only study site where there were consistent significant positive relationships between N_{area}, P_{area} and K_{area} and V_{cmax}^{25} values (P<0.05), while Ghana forest and Brazil savanna 2 plot did not show any significant relationships between foliar nutrients and V_{cmax}^{25}.

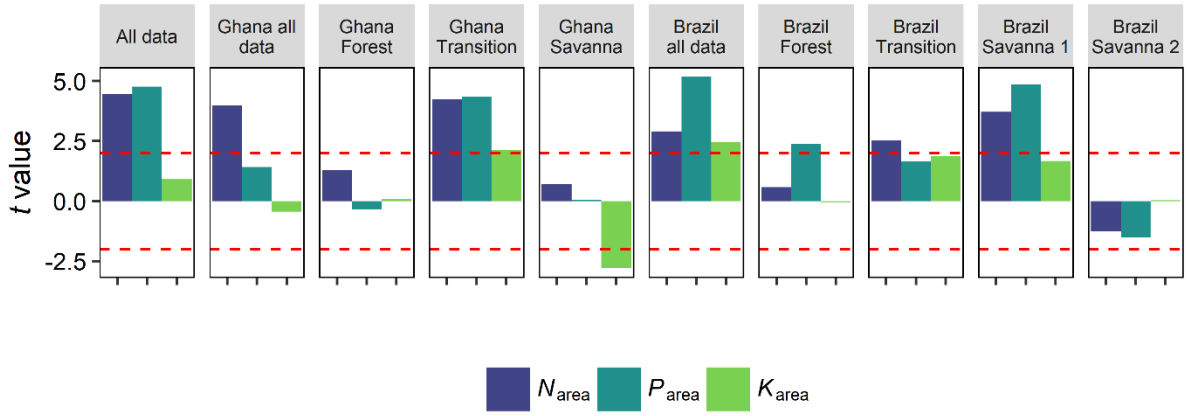


Fig. 4.3 Comparison of foliar nutrient effects on photosynthetic capacity (V_{cmax}^{25}) within plots, ecotones and across the dataset. The effect size is *t* value of a mixed effect model (Table 4.3). The dashed lines show statistically significant effects at α=0.05.

When foliar nutrients were assessed individually, in most cases P_{area} was the best explanatory variable to V_{cmax}^{25} (lowest AIC values and highest r^2 values, Table 4.3). Model fit was not improved by including more than one nutrient with two exceptions (Table S4.2). In the model encompassing data from both ecotones, including all three nutrients resulted in a significantly improved explanatory power of V_{cmax}^{25} ($\Delta AIC=7.3$, $\chi^2=11.34$, $P<0.01$, $r^2=0.08$). Including N_{area} and P_{area} in the model using data from the Ghana transition plot also improved the model fit ($\Delta AIC=4$, $\chi^2=5.99$, $P<0.05$, $r^2= 0.34$). Nevertheless, r^2 values were generally low with more than half of significant relationships having r^2 less than 0.1 (Table 4.3).

Table 4.3 Summary of linear mixed-effects models testing relationships between photosynthetic capacity (V_{cmax}^{25}) and area based foliar nitrogen (N_{area}), phosphorus (P_{area}) and potassium (K_{area}). Family, species and tree were treated as random effects. Akaike information criterion values (AIC) are comparable horizontally. AIC values in bold indicate nutrient providing the best fit for the model. Significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. = $P > 0.05$.

		$V_{cmax}^{25} \sim N_{area}$				$V_{cmax}^{25} \sim P_{area}$				$V_{cmax}^{25} \sim K_{area}$			
	n	*t*	r^2	*P*	AIC	*t*	r^2	*P*	AIC	*t*	r^2	*P*	AIC
All data	576	4.46	0.05	***	1827.4	4.77	0.06	***	**1820.1**	0.91	0.00	n.s.	1843.9
Ghana all data	195	3.99	0.10	***	**641.9**	1.41	0.02	n.s.	650.4	-0.44	0.00	n.s.	655.5
Ghana Forest	82	1.29	0.03	n.s.	267.6	-0.34	0.00	n.s.	**265.0**	0.08	0.00	n.s.	268.7
Ghana Transition	61	4.24	0.26	***	202.4	4.34	0.28	***	**197.7**	2.13	0.09	*	212.4
Ghana Savanna	52	0.70	0.01	n.s.	183.7	0.05	0.00	n.s.	181.0	-2.80	0.16	*	**177.6**
Brazil all data	381	2.90	0.02	**	1186.1	5.17	0.11	***	**1163.8**	2.47	0.02	*	1185.6
Brazil Forest	51	0.58	0.01	n.s.	146.8	2.37	0.12	*	**138.3**	-0.05	0.00	n.s.	145.6
Brazil Transition	78	2.53	0.07	*	264.4	1.64	0.03	n.s.	**262.4**	1.87	0.05	n.s.	264.3
Brazil Savanna 1	168	3.71	0.08	***	514.8	4.86	0.20	***	**501.0**	1.67	0.02	n.s.	523.1
Brazil Savanna 2	84	-1.25	0.02	n.s.	266.2	-1.50	0.04	n.s.	**259.8**	0.04	0.00	n.s.	264.2

Within-leaf N allocation

We calculated the average proportion of total leaf nitrogen that was invested in Rubisco, P_r, to further explore the effect of N_{area} on V_{cmax}^{25}. Threefold variation of P_r was observed across the study plots with significantly higher investment of N in Rubisco in Ghana transect than in Brazil ($F_{53.69}$=4.68, P<0.05). The proportion of total N_{area} that was invested in Rubisco and bioenergetics, decreased significantly with increasing LMA (t=-6.72, r^2=0.09, P<0.001; Fig. 4.4a) and N_{area} (t=-9.14, r^2=0.16, P<0.001; Fig. 4.4b). In particular, the upper envelope of values showed a strong trend, with high P_r only occurring in low LMA and low N_{area} leaves. In low N_{area} leaves up to 30% of N was invested in Rubisco, but in high N_{area} leaves only 10% was invested. Across the datasets, mean P_r was 0.1 in Brazil and 0.13 in Ghana.

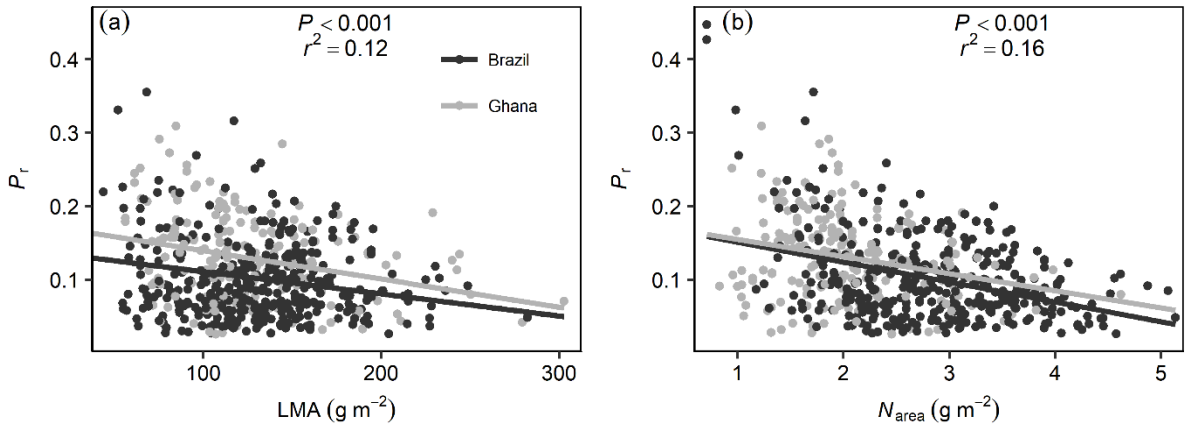


Fig. 4.4 Fraction of total leaf nitrogen invested in Rubisco and bioenergetics, P_r, in relation to (a) leaf mass per area (LMA), and (b) leaf area-based nitrogen (N_{area}) in Ghana and Brazil.

Role of ecological strategies

Foliar nutrient concentrations and V_{cmax}^{25} were not significantly different between evergreen and deciduous species, or among species of different light requirements for germination and establishment (Table S4.3). Leaf mass per area was significantly greater in evergreen species in

Brazil ($F_{50.42}$=5.19, P<0.05), but not in Ghana. Species that are potentially able to fix N had significantly lower K_{area} ($F_{57.65}$=5.59, P<0.05) than species that do not fix N in Ghana (Table S4.3), but N_{area}, P_{area}, V_{cmax}^{25} or LMA were not significantly different between these two groups.

Role of taxonomy

Variance partitioning analysis indicated that majority of the variance in V_{cmax}^{25} (37.1%) occurred within trees, while LMA, N_{area} and P_{area} mostly varied among species. Foliar potassium showed almost 50% of variance summed at intraspecific level (Fig. 4.5).

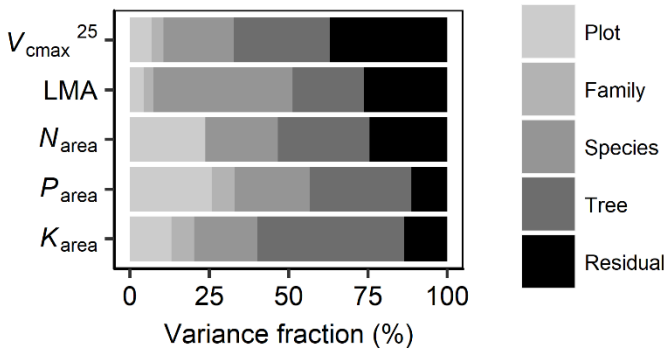


Fig. 4.5 Partitioning of the total variance for photosynthetic capacity (V_{cmax}^{25}), leaf mass per area (LMA), area-based nitrogen (N_{area}), phosphorus (P_{area}) and potassium (K_{area}) into taxonomic (family, species, tree, residual) and environmental (plot) components.

We further investigated the V_{cmax}^{25}↔ N_{area} relationship within and among individual species. We found that this relationship was extremely variable (Fig. S4.3) with some species (8% of all species) having a strong positive V_{cmax}^{25} and N_{area} and others showing strong negative (17% of all species) or no relationship at all. The slope between V_{cmax}^{25} and N_{area} for individual species decreased with increasing LMA (P<0.05; Fig. 4.6), indicating that species with higher LMA have weaker V_{cmax}^{25}↔ N_{area} relationships.

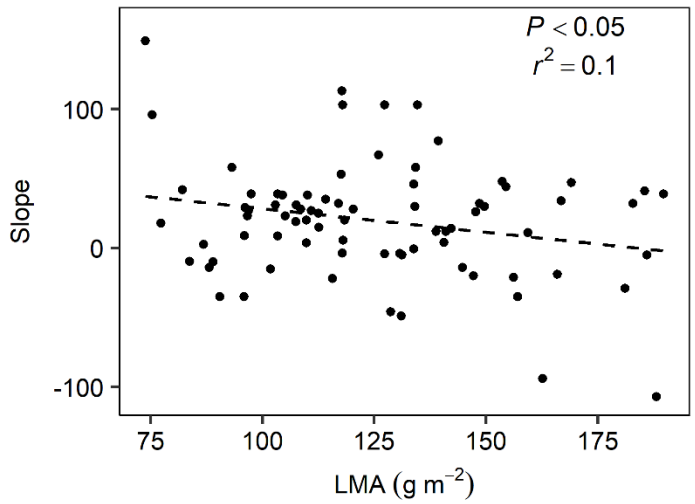


Fig. 4.6 Slope value derived from linear regressions between photosynthetic capacity (V_{cmax}^{25}) and total leaf nitrogen (N_{area}) for each studied species (Fig. S4.3) versus species average leaf mass per area (LMA).

4.5. Discussion

The overall objective of this study was to improve the limited understanding of photosynthetic capacity dynamics within and among forest-savanna ecotones in relation to key nutrients. Our study benefits from a large dataset of measured and derived V_{cmax}^{25} values (for 713 leaves from 85 species) in two forest-savanna vegetation transects in Ghana and Brazil. We found that, despite significant differences in leaf chemistry, V_{cmax}^{25} was less variable among the study sites than expected. Among the possible explanations for these patterns we considered differences in *N* partitioning between photosynthetic machinery and leaf structure, and species ecological strategies. Together, our findings question the assumption that foliar nutrient concentrations are good predictors of photosynthetic capacity at the leaf level.

Soil and canopy nutrients

Soil [P] values in the Ghana transect were at the lower end of the pan-Amazonian values reported by Quesada et al. (2010) for soils across Amazonia, whereas the values for most of the Brazil plots were

in the middle range. Soil [N] was particularly low across the study sites (average 0.6 mg g^{-1}), most likely because of high sand content in the soil (average 77.7%) (Weil & Brady, 2016). Soil [K] values across our study plots were similar to those reported by Lloyd et al. (2015) for the forest-savanna ecotones in Brazil.

We expected that savanna soils will exhibit lower [N], [P] and [K] values than nearby forest and transition plots, but this was the case only in Brazil (savanna 1 plot).This finding suggests that at least in Ghana local edaphic conditions were of lesser importance in determining the patchy distribution of forests and savannas as suggested by other studies on forest-savanna transitions (Gautier & Spichiger, 2004; Silva *et al.*, 2006; Veenendaal *et al.*, 2014; Lloyd *et al.*, 2015). In addition to edaphic factors, water availability in the soil (dependent on soil structure and drainage) and fire frequency can form and maintain the boundaries of studied ecotones (Bueno *et al.*, 2018).

Our observations on soil and leaf nutrient concentrations do not support the assumption that soil environment is the primary determinant of foliar nutrient concentrations in forest-savanna boundaries. This is in contrast to what has previously been observed across lowland tropical forests in Amazonia (Quesada & Lloyd, 2016).

The absence of significant correlations between soil and leaf nutrient concentrations could be explained by irregular water supply as decline in soil moisture impedes nutrient uptake by plants (Hu & Schmidhalter, 2005). Barbosa et al. (2014) found that foliar *N* and *P* decreased in species dominating South African semi-arid savannas in response to uneven water availability. Moreover, soil and leaf *P* showed significant negative relationship, which was driven by the forest plot in Brazil, where total soil *P* concentration was high (0.16 g mg^{-1}) but foliar concentrations were particularly low (0.07 g m^{-2}). One of the possible explanations for this observation is reduction of bioavailable soil *P* for plants due to frequent soil waterlogging (Steffens *et al.*, 2005) which is characteristic of plinthosol (Sehgal *et al.*, 1998). Additionally, high amount of clay in the soil (23.6%) might restrict phosphorus movement in the soil and hamper the nutrient uptake (Wiersum, 1962).

It is also likely that the lack of relationship between soil and leaf nutrient levels observed in our study was due to different allocation of resources among plant organs. Dry semideciduous forests of Ghana are known to allocate more than 50% of carbon to woody production, which is significantly lower in wetter sites (Moore *et al.*, 2017). Respectively, proportion of nutrient allocated to wood than to leaves could also increase. While nutrient levels of wood, roots and leaves are thought to be correlated (Liu *et al.*, 2010), stressful environments can have a strong effect on these relationships. In semi-arid environments especially, wood plays an important role in nutrient storage and supply (He *et al.*, 2015). Additionally, in environments of intermittent water supply nutrients in the wood, such as *N* and *K*, are essential for osmoregulation; *N*-based compounds are used to mobilise starch for osmotic adjustment (Martin *et al.*, 2014), and *K* plays an important osmotic role in cambial cell expansion (Fromm, 2010).

Foliar nutrient constraints on photosynthetic capacity

The studied forest-savanna ecotones had particularly low V_{cmax}^{25} values (average 34.45 μmol m^{-2} s^{-1}) when compared with other biomes with different edaphic conditions including lowland tropical and temperate forests, coniferous trees and crops (Kattge *et al.*, 2009; Bahar *et al.*, 2016). Observed V_{cmax}^{25} values were similar to values reported for 'long-grass savanna woodlands' and dry semideciduous forests in West Africa (Domingues *et al.*, 2010, 2015).

Idiosyncratic relationships between V_{cmax}^{25} and foliar nutrients observed here, have been reported in other studies along forest-savanna transitions (Domingues *et al.*, 2010, 2015; Bloomfield *et al.*, 2014). In West Africa and Australia V_{cmax}^{25} of trees growing in savanna was more limited by leaf *N* and forest species by *P*. In our study *P* had a slightly stronger effect on V_{cmax}^{25} than *N* in most cases. This finding is in agreement with suggestions that *P* can modify, co-limit, or limit V_{cmax}^{25}more than *N* (Domingues *et al.*, 2010; Norby *et al.*, 2017; Walker *et al.*, 2017). Interpretation of $V_{cmax}^{25} \leftrightarrow P$

relationship is however much more difficult than with *N*. It is thought that *P* can co-limit regeneration of ribulose 1,5 biphophate (RuBP) or decrease activation of Rubisco (Norby *et al.*, 2017).

It is possible that the overall lack of significant relationship between V_{cmax}^{25} and K_{area} (except the savanna plot in Ghana) was due to indirect effects of *K* on plant functioning. While *K* is known to have a direct positive effect on stomatal and chloroplast functioning (Tränkner *et al.*, 2018), in sites of intermittent water supply and drought conditions, due to the role that it plays in osmotic adjustment, *K* enables plant with more efficient water retention in plant tissues, improves cell membrane stability and plant hydraulic conductance (Wang *et al.*, 2013b). Indeed, Lloyd et al. (2015) found that it was the soil rather than leaf *K* concentrations that were linked with photosynthetic rates in forest-savanna ecotones across the Amazon Basin.

Despite the significant trends, in most cases area-based foliar nutrient concentrations were not good predictors of the V_{cmax}^{25} values, as very little or none of the variation was explained (the highest r^2 value across the data set was 0.28; Table 4.3). Similarly, weak or no significant relationships were found between V_{cmax}^{25} and leaf *N* or *P* in the TROBIT (Tropical Biomes in Transition) project (Domingues *et al.*, 2010), which spanned a rainfall gradient in West Africa. The same lack of relationship was observed in other tropical forests too, such as montane cloud and rainforests (Coste *et al.*, 2005; van de Weg *et al.*, 2012; Dusenge *et al.*, 2015).

A few explanations have been proposed for the lack of significant $V_{cmax}^{25} \leftrightarrow N$ relationships. Dusenge et al. (2015) suggested that V_{cmax}^{25} was not related to N_{area} because of differences in within leaf *N* allocation to photosynthetic machinery versus light harvesting compounds in pioneer and climax species. This explanation is not consistent with our findings, as classification of species into pioneer and shade tolerant categories did not have a statistically significant effect on the $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship. Coste et al. (2005) suggested that this lack of relationship is because of a proportionally greater investment of *N* in leaf structure in tropical rainforest species of high LMA. This suggestion is particularly fitting to our findings because modelled fraction of *N* allocated towards photosynthetic

machinery (Niinemets & Tenhunen, 1997) declined with increasing N_{area} and LMA across the study sites (Fig. 4.6). This pattern was observed previously: for example, the fraction of *N* allocated to photosynthesis decreased with increasing LMA across *Quercus* species while that to membrane associated proteins increased (Takashima *et al.*, 2004). Higher LMA species require greater *N* allocation towards structure to increase major vein allocation and mesophyll cell layers (Milla-Moreno *et al.*, 2016; John *et al.*, 2017).

High LMA species are characteristic of arid and semi-arid regions as denser leaves have lower water requirements under dry conditions and are more resistant to wilting (Poorter *et al.*, 2009). The mean LMA value observed in our study, 124.9±.2.3 g m^{-2}, is significantly greater than that reported for tropical and temperate forests in a global study (73 and 81 g m^{-2}, respectively) (Poorter *et al.*, 2009). As discussed above, the weak relationship between leaf total N_{area} and V_{cmax}^{25} observed here is potentially due to requirement for higher investment of *N* in high LMA leaves, possibly because of adaptation to intermittent water availability. This pattern was observed in Australia by Prentice et al. (2014) who found that relationship between V_{cmax}^{25} and N_{area} is weaker in drier climates.

In addition to photosynthetic process and leaf structure, *N* is used for other functions. For example, up to 15% of total leaf *N* can be invested into cyanogenic glycosides, alkaloids, and/or glucosinolates, defensive compounds against herbivory (Burns *et al.*, 2002). These compounds can be particularly important in forest-savanna ecotones, as up to 17% of foliar biomass produced annually in Cerrado can be consumed by leaf-cutter ants alone (Costa *et al.*, 2008). Van de Weg et al. (2012) attributed the weak relationship between total N_{area} and V_{cmax}^{25} to large investment of leaf *N* in cyanogenic glycosides.

Finally, the lack of strong relationships between V_{cmax}^{25} and foliar nutrients observed in our study can be partially explained by variability in stomatal conductance. Despite V_{cmax}^{25} being indicative of biochemical rather than stomatal limitations on gas exchange, the relationship between V_{cmax}^{25} and stomatal conductance was significant (r^2=0.26, P<0.001; data not shown). Stomatal limitation on

V_{cmax}^{25} might be emphasised when performing photosynthesis measurements on cut branches *(in situ* measurements were not feasible). Nevertheless, we allowed cut branches to rehydrate and for stomatal conductance to stabilise after removal from the tree. This methodology generally provides reliable estimates of gas exchange (Dang *et al.*, 1997; Cavaleri *et al.*, 2008).

Effects of taxonomy and ecological strategies on photosynthetic capacity

N_2-fixation

As the soils in our study sites were poor in [N], we expected that N_2-fixing plants would have higher concentrations of N_{area} and, consequently, higher V_{cmax}^{25}, which was not the case. Nardoto et al. (2008) suggested that in most cases legumes within mature forests of Amazonia do not fix *N*, even when physiologically capable of doing so. Additionally, in both transects *N* fixation ability did not correlate with V_{cmax}^{25}. These findings are in agreement with global meta-analysis by Adams et al. (2016) who found that N-fixers, especially, in semi-arid climates, do not have higher photosynthesis rates, than non-fixing plants.

Deciduousness

Our expectation for higher V_{cmax}^{25} in deciduous species was not supported by the results. . Cianciaruso et al. (2013) suggested that leaf habit does not constitute different functional groups in forest-savanna ecotones as a number of strong abiotic and biotic pressures, such as fire and herbivory, increases the plasticity of traits. Additionally, it is thought that deciduous and evergreen species differ in water uptake from the soils (Hasselquist *et al.*, 2010) as well as crown structural traits (Esteso-Martinez *et al.*, 2006). It is possible that the role of deciduousness is not to maximise carbon gain but to alleviate the effects of intermittent water supply. Clearly, more focused studies are required to uncover the relationship among foliar nutrient concentrations, photosynthesis and leaf habit in forest-savanna ecotones.

Variance of photosynthetic capacity and foliar nutrients in different taxonomic levels

A variance partitioning analysis indicated high levels of trait variance within species and within trees, rather than among species. This is also supported by a low phylogenetic control over photosynthetic and leaf chemistry traits here (Appendix 4.2) and in other studies (Oliveras *et al.*; Messier *et al.*, 2017). These findings suggest the importance of studying variability of photosynthetic traits at lower taxonomic levels.

. In many cases, considering species life history strategy was useful in explaining observed patterns in the trait values. For example, *Tachigali vulgaris*, a pioneer with one of the highest V_{cmax}^{25} values across the studied ecotone in Brazil (Fig. S4.4), is an extremely fast-growing and successful species in terms of recruitment in Cerrado (Morandi *et al.*, 2016). *Bombax buonopozense*, a deciduous pioneer species with the highest V_{cmax}^{25} values in Ghana, is known for its' adaptation to harsh savanna environments, specifically droughts and fires (Cardoso *et al.*, 2016). *B. buonopozense* is also known for high *N* investment in herbivory defences (Iroka *et al.*, 2016), which could be a possible explanation for the lack of $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship observed in this species (Fig. S4.3). The low V_{cmax}^{25} and nutrient values from *Vitellaria paradoxa*, a tree native to the savanna zone in Ghana, could be explained by high nutrient requirements for fruiting, as each tree can produce around ~20 kg a^{-1}. This species is also known for its slow growth and long juvenile phase. Despite low values for studied traits this species is one of the most abundant trees across the West African landscapes.

Conclusions

The proximity of the study plots within each region enabled us to investigate variation of V_{cmax}^{25} in different vegetation formations and its links with foliar nutrient concentrations without the added complexity of variation in climate, temperature or altitude. We observed a large scatter of V_{cmax}^{25} values in relation to N_{area}, P_{area} and K_{area} which reflects different allocation of resources among leaf

structure and photosynthetic machinery, multiple ecological strategies and phenological adaptations among species. Further, we can speculate that for species existing in forest-savanna ecotones it is not necessary to maximise photosynthetic capacity for a given leaf *N* to survive. A more important selective pressure may be the ability to cope with irregular availability of water and long periods of drought, which might require greater investment in *N*-osmolytes to leaf turgor maintenance. This work has demonstrated that variability in V_{cmax}^{25} values as well as $V_{cmax}^{25} \leftrightarrow N_{area}$ relationships along the forest-savanna ecotones is large, and that the leaf nitrogen-photosynthesis paradigm employed in many biosphere models needs to be revised in the context of tropical ecosystems.

4.6. Supplementary Information

The following Supplementary Information is available for this article:

Table S4.1 Species list.

Table S4.2 Model selection.

Table S4.3 Mean trait values among different ecological strategies.

Fig. S4.1 Estimated $V_{cmax,a}$ values against $V_{cmax,a}$ calculated from A-C_i curves

Fig. S4.2 Plots linking mass based foliar and soil nutrient concentrations.

Fig. S4.3 $V_{cmax,a}^{25} \leftrightarrow N_a$ relationship for individual species.

Appendix 4.1 Community weighted means

Appendix 4.2 Phylogenetic analyses

Table S4.1 Sampled species list with leaf habit, guild and nitrogen fixation classifications. + is for species able to fix nitrogen. N.P.L.D. stand for non-pioneer light demander.

Country	Family	Species	Deciduousness	N fixer	Guild
Ghana					
	Fabaceae	*Afzelia africana*	Deciduous	-	N.P.L.D.
	Combretaceae	*Anogeissus leiocarpus*	Deciduous	-	Pioneer
	Malvaceae	*Bombax buonopozense*	Deciduous	-	Pioneer
	Phyllanthaceae	*Bridelia ferruginea*	Evergreen	-	Pioneer
	Malvaceae	*Ceiba pentandra*	Deciduous	-	Pioneer
	Malvaceae	*Cola gigantea*	Deciduous	-	N.P.L.D.
	Burseraceae	*Dacryodes klaineana*	Evergreen	-	Shade-bearer
	Fabaceae	*Daniellia oliveri*	Deciduous	+	Pioneer
	Ebenaceae	*Diospyros mespiliformis*	Deciduous	-	N.P.L.D.
	Fabaceae	*Erythrophleum suaveolens*	Deciduous	+	N.P.L.D.
	Meliaceae	*Khaya grandifoliola*	Deciduous	-	N.P.L.D.
	Meliaceae	*Khaya senegalensis*	Evergreen	-	N.P.L.D.
	Meliaceae	*Khaya* spp.	?	-	?
	Anacardiaceae	*Lannea velutina*	Deciduous	-	Pioneer
	Sapotaceae	*Manilkara multinervis*	Evergreen	-	?
	Chrysobalanaceae	*Maranthes polyandra*	Evergreen	-	Shade-bearer
	Moraceae	*Milicia excelsa*	Deciduous	-	Pioneer
	Sapotaceae	*Pouteria alnifolia*	Deciduous	-	Pioneer
	Fabaceae	*Pterocarpus erinaceus*	Deciduous	-	Pioneer
	Bignoniaceae	*Spathodea campanulata*	Deciduous	-	Pioneer
	Malvaceae	*Sterculia tragacantha*	Deciduous	-	Pioneer

	Combretaceae	*Terminalia glaucescens*	Deciduous	-	Pioneer
	Combretaceae	*Terminalia* spp.	?	-	?
	Sapotaceae	*Vitellaria paradoxa*	Deciduous	-	N.P.L.D.
Brazil					
	Rubiaceae	*Amaioua guianensis*	Evergreen	-	
	Anacardiaceae	*Anacardium occidentale*	Evergreen	-	
	Fabaceae	*Apuleia leiocarpa*	Deciduous	+	
	Apocynaceae	*Aspidosperma macrocarpon*	Deciduous	-	
	Apocynaceae	*Aspidosperma multiflorum*	Deciduous	-	
	Apocynaceae	*Aspidosperma tomentosum*	Deciduous	-	
	Anacardiaceae	*Astronium fraxinifolium*	Deciduous	-	
	Fabaceae	*Bowdichia virgilioides*	Deciduous	+	
	Moraceae	*Brosimum rubescens*	Deciduous	-	
	Combretaceae	*Buchenavia capitata*	Deciduous	-	
	Combretaceae	*Buchenavia tomentosa*	Deciduous	-	
	Malpighiaceae	*Byrsonima coccolobifolia*	Deciduous	-	
	Malpighiaceae	*Byrsonima pachyphylla*	Evergreen	-	
	Caryocaraceae	*Caryocar brasiliense*	Deciduous	-	
	Euphorbiaceae	*Chaetocarpus echinocarpus*	Evergreen	-	
	Celastraceae	*Cheiloclinium cognatum*	Deciduous	-	
	Fabaceae	*Dalbergia miscolobium*	Deciduous	+	
	Dilleniaceae	*Davilla elliptica*	Deciduous	-	
	Fabaceae	*Dimorphandra mollis*	Deciduous	+	
	Fabaceae	*Dipteryx alata*	Deciduous	-	
	Icacinaceae	*Emmotum nitens*	Evergreen	-	
	Annonaceae	*Ephedranthus parviflorus*	Evergreen	-	
	Malvaceae	*Eriotheca gracilipes*	Deciduous	-	
	Erythroxylaceae	*Erythroxylum suberosum*	Deciduous	-	
	Myrtaceae	*Eugenia aurata*	Deciduous	-	
	Euplassa	*Euplassa inaequalis*	Deciduous	-	
	Nyctaginaceae	*Guapira graciliflora*	Deciduous	-	
	Olacaceae	*Heisteria ovata*	Evergreen	-	
	Malpighiaceae	*Heteropterys byrsonimifolia*	Deciduous	-	
	Chrysobalanaceae	*Hirtella glandulosa*	Evergreen	-	
	Fabaceae	*Hymenaea courbaril*	Deciduous	+	
	Fabaceae	*Hymenaea stigonocarpa*	Deciduous	-	
	Clusiaceae	*Kielmeyera rubriflora*	Deciduous	-	
	Lythraceae	*Lafoensia pacari*	Deciduous	-	
	Euphorbiaceae	*Mabea fistulifera*	Evergreen	-	
	Sapindaceae	*Magonia pubescens*	Deciduous	-	
	Euphorbiaceae	*Maprounea guianensis*	Deciduous	-	
	Sapindaceae	*Matayba guianensis*	Evergreen	-	
	Lauraceae	*Mezilaurus crassiramea*	Deciduous	-	
	Myrtaceae	*Myrcia splendens*	Evergreen	-	

Lauraceae	*Ocotea hoehneii*	Deciduous	-
Ochnaceae	*Ouratea hexasperma*	Evergreen	-
Sapotaceae	*Pouteria ramiflora*	Deciduous	-
Burseraceae	*Protium heptaphyllum*	Deciduous	-
Malvaceae	*Pseudobombax longiflorum*	Deciduous	-
Fabaceae	*Pterodon pubescens*	Deciduous	-
Vochysiaceae	*Qualea grandiflora*	Deciduous	-
Vochysiaceae	*Qualea multiflora*	Deciduous	-
Vochysiaceae	*Qualea parviflora*	Deciduous	-
Proteaceae	*Roupala montana*	Evergreen	-
Vochysiaceae	*Salvertia convallariodora*	Evergreen	-
Siparunaceae	*Siparuna guianensis*	Evergreen	-
Moraceae	*Sorocea klotzschiana*	Deciduous	-
Loganiaceae	*Strychnos pseudoquina*	Evergreen	-
Arecaceae	*Syagrus flexuosa*	Evergreen	-
Bignoniaceae	*Tabebuia serratifolia*	Deciduous	-
Fabaceae	*Tachigali aurea*	Deciduous	+
Fabaceae	*Tachigali vulgaris*	Evergreen	+
Anacardiaceae	*Tapirira guianensis*	Deciduous	-
Combretaceae	*Terminalia argentea*	Deciduous	-
Burseraceae	*Tetragastris altissima*	Evergreen	-
Fabaceae	*Vatairea macrocarpa*	Evergreen	-
Vochysiaceae	*Vochysia haenkeana*	Deciduous	-
Vochysiaceae	*Vochysia rufa*	Evergreen	-
Annonaceae	*Xylopia aromatica*	Evergreen	-

Table S4.2 Selection of explanatory terms in mixed effect models with $V_{cmax}{}^{25}$ as the response variable and foliar nutrient as predictor variables. Models were run using the maximum likelihood method. Test parameters and statistics are Akaike information criteria (AIC); Bayesian information criteria (BIC); maximum likelihood (logLik); the likelihood ratio statistic ($\chi 2$) and *P* value. Significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. = not significant.

Model structure	AIC	BIC	logLik	$\chi 2$	*P*
		All data			
$V_{cmax}{}^{25} \sim N_{area}$	1821.70	1847.80	-904.86		
$V_{cmax}{}^{25} \sim P_{area}$	1819.40	1845.60	-903.72	2.28	***
$V_{cmax}{}^{25} \sim K_{area}$	1840.20	1866.40	-914.12	0.00	n.s.
$V_{cmax}{}^{25} \sim N_{area}+P_{area}$	1814.90	1845.40	-900.43	27.37	***
$V_{cmax}{}^{25} \sim N_{area}+K_{area}$	1823.10	1853.60	-904.54	0.00	n.s.
$V_{cmax}{}^{25} \sim P_{area}+K_{area}$	1819.60	1850.10	-902.82	3.43	***
$V_{cmax}{}^{25} \sim N_{area}+P_{area}+K_{area}$	1813.00	1847.90	-898.51	8.62	**

Ghana all data					
$V_{cmax}^{25} \sim N_{area}$	638.28	657.92	-313.14		
$V_{cmax}^{25} \sim P_{area}$	651.34	670.98	-319.67	0.00	n.s.
$V_{cmax}^{25} \sim K_{area}$	653.15	672.78	-320.57	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}$	640.28	663.19	-313.14	14.87	***
$V_{cmax}^{25} \sim N_{area}+K_{area}$	637.25	660.16	-311.63	3.03	***
$V_{cmax}^{25} \sim P_{area}+K_{area}$	652.33	675.24	-319.17	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	639.06	665.24	-311.53	15.27	***
Ghana Forest					
$V_{cmax}^{25} \sim N_{area}$	266.25	280.69	-127.12		
$V_{cmax}^{25} \sim P_{area}$	267.85	282.29	-127.92	0.00	n.s.
$V_{cmax}^{25} \sim K_{area}$	267.96	282.40	-127.98	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}$	266.56	283.41	-126.28	3.40	n.s.
$V_{cmax}^{25} \sim N_{area}+K_{area}$	268.11	284.95	-127.05	0.00	n.s.
$V_{cmax}^{25} \sim P_{area}+K_{area}$	269.79	286.64	-127.90	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	268.77	288.02	-126.38	3.02	n.s.
Ghana Transition					
$V_{cmax}^{25} \sim N_{area}$	200.08	212.75	-94.04		
$V_{cmax}^{25} \sim P_{area}$	199.59	212.25	-93.79	0.50	***
$V_{cmax}^{25} \sim K_{area}$	211.67	224.34	-99.84	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}$	195.59	210.36	-90.79	18.08	***
$V_{cmax}^{25} \sim N_{area}+K_{area}$	201.24	216.02	-93.62	0.00	n.s.
$V_{cmax}^{25} \sim P_{area}+K_{area}$	201.07	215.85	-93.54	0.17	***
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	197.37	214.25	-90.68	5.71	*
Ghana Savanna					
$V_{cmax}^{25} \sim N_{area}$	183.22	194.93	-85.61		
$V_{cmax}^{25} \sim P_{area}$	184.11	195.82	-86.06	0.00	n.s.
$V_{cmax}^{25} \sim K_{area}$	176.79	188.50	-82.39	7.33	***
$V_{cmax}^{25} \sim N_{area}+P_{area}$	185.08	198.74	-85.54	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+K_{area}$	175.24	188.90	-80.62	9.85	***
$V_{cmax}^{25} \sim P_{area}+K_{area}$	178.52	192.18	-82.26	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	177.07	192.68	-80.54	3.45	n.s.
Brazil all data					
$V_{cmax}^{25} \sim N_{area}$	1181.10	1204.80	-584.56		
$V_{cmax}^{25} \sim P_{area}$	1163.90	1187.60	-575.96	17.21	***
$V_{cmax}^{25} \sim K_{area}$	1183.20	1206.80	-585.59	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}$	1165.80	1193.40	-575.91	19.36	***
$V_{cmax}^{25} \sim N_{area}+K_{area}$	1182.00	1209.60	-584.01	0.00	n.s.
$V_{cmax}^{25} \sim P_{area}+K_{area}$	1165.80	1193.40	-575.89	16.24	***
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	1167.80	1199.30	-575.87	0.04	n.s.
Brazil Forest					
$V_{cmax}^{25} \sim N_{area}$	144.66	156.25	-66.33		
$V_{cmax}^{25} \sim P_{area}$	141.21	152.80	-64.61	3.45	***
$V_{cmax}^{25} \sim K_{area}$	144.87	156.46	-66.44	0.00	n.s.

$V_{cmax}^{25} \sim N_{area}+P_{area}$	141.86	155.38	-63.93	5.01	*
$V_{cmax}^{25} \sim N_{area}+K_{area}$	146.17	159.69	-66.08	0.00	n.s.
$V_{cmax}^{25} \sim P_{area}+K_{area}$	141.72	155.24	-63.86	4.45	***
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	143.10	158.56	-63.55	0.61	n.s.
Brazil Transition					
$V_{cmax}^{25} \sim N_{area}$	262.52	276.66	-125.26		
$V_{cmax}^{25} \sim P_{area}$	265.81	279.95	-126.90	0.00	n.s.
$V_{cmax}^{25} \sim K_{area}$	265.17	279.31	-126.58	0.64	***
$V_{cmax}^{25} \sim N_{area}+P_{area}$	263.14	279.63	-124.57	4.03	*
$V_{cmax}^{25} \sim N_{area}+K_{area}$	264.46	280.95	-125.23	0.00	n.s.
$V_{cmax}^{25} \sim P_{area}+K_{area}$	267.06	283.56	-126.53	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	265.13	283.98	-124.56	3.93	*
Brazil Savanna 1					
$V_{cmax}^{25} \sim N_{area}$	511.17	529.91	-249.59		
$V_{cmax}^{25} \sim K_{area}$	502.34	521.08	-245.17	8.83	***
$V_{cmax}^{25} \sim N_{area}+K_{area}$	521.91	540.65	-254.95	0.00	n.s.
$V_{cmax}^{25} \sim P_{area}+K_{area}$	502.45	524.32	-244.23	21.45	***
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	513.14	535.01	-249.57	0.00	n.s.
$V_{cmax}^{25} \sim N_{area}+P_{area}$	504.00	525.87	-245.00	9.14	***
$V_{cmax}^{25} \sim P_{area}$	503.68	528.67	-243.84	2.32	n.s.
Brazil Savanna 2					
$V_{cmax}^{25} \sim N_{area}$	265.07	279.66	-126.54		
$V_{cmax}^{25} \sim P_{area}$	263.86	278.44	-125.93	1.21	***
$V_{cmax}^{25} \sim K_{area}$	263.20	277.78	-125.60	0.66	***
$V_{cmax}^{25} \sim N_{area}+P_{area}$	265.14	282.15	-125.57	0.06	n.s.
$V_{cmax}^{25} \sim N_{area}+K_{area}$	263.20	280.22	-124.60	1.94	***
$V_{cmax}^{25} \sim P_{area}+K_{area}$	263.12	280.14	-124.56	0.08	***
$V_{cmax}^{25} \sim N_{area}+P_{area}+K_{area}$	263.14	282.58	-123.57	1.98	n.s.

Table S4.3 Mean leaf mass per area, LMA (g m^{-2}), and area based foliar nutrient concentrations (g m^{-2}), and photosynthetic capacity (V_{cmax}^{25}) in species of different ecological strategies. S.E. represent standard errors (±1). The denominator degrees of freedom (*Den. d.f.*) and *F* values are for mixed effect models comparing trait averages among the strategies. Significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. = *P* value.

		n (leaves)	LMA (g m^{-2})		N_{area} (g m^{-2})		P_{area} (g m^{-2})		K_{area} (g m^{-2})		V_{cmax}^{25} (μmol m^{-2} s^{-1})	
Country			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Ghana	Deciduous	163	124.08	3.12	2.12	0.05	0.19	0.01	0.78	0.03	34.63	1.19
	Evergreen	28	126.35	5.93	1.84	0.09	0.16	0.01	0.71	0.06	31.35	2.68
	F		0.02		2.81		0.88		0.07		0.68	
	P		0.88		0.11		0.36		0.79		0.41	
Brazil	Deciduous	263	123.58	2.33	2.75	0.05	0.15	0.00	0.57	0.02	33.54	0.98
	Evergreen	118	139.29	4.06	2.91	0.07	0.14	0.01	0.63	0.02	38.32	1.68
	F		5.19		3.21		0.37		1.71		0.99	
	P		*		0.08		0.85		0.19		0.32	
Ghana	Pioneer	65	127.28	6.02	2.11	0.08	0.20	0.01	0.81	0.05	35.32	1.98
	N.P.L.D.	46	121.32	4.18	2.49	0.09	0.17	0.01	0.80	0.05	37.51	1.93
	Shade-bearer	10	139.16	7.71	2.03	0.15	0.21	0.03	0.58	0.04	34.99	5.97
	F		0.16		2.59		0.05		0.38		0.17	
	P		0.85		0.12		0.94		0.68		0.85	
Ghana	N-fixer	20	107.40	5.63	2.42	0.13	0.11	0.01	0.48	0.05	30.33	2.90
	not fixer	177	125.55	2.97	2.03	0.05	0.19	0.01	0.81	0.03	34.13	1.16
	F		0.18		0.02		2.41		4.77		3.09	
	P		0.67		0.9		0.14		*		0.15	
Brazil	N-fixer	64	113.33	4.62	2.70	0.11	0.12	0.01	0.45	0.03	34.23	2.07
	not fixer	317	131.50	2.27	2.82	0.04	0.15	0.00	0.62	0.02	35.18	0.95
	F		0.06		0.01		0.56		1.77		1.34	
	P		0.8		0.95		0.47		0.19		0.25	

Fig. S4.1 Comparison between values of the maximum carboxylation rate, $V_{cmax,a}$, measured from *A*–*C*i curves and $V_{cmax,a}$ estimated from one-point method according to De Kauwe *et al.* (2016).

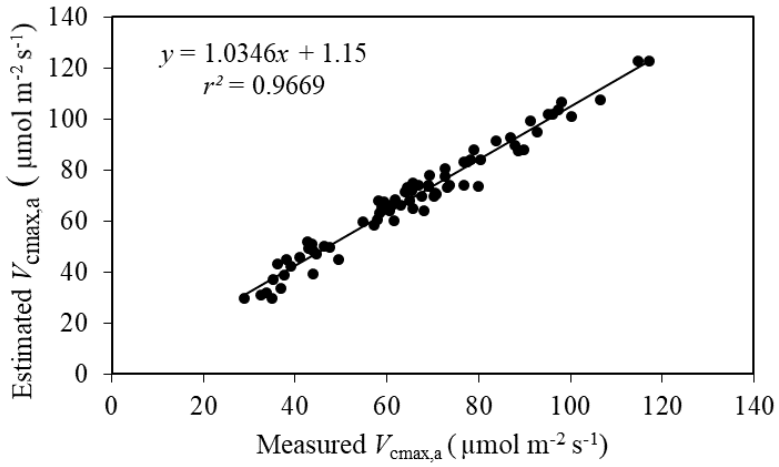


Fig. S4.2 Relationship between basal area weighted mass based foliar nutrient concentrations (g kg^{-1}) and soil nutrient concentrations (top 20 cm; g kg^{-1}) with standard errors: (a) nitrogen (*N*) (b) phosphorus (*P*) and (c) potassium (*K*). Triangles indicate study sites in Ghana and circles sites in Brazil. *P* and r^2 values are for standardized major axis tests.

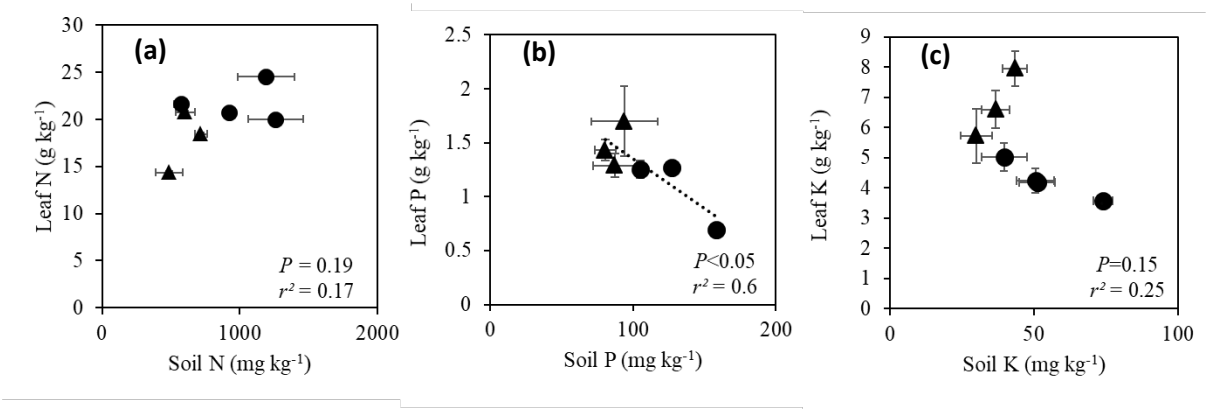
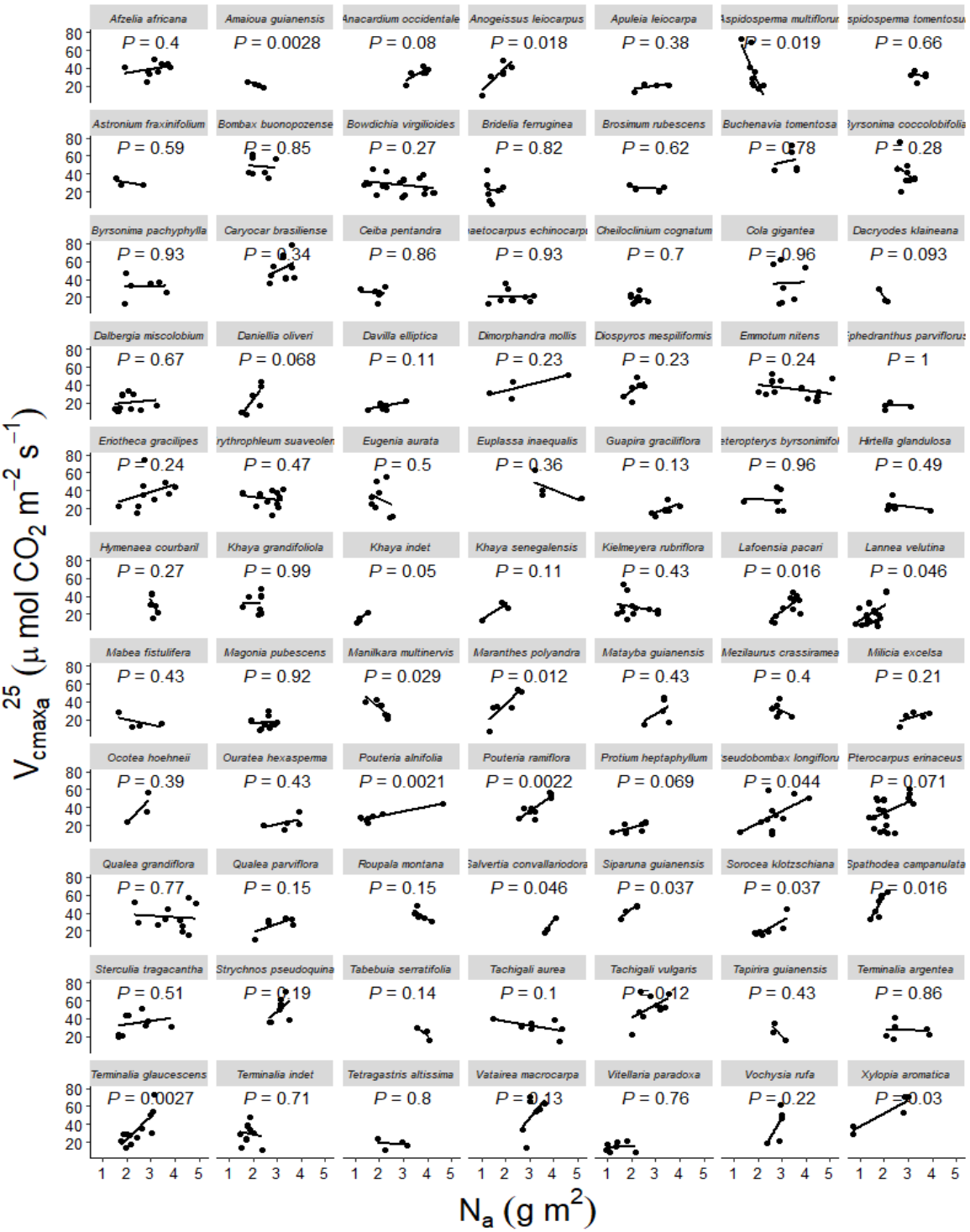


Fig. S4.3 Relationship between photosynthetic capacity, $V_{cmax,a}^{25}$, and leaf nitrogen concentrations, N_a, for each sampled species in Ghana and Brazil. *P* values indicate the significance of the relationship.



Appendix 4.1 Community weighted means

In order to compare traits among study sites while accounting for species abundance, we derived community weighted means (CWM; Violle *et al.* 2007) and standard errors following:

$$\mathrm{CWM}k = \sum \cdot i \cdot \mu_i \cdot f_i \qquad \text{Eqn S10}$$

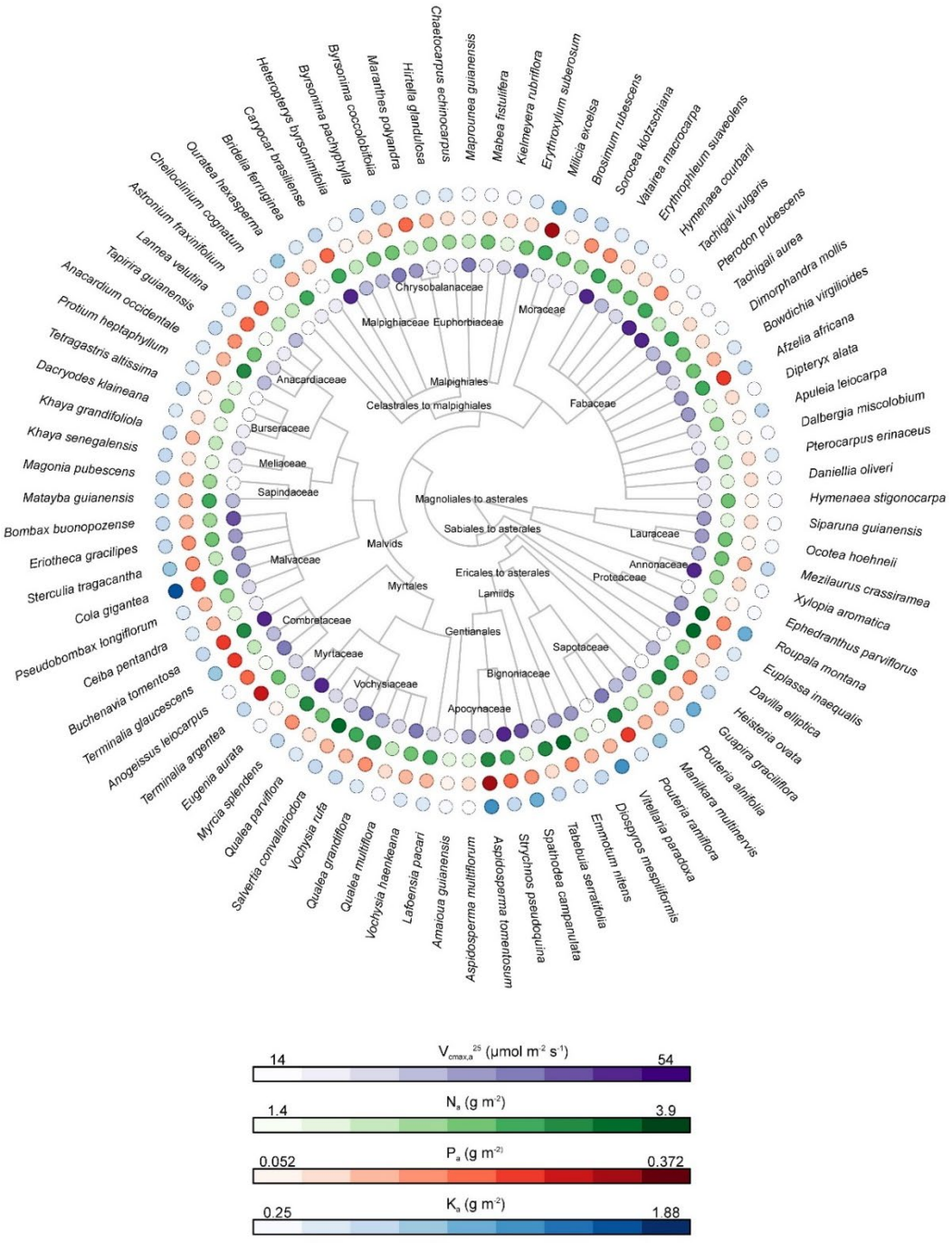
where k is a study plot, i is species, and μ_i and f_i are the average trait value and abundance of the species based on basal area, respectively.

Appendix 4.2 Phylogenetic analyses

We conducted a phylogenetic analysis of $V_{cmax,a}{}^{25}$ and foliar nutrients using species-mean values and a phylogeny constructed with the *phytools* package (Revell, 2012). The ultrametric tree was based on the R20100701 angiosperm supertree proposed in The Angiosperm Phylogeny Group III (2009) (available at https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20100701.new). Branch lengths were defined using the clade age estimates proposed by (Wikström *et al.*, 2001). The branch lengths were adjusted using BLADJ algorithm (Webb *et al.*, 2008). The methodology we use has its drawbacks but we believe it provides working estimates of branching times appropriate for our purposes. Blomberg's K statistic (Blomberg *et al.*, 2003) was calculated using species-mean values.

The phylogeny tree based on species studied in each forest-savanna transect and the average $V_{cmax,a}{}^{25}$ and leaf nutrient values for each taxon is shown in Figure S4. Visually there was no obvious pattern of certain clades or families having consistently higher or lower $V_{cmax,a}{}^{25}$, N_{area}, P_{area} or K_{area} values. This observation was supported by low Blomberg's K (0.34-0.38) indicating a lack of phylogenetic signal in the variability of $V_{cmax,a}{}^{25}$ and foliar nutrient concentrations.

Fig. S4.4 Ultrametric phylogenetic tree with mean area based photosynthetic capacity, $V_{cmax,a}^{25}$, and leaf nitrogen (N_a), phosphorus (P_a) and potassium (K_a) variations across clades. The tip colour intensity indicates the trait values. This phylogenetic tree includes species from Ghana and Brazil plots.



Chapter 5

The second data chapter addresses **Question 2** of the thesis: *How and why does leaf shape vary along the studied environmental gradient?* The findings of **Chapter 4** suggested that leaf morphological traits play an important adaptive role in environments of high evaporative demand and might one of the reasons behind the lack of observed relationship between photosynthesis and leaf nutrients. Here, I further explored this idea by investigating functional role of leaf shape in 72 woody angiosperm species along the entire gradient in Ghana, including study plots situated in moist semideciduous and wet evergreen forests in addition to forest-savanna ecotone plots. I measured leaf shape for 1753 leaves, estimated leaf thermoregulation according to the leaf energy budget theory and linked these with environmental variables, species light requirements, gas exchange traits and other morphological traits. I focused on local and regional variability of midday temperature and light as potential drivers of leaf shape. The findings of the study are presented in a paper entitled "Significance of leaf shape dynamics along light and temperature gradients in the tropics" which is under review for publication in *Oecologia*.

I was the main author and wrote the paper, conducted the fieldwork and carried out all of the data analysis with guidance from my supervisors Imma Oliveras and Yadvinder Malhi. The field campaigns were planned by Yadvinder Malhi and Imma Oliveras and coordinated by Imma Oliveras and collaborators in Ghana: Theresa Peprah and Stephen Adu-Bredu. In addition to data collection assistants (Mickey Boakye, Lydia Afriyie, Martini David and Jodra Miguel), Sami Rifai, Sophie Fauset and Richard Waites thought me the processing of leaf shape and thermoregulation data and Sam Moore set up and coordination of the plots in Ghana.

Chapter 5:

Significance of leaf shape dynamics along light and temperature gradients in the tropics

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5.1. Abstract

The causes behind interspecific leaf shape diversity are yet to be fully understood, especially in the tropics. We addressed this situation by quantifying leaf shape variability along large environmental gradients and by linking it with leaf functioning. Using quantitative geometric morphometric methods, we derived a leaf elongation parameter for 1753 leaves from 72 angiosperm tree species along gradients of rainfall (1,030 to 2,200 mm a^{-1}), midday temperature (30.7- 36.0 °C) and local light (sunlit and shaded branches) in Ghana, West Africa. We also measured leaf effective width and estimated leaf-to-air temperature differences (ΔT)**,** and thermal constants (τ) according to leaf energy budget theory. Elongated and narrow leaves were present at sites at both ends of the environmental gradient. Narrow leaves in the savanna woodland study site potentially enabled significantly lower ΔT and τ and greater maximum photosynthetic rates. We also observed significant differences in leaf shape among species with different light requirements for germination and establishment as well as sunlit and shaded leaves. Our results suggest that narrow and elongated leaf blades are multifunctional; They are important for optimising canopy light capture in shaded canopies and enable efficient thermoregulation possibly to maintain photosynthesis in sites of high evaporative demand and midday stomatal depression. Including leaf shape metrics in plant performance modelling can improve our understanding of ecosystem response to environmental change.

Keywords Lamina shape, thermoregulation, boundary layer, light interception, functional traits.

5.2. Introduction

Leaf shape is extremely diverse and due to its functional role this diversity can be indicative of local and global environments (Nicotra *et al.*, 2011). In palaeobotany, climate reconstructions are often based on shape of fossil leaves (Greenwood, 2005). For example, serration of the leaf margin is used as an indicator for temperature (Royer & Wilf, 2006), presence of drip-tips is a sign of high rainfall (Johnson & Ellis, 2002) and large leaves are indicative of wet and hot environments with little frost risk (Wright *et al.*, 2017). Relationships between leaf form and climate are much stronger when large climatic gradients are considered (e.g. Peppe *et al.*, 2011; Wright *et al.*, 2017), while findings of regional studies show more uncertainty (Malhado *et al.*, 2009b,a). This can partially be attributed to limited understanding of the role that leaf shape plays in the functioning of a plant, especially in complex tropical ecosystems.

One of the most prominent and recent topical suggestions for the functional role of leaf shape is thermoregulation (Penman, 1948; Michaletz *et al.*, 2016; Fauset *et al.*, 2018). Narrow leaves generate smaller boundary layers which results in faster heat transfer across the leaf (Vogel, 2009) and, consequently, smaller leaf-to-air temperature differences (ΔT) (Leigh *et al.*, 2017). This is particularly important in cold regions where vegetation is prone to frost damage and in hot and arid tropical and sub-tropical forests where leaves are at risk of overheating (Wright *et al.*, 2017).

The importance of leaf thermoregulation in the tropics is likely to become greater under increasing global temperatures, seasonality and aridity (Coumou & Robinson, 2013; Harris *et al.*, 2014). While most tropical species have high optimal photosynthesis temperatures and broad thermal tolerances, extremely high temperatures can affect carbon gain (Mathur *et al.*, 2014). Slot et al. (2018) demonstrated that optimum photosynthesis and stomatal conductance (g_s) temperature for evergreen species growing in Panama were 31.4-31.6 °C, approximately 1 °C and 4 °C lower than average midday and mean annual temperature, respectively. Moderate increases in above-optimum photosynthetic temperature are primarily dealt with g_s (Slot & Winter, 2017) and are thus reversible.

In situations where high temperature and high vapour pressure deficit result in stomatal closure, exceeding the upper temperature limit of heat tolerance leads to irreversible photosynthetic machinery damage. Midday g_s depression is particularly typical of tropical forest-savanna boundaries (Franco & Lüttge, 2002) which can potentially explain the prevalence of small and narrow leaves in arid and hot environments (McDonald *et al.*, 2003).

In theory, increased efficiency of cooling in leaves with smaller boundary layers can have a positive effect on gas exchange rates in adversely hot and dry conditions. To the best of our knowledge, there are no empirical studies that investigate links between leaf shape and photosynthesis, Nicotra et al. (2008) who assessed the effect of leaf dissection on gas exchange in African *Pelargonium* species. Here, lobed leaves had higher rates of carbon gain, but the authors suggested that this is due to correlated evolutionary responses of traits to high temperatures rather than the direct effect of smaller boundary layers. It is evident that the relationship between gas exchange and leaf shape is yet to be fully established.

Leaf shape also plays a significant role in light capture (Falster & Westoby, 2003; Kern *et al.*, 2004). Shade-avoidance syndrome is an elongation of the leaf lamina and/or petiole in response to adverse light conditions and it is a widely recognised phenomenon in the model plant *Arabidopsis thaliana* (Tsukaya, 2006). In large elongated leaves, for a given area a greater fraction of leaf area is located further from stem, which enhances light-interception efficiency by reducing self-shading (Pearcy & Yang, 1998). Within the wider context, leaf morphology adaptations to light availability are evident in studies comparing traits between sunlit and shaded leaves, with the latter exhibiting larger, thinner leaves with lower leaf mass per area (LMA) (Rozendaal *et al.*, 2006). The effect of light on leaf dimensions can be even stronger than that of other resources such as water (Xu *et al.*, 2009).

Modification of leaf shape comes with a trade-off in resource allocation between leaf support and functioning (Niinemets *et al.*, 2006). Larger and wider leaves require larger petioles to avoid leaf bending and larger greater minor vein density (*VD*) to avoid leaf margin curling (Xu *et al.*, 2009).

Large vein networks supply cells less efficiently and can result in large leaves photosynthesizing slower (Price & Enquist, 2007). On the other hand, Niinemets *et al.* (2007) suggested that leaves of different shapes do not require different biomass investments; rather, they fulfil their support requirements by adjusting biomass allocation between midrib, major and minor vein densities. These findings were based on temperate herbaceous and woody seedlings and might not hold true for tropical trees, which have higher canopy biomass investments (Malhi *et al.*, 1999).

While quantitative leaf shape measurements are well developed and widely employed in developmental and evolutionary plant research (e.g. Chitwood et al. 2014; Chitwood and Sinha 2016), studies that consider leaf shape across a large number of tropical species mostly use qualitative categorical leaf lamina descriptions (Hickey, 1973). Occasionally, numerical values are given to the leaf shape traits, for example, the angle of leaf apex, the length and width ratios of the leaf and length of drip-tips are measured (e.g. Panditharathna *et al.*, 2008; Malhado *et al.*, 2009b; Goldsmith *et al.*, 2017), but these methods are not widely employed. Qualitative leaf shape descriptors are difficult to apply across many species, cannot describe continuous variation of leaf shape, and have low statistical power. Our study uses quantitative determination of leaf shape based on geometric morphometrics, a method that provides coordinates for selected points on the leaf margin while preserving the geometry of the landmark configurations throughout the analysis (Zelditch *et al.*, 2012).

Understanding leaf shape variability along tropical forest-savanna vegetation gradients can offer insights to species and ecosystem adaptations to given environmental conditions. This is particularly significant considering the sensitivity of these transitions to current and prospective climatic changes (Aguirre-Gutiérrez *et al.*, 2019), specifically, increases in average and extreme temperatures, light availability due to changes in cloud density, global dimming due to areosols (Wild, 2009) and increases in forest gaps and edge effects. Therefore, in this study, we investigate the variability of leaf shape, specifically, leaf elongation and leaf effective width (e.g., Leigh *et al.*, 2017), at local and regional scales and the effect it has on leaf thermoregulation and photosynthetic traits. We base the

analyses on a large dataset (72 species, 203 trees) originating from different types of forests along a tropical rainfall of 1,030-2,200 mm a^{-1} and midday temperature (30.7- 36.0° C) gradient in Ghana, West Africa. We asked the following questions: how does leaf shape vary along the environmental gradient? Is leaf shape different among species of different ecological strategies? What effect does leaf shape have on leaf thermoregulation and photosynthesis? And what are the costs associated with adjustments in leaf shape?

Specifically, we hypothesise that:

H1: leaf laminas become narrower with decreasing rainfall and increasing midday temperatures. This is due to increased need for leaf thermoregulation to maintain photosynthetic rates in hot and arid conditions.

H2: light has a significant effect on leaf shape. Specifically, leaf shape differs a) between sunlit and shaded branches, and b) among species with different light requirements for germination and establishment (guilds).

H3: leaves of different shapes require different biomass investments. Specifically, this is evident in different *VD*, petiole size and leaf structural traits among different leaf shapes.

5.3. Materials and Methods

Study sites

Data were collected in seven 1 ha study plots which are part of an ongoing research project coordinated by the Global Ecosystems Monitoring Network (http://gem.tropicalforests.ox.ac.uk/) in Ghana, West Africa. The plots are situated along a vegetation gradient of three tropical forest types, each associated with distinct mean annual precipitation (MAP) and mean noon temperature (MNT).Three are located in dry semideciduous forest (MAP of 1,030 mm a^{-1} and MNT of 36.0° C), two in moist semideciduous forest (MAP of 1,500 mm a^{-1} and MNT of 34.0° C) and two in wet evergreen forest (MAP of 2,200 mm a^{-1} and MNT of 30.7° C) (Table 5.1). Within the different forest zones, plots are situated in close proximity to one another, usually, between a few hundred metres to a few kilometres apart, however, they differ in their vegetation composition (Table 5.1). Plots within the driest and hottest site are situated along a dry forest-savanna ecotone. One of the plots in the moist semideciduous zone exhibits more gaps in the canopy than the other study site, potentially due to disturbance as it is situated on the edge of the forest reserve. One of the plots in the wet evergreen forest zone is classified as a swamp due to seasonal flooding, whereas the other plot is an upland forest on a ridge top. Detailed descriptions of the edaphic conditions, productivity and carbon cycling of each plot are given in Moore *et al.* (2017).

Table 5.1 Key study site characteristics. Abbreviations: plot identification code that relates to other studies (Plot ID); mean annual precipitation (MAP); mean annual temperature (MAT); mean noon temperature (MNT; incoming short-wave radiation (SW); leaf area index (LAI);; total number of species, and number of species sampled.

Forest type	Wet evergreen		Moist semideciduous		Dry semideciduous		
Plot ID	ANK01	ANK03	BOB01	BOB02	KOG01	KOG03	KOG04
Vegetation type	Upland evergreen forest	Seasonal swamp forest	Moist semideciduous forest (edge effect)	Moist semideciduous forest	Dry forest	Transitional forest	Savanna woodland
Latitude	5.27	5.27	6.69	6.69	7.26	7.30	7.30
Longitude	-2.70	-2.70	-1.34	-1.34	-1.15	-1.16	-1.16
MAP (mm)	2200	2200	1500	1500	1030	1030	1030
MAT (°C)	25.0	25.0	25.7	25.7	26.4	26.4	26.4
MNT (°C)	30.7	30.7	34.0	34.0	36.0	36.0	36.0
SW (W m^{-2})	201.9	201.9	207.8	207.8	214.2	214.2	214.2
LAI	5.7	6.4	4.1	4.4	3.7	4.5	2.0
Wind speed (m s^{-1})	1.30	1.30	1.28	1.28	1.21	1.21	1.21
Basal area (m^2 ha^{-1})	28.81	25.65	22.60	30.20	20.65	14.55	8.79
Total no. species	106	93	87	83	40	35	29
Stem density (no. ha^{-1})	466	497	474	756	197	234	193
No. species sampled	38	13	22	14	15	11	7

Environmental variables

Climatic parameters listed in Table 5.1 were derived for each forest type over 1991-2016 using monthly time series from the Climate Hazards Group InfraRed Precipitation with Stations (CHIRPS) data archive version 2.0 at 0.05° resolution. Leaf area index (LAI) was estimated from 25 hemispherical photographs taken monthly in each 1 ha plot between 2012-2015 with a Nikon 5100 camera and a Nikon FishEye Lens of 8 mm (Nikon U.K. Ltd, Surrey, U.K). Images were analysed using the software Hemisfer (Thimonier *et al.*, 2010). We derived short-wave radiation parameter

(SW), an indicator of solar irradiance, from Global Land Data Assimilation System Version 2.1 (GLDAS) (Rodell *et al.*, 2004).

Leaf structural and functional traits

Field methods

In total we sampled 72 angiosperm tree species (Appendix 5.1) that constituted approximately 80% of the plot basal area (tree diameter at breast height > 10cm). All sampled trees were mature and canopy emergent. For each selected species within a plot, using a single rope climbing technique we sampled three trees; and within each tree we collected one fully sunlit and one shaded branch.

The cut branch was immediately placed and recut under water. Leaf gas exchange traits were measured using an open flow gas exchange system (LI-6400XT, Li-Cor Inc., Lincoln, NE, USA). Three leaves from each tree were sampled for maximum rate of net CO_2 assimilation at 400 ppm CO_2 (A_{sat}) and 2000 ppm CO_2 (A_{max}) transpiration at 400 ppm and 2000 ppm CO_2 (E_{sat} and E_{max}, respectively), average stomatal conductance (g_s) and dark respiration (D_{resp}) (μmol m^{-2} s^{-1} for all photosynthesis traits). The photosynthetic photon flux density was set to 2000 μmol m^{-2} s^{-1}, except for dark respiration measurements (0 μmol m^{-2} s^{-1}) and block temperature was kept constant throughout the sampling period at 30º C which was close to ambient air temperature.

Leaf thickness was measured using a micrometer (Tresna, 211- 101F). To determine leaf area (*LA*), leaves were scanned at 300 dpi resolution with a digital flatbed scanner (Canon LiDE 110) and weighed. Leaves were then placed in an oven at 70°C and dried to constant mass. Effective leaf width was defined as the diameter of the largest circle that could be fitted within the leaf's margin. Total *LA* (cm^2) not including the petiole or rachis and effective leaf width were measured using ImageJ and a custom MATLAB script (https://github.com/bblonder/leafarea). Leaf dry matter content

(LDMC; g g^{-1}), was calculated by dividing leaf dry weight by fresh weight. Photosynthetic trait values were averaged to branch level (*n*=255) for further analyses.

Leaf shape analysis

Leaf shape (or leaflet if compound) was determined from 1753 leaves using LeafAnalyser software (Weight *et al.*, 2008). The software evenly distributed 50 landmark points along the margin of each leaf and assigned coordinates (x;y) to each point. The coordinates were used to calculate eigenvalues for each leaf using Principal Component Analysis (PCA) in LeafAnalyser. The first principal component axis of leaf shape variation was identified as leaf size. As leaf area analysis was not the focus of our study we removed the size variation from the coordinates within the individual leaves using Procrustes superimposition method with *gpagen* function in *geomorph* package in R (Adams & Otárola-Castillo, 2013). Superimposition method scales raw coordinates to unit centroid size and rotates them until the sum of the squared distances between corresponding landmarks is minimized. Removing size variation of leaf shape allowed us to investigate leaf shape and leaf width as two separate leaf morphological traits. The scaled coordinates were again used to calculate eigenvalues for each leaf, that were further used in PCA to identify the main axes of leaf shape variation. The resulting principal component scores were averaged to branch level (*n*=299) for further analyses.

Venation

To expose the vein networks, 1 cm^2 pieces of leaf (avoiding major and secondary veins) were cleared using 5% NaOH solution, stained with safranin and mounted on a glass slide with a toluene based mounting medium. Values for venation traits were determined from hand-traced images following Blonder et al. (2017). Values of minor vein density were corrected for the leaf area that was lost during drying processes using random forest regression model in the *randomForest* package in R (Liaw & Wiener, 2002) using leaf thickness and fresh *LA* as predictors (Blonder *et al.*, 2012).

Thermoregulation

We estimated leaf-to-air temperature difference, ΔT, following the methodology described in Fauset *et al.* (2018). The calculation of ΔT (Eqn 1) is based on steady state leaf energy budget theory (Penman, 1948) and is modelled as a function of air temperature, leaf radiation load, evaporative energy exchange and boundary layer conductance (Jones, 2014):

$$\Delta T = \frac{r_{b,HR} \cdot (r_{b,W}+r_{l,W}) \cdot \gamma \cdot R_{ni}}{\rho_a \cdot c_{pa} \cdot [\gamma \cdot (r_{b,W}+r_{l,W})+s \cdot r_{b,HR}]} - \frac{r_{b,HR} \cdot \mathrm{NVPD}}{\gamma \cdot (r_{b,W}+r_{l,W})+s \cdot r_{b,HR}} \quad \text{Eqn 1}$$

Here, $r_{b,HR}$, $r_{b,W}$, $r_{l,W}$ (s m^{-1}) are boundary layer resistance to heat and radiation (Eqn 2), water (Eqn 5) and leaf resistance to water (Eqn 6), respectively:

$$r_{b,HR} = 1/(g_{b,H} + g_{b,R}) \quad \text{Eqn 2}$$

$g_{b,R}$ is boundary layer conductance to radiation (Eqn 3x):

$$g_{b,R} = 4 \cdot \varepsilon_{leaf} \cdot \sigma \cdot {T_a}^3 / \rho_a \cdot c_{pa} \quad \text{Eqn 3}$$

ε_{leaf} accounts for emissivity of the leaf (0.95), σ (W m^{-2} K^{-4}) is Stephan-Boltzman constant, ρ_a (kg m^{-3}) is temperature dependent density of dry air, c_{pa} (J kg^{-1} K^{-1}) is a specific heat capacity of dry air (Jones, 2014) and T_a is air temperature.

Boundary layer conductance, $g_{b,H}$ (s m^{-1}) is inversely correlated with boundary layer thickness (δ mm) which is determined by leaf dimensions and wind speed (Eqn 4), although other leaf morphology traits, such as trichomes, can also have an effect. Specifically:

$$\delta = \alpha \sqrt{W/U} \quad \text{Eqn 4}$$

where α=4 for flat leaves, U is wind speed (m s^{-1}) and W is leaf width (m).

$$r_{b,W} = 2/g_{b,H} \quad \text{Eqn 5}$$

$$r_{l,W} = 1/(g_s/d) \quad \text{Eqn 6}$$

g_s is stomatal conductance (mmol m^{-2} s^{-1}) and d is a temperature dependent conversion factor for g_s (Jones, 2014).

The leaf radiation load can vary in response to many factors that affect leaf temperature, therefore, in the leaf energy balance model it was calculated as net isothermal radiation (R_{ni}, W m^{-2}, Eqn 7).

$$R_{ni} = R_S \cdot (1 + \rho_S) \cdot \alpha_S + \varepsilon_{sky} \cdot \sigma \cdot T_{sky}^4 - \varepsilon_{leaf} \cdot \sigma \cdot T_a^4 \quad \text{Eqn 7}$$

Here, R_s (W m^{-2}) is for incoming shortwave radiation, ρ_s is reflection coefficient of the forest, α_S leaf absorptivity to total solar radiation, ε_{sky} is sky emissivity T_{sky} is sky temperature. Evaporative energy exchange also depends on the difference in vapour pressure, s (kPa °C^{-1}), between leaf and air which is a temperature dependent constant (Jones, 2014). Values and annotations for all variables required for the calculations are listed in Appendix 5.2.

Studies that have estimated leaf energy budgets have used thermocouple or infra-red thermometer data to validate the models (Dong *et al.*, 2017; Fauset *et al.*, 2018). These studies found that ΔT estimated from leaf energy balance and using the spot measurements matched well, only with small underestimation at higher actual leaf temperatures. Measuring leaf temperature *in situ* was beyond the scope of our study, therefore we substituted leaf level data with average day time conditions. Rather than deriving precise ΔT, with these estimations we aimed to demonstrate the potential importance of leaf shape in thermoregulation in tropical environments. While average air temperature varied minimally along the study gradient, there was a 6°C difference in midday temperatures among the study sites (Table 5.1). To best reflect limitations of air temperature on plant functioning, we used average midday air temperatures to estimate ΔT.

Thermal time constant (τ, s) is a trait that quantifies the cooling time of a leaf and it was estimated following Michaletz *et al.* (2016) (Eqn 8). Low values of τ indicate fast acclimation to changes in air temperature, whereas high τ is for leaves that respond slowly to changes in temperature. Thermal time constant depends on LMA, LDMC, heat transfer coefficient (H, Eqn 9) and specific heat capacity constants listed in Appendix 5.2.

$$\tau = \varphi \cdot LMA \cdot \left(\frac{c_{pw}}{LDMC \cdot H} + \frac{c_{pd} - c_{pw}}{H}\right) \quad \text{Eqn 8}$$

Where c_{pw} and c_{pd} (J kg^{-1} K^{-1}) are specific heat capacity of water and of dry leaf matter, respectively.

$$H = \rho_a \cdot c_{pa} \cdot \left(g_{b,H} + g_{b,R} + g_s/d \cdot s/\gamma\right) \quad \text{Eqn 9}$$

Ecological guilds

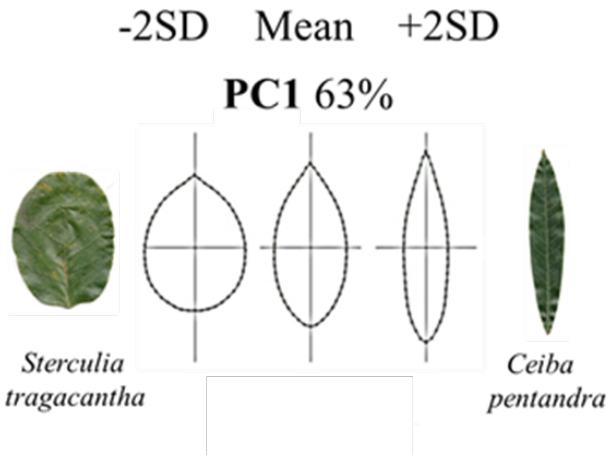
All studied species were classified into three ecological guilds according to their light requirements for germination and seedling establishment: pioneers, non-pioneer light demanders (N.P.L.D.), and shade bearers (Hawthorne, 1995). Distribution of species guilds across the study plots can be seen in Fig. S5.1. Pioneers have the greatest light requirements for germination and establishment, non-pioneer light demanders require light to develop beyond sapling stage and shade bearers can germinate and survive in the shaded parts of the forest.

Data analysis

All statistical analyses were conducted in R (version 3.4.0) using the R Studio interface (RStudio Team, 2018). One way analysis of variance (ANOVA) was used to explore differences in leaf shape and thermoregulation traits among study plots followed by post hoc Tukey's HSD test in the *agricolae* package (de Mendiburu, 2017), while linear regressions were used to test for relationships between leaf shape and climatic variables (H1) (Legendre & Legendre, 1998). Shapiro-Wilk and Flinger-Killeen tests were used to assess normality and homoscedasticity of model residuals. The results were illustrated using community weighted means (CWM) (Appendix 5.3), to account for uneven abundance of species and trees along the study gradient.

To allow for hierarchical nested structure of our dataset and potential phylogenetic non-independence of data, we tested relationships between leaf shape and other leaf functional traits using linear mixed-effects models (LMMs). Model assumptions were checked using protocols outlined in Zuur *et al.* (2009) and fitted using the *lme4* package in R (Bates *et al.*, 2015). For H1, we used leaf shape traits as predictors and leaf photosynthetic traits as response variables and species taxonomy and study site as random nested factors (plot+family/genus/species). The model structure for H2 testing was based on leaf shape traits as response variables, sunlit or shaded branch and light guild as categorical fixed effects and, and study site (plot) (and family/genus/species in the case of sun/shade analysis) as

random variable. Model significance was derived using "anova" function with Satterthwaite's method implemented in *lmerTest* package (Kuznetsova *et al.*, 2017). We report *t*, *P* values and marginalised r^2 for fixed effects (derived using *MuMin* package (Barton, 2018)). We used Pearson's Product-Moment Correlation (*r*) to test for relationship between leaf shape and morphological traits (H3).



5.4.Results

Leaf elongation

The first five principal components of the PCA performed on the coordinates of points placed along the margin of each studied leaf explained 94% of total leaf shape variability (Fig. S5.2). The average leaf shape across the studied species was elliptical. The first principal component (PC1) represented leaf lamina elongation (tip-to-base curvature) and explained 64% of the leaf shape variability (Fig. 5.1). The second principal component (PC2) explained 12% of leaf shape variance with high PC2 values suggesting presence of drip-tips (data not shown). Principal components 3-5 represented leaf asymmetry at the base, apex and across the leaf. We retained PC1 for further data analysis as it explained the majority of leaf shape variability. High values of PC1 indicated narrow oblong leaves and low values wide obovate leaves. The leaf shape PCA did not capture significant variability in leaf margin as only two species out of 76, *Triplochiton scleroxylon* and *Cola lateritia*, had lobed leaf margins.

Fig. 5.1 Leaf shape principal component indicating leaf elongation (PC1). The average leaf shape is shown in the middle, together with leaves for two standard deviations either side and example species of particular leaf shape. The analysis was based on landmark data derived from 1750 leaves from 72 species.

Leaf shape dynamics along the climatic gradient

Leaves generally became more elongated with increasing MAP and decreasing MNT (Fig. 5.2a). Wet evergreen forests displayed particularly elongated leaves while the semideciduous forests had much rounder leaves ($F_{2, 297}$=35.9, P<0.001). Tukey's post-hoc test revealed that, on average, leaf elongation was similar between study plots situated within each forest type, with the exception of the dry site plots (Fig. 5.2a). The savanna plot had oblong leaves of similar shape to leaves in wet evergreen plots and the transitional forest, had significantly rounder leaves than any other study plot.

Average leaf width across the study sites was 5.9 cm (±0.1 cm) with the narrowest leaves present in the wet evergreen seasonal swamp plot, as well as the savanna woodland plot (Fig. 5.2b). Despite being negatively correlated to lamina elongation (P<0.001, Pearson's r= -0.28), leaf width did not differ significantly among the forest types or the study plots (P=0.83 and P=0.06, respectively).

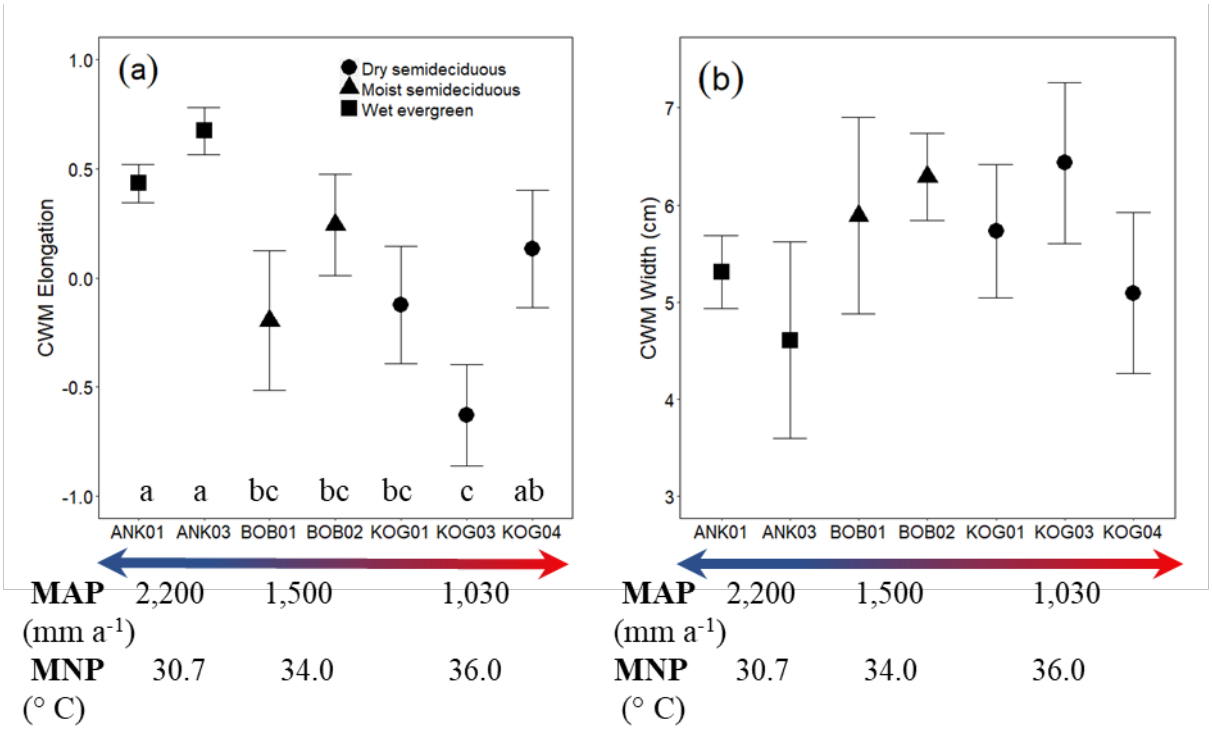


Fig. 5.2 Community weighted mean (CWM) leaf (a) elongation and (b) width (± 1 s.e.) across the study plots in three forest types denoted by different symbols (wet evergreen, moist semi-deciduous and dry semideciduous). The arrows indicate the direction of environmental gradient: mean annual precipitation (MAP) and mean noon temperature (MNT). Letters indicate significant results of post-hoc tests.

Estimation of leaf thermoregulation along the gradient

Leaf-to-air temperature differences estimated from leaf energy balance ranged between 0.1 and 5.1 °C and reduced gradually along the temperature and aridity gradient, being, on average, the highest in wet evergreen forests (3.0±0.3 °C) and the lowest in the dry semideciduous forest zone (2.4±0.2°C) ($F_{2, 247}$=73.2, P<0.001; Fig. 5.3a). This pattern was also evident in leaf boundary thickness, δ, which ranged from 11.9 to 28.5 mm across the dry-to-wet gradient. Estimated typical midday leaf temperatures ranged between 41.1°C in dry semideciduous forests and 33.4 °C in wet evergreen forests (Fig. S5.3).

Average thermal time constant, τ, was 32 s and ranged from 2 to 125 s. Similarly to ΔT, it was significantly different among the forest types ($F_{2, 235}$=82.7, P<0.001) as well as study plots ($F_{2, 231}$=29.1, P<0.001) with the highest values present in the upland wet evergreen forest (51.3±2.5 s) and the lowest in the savanna study plot (20.6±3.7 s) (Fig. 5.3b). Thermal time constants in the seasonal swamp plot were significantly lower than in the nearby situated plot, due to a lower average width (Fig. 5.2b).

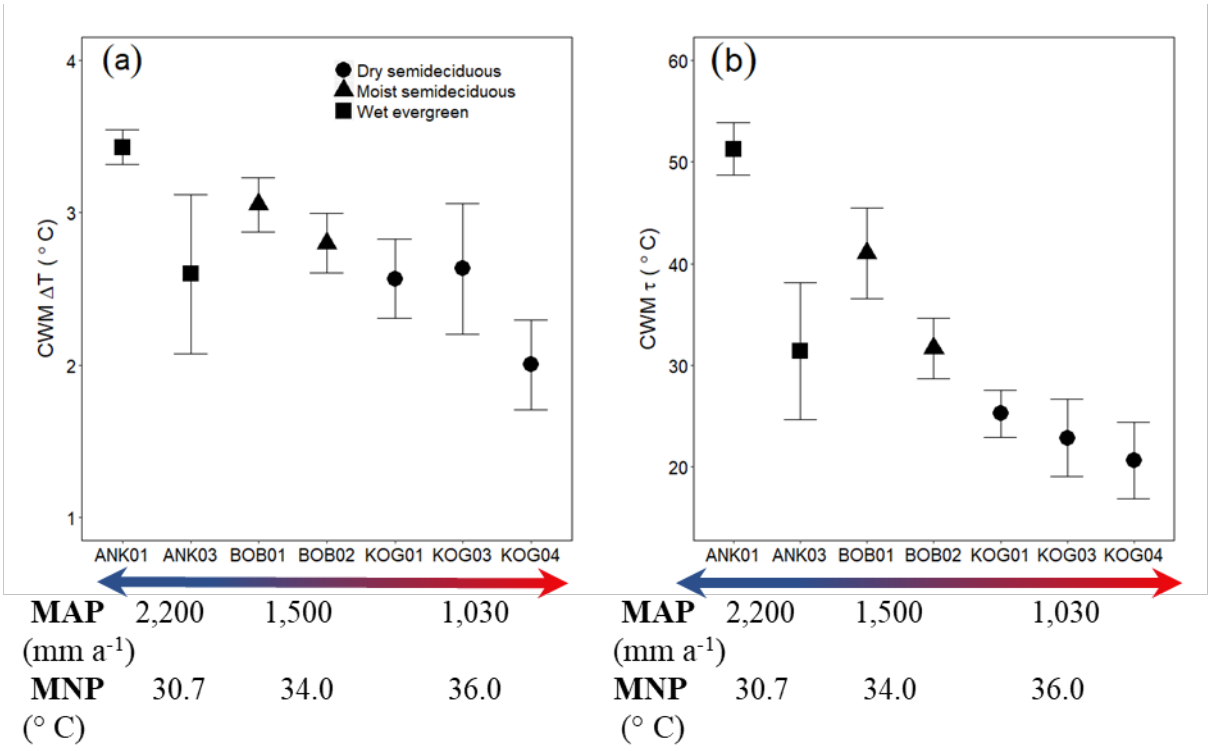


Fig. 5.3 Community weighted mean (a) leaf to air temperature difference (ΔT) from energy balance estimations and **(**b) thermal time constants (τ) with standard errors across the study plots in three forest types denoted by different symbols (wet evergreen, moist semi-deciduous and dry semideciduous). The arrows indicate the direction of environmental gradient: mean annual precipitation (MAP) and mean noon temperature (MNT). Letters indicate significant results of post-hoc tests.

Links between leaf shape and photosynthetic traits

We investigated the links between leaf shape and photosynthetic traits to better understand the benefits of having certain leaf shapes. We did observe an increase in gas exchange rates with increasing aridity and MNT along the study gradient (Table S5.1) as well as leaf elongation (Fig. 5.2a), however, leaf shape did not have a significant effect on photosynthesis when study site and taxonomic information were included as random effects in the models (Table 5.2). Leaf width showed a negative relationship with A_{max} across the gradient, however with very low marginalised r^2 value (0.02) (Table 5.2).

Table 5.2 Summary of linear mixed-effects models testing relationships between leaf shape (elongation and width) and gas exchange traits: saturated photosynthesis (A_{sat}), maximum photosynthesis (A_{max}), average stomatal conductance (g_s), dark respiration (D_{resp}), saturated transpiration (E_{sat}) and maximum transpiration (E_{max}). Plot, family, genus and species were treated as random effects.

Predictor	Elongation				Width			
Response	*n*	*t*	r^2	*P*	*n*	*t*	r^2	*P*
A_{sat}	255	-1.00	0.01	0.32	250	-1.06	0.00	0.29
A_{max}	255	-0.34	0.00	0.73	250	-2.42	0.02	0.02
g_s	255	-0.11	0.00	0.91	250	-1.28	0.01	0.20
D_{resp}	254	-1.64	0.01	0.10	250	-1.15	0.01	0.25
E_{sat}	255	-0.30	0.00	0.77	250	-0.87	0.00	0.39
E_{max}	255	-0.07	0.00	0.95	250	-0.68	0.00	0.50

We further investigated the relationship between leaf shape and photosynthesis within study plot levels. In this case, the savanna woodland plot stood out: there was a significant positive relationship between A_{max}, g_s and leaf elongation in this plot (t=2.3, r^2=0.32 and t=2.2, r^2=0.22, respectively, P<0.05, n=21). Leaf width showed even stronger relationship with A_{max} in the savanna woodland plot than across the gradient (t=-3.5, r^2=0.34, P<0.01).

Leaf shape and light requirements

Overall, leaves of pioneer and light demanding species were significantly rounder than those of species that do not require light for seed germination and seedling establishment (n=263, F=7.61, P<0.001) (Fig. 5.4). However, this pattern was not applicable for dry semideciduous forest zone where leaves of pioneer species were more elongated than in other guilds (Fig. S5.4). Leaf width was significantly lower in shade-bearing species than light demanders (n=256, F=3.21, P<0.05).

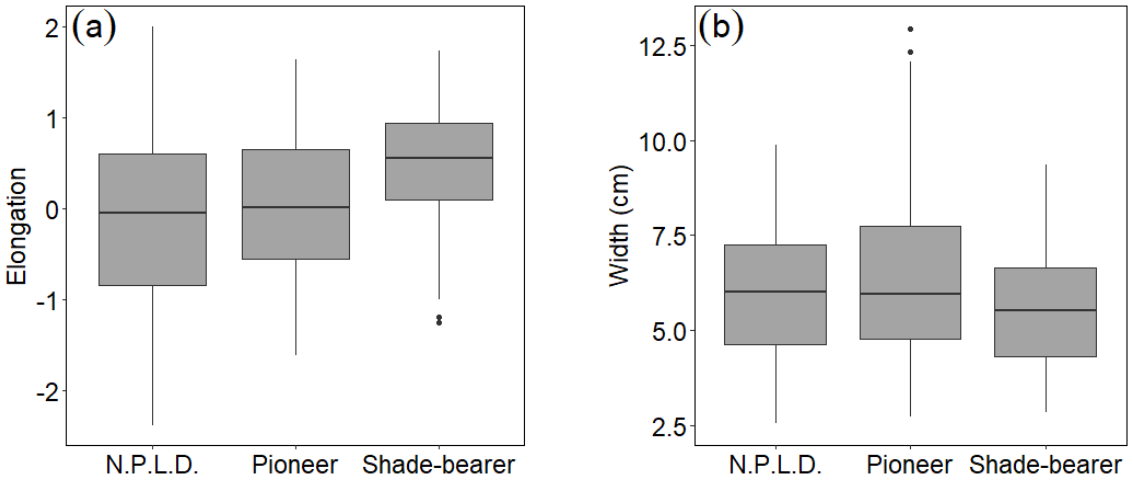


Fig. 5.4 Differences in a) leaf elongation and b) leaf width among species of different light requirements for germination and establishment (non-pioneer light demanders (N.P.L.D.), pioneers and shade-bearers).

Additionally, there was no statistically significant difference in leaf elongation between sunlit and shaded branches (n=299, F=0.12, P=0.73), however, shade leaves were on average 5% wider than sunlit leaves (n=291, F=14.16, P<0.001) with the greatest differences observed in the wet evergreen forests (Table S5.3).

Leaf shape and biomass investments

Leaf elongation was positively correlated with *LA* and negatively correlated with leaf thickness meaning that elongated leaves were, on average, larger and thinner than round leaves (Table 5.3). Additionally, elongated leaves tended to have lower *VD*. Correlations between leaf width and leaf size metrics (*LA*, thickness and petiole width) were positive and stronger than for leaf elongation. Additionally, wider leaves tended to have lower LDMC and greater *VD*. Both leaf shape metrics did not show any correlation with LMA. A particularly strong correlation was found between leaf width and petiole width.

Leaf shape trait	Trait	ρ	*P*
Elongation	*LA*	0.28	**<0.001**
	Thickness	-0.15	**<0.05**
	Petiole width	0.08	0.14
	LMA	-0.06	0.29
	LDMC	-0.11	0.06
	VD	-0.33	**<0.001**
Width	*LA*	0.93	**<0.001**
	Thickness	0.3	**<0.001**
	Petiole width	0.76	**<0.001**
	LMA	0.09	0.11
	LDMC	-0.20	**<0.05**
	VD	0.18	**<0.05**

Table 5.3 Spearman's correlations (ρ) among leaf shape features and leaf structural traits. The statistical significance is indicated in bold. Abbreviations: *LA*: leaf area; LMA: leaf mass per area; LDMC: leaf dry matter content; *VD*: minor vein density

5.5. Discussion

Leaf shape variability along the studied gradient

On average, leaves across the study sites were elliptical, which is known to be the dominant leaf shape in temperate (Traiser *et al.*, 2005) and tropical forests (Malhado *et al.*, 2009b). The first principal component axis of leaf shape variation, leaf elongation, in our study explained 64% of total lamina shape variance, which is similar as reported by Krieger *et al.* (2007) for 400 leaves from temperate deciduous, tropical wet and tropical dry forest ecosystems across an extensive temperature and rainfall range (9.5-25.9° C, 440-5000 mm a^{-1}). This suggests that, at least in unlobed angiosperm leaves, leaf lamina shape varies in the same way across temperate and tropical forest ecosystems with the main sources of variation being leaf roundness.

We hypothesized that leaves will become narrower and more elongated with increasing aridity and MNT as narrow leaves are beneficial in hot, arid and sunny sites due to smaller boundary layers and more effective leaf cooling (Leigh *et al.*, 2017). Contrary to this expectation, we found that leaves became more elongated with decreasing MNT and irradiance, with one exception, the savanna woodland plot, which also had elongated leaves. Leaf width did not vary significantly along the studied gradient, however, similarly to leaf elongation, the savanna woodland plot had the second lowest leaf width after the wet evergreen forest plots. This pattern of narrow and elongated leaves at sites of high as well as low evaporative demand has been observed across Amazonia and equatorial Africa (Jacobs, 1999; Malhado *et al.*, 2009b). Therefore, we propose that elongated and narrow leaf blades may be multi-functional and are adaptations to more efficient thermoregulation to support photosynthesis and to increase levels of light intercepting the canopy. Suggestions for both roles of leaf shape are discussed in the paragraphs below.

Role of leaf shape in thermoregulation and photosynthetic rates

Our findings suggest that the savanna woodland plot in the forest-savanna ecotone had elongated and narrow leaves to increase efficiency of thermoregulation in order to cope with extreme maximum temperatures when g_s declines due to midday depression which characteristic of forest-savanna boundaries (Franco & Lüttge, 2002). Lower species diversity, tree density, LAI, and shallower crowns in this plot (Table 5.1; Shenkin *et al.*, 2018, submitted) would result in greater exposure of the canopy to extreme irradiances, high within-canopy temperatures and low relative humidities (Leuzinger *et al.*, 2007; Rambo & North, 2009). Indeed, estimated mean daytime leaf temperature was significantly higher in these plots, in some species reaching 41°C (Fig. S5.3). Additionally, it is very likely that our estimations for ΔT are underestimated as studies that have measured leaf surface temperature in the tropics suggest the range of ΔT between 7 and 18 °C (Dong *et al.*, 2017; Fauset *et al.*, 2018). To avoid photoinhibition and even permanent damage to photosynthetic machinery efficient thermal regulation of the leaves is essential (Mathur *et al.*, 2014), which narrow, small leaves with resultant smaller boundary layers are potentially capable of achieving (Yates *et al.*, 2010). Leaf energy balance modelling suggested that ΔT (2.0±0.3°C) and thermal time constants (20.6±3.7 s) were lowest in the savanna woodland plot (Fig. 5.3).

It is also thought that the purpose of more efficient thermoregulation facilitated by narrower and elongated leaves is to enable greater photosynthetic rates (Yabuki, 2004). This suggestion was partially supported by our results as we found no overall relationship between leaf shape and photosynthesis, with one exception – the savanna woodland plot. Here, we observed a statistically significant relationship between both leaf shape metrics, elongation and width and A_{max}. Nicotra *et al.* (2008) also observed a positive link between leaf shape and photosynthesis in *Pelargonium* species. However, they proposed an alternative suggestion: environmental filtering favours a more narrow leaf shape in hot dry conditions, which are the same conditions that favour high gas exchange rates (Wright *et al.*, 2001). In our study, mixed effect models included plot and species taxonomic information as random effects and utilised large enough sample size to allow for site specific analysis

for links between leaf shape and photosynthesis traits. While our results were not statistically very strong, they provide some empirical support that leaf shape can facilitate greater maximum photosynthetic rates in hot and seasonally arid conditions, typical of forest-savanna ecotones (Oliveras & Malhi, 2016).

Support for links between light availability and leaf shape

Leaves in the wet evergreen forest study sites were, on average, significantly more elongated than in deciduous forests potentially to cope with adverse light conditions in the canopy. The stature of the forests changed significantly along the study vegetation gradient, with greater tree density, species richness, LAI, and depth of crowns evident in forests of higher rainfall (Table 5.1; Shenkin *et al.*, 2018, submitted). These factors are known to reduce the light availability and distribution within the canopy (Niinemets, 2010). Narrow and elongated leaves are more efficient at light interception as they reduce self-shading (Takenaka, 1994; Xu *et al.*, 2009), making leaf elongation particularly important in forests with layered canopy architecture and deeper levels of shading.

Effect of light availability on leaf shape was also evident when traits were compared between sunlit and shaded leaves with the strongest effects seen in the wet evergreen forest plots. The pattern of greater leaf width in shaded leaves is seen in other leaf morphological traits. For example, leaves tend to be larger and thinner in shaded environments due to reduced need for leaf-to-air temperature regulation (Rozendaal *et al.*, 2006; Wright *et al.*, 2017). Leaf elongation, however, was not significantly different between sunlit and shaded leaves. This trait is potentially more genetically conserved than leaf width as indicated by the variance partitioning (Appendix 5.4). Similarly, in the lowland tropical moist forests of Bolivia, it was found that trees avoided overheating by altering their leaf size rather than their leaf shape (Rozendaal *et al.*, 2006). Changes in petiole size while maintaining the same leaf shape can also enable plants to achieve favourable light conditions as seen in *Arabidopsis* (Kozuka *et al.*, 2005). Indeed, leaves had widest petioles in the seasonal swamp plot

(Table S5.3) where canopies were the most shaded as indicated by the highest LAI along the study gradient (Table 5.1).

Strong links between species with different light requirements for seedling germination and establishment and leaf shape further supports our finding that light availability plays an important role in leaf shape determination. Shade tolerant species in the wet evergreen forest study sites had on average more elongated and narrower leaf shapes than pioneers (Fig. S5.4) to deal with prolonged periods of shade due to slow growth. This pattern was also observed between pioneers and canopy gap-independent species in wet evergreen forests of Veracruz, Mexico (Pompa *et al.*, 1992).

Leaf shape and structural leaf investment

We hypothesised that larger, more elongated leaves would have greater requirements for support to withstand leaf bending (Takenaka, 1994). However, we found that elongated leaves were not thicker, did not have larger petioles or greater minor vein densities than round leaves, which suggests that leaf bending was not an undesirable effect. Bending can be advantageous for light interception as smaller investment in petiole results in steeper foliar inclination angles, more uniform dispersal of light within the canopy, and therefore greater exposure of photosynthesizing leaf area to light (Niinemets & Fleck, 2002). Additionally, both studied leaf shape metrics did not show any correlation with LMA. As LMA is strongly correlated with photosynthetic rates within species (Niinemets *et al.*, 2015) our findings suggest that trees can alter leaf shape to adjust to environmental conditions without compromising leaf functioning.

Conclusion

While there are few studies that assess the variability of leaf shape in tropical regions (e.g., Malhado *et al.*, 2009b; Goldsmith *et al.*, 2017) our study is the first that employs geometric morphometrics, a quantitative leaf shape assessment method, for a wide set of tropical forest taxa along a rainfall

gradient in West Africa. Additionally, our study is the first to estimate leaf thermoregulation efficiency along a large tropical gradient. Collectively, our findings suggest that leaf shape is multifunctional; it is an important adaptation to optimising canopy light capture in dense and shaded canopies as well as to enable more efficient thermoregulation in sites where differences between leaf and air temperatures are the greatest. In the latter conditions, more elongated and narrower leaves potentially have a positive effect on photosynthetic rates due to more efficient thermoregulation. Further research should asses the links between leaf shape and photosynthesis while accounting for species ability to cope with midday stomatal depression. The novel methodology used in our study can be applied to large datasets and expand the understanding of leaf morphology variation beyond leaf size.

Acknowledgements

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5.6. Supplementary Information

The following Supplementary Information is available for this article:

Fig. S5.1 Number of species of different deciduousness and guilds.

Fig. S5.2 Variances associated with leaf shape principal components.

Fig. S5.3 Estimated midday leaf temperatures.

Fig. S5.4 Elongation and width among guilds in each forest type.

Table S5.1 Means and differences for morphological and photosynthetic traits among the plots.

Table S5.3 Average elongation and width for sunlit and shaded leaves.

Appendix 5.1 Species list

Appendix 5.2 Leaf energy balance methods

Appendix 5.3 Community weighted means

Appendix 5.4 Variance partitioning of leaf shape traits

Fig. S5.1 Number of species of different light guilds in each study plot.

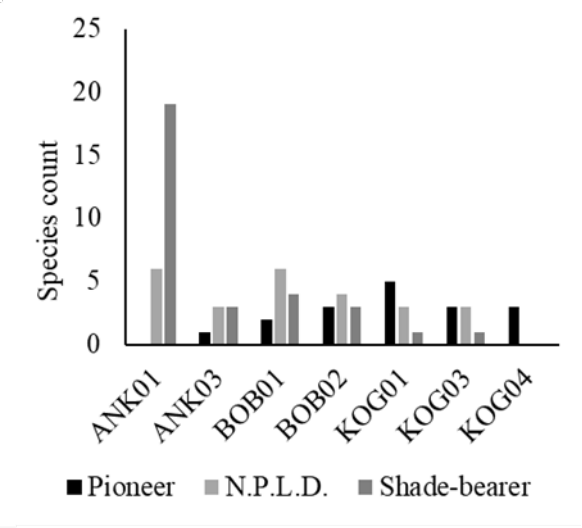


Fig. S5.2 Variances associated with principal components (PC1-PC5) resulting from the PCA on the coordinates of leaf shape.

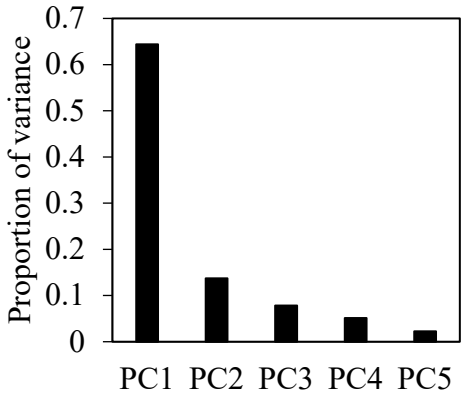


Fig. S5.3 Community weighted mean estimated midday leaf temperatures with standard errors across the study plots and vegetation types.

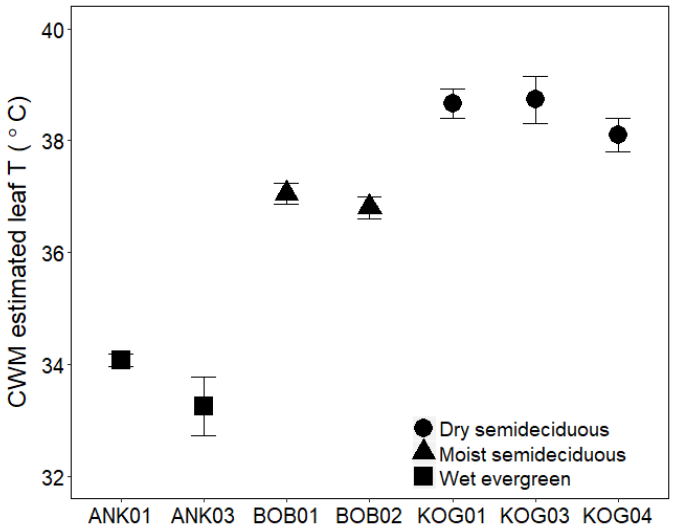


Fig. S5.4 Species abundance weighted a) elongation and b) width values among guilds in each forest type.

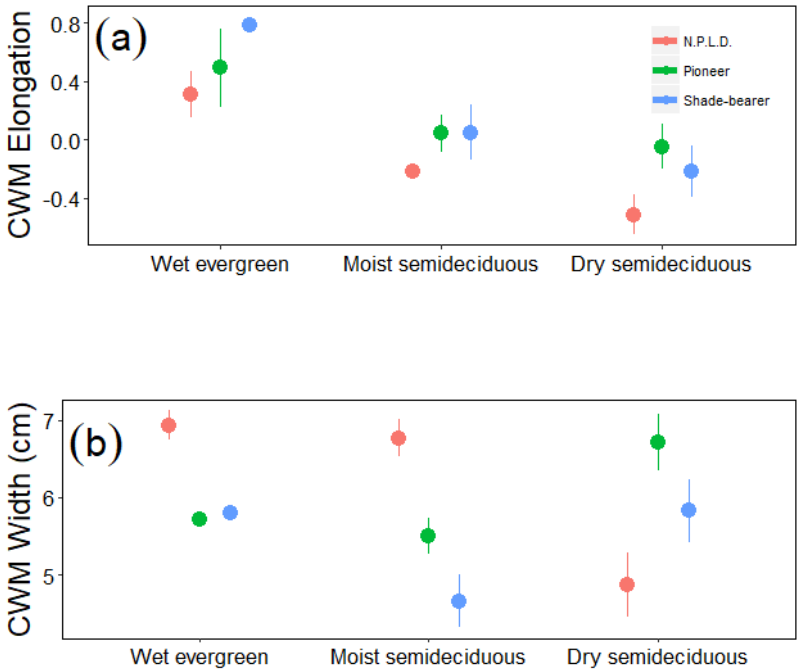


Table S5.1 Means and standard errors for leaf morphological traits for each study plot. Statistical differences for traits between the plots were tested using Kruskal-Wallis. All traits were significantly different between the plots. Post-hoc results are indicated by letters.

Trait		Plot						
		ANK01	ANK03	BOB01	BOB02	KOG01	KOG03	KOG04
	n	94	28	25	42	47	40	23
Petiole width (mm)	Mean	2.08	2.51	1.78	2.21	2.06	2.14	2.36
	± Standard error	0.09	0.13	0.13	0.13	0.11	0.11	0.15
	Significance	cd	a	d	abc	bcd	bc	ab
Leaf thickness (mm)	Mean	0.25	0.26	0.25	0.26	0.30	0.27	0.35
	± Standard error	0.01	0.01	0.02	0.01	0.01	0.02	0.02
	Significance	c	bc	c	c	b	bc	a
LMA ($g\ m^{-2}$)	Mean	86.23	90.98	114.71	86.36	102.15	112.46	133.47
	± Standard error	2.83	4.57	10.37	4.43	4.37	7.27	9.34
	Significance	c	bc	ab	c	b	ab	a
LDMC ($g\ g^{-1}$)	Mean	0.41	0.39	0.46	0.38	0.45	0.47	0.45
	± Standard error	0.01	0.02	0.02	0.01	0.01	0.02	0.01
	Significance	b	bc	a	b	a	a	a
A_{max} (µmol m-2 s^{-1})	Mean	13.31	18.14	21.67	21.04	22.04	20.78	19.17
	± Standard error	0.37	1.21	1.45	0.73	1.07	1.01	0.76
	Significance	c	b	a	a	a	ab	ab
g_s (µmol m-2 s^{-1})	Mean	0.06	0.08	0.09	0.13	0.17	0.17	0.17
	± Standard error	0.00	0.00	0.01	0.01	0.01	0.02	0.02
	Significance	d	c	c	b	ab	ab	a
Leaf size (cm^2)	Mean	63.64	86.47	51.25	67.75	57.91	56.94	74.12
	± Standard error	4.00	7.21	8.66	5.50	5.92	5.95	9.79
	Significance	b	a	c	ab	bc	bc	ab

Table S5.3 Means and standard errors for leaf elongation and width for sunlit and shaded leaves in each study plot.

		Elongation		Width (cm)	
		Mean	± Standard Error	Mean	± Standard Error
ANK01	Sun	0.50	0.08	5.38	0.26
	Shade	0.52	0.09	5.90	0.24
ANK03	Sun	0.62	0.15	6.61	0.47
	Shade	0.67	0.11	6.97	0.45
BOB01	Sun	-0.26	0.23	5.80	0.85
	Shade	0.05	0.25	4.95	0.35
BOB02	Sun	-0.13	0.19	6.16	0.37
	Shade	-0.12	0.16	6.44	0.32
KOG01	Sun	-0.15	0.20	5.88	0.50
	Shade	-0.06	0.18	5.87	0.49
KOG03	Sun	-0.52	0.17	5.67	0.41
	Shade	-0.61	0.17	6.63	0.53
KOG04	Sun	0.05	0.23	6.12	0.64
	Shade	0.25	0.23	6.56	0.64

Appendix 5.1 Species list

Family	**Genus**	**Species**
Achariaceae	Scottellia	*Scottellia klaineana*
Anacardiaceae	Lannea	*Lannea velutina*
Anacardiaceae	Lannea	*Lannea welwitschii*
Annonaceae	Cleistopholis	*Cleistopholis patens*
Apocynaceae	Funtumia	*Funtumia elastica*
Bignoniaceae	Spathodea	*Spathodea campanulata*
Burseraceae	Dacryodes	*Dacryodes klaineana*
Calophyllaceae	Mammea	*Mammea africana*
Cannabaceae	Celtis	*Celtis mildbraedii*
Cannabaceae	Celtis	*Celtis zenkeri*
Cardiopteridaceae	Leptaulus	*Leptaulus daphnoides*
Chrysobalanaceae	Maranthes	*Maranthes aubrevillei*
Chrysobalanaceae	Maranthes	*Maranthes glabra*
Chrysobalanaceae	Maranthes	*Maranthes polyandra*
Clusiaceae	Pentadesma	*Pentadesma butyracea*

Combretaceae	Anogeissus	*Anogeissus leiocarpus*
Combretaceae	Terminalia	*Terminalia indet*
Combretaceae	Terminalia	*Terminalia schimperiana*
Combretaceae	Terminalia	*Terminalia superba*
Ebenaceae	Diospyros	*Diospyros heudelotii*
Ebenaceae	Diospyros	*Diospyros mespiliformis*
Fabaceae	Afzelia	*Afzelia africana*
Fabaceae	Albizia	*Albizia zygia*
Fabaceae	Berlinia	*Berlinia indet*
Fabaceae	Berlinia	*Berlinia occidentalis*
Fabaceae	Bussea	*Bussea occidentalis*
Fabaceae	Calpocalyx	*Calpocalyx brevibracteatus*
Fabaceae	Cylicodiscus	*Cylicodiscus gabunensis*
Fabaceae	Cynometra	*Cynometra ananta*
Fabaceae	Daniellia	*Daniellia oliveri*
Fabaceae	Erythrophleum	*Erythrophleum suaveolens*
Fabaceae	Hymenostegia	*Hymenostegia afzelii*
Fabaceae	Pterocarpus	*Pterocarpus erinaceus*
Gentianaceae	Anthocleista	*Anthocleista vogelii*
Lamiaceae	Vitex	*Vitex indet*
Lamiaceae	Vitex	*Vitex micrantha*
Lecythidaceae	Petersianthus	*Petersianthus macrocarpus*
Malvaceae	Bombax	*Bombax buonopozense*
Malvaceae	Ceiba	*Ceiba pentandra*
Malvaceae	Cola	*Cola nitida*
Malvaceae	Heritiera	*Heritiera utilis*
Malvaceae	Nesogordonia	*Nesogordonia papaverifera*
Malvaceae	Pterygota	*Pterygota macrocarpa*
Malvaceae	Scaphopetalum	*Scaphopetalum amoenum*
Malvaceae	Sterculia	*Sterculia oblonga*
Malvaceae	Sterculia	*Sterculia rhinopetala*
Malvaceae	Sterculia	*Sterculia tragacantha*
Melastomataceae	Memecylon	*Memecylon afzelii*
Meliaceae	Khaya	*Khaya indet*
Meliaceae	Khaya	*Khaya senegalensis*
Meliaceae	Lovoa	*Lovoa trichilioides*
Meliaceae	Trichilia	*Trichilia indet*
Meliaceae	Trichilia	*Trichilia prieuriana*
Meliaceae	Turraeanthus	*Turraeanthus africanus*
Myristicaceae	Pycnanthus	*Pycnanthus angolensis*
Myrtaceae	Eugenia	*Eugenia whytei*
Olacaceae	Coula	*Coula edulis*
Olacaceae	Strombosia	*Strombosia pustulata*
Phyllanthaceae	Bridelia	*Bridelia ferruginea*
Phyllanthaceae	Protomegabaria	*Protomegabaria macrophylla*
Phyllanthaceae	Uapaca	*Uapaca corbisieri*

Putranjivaceae	Drypetes	*Drypetes aylmeri*
Rutaceae	Vepris	*Vepris indet*
Sapotaceae	Chrysophyllum	*Chrysophyllum pruniforme*
Sapotaceae	Chrysophyllum	*Chrysophyllum subnudum*
Sapotaceae	Manilkara	*Manilkara obovata*
Sapotaceae	Pouteria	*Pouteria alnifolia*
Sapotaceae	Vitellaria	*Vitellaria paradoxa*
Simaroubaceae	Hannoa	*Hannoa klaineana*

Appendix 5.2 Leaf energy balance methods

Table S5.4 Abbreviations, acronyms and symbols of variables used in leaf energy balance equations (Eqn 1-9) with values for constants when available.

Symbol	Units	Description in full	Value, if available
c_{pa}	J kg^{-1} K^{-1}	specific heat capacity of dry air	1012
c_{pd}	J kg^{-1} K^{-1}	specific heat capacity of dry leaf matter	2814
c_{pw}	J kg^{-1} K^{-1}	specific heat capacity of water	4181
d	dimensionless	unit conversion factor for g_s	40.3 at 25°C, 39.7 at 30°C, 39.0 at 35°C (Jones, 2014)
$g_{b,H}$	m s^{-1}	boundary layer conductance to heat	Eqn S4
$g_{b,R}$	m s^{-1}	boundary layer conductance to radiation	Eqn S5
g_s	mmol m^{-2} s^{-1}	stomatal conductance	
H	W m^{-2} K^{-1}	heat transfer coefficient	Eqn S9
LDMC	kg kg^{-1}	leaf dry matter content	
LMA	kg m^{-2}	leaf mass-to-area ratio	
NVPD	Pa	midday vapour pressure deficit of the air	
ρ_a	kg m^{-3}	density of dry air	1.183 at 25°C, 1.164 at 30°C, 1.146 at 35°C (Jones, 2014)
ρ_s	dimensionless	reflection coefficient of the forest	0.12 (Jones, 2014)
$r_{b,HR}$	s m^{-1}	boundary layer resistance to heat and radiation	Eqn S3

$r_{b,W}$	s m^{-1}	boundary layer resistance to water	Eqn S6
$r_{l,W}$	s m^{-1}	leaf resistance to water	Eqn S7
R_{ni}	W m^{-2}	net isotropic radiation	Eqn S2
R_s	W m^{-2}	incoming shortwave radiation	
s	kPa $°C^{-1}$	slope of the saturation vapor pressure curve versus temperature evaluated at T_a	257 at 31°C, 327 at 36°C, 297 at 34°C (Jones, 2014)
T_a	K	air temperature	
T_{sky}	K	sky temperature	assumed equal to T_a
U	m s^{-1}	wind velocity	
W	m	leaf width	
α_S	dimensionless	leaf absorptivity to total solar radiation	0.5 (Michaletz *et al.*, 2016)
γ	Pa K^{-1}	psychrometric constant	66.5 at 25°C, 66.8 at 30°C, 67.2 at 35°C
ΔT	°C	leaf-to-air temperature difference	Eqn S1
ε_{leaf}	dimensionless	emissivity of the leaf	0.95
ε_{sky}	dimensionless	sky emissivity	0.97
σ	W m^{-2} K^{-4}	Stephan-Boltzman constant	5.67 x 10^{-8}
τ	s	thermal time constant	Eqn S8
φ	dimensionless	ratio of projected to total leaf area	0.5 for flat leaves

Appendix 5.3 Community weighted means

In order to compare traits among study sites while accounting for species abundance, we derived community weighted means (CWM; Violle *et al.* 2007) and standard errors following:

$$\mathrm{CWM}k = \sum \cdot i \cdot \mu_i \cdot f_i \qquad \text{Eqn S10}$$

where k is a study plot, i is species, and μ_i and f_i are the average trait value and abundance of the species based on basal area, respectively.

Appendix 5.4 Variance partitioning of leaf shape traits

To explore the variation observed within each of the random terms (plot/family/genus/species/within species) we partitioned the variance based on a null model for each leaf shape trait (no fixed effects).

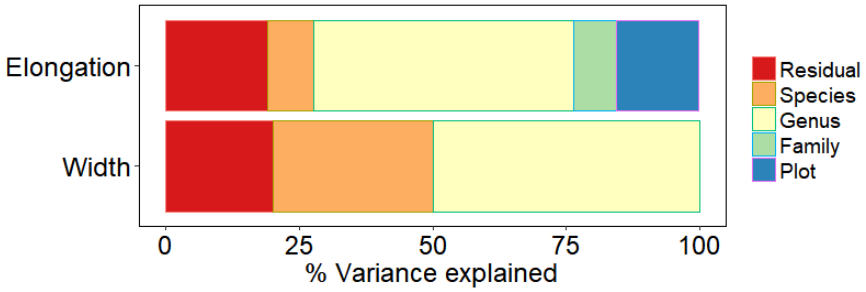


Fig. S5.5 Partitioning of the total variance for leaf elongation and width into phylogenetic (family, genus, species), environmental (plot) and residual components.

Variance decomposition of mixed effect models indicated that the variability of leaf elongation and width were controlled at different phylogenetic levels (Fig. S5). Leaf elongation was primarily determined at a genus level (50% variation), with a considerable environment (plot) effect (15.5%). Leaf width was mostly determined at species and genus level (50% and 29%, respectively) with no environmental or family effect. Around one fifth of variation in both leaf elongation and width was accounted by intraspecific and tree level variation.

Chapter 6

From the two previous chapters it is evident that species at the drier end of the gradient are in some ways adapted to functioning in environments of high evaporative demand. Additionally, in **section 2.4** of the literature review I highlighted the control that leaf venation networks play and important part in gas exchange regulation. Therefore, the third data chapter addresses **Question 3** of the thesis: *Does venation architecture play an important role in plant's adaptation to intermittent water availability?* The data used in this chapter uses venation, leaf morphology, nutrient, gas exchange and wood anatomy data for 66 species along the Ghana gradient.

The findings of the study are presented in the paper entitled "Water availability drives venation architecture along a tropical rainfall gradient in West Africa." which will in review in *Functional Ecology*.

I was the main author and wrote the paper, conducted the fieldwork and carried out all of the data analysis with guidance from my supervisors Imma Oliveras and Yadvinder Malhi. The field campaigns were planned by Yadvinder Malhi and Imma Oliveras and coordinated by Imma Oliveras and collaborators in Ghana: Theresa Peprah and Stephen Adu-Bredu. In addition to data collection assistants (Mickey Boakye, Lydia Afriyie, Martini David and Jodra Miguel), Benjamin Blonder thought me the processing of leaf venation data and Kasia Ziemińska provided wood anatomy data.

Chapter 6:

Water availability drives venation architecture along a tropical rainfall gradient in West Africa

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6.1.Abstract

Leaf vascular networks play a vital role in water transport, but the response of leaf veins to water availability remain poorly understood. Here, we tested a hypothesis that minor vein traits play an important hydraulic function that underlies plants adaptation/acclimation to intermittent water availability. We examined the variability of seven minor vein traits from for 66 woody angiosperm species along a precipitation gradient (1,030 mm – 2,200 mm a^{-1}) in Ghana, West Africa and in relation to leaf and wood functional traits that have previously been suggested to be correlates or predictors of species acclimation/adaptation to drought. We used phylogenetic comparative methods to understand if species evolutionary relatedness and/or environmental conditions were the main drivers of trait-trait relationships. We found that minor vein density (*VD*), vein areole loopiness and circularity varied significantly along the transect, with denser and more reticulated networks present at the driest sites. Venation showed the strongest links with leaf potassium (K_{mass}) and leaf area (*LA*), traits known to be important for osmotic adjustment and thermoregulation, respectively, in environments of high evaporative demand. Coordination between venation and morphological leaf traits was significantly stronger in drier sites vein and wood trait relationships were substantially stronger in more evolutionary related species. Our observations suggest that leaf vascular architecture varies in response to water availability and, potentially, is an import feature of a whole plant strategy in response to drought.

Keywords: vein density, vein reticulation, drought, functional traits, rainfall gradient.

6.2. Introduction

Current changes in rainfall regimes and widespread droughts are having a significant effect on tropical forest ecosystems as many species are already living at the edge of their functional hydraulic limits (Phillips *et al.*, 2010). These effects range from reduction of photosynthesis to reduction in the aboveground biomass, increased tree mortality or even large scale forest die-backs (Lewis *et al.*, 2011; Esquivel-Muelbert *et al.*, 2017; Yang *et al.*, 2018). Ecosystems that are particularly sensitive to short- and long-term changes in precipitation are tropical forest-savanna transitions as the stability of the boundaries between the two biomes is usually weak (Hirota *et al.*, 2011; Staver *et al.*, 2011a; Oliveras & Malhi, 2016). However, the diversity of tree hydraulic traits can mediate ecosystem resilience to drought and potentially maintain the stability of the ecotones (Oliveras & Malhi, 2016; Anderegg *et al.*, 2018b). With further predictions of changes in precipitation extremes across most of tropical forests (James *et al.*, 2013; Harris *et al.*, 2014) identifying and understanding traits that promote ecosystem resistance to moisture stress is a significant challenge for predicting climate change impacts on these tropical forest ecosystems.

Leaves are particularly important plant organs in environments prone to drought. During periods of water stress leaves can act as 'hydraulic fuses' (Wolfe *et al.*, 2016) and prevent embolism in stem and whole-plant hydraulic failure. This can be achieved by breaking the soil–plant–atmosphere continuum by leaf shedding before stems incur damage (Tyree & Ewers, 1991) and/or by having an anatomy able to maintain high levels of hydraulic resistance and resilience to hydraulic cavitation. Many leaf hydraulic traits are associated with drought tolerance or avoidance strategies (Marechaux *et al.*, 2015; Sack *et al.*, 2015; Anderegg *et al.*, 2018b). They are often linked to leaf phenology, specifically, evergreen species tend to exhibit traits that are tolerant to drought while deciduous species often have drought-avoidance traits (Poorter & Markesteijn, 2008). Thus, leaf morphology and anatomy vary significantly along rainfall gradients because of the pivotal role they play in tree and ecosystem response to water availability (e.g. Brewer & Nuñez, 2007; Ramírez-Valiente *et al.*, 2010; Maharjan *et al.*, 2011).

Architectural features of leaf venation are known to affect plant productivity and survival across species and ecosystems partially due to their impact on leaf hydraulic functioning (Sack & Scoffoni, 2013; Blackman *et al.*, 2018). Specifically, minor vein density (*VD*, length of minor veins per unit leaf area, units mm $^{-}$1) strongly influences leaf hydraulic conductance as it facilitates water movement inside and outside of xylem (Sack & Frole, 2006; Scoffoni *et al.*, 2011b; Buckley *et al.*, 2015). For this reason, certain venation traits are often considered to be indicative of plant drought tolerance (Sack & Scoffoni, 2013). Leaves with greater *VD* and reticulation (more loops and alternative pathways (Roth-Nebelsick *et al.*, 2001)) are less vulnerable to drought induced leaf xylem embolism as they provide more alternative routes for flow and more efficient water transport (Katifori *et al.*, 2010). The role of venation in leaf and plant hydraulics is also reflected in positive correlations between *VD* and aridity/temperature at smaller as well as cross-biome studies (Blonder *et al.*, 2013; Sack & Scoffoni, 2013), however, studies investigating the distribution of venation traits along rainfall gradients are very few. With expanding research into the functional significance of leaf vasculature, conflicting reports on the hydraulic relationships are also increasing. For example, Blonder *et al.* (2017, 2018) found no relationship between mean annual precipitation (MAP) and *VD* or other vein traits along a large Amazon-Andes transect.

Venation traits are often correlated with other traits linked with drier environments and thus proposed as potentially indicative of plant drought tolerance, such as leaf mass per area (LMA) and/or leaf area (*LA*) (Blonder *et al.*, 2013; Li *et al.*, 2015). Leaf mass per area is suggested to be correlate or predictor of drought tolerance, because it can be associated with sclerophylly and bulk modulus of elasticity which reduces leaf shrinkage and dehydration under drought (Wright *et al.*, 2005; Poorter *et al.*, 2009; Blonder *et al.*, 2012; Males & Griffiths, 2017). Additionally, small leaves are beneficial in environments of high evaporative demand due to smaller boundary layers (Peppe *et al.*, 2011; Nardini & Luglio, 2014; Leigh *et al.*, 2017; Gvozdevaite *et al.*, in prep.). Research exploring links between venation traits and other traits linked with drought tolerance or avoidance strategies is,

however, very limited. For example, no studies have compared the variability of venation architecture in species of different wood density and anatomy, different leaf habit, or varying foliar nutrient concentrations.

Linked with hydraulic functioning, pivotal role of venation is to maintain gas exchange rates (Brodribb *et al.*, 2007), especially, at sites of high evaporative demand (Scoffoni *et al.*, 2011a). The ability of the stomata to remain open when loosing water due to transpiration depends on the plant's capacity to replace that water. High *VD* can enable greater g_s, photosynthetic rates and less of vein blockage (Brodribb *et al.*, 2007; Sack & Scoffoni, 2013) which are particularly important in sites of intermittent water supply. Venation architecture has been proposed to play a key role in the evolution of high leaf-level stomatal conductance (g_s) in angiosperms (Boyce *et al.*, 2009; Brodribb *et al.*, 2010; Zwieniecki & Boyce, 2014) which is evident in strong empirical links between *VD* and g_s (McElwain *et al.*, 2016). However, some studies are suggesting decoupling of leaf hydraulic and economic strategies (leaf economic spectrum, (Wright *et al.*, 2004)), especially in tropical regions when a large number of species is considered (Li *et al.*, 2015). Denser venation networks can displace photosynthetic tissue and incur greater carbon costs (John *et al.*, 2017).

To improve understanding of the role that venation geometry plays in drought prone tropical ecosystems, our study measured the variability of venation in 66 angiosperm tree species growing along a strong rainfall gradient of 1,030-2,200 mm a^{-1} in Ghana, West Africa. We conducted the research in this region as tropical forests of Central and West Africa are also demonstrating drying trends (Malhi & Wright, 2004; Fauset, 2012), but in contrast to the Amazon, are much less studied. We used seven minor venation traits (described in detail in section 2.3. and Blonder *et al.* (2018): minor *VD* (mm^{-1}), areole loopiness (mm^{-2}) and circularity (mm), vein radius (mm), freely ending veinlet (FEV), minimum spanning tree (MST), and meshedness ratios. Higher FEV, MST and lower loopiness and meshedness values indicate lesser reticulation of the vein network: more redundant and tree-like pathways (Ronellenfitsch & Katifori, 2017). In addition to the variability of venation architecture, we also explored links between venation traits and other functional traits correlated with

drought performance strategies. As many trait-trait and trait-environment relationships are significantly affected by species phylogenetic relatedness (Cavender-Bares *et al.*, 2009; Reich, 2014), we analysed these relationships using phylogenetic comparative methods. Species that are closely related tend to have more similar suites of traits than would occur randomly (Cavender-Bares *et al.* (2009) and not accounting for this factor can skew the interpretation of our findings.

Specifically, we hypothesized that:

H1. minor vein density and reticulation traits (more loops and branching) will increase with decreasing precipitation.

H2. minor vein density and reticulation traits will be correlated with functional traits indicative of drought strategies, specifically, a) higher in evergreen than in deciduous species, b) positively correlated with wood density, LMA, leaf potassium concentration, stomatal conductance and negatively with leaf size.

H3. evolutionary distant species will have similar trait values due to strong abiotic filtering at the drier end of the gradient.

6.3. Materials and Methods

Study sites

Data were collected in seven 1 ha study plots situated along a precipitation gradient in Ghana (1,030 mm -2,200 mm a^{-1}). These plots are part of an ongoing research project coordinated by the Global Ecosystems Monitoring Network (GEM; http://gem.tropicalforests.ox.ac.uk/). The study plots are situated in three distinct forest types; three are located in dry semideciduous forest (1,030 mm a^{-1}), two in moist semideciduous forest (1,500 mm a^{-1}) and two in wet evergreen forest (2,200 mm a^{-1})

(Table 6.2; Hall & Swaine (1981)). Plots within each forest type were located in close proximity, presented relatively homogeneous soil substrates and the same climatic conditions.

Table 6.2 Key study site characteristics. Abbreviations: mean annual precipitation (MAP); mean climatic water deficit (MCWD); mean annual temperature (MAT); maximum noon temperature (MNT) (based on monthly averages); short-wave radiation (*SW*); leaf area index (LAI); total soil phosphorus (*P*); nitrogen (*N*); carbon (*C*); calcium (*Ca*); potassium (*K*); magnesium (*Mg*); cation exchange capacity (CEC). Climate data were derived using monthly time series from the Climate Hazards Group InfraRed Precipitation with Stations data archive version 2.0 at 0.05° resolution (Funk *et al.*, 2015). Soil nutrient concentrations are for the soil depth of 0-0.3m. Soil chemistry data were provided by Moore *et al.* (2017).

Study site	Wet evergreen	Moist semideciduous	Dry semideciduous
Latitude (°)	5.27	6.69	7.30
Longitude (°)	-2.70	-1.34	-1.16
MAP (mm)	2200	1500	1030
MCWD (mm)	-189.60	-308.90	-383.10
MAT (°C)	25.00	25.70	26.40
MNT (°C)	30.70	34.00	36.00
SW (W m^{-2})	201.90	207.80	214.20
LAI	6.05	4.40	4.50
P (mg kg^{-1})	128.25	258.28	74.60
N %	0.15	0.16	0.05
C %	2.27	1.71	0.67
Ca (mg kg^{-1})	33.41	657.55	307.90
K (mg kg^{-1})	32.99	49.01	35.60
Mg (mg kg^{-1})	35.61	133.65	78.60
CEC ($mmol^{+}$ kg^{-1})	19.58	45.99	22.84
Sand (%)	69.49	46.74	79.70
Clay (%)	17.18	28.77	3.30
Silt (%)	13.32	24.47	17.02

The sites exhibit strongly seasonal distribution of rainfall with two wet seasons and two dry seasons per year (Fig. S6.1). The main wet season lasts from April through June with a secondary wet season from September to November. The first wet season in the wet evergreen forests is significantly more pronounced than the second one.

Field methods

In the seven 1 ha plots we sampled 66 angiosperm tree species (Table S6.1) that accounted for approximately 80% of the plot basal area (tree diameter at breast height > 10cm). Around 60% of species were evergreen. For each selected species within a plot, we sampled three trees; and within each tree we collected one fully sunlit and one shaded branch. All sampled trees were mature canopy trees.

Leaf gas exchange traits were measured using an open flow gas exchange system (LI-6400XT, Li-Cor Inc., Lincoln, NE, USA). Three leaves from each branch were sampled for light-saturated rate of net CO_2 assimilation at ambient (400 ppm) CO_2 (A_{sat}) and g_s (μmol m^{-2} s^{-1}) at 400 ppm CO_2 from which $_i$WUE was calculated (A_{sat}/g_s). The photosynthetic photon flux density was set to 2000 μmol m^{-2} s^{-1} and block temperature was kept constant throughout the sampling period at 30ºC which was close to ambient air temperature.

Leaves, or all leaflets if compound, were collected immediately following the gas exchange measurements, had petioles and rachis removed, were scanned at 300 pixels per inch resolution with a digital flatbed scanner (Canon LiDE 110) and weighed. Leaves were then placed in an oven at 70°C and dried until the mass reached constant mass. Total leaf lamina area (cm^2) was calculated from scanned images using NIH ImageJ (http://rsbweb.nih.gov/ij/) with a custom MATLAB script (https://github.com/bblonder/leafarea). Dried leaf mass was divided by leaf area to calculate LMA.

Wood density and anatomy

Stem wood density measurements were performed on twigs of the terminal branches. From each branch we cut three wood segments (± 1-2 cm diameter), removed the bark, and suspended in water and measured the mass of displaced water. Wood segments were then dried at 70 °C until they reached constant weight. Wood density was calculated as the bark-free dry mass divided by bark-free volume (g cm^{-3}) (Pérez-Harguindeguy *et al.*, 2013).

Wood anatomical traits were measured on ~8-10mm diameter twigs, on three replicates per species. Cross sections, about 20-50µm were made using a sliding microtome, stained in safranin O and alcian blue, and permanently mounted on a microscopic slide. A pie shaped segment stretching from pith to cambium was photographed using OptronicsMicrofire camera mounted on Olympus BX-50 microscope and PictureFrame software. All vessels within a pie region were marked and coloured using magic wand tool (GIMP, http://gimp.org) and interactive pen display (Wacom Cintiq 22HD). Next, vessel area, average diameter (average of minimum and maximum diameters) and pie region area were measured using ImageJ (Rasband, 1997). Vessel density (mm^{-2}) was calculated as total number of vessels within a pie region divided by the pie region area. Parenchyma fraction was measured using a grid method (Ziemińska *et al.*, 2015); A grid was overlaid over a pie region and all points that fell within parenchyma tissue were counted. Parenchyma fraction was calculated as sum of all point that fell within parenchyma tissue divided by total number of points overlaid over the pie region.

Venation traits

Detailed methodology for leaf vein clearing and processing is available at https://github.com/bblonder/venation_programs (Blonder *et al.*, 2017). In summary, to expose the vein networks, 1 cm^2 pieces of dried leaf were submerged in aqueous 5% NaOH solution and left in

it for 1-4 days until transparent. In some cases, thick epidermis was removed using a soft brush to aid the clearing process. The samples were then bleached, washed and placed in ethanol for 30 min before being stained with safranin and mounted on a glass slide with a toluene based mounting medium. Images were taken using OptronicsMicrofire camera mounted on Olympus BX-50 microscope. Values for venation traits were determined from hand-traced images following (Blonder & Enquist, 2014). Values of scale-dependent traits (*VD*, areole loopiness, circularity and vein radius) were corrected for the leaf area that was lost during drying processes using the random forest regression model in the "randomForest*"* package in R (Liaw & Wiener, 2002) using leaf thickness and fresh leaf area as predictors (Blonder *et al.*, 2012).

Phylogenetic tree

To account for the effect evolutionary relatedness on the relationships between vein and other traits we analysed the data using phylogenetic comparative methods, specifically, phylogenetic least-squares (PGLS) regressions. A phylogenetic tree used in the PGLS analysis was constructed with the Phylocom software (Webb *et al.*, 2008) which was accessed in RStudio using "phylocomr" package (Ooms & Chamberlain, 2016). The tree was built using the most recent R20160415 angiosperm phylogeny megatree (Gastauer & Meira Neto, 2017) with branch lengths adjusted using ages file estimated by (Magallon *et al.*, 2015). For phylogenetic analysis to be possible, data analysis was performed on site-specific species trait averages. Prior to averaging the data, we tested if venation traits were significantly different between sunlit and shaded branches (Table S6.2) or if proportion of trait variance (Fig. S6.2) was greater within species rather than among species, which was not the case. Similar findings were also observed by Blonder *et al.* (2018).

Data analysis

All statistical analyses were conducted in R (version 3.4.0) using the R Studio interface (RStudio Team, 2018). Differences in venation traits along the rainfall gradient (all species together and

evergreen and deciduous species separated) were tested using one-way analysis of variance (ANOVA) followed by post hoc Tukey's HSD test in the "agricolae" package (de Mendiburu, 2017) (H1). Model residuals were tested for non-normality and heteroscedasticity and predictors were natural-log or squared root transformed if applicable.

To test the differences in traits between deciduous and evergreen species (H2a) we used linear mixed-effects models. This method was chosen to account for uneven number of samples within each group in each forest type. We used venation traits as response and leaf habit (deciduous or evergreen) as predictor variables and forest type as a random factor. Model assumptions were checked using protocols outlined in Zuur *et al.* (2009) and fitted using the "lme4" package (Bates *et al.*, 2015). Model significance was derived using "anova" function with Satterthwaite's method implemented in "lmerTest" package (Kuznetsova *et al.*, 2017).

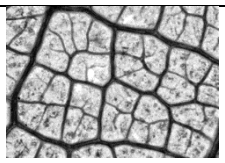
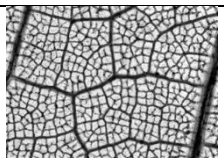
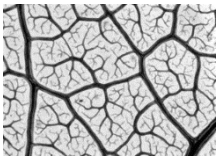
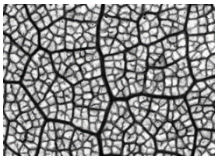
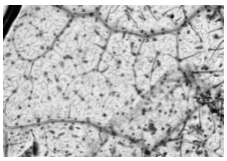
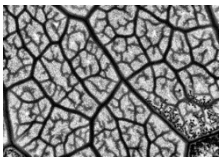
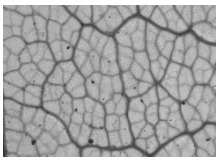
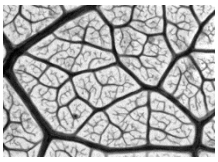
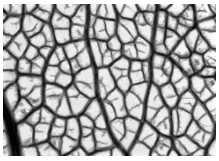
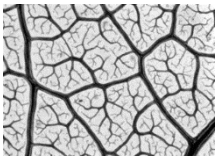
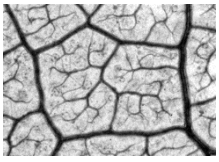
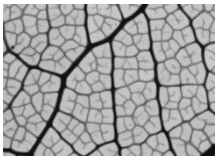
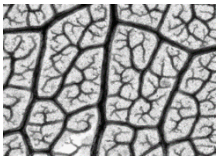
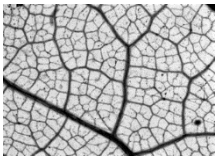
We tested relationships between venation and other leaf and wood functional traits using phylogenetic least-squares (PGLS) regressions using "caper" package (Orme *et al.*, 2018) (H2b). We performed six different sets of PGLS models: one including all species along the transect, three including species specific to each forest type (wet evergreen, moist semideciduous and dry semideciduous) and two including deciduous and evergreen species only. The splitting of the dataset allowed us to investigate the trait-trait relationships within each group and, followingly, compare them. Model fit by maximum likelihood estimates Pagel's λ that provides the best fit of the data. Values of the λ vary from 0 to 1; when λ is equal to 0, all phylogenetic structure is removed, and all species are effectively phylogenetically independent (Pagel, 1999). When λ is 1, traits covary in direct proportion to shared evolutionary history (i.e. they conform to a Brownian model of trait evolution). This analysis allowed us to test the effect that phylogenetic relatedness of species has on trait-trait relationships (H3).

6.4.Results

Distribution of vein traits

Minor venation traits varied substantially among the species with loopiness showing greatest range of values (Table 6.3).

Table 6.3 Variation of minor venation traits among 66 sampled tree species. Min- minimum value, max-maximum value, S.E. – standard error, *VD* – minor vein density, FEV – freely ending veinlet ratio, MST - minimum spanning tree. Images of venation patterns represent species with the lowest and the highest value of each trait. All images are of the same scale.

	Min.	Mean	Max.	S.E.	Example of min. value	Example of max. value
VD (mm^{-1})	8.89	18.21	28.76	0.52		
Loopiness (mm^{-2})	1.91	14.51	48.08	1.18		
Radius (mm)	0.01	0.02	0.04	0.00		
FEV	0.15	0.36	0.55	0.01		
MST	0.64	0.78	0.94	0.01		
Circularity (mm)	0.62	0.71	0.79	0.00		
Meshedness	0.95	1.02	1.10	0.00		

All dimensional venation traits varied significantly along the rainfall gradient. Vein density, areole loopiness and circularity were significantly different among the evergreen and deciduous forests (P<0.05, $F_{2,65}$=11.92, $F_{2,65}$=6.97 and $F_{2,65}$=3.15, respectively). Values of these traits were the lowest in the wet evergreen forests at the wettest end of the transect and the highest in the semideciduous forests (slightly higher in dry than moist forests) (Fig. 6.1). Similarly, minor veins had significantly larger diameters in evergreen forests than in semideciduous ($F_{2,65}$=3.18, P<0.05). Scale independent traits (FEV, MST and meshedness) did not vary significantly along the rainfall gradient.

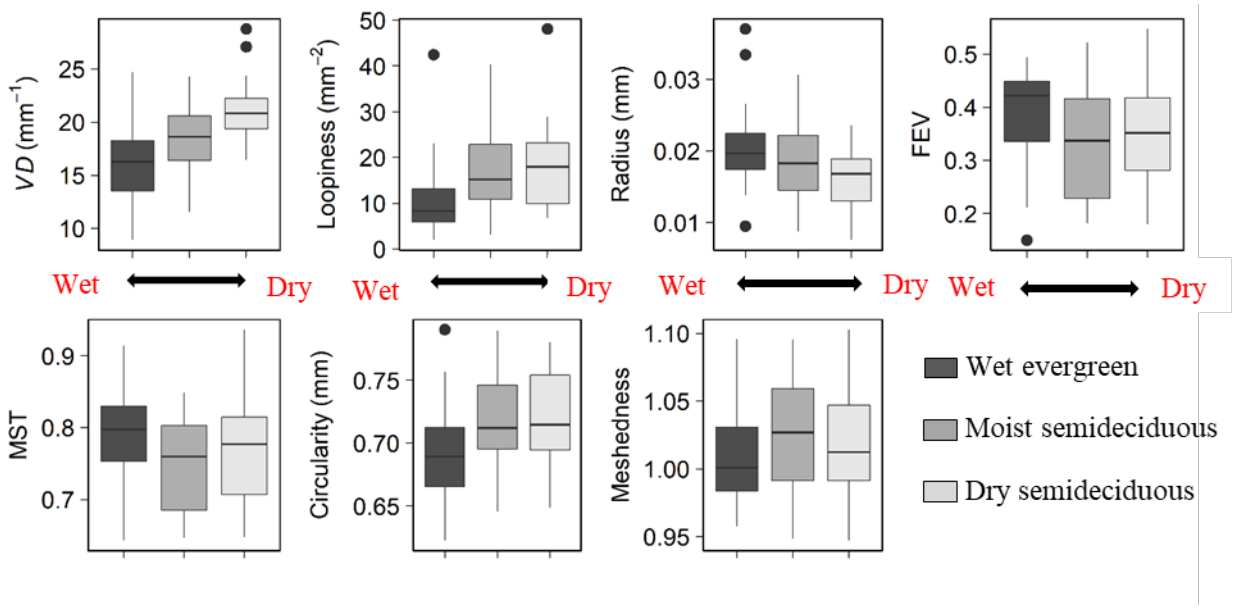


Fig. 6.1 Distribution of minor venation traits in three forest types situated along the rainfall gradient. The arrows indicate the direction of the rainfall gradient. The box boundaries represent 25th and 75th percentiles; the median (solid line); whiskers: 10th and 90th percentiles.

Vein traits between species of different leaf habit along the rainfall gradient

All measured minor vein traits, with the exception of radius, were significantly different between species of different leaf habit (P<0.05). In most cases, vein density, areole loopiness, circularity and meshedness values were higher in deciduous species, while MST and FEV ratios were higher in evergreen species (Fig. 6.2). The greatest differences were observed in areole loopiness, which was

almost 75% higher in deciduous than in evergreen species ($F_{1,65}$= 20.2, P<0.001). When assessed separately, species of different leaf habit did not vary in vein traits along the rainfall gradient, except for *VD* which was significantly higher in dry semideciduous than moist and evergreen forests in evergreen species ($F_{2,36}$=3.79, P<0.05). Even though not significant, deciduous species showed stronger rainfall gradient related trends.

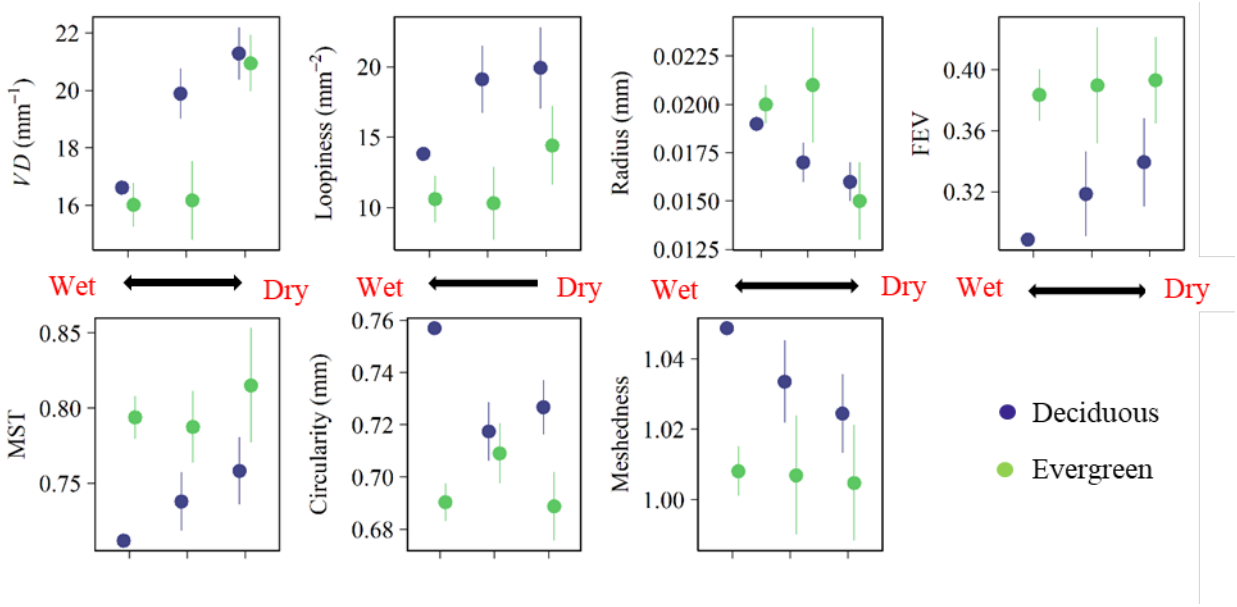


Fig. 6.2 Distribution of minor venation traits between deciduous and evergreen species along the rainfall gradient. The arrows indicate the direction of the rainfall gradient. Circles indicate mean values and lines are for ±1 S.E.

Trait-trait relationships along the rainfall gradient

Results for PGLS models between venation traits and other leaf and wood functional traits across all studied species are presented in Table 6.4. Overall, *VD* and areole circularity showed the strongest relationships with functional traits. The greatest association was observed between *VD* and stomatal conductance (t=4.16, r^2=0.21, P<0.001). Interestingly, K_{mass} had a significant relationship with five out of seven vein traits, the strongest observed with areole circularity (t=3.23, r^2=0.13, P<0.001).

Table 6.4 Summary of Phylogenetic Generalized Least Squares models, with leaf and wood functional traits as response variables, and venation traits as predictor variables. Abbreviations: *WD* – wood specific density, *VP* -vessel perimeter, $_{i}$WUE – intrinsic water use efficiency, g_s – stomatal conductance, K_{mass} – mass based foliar potassium concentration, LMA- leaf mass per area, LA – leaf area. Values are for *t* for a model predictor variable, overall model *P* value and r^2 and Pagel's λ based on a maximum likelihood calculation. * -*P*<0.05, **- <0.01, ***<0.001, n.s. – not significant.

		WD	*VP*	Parenchyma	$_{i}$WUE	g_s	K_{mass}	LMA	*LA*
	n	63	63	30	63	63	63	63	63
	t	-1.06	1.55	-1.94	-2.04	4.16	-2.40	2.97	-0.54
	r^2	0.00	0.02	0.08	0.05	0.21	0.07	0.11	-0.01
VD	*P*	n.s.	n.s.	n.s.	*	***	*	**	n.s.
	Pagel's λ	0.76	0.82	0.86	0.14	0.00	0.26	0.09	0.63
	t	-2.36	2.39	-0.27	0.33	3.31	0.86	0.61	1.28
	r^2	0.07	0.07	-0.03	-0.01	0.14	0.00	-0.01	0.02
Loopiness	*P*	**	**	n.s.	n.s.	***	n.s.	n.s.	n.s.
	Pagel's λ	0.74	0.84	0.81	0.00	0.00	0.05	0.07	0.28
	t	1.95	-1.31	1.09	-0.68	-2.58	0.13	1.39	0.32
	r^2	0.04	0.01	0.01	-0.01	0.08	-0.02	0.01	-0.01
Radius	*P*	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
	Pagel's λ	0.72	0.85	0.79	0.00	0.00	0.12	0.04	0.63
	t	1.71	-1.31	-1.09	-1.42	-1.23	-3.05	0.95	-2.40
	r^2	0.03	0.01	0.01	0.02	0.01	0.12	0.00	0.07
FEV	*P*	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	**
	Pagel's λ	0.78	0.83	0.69	0.00	0.00	0.00	0.05	0.45
	t	1.75	-1.87	-1.45	-1.31	-1.07	-2.81	0.56	-2.00
	r^2	0.03	0.04	0.04	0.01	0.00	0.10	-0.01	0.05
MST	*P*	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	*
	Pagel's λ	0.81	0.88	0.62	0.00	0.00	0.00	0.00	0.49
	t	-2.50	2.58	0.75	1.30	2.19	3.23	-0.14	1.72
	r^2	0.08	0.08	-0.01	0.01	0.06	0.13	-0.01	0.03
Circularity	*P*	*	**	n.s.	n.s.	*	**	n.s.	n.s.
	Pagel's λ	0.84	0.87	0.69	0.00	0.00	0.00	0.05	0.58
	t	-1.73	1.41	1.38	1.38	1.20	3.05	-1.11	2.01
	r^2	0.03	0.01	0.03	0.01	0.01	0.12	0.00	0.05
Meshedness	*P*	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	**
	Pagel's λ	0.76	0.81	0.66	0.00	0.00	0.00	0.05	0.44

When PGLS models were performed on species present within each vegetation type (Table S6.3, S6.4 and S6.5 for wet evergreen, moist and dry semideciduous forests, respectively), the results were different. At the wettest end of the rainfall gradient in evergreen sites, only two models out 49 were significant (comparing to 18 out of 56 when all sites were included). Only K_{mass} showed significant associations with vein radius and areole circularity. In moist semideciduous forest, there were 14 significant trait-trait associations seen in *VP*, K_{mass}, LMA and *LA*. PGLS analyses in dry semideciduous forests resulted in 16 significant relationships, mostly with *LA* and K_{mass}. Here, larger leaves had greater proportion of free ending veins and lower MST ratios, more infolding areoles and more tree like pathways. This pattern was also seen across the gradient and in moist semideciduous forests but was not observed in the wet evergreen forests.

While *t* values derived from the PGLS models were not significant among the vegetation types (*Chisq* = 5.58, *P*=0.06), dry semideciduous forests had more significant *t* values (≥2) than other study sites (Fig. 6.3a).

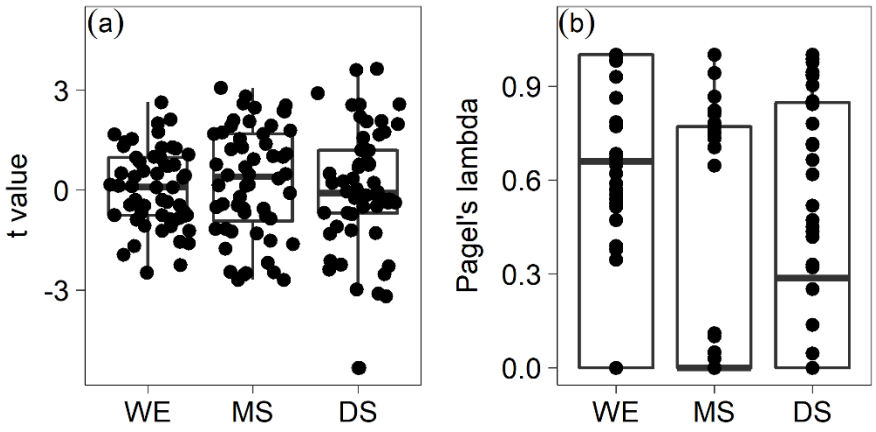


Fig. 6.3 Model values taken from PGLS models between venation and other functional leaf and wood traits. a) *t* values for predictor (minor venation trait), and b) Pagel's λ based on the maximum likelihood fit. Higher values of λ suggest greater effect of phylogeny on the trait-trait relationships. WE – wet evergreen, MS – moist semideciduous, DS – dry semideciduous.

The effect of phylogeny on trait-trait relationships

Average Pagel's λ values were on average higher in wet evergreen forests than in moist and dry semideciduous forests (Fig. 6.3b). However, we found that environment, and not the phylogeny, was the primary factor structuring the relationships between venation trait and leaf traits along the gradient (Pagel's λ=0.13; Table 6.4) and within each forest site (λ=0.43, λ=0.23 and λ=0.25 in evergreen, moist and dry semideciduous forests, respectively; Table S6.3, S6.4, S6.5). In contrast to leaf trait relationships, in links between wood and venation traits phylogenetic relatedness was an important factor underlining these relationships (λ=0.8-0.9) except for species in moist semideciduous forest where λ=0.35.

6.5. Discussion

Our study examined minor venation traits (*VD* and less explored reticulation traits) dynamics along a strong tropical rainfall gradient in a large number of species (*n*=66) in West Africa. While these traits have been investigated before along the Andes-Amazon transect (Blonder *et al.*, 2018), our study did not have the added complexity of elevation and a strong temperature gradient, enabling us to investigate changes in vein architecture in relation to water availability. Additionally, here we for the first time tested the relationships between leaf venation and deciduousness and wood density and anatomy. The significant relationships that we observed between vein, leaf and wood traits that are considered to be correlated with drought stress, suggest that leaf vascular architecture is an import feature of a whole plant strategy in response to intermittent water availability. This is also supported by phylogeny having a less significant influence on trait-trait relationships in drier sites where environment is overriding these evolutionary relationships.

Global context

The average transect values of *VD*, areole loopiness, and FEV ratio were consistently higher (almost 50%) and lower in the case of vein radius than those reported for an Amazon-Andes transect (Blonder *et al.*, 2017, 2018) and differed only slightly in MST and areole circularity. Our venation trait values from the wet evergreen forests were the most similar to the values of Amazonia sites. The range of our *VD* values were similar to those of 217 taxa from West African Sahelian-Sudanian zone (range from 2.7 to 26.7 mm^2) (Schneider *et al.*, 2018). The difference in average vein trait values among the studies can be anticipated if we consider the differences in MAP; our sites receive almost three quarters less of annual precipitation than plots in the Andes and approximately twice as much as in the Sahelian-Sudanian study.

Venation dynamics along the rainfall gradient

As expected, average scale-dependent minor venation traits varied significantly along the precipitation gradient, suggesting a hydraulic role for vein architecture. This is not surprising as rainfall in Ghana is considered to be the most significant factor determining plant species distribution (Swaine, 1996; Fauset *et al.*, 2012), carbon stocks (Moore *et al.*, 2017) and other functional traits (Maharjan *et al.*, 2011; Schrodt *et al.*, 2015; Gvozdevaite *et al.*, pers. comm.). A large meta-analysis at other sites also observed that minor *VD* increases in response to aridity (Sack & Scoffoni, 2013). Conversely, minor venation traits did not vary significantly in response to rainfall in the Andes-Amazon transect (Blonder *et al.*, 2018). It is possible that in the Andean study, water was not the limiting factor to plant functioning as the lowest MAP at the study sites was 1,560 mm a^{-1}, and transpiration rates were low in the montane forest ecosystems. The authors suggested that structural and defensive roles of venation reticulation were of greater importance than hydraulic function. This might be an explanation for our findings as well, as scale-independent traits did not vary along the rainfall gradient. Similarly, Schneider *et al.* (2018) found weak vein–climate relationships even in the sites where water is a strong limiting factor. It was proposed that different adaptive strategies

such as leaf deciduousness might circumvent the problem of maintaining the leaf hydraulic functioning during periods of water stress.

Relationships between venation and drought tolerance or avoidance indicative traits

Venation traits varied significantly between species of deciduous and evergreen leaf habit, but in the opposite direction than expected; vein networks were denser and more reticulated in deciduous species. One explanation for this observation could be the lower *WD* seen in deciduous species (Choat *et al.*, 2005), which is an indication of lower drought tolerance (O'Brien *et al.*, 2017). Investment in greater *VD* and reticulation might prevent stem hydraulic damage. Indeed, species with higher *WD* had lower *VD* at the driest end of the rainfall gradient. No significant relationships between leaf and wood vessels were observed in evergreen species or wet evergreen forests pointing towards water stress having an effect on these relationships. Other studies also have observed a strong coordination between leaf and wood traits being significantly stronger in drier than wetter forests (Méndez-Alonzo *et al.*, 2012)

Another possible reason for deciduous species having denser venation networks could be due to requirements for higher gas exchange rates, which are characteristic of deciduous species. Previously, vein density has been demonstrated to have a positive effect on photosynthetic rates (Brodribb *et al.*, 2007) but this was not the case here (data not shown). Additionally, in our earlier study (Gvozdevaite *et al.*, in press), deciduous and evergreen species did not differ in photosynthetic capacity. This finding further supports our suggestion that venation networks plays a vital role in species response to intermittent water availability and drought.

The relationships between venation and leaf morphological traits

In theory, minor venation traits and leaf size should not show intrinsic correlation. While the development of higher order veins coincides with leaf expansion during morphogenesis, minor

venation continues to form after the leaf stops growing (Dengler & Kang, 2001; Ruonala *et al.*, 2017). Therefore, the lack of the relationship between leaf size and minor venation traits has been observed (Sack *et al.*, 2012; Price *et al.*, 2014). However, (Murphy *et al.*, 2014) found a weak correlation between vein density and leaf size in subtropical woody angiosperm at sites of high evaporative demand which suggests functional coordination. Similarly, here we found significant relationships between leaf size and dimensionless reticulation traits, in semideciduous but not in evergreen forests. Additionally, in the dry semideciduous forests PGLS models suggested that these relationships were not due to phylogenetic relatedness as all λ were near 0. Leaf shape is known to be of significant importance to temperature regulation in hot and humid environments (Wright *et al.*, 2017; Gvozdevaite *et al.*, in prep.) which might explain these relationships. Small leaves are also considered to be an adaptation to drought stress (Farooq *et al.*, 2012). Indeed, Scoffoni *et al.* (2011) found that major vein density together with leaf size have a significant effect on leaf and whole plant hydraulic vulnerability.

It has been observed that species with low LMA are more susceptible to drought and drought-induced mortality (Greenwood *et al.*, 2017). While the physiological relationships are complex, high LMA species often display higher modulus of elasticity, more negative osmotic potentials and lower hydraulic vulnerability (Scoffoni *et al.*, 2011a). High LMA and thick leaves are often characteristic of xeromorphic plants that are tolerant to high water stress (Groom & Lamont, 1999) and is found in hot and dry environments (Costa-Saura *et al.*, 2016). However, in tropical forests many species have high LMA yet are comparatively intolerant of drought (Bartlett *et al.*, 2012), suggesting that the utility of LMA as a drought resistance index may be rather context dependent. In our case, LMA was positively corelated with vein density along the gradient, however not within each vegetation type.Other studies have demonstrated weak correlation between leaf economic and hydraulic traits (Li *et al.*, 2015).

Potassium and veins

One striking pattern that we observed and expected was the strong positive relationship between vein reticulation and potassium (K_{mass}). One potential explanation for this findings could be due to the fact that most of the total K^+ is absorbed by the roots and transported to the leaves via xylem, translocated to phloem and circulated within the venation network (Shabala *et al.*, 2002). Greater reticulation would provide more "space" for K^+ circulation. Nevertheless, the relationships between reticulation and K_{mass} were the strongest in the dry semideciduous forests zone. Soil and leaf *K* concentrations cannot be used to explain the relationships being stronger in the dry semideciduous forests, because it was the moist semideciduous forests that had significantly higher soil and foliar *K* values than other forest types (Table 6.1). Therefore, we assume that minor vein reticulation was linked with K_{mass} because these traits are important for leaf and plant osmoregulation, especially in the environments of intermittent water availability. Potassium is known to affect leaf growth, stomatal conductance, photosynthetic capacity, plant resistance to stress and is especially important nutrient during drought conditions (Cakmak, 2005; Lloyd *et al.*, 2015; Tränkner *et al.*, 2018).

The effect of venation on stomatal conductance and water use efficiency

As expected, we found that greater *VD* was linked with higher g_s along the transect, however this relationship was not observed when assessed at the level of individual forest types. We suggest that the overall link between *VD* and g_s is the outcome of correlated responses to selective environmental conditions as both trait varied in the same direction along the rainfall gradient. This interpretation is supported by the Pagel's λ of 0. Similarly, significant effect of *VD* on g_s has been observed across tropical and temperate fern, conifer and angiosperm lineages (Boyce *et al.*, 2009; McElwain *et al.*, 2016), however this relationship was not true when only angiosperm or closely related species were examined (Gleason *et al.*, 2015; Scoffoni *et al.*, 2016a).

In addition, venation traits are highly conservative (with the exception of *VD* along our transect) while this is not the case for g_s. Stomatal conductance will vary significantly depending on the time of the day, light and water availability, and many other reasons. This is especially important to consider when interpreting these relationships on data measured in tropical environments in contrast to greenhouses (e.g. Gleason et al. 2015). While we interpolated g_s values from a large number of samples, it is possible that it was not optimal due to abiotic factors. Additionally, we performed gas exchange measurements on the cut (but rehydrated) branches. This methodology generally provides reliable estimates of gas exchange (Dang *et al.*, 1997; Cavaleri *et al.*, 2008).

In contrast to insignificant $VD \leftrightarrow g_s$ relationship, in the dry semideciduous forest zone *VD* had a significant negative effect on the $_i$WUE, which suggests that here higher vein density resulted in greater water loss. One of the explanations for this observation could be that greater water supply (enabled by higher *VD*) meant that species did not need to use water resources efficiently. This is in contrast to other studies finding positive relationship between water use efficiency and *VD* (Lei *et al.*, 2018) or not finding the relationship at all (Blonder *et al.*, 2018). The reason why this did not result into higher photosynthetic capacity could be because maximising photosynthesis is not the main goal in these environments. We previously found that in these forest-savanna ecotones gas exchange was not limited by nutrients, did not vary among different sites or deciduous and evergreen species (Gvozdevaite *et al.*, in press).

The effect of phylogeny on trait-trait relationships

Overall, our findings suggest that vein trait and other leaf trait evolutionary relationships could be over-ridden by strong environmental pressures in species at the driest end of the transect. This was not the case for vein-wood trait relationships which showed strong phylogenetic signal. Similarly, Lonzo *et al.* (2012) suggested that the coordination between wood and leaf drought traits is evolutionary. Strong phylogenetic correlations have also been observed between root and leaf traits (Valverde-Barrantes *et al.*, 2017). One exception were species in the moist semideciduous forests,

where vein-wood trait relationships were mostly driven by environment. It seems that there integrating resource economy across leaves and wood is of less important. Indeed this plot is known for incredibly high investments in canopy biomass, which is potentially an indication of double leaf-flushing in each year (Moore *et al.*, 2017).

Conclusion

In the present study, we demonstrated that minor venation traits vary significantly along a strong precipitation gradient, correlate with other leaf and wood traits that previously have been linked as predictors or correlates of drought tolerance or avoidance, and are different among deciduous and evergreen species. Generally, these relationships were stronger in sites at the driest end of the transect and in deciduous species, suggesting the importance of denser and more reticulated venation networks in sites of intermittent water availability. We also show that incorporating phylogenetic affiliations in trait-trait relationship analyses can help to better understand the effects of environmental conditions on species functioning.

6.6. Supplementary Information

The following Supplementary Information is available for this article:

Table S6.1 Species list

Table S6.2 Differences in vein traits among sunlit and shaded branches

Table S6.3 PGLS models for wet evergreen forests

Table S6.4 PGLS models for moist semideciduous forests

Table S6.5 PGLS models for dry semideciduous forests

Fig. S6.1 Seasonality and precipitation along the gradient

Fig. S6.2 Variance partitioning of vein traits into taxonomic units

Table S6.1 Species and their leaf habit sampled in the present study at each site (wet evergreen, moist and dry semideciduous forests).

Family	Species	Leaf habit
Wet evergreen		
Achariaceae	*Scottellia klaineana*	Evergreen
	Cleistopholis patens	Evergreen
Burseraceae	*Dacryodes klaineana*	Evergreen
Calophyllaceae	*Mammea africana*	Evergreen
Cardiopteridaceae	*Leptaulus daphnoides*	Evergreen
Chrysobalanaceae	*Maranthes aubrevillei*	Evergreen
	Maranthes glabra	Evergreen
Clusiaceae	*Pentadesma butyracea*	Evergreen
Ebenaceae	*Diospyros heudelotii*	Evergreen
Fabaceae	*Berlinia occidentalis*	Evergreen
	Bussea occidentalis	Evergreen
	Calpocalyx brevibracteatus	Deciduous
Lamiaceae	*Cynometra ananta*	Evergreen
	Vitex micrantha	Evergreen
Malvaceae	*Cola lateritia*	Evergreen
	Cola nitida	Evergreen
	Heritiera utilis	Evergreen
Melastomataceae	*Scaphopetalum ameonum*	Evergreen
	Memecylon afzelii	Evergreen
Meliaceae	*Lovoa trichilioides*	Evergreen
Myrtaceae	*Eugenia whytei*	Evergreen
Olacaceae	*Coula edulis*	Evergreen
	Strombosia pustulata	Evergreen
Phyllanthaceae	*Protomegabaria macrophylla*	Evergreen
	Uapaca corbisieri	Evergreen
Sapotaceae	*Chrysophyllum pruniforme*	Evergreen
	Chrysophyllum subnudum	Evergreen
	Manilkara obovata	Evergreen
Moist semideciduous		
Anacardiaceae	*Lannea welwitschii*	Deciduous
Apocynaceae	*Funtumia elastica*	Evergreen
Cannabaceae	*Celtis mildbraedii*	Evergreen
	Celtis zenkeri	Evergreen
Combretaceae	*Terminalia superba*	Deciduous
Fabaceae	*Albizia zygia*	Deciduous
	Cylicodiscus gabunensis	Deciduous

Lecythidaceae	*Hymenostegia afzelii*	Evergreen
	Petersianthus macrocarpus	Deciduous
Malvaceae	*Ceiba pentandra*	Deciduous
	Cola caricifolia	?
	Cola gigantea	Deciduous
	Nesogordonia papaverifera	Deciduous
	Pterygota macrocarpa	Deciduous
	Sterculia oblonga	Deciduous
	Sterculia rhinopetala	Deciduous
	Triplochiton scleroxylon	Deciduous
Meliaceae	*Trichilia prieuriana*	Evergreen
	Turraeanthus africanus	Evergreen
Myristicaceae	*Pycnanthus angolensis*	Evergreen
Dry semideciduous		
Simaroubaceae	*Hannoa klaineana*	Deciduous
Anacardiaceae	*Lannea velutina*	Deciduous
Bignoniaceae	*Spathodea campanulata*	Deciduous
Burseraceae	*Dacryodes klaineana*	Evergreen
Chrysobalanaceae	*Maranthes polyandra*	Evergreen
Combretaceae	*Anogeissus leiocarpus*	Evergreen
	Terminalia schimperiana	Deciduous
Ebenaceae	*Diospyros mespiliformis*	Deciduous
Fabaceae	*Afzelia africana*	Deciduous
	Daniellia oliveri	Deciduous
	Erythrophleum suaveolens	Deciduous
	Pterocarpus erinaceus	Deciduous
Malvaceae	*Bombax buonopozense*	Deciduous
	Ceiba pentandra	Deciduous
	Cola gigantea	Deciduous
	Sterculia tragacantha	Deciduous
Meliaceae	*Khaya senegalensis*	Evergreen
Phyllanthaceae	*Bridelia ferruginea*	Evergreen
Sapotaceae	*Pouteria alnifolia*	Deciduous
	Vitellaria paradoxa	Deciduous

Table S6.2 Differences in venation trait values between leaves from sun and shade branches. The differences were tested using mixed-effect models, including forest type as a random effect to account for uneven number of samples within each study site.

	Mixed model results			Wet evergreen		Moist semideciduous		Dry semideciduous	
Trait	*n*	*t*	*P*	Shade	Sun	Shade	Sun	Shade	Sun
VD	335	1.53	0.13	16.08	17.09	18.70	18.67	20.90	21.76
Loopiness	335	0.53	0.59	11.67	13.61	17.68	16.45	19.24	20.05
Radius	241	1.90	0.06	0.02	0.02	0.02	0.02	0.02	0.02
FEV	360	0.41	0.68	0.36	0.37	0.32	0.33	0.35	0.35
MST	360	0.46	0.64	0.78	0.79	0.74	0.75	0.77	0.77
Circularity	360	-0.64	0.52	0.70	0.70	0.72	0.71	0.72	0.72
Meshedness	360	-0.17	0.85	1.01	1.02	1.03	1.03	1.02	1.02

Table S6.3 Summary of Phylogenetic Generalized Least Squares models in wet evergreen forests, with leaf and wood functional traits as response variables, and venation traits as predictor variables. Abbreviations: *WD* – wood specific density, *VP* -vessel perimeter, $_{i}$WUE – intrinsic water use efficiency, g_s – stomatal conductance, K_{mass} – mass based foliar potassium concentration, LMA- leaf mass per area, LA – leaf area. Values are for *t* for a model predictor variable, overall model *P* value and r^2 and Pagel's λ based on a maximum likelihood calculation. * -*P*<0.05, **- <0.01, ***<0.001, n.s. – not significant.

		WD	*VP*	$_{i}$WUE	g_s	K_{mass}	LMA	*LA*
	n	27	27	27	27	27	27	27
VD	*t*	-0.58	0.08	0.14	1.45	-0.29	1.27	-0.29
	r^2	-0.03	-0.04	-0.04	0.04	-0.04	0.02	-0.04
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	1.00	0.65	0.00	0.00	0.99	1.00	0.59
Loopiness	*t*	-1.07	0.50	0.76	1.32	0.44	1.06	0.58
	r^2	0.01	-0.03	-0.02	0.03	-0.03	0.00	-0.03
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	1.00	0.67	0.00	0.00	1.00	0.98	0.52
Radius	*t*	1.26	-0.89	-1.59	-1.94	-2.25	2.01	0.16
	r^2	0.02	-0.01	-1.59	0.10	0.14	0.10	-0.04
	P	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.
	Pagel's λ	1.00	0.38	0.00	0.00	1.00	0.00	0.57
FEV	*t*	1.00	-0.85	-0.63	-0.75	-0.75	-0.44	-1.22
	r^2	0.00	-0.01	-0.02	-0.02	-0.02	-0.03	0.02
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	1.00	0.62	0.00	1.00	1.00	0.00	0.35
MST	*t*	0.50	-0.67	-0.35	0.41	-0.46	-0.45	-0.79
	r^2	-0.03	-0.02	-0.35	-0.03	-0.03	-0.03	-0.01
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	1.00	0.67	0.00	0.00	1.00	0.00	0.47
Circularity	*t*	-1.08	0.86	1.53	0.08	2.13	-0.75	0.74
	r^2	0.01	-0.01	0.05	-0.04	0.12	-0.02	-0.02
	P	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
	Pagel's λ	1.00	0.55	0.00	0.00	1.00	0.93	0.52
Meshedness	*t*	-0.87	1.28	0.72	0.12	0.37	0.98	0.98
	r^2	-0.01	0.02	-0.02	-0.04	-0.03	-0.02	0.00
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	1.00	0.54	0.00	0.00	0.98	0.68	0.39

Table S6.4 Summary of Phylogenetic Generalized Least Squares models in moist semideciduous forests, with leaf and wood functional traits as response variables, and venation traits as predictor variables. Abbreviations: *WD* – wood specific density, *VP* -vessel perimeter, $_{i}$WUE – intrinsic water use efficiency, g_s – stomatal conductance, K_{mass} – mass based foliar potassium concentration, LMA-leaf mass per area, LA – leaf area. Values are for *t* for a model predictor variable, overall model *P* value and r^2 and Pagel's λ based on a maximum likelihood calculation. * -*P*<0.05, **- <0.01, ***<0.001, n.s. – not significant.

		WD	*VP*	$_{i}$WUE	g_s	K_{mass}	LMA	*LA*
	n	20	20	20	20	20	20	20
VD	*t*	-0.51	1.29	0.14	0.17	-1.25	1.03	-0.20
	r^2	-0.04	0.03	-0.05	-0.05	-1.25	0.00	-0.05
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	0.82	0.00	0.00	0.00	0.00	0.65	0.94
Loopiness	*t*	-1.76	2.38	1.00	0.77	0.69	-1.17	1.23
	r^2	0.10	0.18	0.00	0.77	-0.03	0.02	0.02
	P	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	0.78	0.00	0.00	0.00	0.00	0.00	0.78
Radius	*t*	-0.56	-0.56	-1.62	1.69	0.47	0.94	-0.67
	r^2	-0.04	-0.04	0.08	0.08	-0.04	-0.01	-0.03
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pagel's λ	0.00	0.00	0.00	1.00	0.00	0.00	0.70
FEV	*t*	1.68	-2.18	-0.77	-0.45	-2.70	2.82	-2.49
	r^2	0.08	0.15	-0.02	-0.04	0.24	0.26	0.21
	P	n.s.	*	n.s.	n.s.	*	*	**
	Pagel's λ	0.82	0.00	0.00	0.00	0.00	0.00	1.00
MST	*t*	1.78	-2.53	-0.86	-0.42	-2.69	2.36	-2.46
	r^2	1.78	0.21	-0.01	-0.04	0.24	0.19	0.20
	P	n.s.	*	n.s.	n.s.	*	*	*
	Pagel's λ	0.81	0.00	0.00	0.00	0.00	0.00	1.00
Circularity	*t*	-1.30	2.47	0.48	0.35	2.54	-0.09	2.10
	r^2	0.03	0.20	-0.04	-0.05	0.21	-0.05	0.15
	P	n.s.	n.s.	n.s.	n.s.	*	n.s.	*
	Pagel's λ	0.87	0.00	0.00	0.00	0.00	0.77	1.00
Meshedness	*t*	-1.16	1.94	1.09	0.45	3.07	-2.45	2.06
	r^2	0.02	0.12	0.01	-0.04	0.30	0.20	0.14
	P	n.s.	n.s.	n.s.	n.s.	**	*	*
	Pagel's λ	0.73	0.00	0.00	0.00	0.00	0.00	0.77

Table S6.5 Summary of Phylogenetic Generalized Least Squares models in dry semideciduous forests, with leaf and wood functional traits as response variables, and venation traits as predictor variables. Abbreviations: *WD* – wood specific density, *VP* -vessel perimeter, $_{i}$WUE – intrinsic water use efficiency, g_s – stomatal conductance, K_{mass} – mass based foliar potassium concentration, LMA- leaf mass per area, LA – leaf area. Values are for *t* for a model predictor variable, overall model *P* value and r^2 and Pagel's λ based on a maximum likelihood calculation. * -*P*<0.05, **- <0.01, ***<0.001, n.s. – not significant.

		WD	*VP*	$_{i}$WUE	g_s	K_{mass}	LMA	*LA*
	n	19	19	19	19	19	19	19
VD	*t*	-3.11	0.76	-2.99	0.22	-0.68	-0.06	0.78
	r^2	0.32	-0.02	0.31	-0.06	-0.03	-0.06	-0.02
	P	**	n.s	**	n.s	n.s	n.s	n.s
	Pagel's λ	1.00	0.84	0.71	0.95	0.67	0.00	0.00
Loopiness	*t*	-2.52	-0.37	-0.23	0.27	2.56	-0.50	2.58
	r^2	0.23	-0.05	-0.06	-0.05	0.24	-0.04	0.24
	P	*	n.s	n.s	n.s	*	n.s	*
	Pagel's λ	0.78	0.94	0.00	0.72	0.00	0.00	0.00
Radius	*t*	1.98	-0.37	0.03	-0.15	-0.48	2.07	0.69
	r^2	0.14	-0.05	-0.06	-0.05	-0.04	0.15	-0.03
	P	n.s.	n.s	n.s	n.s	n.s	*	n.s
	Pagel's λ	1.00	0.94	0.00	0.44	0.42	0.00	0.05
FEV	*t*	1.19	-0.13	-0.68	-0.04	-5.33	-0.11	-2.39
	r^2	0.02	-0.06	-0.03	-0.06	0.57	-0.06	0.21
	P	n.s	n.s	n.s	n.s	***	n.s	*
	Pagel's λ	0.93	0.94	0.00	0.33	0.85	0.00	0.00
MST	*t*	1.74	-0.72	-0.28	-1.20	-3.19	-0.20	-2.12
	r^2	0.10	-0.03	-0.05	0.02	0.34	-0.06	0.16
	P	n.s.	n.s	n.s	n.s	**	n.s	*
	Pagel's λ	0.90	1.00	0.00	0.00	0.90	0.00	0.00
Circularity	*t*	-2.28	0.77	0.23	0.87	3.60	-0.12	2.57
	r^2	0.19	-0.02	-0.06	-0.01	0.40	-0.06	0.24
	P	*	n.s	n.s	n.s	**	n.s	*
	Pagel's λ	0.98	0.99	0.00	0.00	0.62	0.00	0.00
Meshedness	*t*	-2.23	0.49	-0.28	1.20	3.64	0.35	2.91
	r^2	0.18	-0.04	-0.05	0.02	0.41	-0.05	0.29
	P	*	n.s	n.s	n.s	**	n.s	**
	Pagel's λ	0.85	0.52	1.00	0.00	0.45	0.00	0.00

Fig. S6.1 Mean monthly precipitation (1991-2016) with standard errors (±1) in each of the study sites: wet evergreen, moist semideciduous and dry semideciduous forests. Data were derived using monthly time series from the Climate Hazards Group InfraRed Precipitation with Stations data archive version 2.0 at 0.05° resolution (Funk *et al.*, 2015).

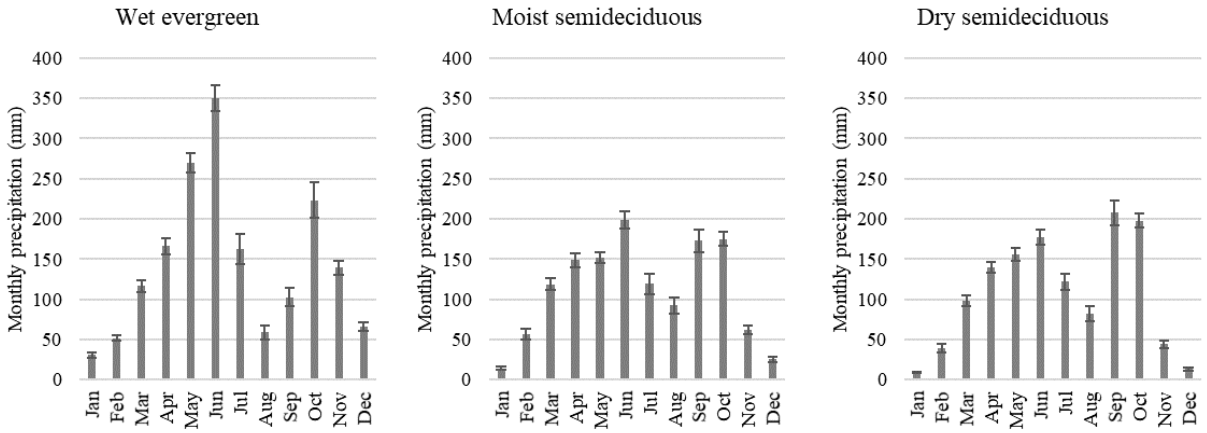
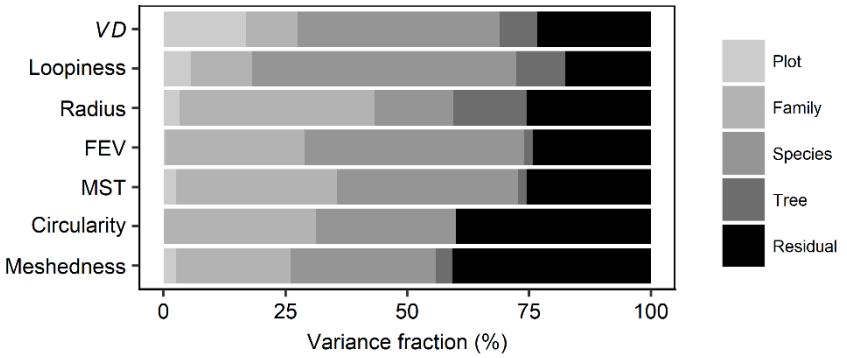


Fig. S6.2 Partitioning of the total variance for venation traits into taxonomic (family, species, tree, residual) and environmental (plot) components.



Chapter 7

Previous data chapters assessed economic, morphological and venation leaf functional trait variability in response to specific environmental variables. The main purpose of this final data chapter was to bring together the aforementioned leaf trait dimensions without previous assumptions of their functional importance, identify the most significant ones for community assembly along the environmental gradient and accordingly formulate a functional trait space which can further be linked to ecosystem functioning. This data chapter addresses **Question 4** of the thesis: *How does multivariate leaf trait space affect plant and ecosystem performance?* The data used in this chapter includes all leaf functional trait data for 80 species in Ghana, their distribution, all measured and estimated environmental variables and values for net primary productivity for each study plot.

The findings of the study are presented in the paper entitled "Leaf functional trait space reflects species ecological strategies and ecosystem productivity" which will be submitted for publication at a later stage in 2019.

I was the main author and wrote the paper, conducted the fieldwork and carried out all of the data analysis with guidance from my supervisors Imma Oliveras and Yadvinder Malhi. The field campaigns were planned by Yadvinder Malhi and Imma Oliveras and coordinated by Imma Oliveras and collaborators in Ghana: Theresa Peprah and Stephen Adu-Bredu. Data collection was assisted by Mickey Boakye, Lydia Afriyie, Martini David and Jodra Miguel. Sam Moore provided net primary productivity data (Moore *et al.*, 2017) and assisted with interpretation of relevant findings.

Chapter 7:

Leaf functional trait space reflects species ecological strategies and ecosystem productivity

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7.1. Abstract

Determining the variability of ecosystem functional composition along environmental gradients can provide a more mechanistic understanding of the impacts that climate change will have on the forest structure and functioning. Numerous studies have addressed this idea, but due to univariate approaches and small sample sizes the relevance of these studies to the understanding functioning of the complex tropical forest ecosystems is currently limited. For this reason, we defined a multivariate leaf functional trait space (species *n*=80) along a climatic vegetation gradient in Ghana, West Africa. We also assessed the links between the trait space, environmental conditions, species ecological strategies and ecosystem function Using a novel fourth-corner methodology, we identified 16 leaf traits that defined photosynthetic, structure and venation trait space dimensions. The photosynthetic axis varied significantly along the climatic gradient, while the structure axis values were the most responsive to soil fertility. Mean noon temperature (MNT) and annual precipitation (MAP) had the greatest effect on trait distributions, while the most important edaphic parameters were soil carbon (*C)*, aluminium (*Al)* and sodium (*Na).* Species ecological strategies (deciduousness and light guilds, wood density (*WD*)) were significantly linked with leaf trait dimensions along the "fast-slow" trait spectrum. Additionally, there were strong positive correlations between net primary productivity (NPP) components, canopy biomass and all three leaf trait dimensions. Our findings suggest that leaf trait space directly link the performance of trees and species to the environment and can be used to scale up to effects on the overall functioning of these tropical forest ecosystems. Identification of changes in multivariate plant trait space is needed to further advance our understanding of tropical forest ecosystem functioning.

Keywords: trait dimensions, functional traits, tropical forest ecosystems.

7.2. Introduction

Research focusing on plant functional traits, rather than species identities, has greatly improved the ability to understand the structure and dynamics of ecological communities and potentially predict their response to anthropogenic and natural changes (Díaz *et al.*, 2013). Specifically, relationships between functional traits and plant functioning are used to explain species and ecosystem distributions along geographical gradients (Westoby & Wright, 2006; Kraft *et al.*, 2008). Resultantly, the range of functional traits space at certain environmental conditions can be used to infer the plasticity of niche that the species is able to occupy at current and future climatic conditions (e.g. Pellissier *et al.*, 2018).

Studies exploring bivariate relationships between specific functional traits and plant function can be mechanistically informative; however, it is a combination of ecologically relevant traits that allow plants to grow and survive in a defined set of environments (Lebrija-Trejos *et al.*, 2010). Multivariate trait analyses are useful tools for investigating relationships between multiple traits and environment to get a better understanding of the whole plant functioning strategy. One of the key challenges is, however, to identify which of many functional traits are the most useful in predicting plant functioning and survival in specific environments. Certain traits, or combination of traits, may be of greater functional significance in ecosystems of specific environmental pressures. For example, water deficit, high temperatures and fire in savannas might filter species with drought avoidance, reduced flammability or regeneration traits (Cardoso *et al.*, 2016), while dense and shaded wet evergreen forests would require traits important for shade adaptation (Niinemets & Sack, 2006). Hence, inclusion of multiple correlated and/or irrelevant traits in the analyses may lead to spurious results and wasted efforts (Laughlin, 2014).

All functional traits are filtered by the environment at local and global scales; however, a set of traits are most commonly measured as proxies to species growth, survival and distribution. For woody angiosperms, these are, plant height, wood density, leaf shape, LMA, foliar mass-based nitrogen

content (N_{mass}) and seed mass (Wright *et al.*, 2010b; Díaz *et al.*, 2015). Tree height is linked with light acquisition (Stark *et al.*, 2015) and wood density (*WD),* which is informative of plant growth and survival under mechanical and drought stress (Phillips *et al.*, 2010; O'Brien *et al.*, 2017). Leaf shape, specifically size and effective width, has recently gained importance as a trait that regulates thermal dynamics and is an essential adaptation to hot and dry climates (Leigh *et al.*, 2017; Wright *et al.*, 2017; Gvozdevaite *et al.*, in prep). Leaf mass per area and N_{mass} are linked with plant carbon gain and defense mechanisms against herbivory (Wright *et al.*, 2004), while seed mass is important for seedling survival and colonization (Moles & Westoby, 2004).

The assumptions for trait-trait relationships and trade-offs are, however, not always universal, especially when investigated within plant communities, across smaller environmental gradients and in varying light environments (Niinemets *et al.*, 2015; Keenan & Niinemets, 2016). Gas exchange (e.g. photosynthetic capacity) has been shown to vary independently of *N* and be more limited by leaf phosphorus (*P*) (Domingues *et al.*, 2010; Gvozdevaite *et al.*, in press) or linked to soil potassium (*K*) (Lloyd *et al.*, 2015). Photosynthetic rates and stomatal conductance are also strongly limited by leaf hydraulic supply capacity which is related to minor vein density (*VD*) (Brodribb *et al.*, 2007). The lack of expected trait-trait relationships suggests that these relationships are more complex and not always one-dimensional as suggested by leaf economic spectrum theory (Wright *et al.*, 2004; Li *et al.*, 2015).

The trade-offs and relationships among these traits represent species growth and survival strategies along the "fast-slow" plant economics spectrum (Reich, 2014). Species with "fast" leaf traits, such as high concentrations of N_{mass} have higher rates of gas exchange but shorter life spans than species with "slow" traits (Wright *et al.*, 2004). This trade-off is reflected in other plant organs, such as roots (McCormack *et al.*, 2012) and wood (Chave *et al.*, 2009). Based on these trade-offs of resource acquisition and allocations, assumptions may enable inferences about the whole plant strategy. For example, deciduous species with "fast" traits grow faster but are more susceptible to drought and high temperatures than "slow" evergreen species (Sastry & Barua, 2017).

Previous studies have considered relationships between leaf functional traits and growth-survival strategies (Poorter & Markesteijn, 2008; Wright *et al.*, 2010b). For example, greater LMA is positively correlated with greater survival rates and growth (Finegan *et al.*, 2015; Visser *et al.*, 2016). Conversely, a number of studies do not find significant or strong correlations in the same variables (e.g. Easedale & Healey, 2009; Wright *et al.*, 2010). These observations for LMA and for other traits can be explained by accounting for tree size and ontogenenetic stage; significant relationships between LMA, photosynthesis, area-based foliar nitrogen (N_{area}) and growth rate are more common in seedlings, while correlation between *WD*, mass-based leaf phosphorus (P_{mass}), N_{mass}, tree height and growth are stronger in mature trees (Gilbert *et al.*, 2016).

The magnitude and direction of relationships between traits, growth and survival, varies with life-history strategy (successional stage) and drought response (e.g. drought deciduous and evergreen) (O'Brien *et al.*, 2017). For example, in tropical gap-dependent pioneer species, fast growth rates are associated with high mortality rates in the understorey (up to 30%), while shade bearing species grow > 1 mm in diameter but their understory survival exceeds 99% (Ruger *et al.*, 2018). However, the variation of leaf trait spectra among different ecological strategies remains to be fully understood.

Determining the variability of ecosystem functional composition along environmental gradients can provide a more mechanistic understanding of the impacts that climate change will have on the forest structure and functioning. Considering the projected climate change, especially in Africa (James *et al.*, 2013; Harris *et al.*, 2014), the main of our study was to assesses the variability of leaf functional traits space along a strong climatic(rainfall 1000-2200 mm a^{-1}), soil fertility and vegetation (wet evergreen forest to forest-savanna ecotone) gradient in Ghana, West Africa. This study benefits from a large leaf functional trait dataset, spanning 80 species, 210 trees and data available at a leaf level. Specifically, we consider photosynthetic traits, , key foliar nutrient concentrations, leaf shape metrics

such as width, size and elongation, and minor venation traits (Blonder *et al.*, 2018). After relevant trait selection, we link these with plant performance metrics, such as *WD*, light guilds, leaf habit (deciduous or evergreen) and plot level properties, specifically, NPP and canopy and aboveground biomass (ABG).

We aim to answer the following questions:

Q1. Which leaf traits are the most responsive to environmental filtering?

Q2. Which traits define leaf functional trait space?

Q3. How does leaf trait space link with plant ecological strategies, such as deciduousness, light requirements and *WD*?

Q4. Is leaf functional trait space linked with net primary productivity and biomass?

7.3. Materials and Methods

Study area

Data were collected in seven 1 ha study plots situated along a rainfall gradient (1,030 mm -2,200 mm a^{-1}) in Ghana, West Africa. These plots are part of an ongoing research project coordinated by the Global Ecosystems Monitoring Network (GEM; http://gem.tropicalforests.ox.ac.uk/). The study plots are situated in three distinct forest types; three are located in dry semideciduous forest (1,030 mm a^{-1}), two in moist semideciduous forest (1,500 mm a^{-1}) and two in wet evergreen forest (2,200 mm a^{-1}). Plots within each forest type were located in close proximity, presented relatively homogeneous soil substrates and the same climatic conditions (Table 7.1).

Table 7.1 Key study site characteristics. Abbreviations: incoming shortwave radiation (SW); mean annual precipitation (MAP); mean climatic water deficit (MCWD); mean annual temperature (MAT); maximum noon temperature (MNT) (based on monthly averages); short-wave radiation (SW); leaf area index (LAI); total soil phosphorus (P); nitrogen (N); carbon (C); cation exchange capacity (CEC); total number of species, and number of species sampled. Climate data were derived using monthly time series from the Climate Hazards Group InfraRed Precipitation with Stations data archive version 2.0 at 0.05° resolution (Funk *et al.*, 2015). Soil nutrient concentrations are for the soil depth of 0 - 0.3 m. Soil data was not available for the BOB01 plot. Soil chemistry data were taken from Moore *et al.* (2017).

Forest type	Wet evergreen		Moist semideciduous		Dry semideciduous		
Plot ID	ANK01	ANK03	BOB01	BOB02	KOG01	KOG03	KOG04
Vegetation type	Upland evergreen forest	Seasonal swamp	Moist semideciduous (edge effect)	Moist semideciduous	Dry forest	Transitional Forest	Savanna woodland
Latitude	5.27	5.27	6.69	6.69	7.26	7.30	7.30
Longitude	-2.70	-2.70	-1.34	-1.34	-1.15	-1.16	-1.16
MAP (mm)	2200	2200	1500	1500	1030	1030	1030
MCWD (mm)	-189.6	-189.6	-308.9	-308.9	-383.1	-383.1	-383.1
MAT (°C)	25.0	25.0	25.7	25.7	26.4	26.4	26.4
MNT (°C)	30.7	30.7	34.0	34.0	36.0	36.0	36.0
SW ($W\ m^{-2}$)	201.9	201.9	207.8	207.8	214.2	214.2	214.2
LAI	5.7	6.4	4.1	4.4	3.7	4.5	2.0
P ($mg\ kg^{-1}$)	146.76	109.74	-	258.28	67.20	74.60	81.90
N %	0.17	0.12	-	0.16	0.05	0.05	0.04
C %	2.61	1.92	-	1.71	0.71	0.67	0.62
Ca ($mg\ kg^{-1}$)	26.75	40.06	-	657.55	378.80	307.90	237.10
K ($mg\ kg^{-1}$)	32.30	33.68	-	49.01	42.50	35.60	28.60
Mg ($mg\ kg^{-1}$)	41.99	29.22	-	133.65	75.50	78.60	81.80
CEC ($mmol^{+}\ kg^{-1}$)	24.48	14.68	-	45.99	26.27	22.84	19.41
Sand (%)	63.13	75.85	-	46.74	82.40	79.70	76.90
Clay (%)	21.57	12.78	-	28.77	2.30	3.30	4.30
Silt (%)	15.28	11.36	-	24.47	15.34	17.02	18.71
Basal area ($m^2\ ha^{-1}$)	28.81	25.65	22.60	30.20	20.65	14.55	8.79
Total no. species	106	93	87	83	40	35	29
Stem density (no. ha^{-1})	466	497	474	756	197	234	193

Trait measurements

Traits data collection was conducted between September 2014 and March 2015 during an intensive field campaign in Ghana. In the seven 1 ha plots we sampled 80 angiosperm tree species that accounted for approximately 80% of the plot basal area (DBH > 10cm). For each selected species within a plot, we sampled three trees; and within each tree we collected one fully sunlit and one shaded branch. All sampled trees were mature canopy trees. Detailed methodology of specific functional trait measurements is outlined in sections **4.3., 5.3.** and **6.3**.

Net primary productivity and biomass measurements

All NPP and AGB calculations follow those employed in the GEM protocols manual (Marthews *et al.*, 2014) where they are described in detail, as well as in Moore *et al.* (2017) across the same study sites. To summarise, the protocol measures and sums all major components of (aboveground) NPP on monthly or seasonal timescales in each 1 ha plot. This includes canopy litterfall from litterfall traps at bimonthly intervals, estimates of leaf loss to herbivory, aboveground coarse woody productivity of all large trees (≥10 cm DBH) in the plot and small trees (2–10 cm DBH) in subplots via yearly plot censuses and the turnover of branches on live trees by conducting branch fall transects every three months between the years 2011-2015. Biomass calculated using the Chave *et al.* (2015) allometric equation:

$$\mathrm{AGB} = 0.0673 \times (p\, D^2\, H)^{0.976}$$

where AGB is aboveground biomass (kg), p is density (g cm^{-3}) of wood, D is DBH (cm), and H is height (m). Tree height data was plot/site-specific. To convert biomass values into carbon, we assumed that dry stem biomass is 47.3% carbon (Martin & Thomas, 2011). In such calculations, where measurement uncertainties are dominated by systematic uncertainties, large errors which are

consistent with previous analyses (Malhi *et al*., 2009a, 2015, 2016; Girardin *et al*., 2010; Galbraith *et al*., 2014) were assigned to reflect the relative uncertainties.

Data analysis

We acknowledge the big role of functional intra-specific variability, however, this study focused on the species specific trait and therefore leaf trait values were averaged to the species level (Oliveras *et al., in review*). Some species were present in more than one plot, and in these cases trait values were averaged for each species in each study site. For example, *Ceiba pentandra* had trait values from the savanna plot, KOG04, and the savanna woodland plot, KOG01.

Fourth-corner analysis (Legendre *et al.*, 1997; Dray *et al.*, 2008) was used to test the associations between each functional trait and environmental variables while accounting for species distributions among the study plots (Q1). This method measures the link between three tables: a table containing the abundances of species at each study plot, a second table with the measurements of environmental variables for each plot, and a third table describing species traits for each species. The results were based on Pearson's correlation coefficient. To obtain a test with a correct type I error, results of models including permutation of sites and permutation of species were combined. We used a very high number of repetitions (49999) to have enough power in tests. The fourth-corner analysis was performed using "ade4" package in R (Dray & Dufour, 2007). All statistical analyses were conducted in R (version 3.4.0) using the R Studio interface (RStudio Team, 2018).

Leaf functional traits and environmental variables identified as significant by the fourth-corner analysis were used further in the analyses. Leaf functional trait space was defined by significant principal component axes of selected trait principal component analysis (PCA) performed on the selected leaf traits (Q2). We selected a number of significant axes and traits contributing the most to each axis using Broken Stick and Kaiser-Guttman criteria (Jackson, 1993). The variation of leaf trait

space among the study sites was tested using ANOVA with prior log transformation of the data to correct for non-normal distribution of model residuals (Legendre & Legendre, 1998).

Links between trait space and species ecological strategies (Q3) were tested using linear mixed-effect models (LMM). Model assumptions were checked using protocols outlined in Zuur *et al.* (2009) and fitted using the "lme4" package in R (Bates *et al.*, 2015). The model structure for the models was based on leaf trait principal components as predictor variables, *WD*, leaf deciduousness and light guilds as fixed effects, and study plot as a random effect variable. We report *P* values and marginalised r^2 for continuous fixed effects (*WD*) (derived using "MuMin" package (Barton, 2018)). Model significance was derived using "anova" function with Satterthwaite's method implemented in "lmerTest" package (Kuznetsova *et al.*, 2017). Post-hoc Tukey's comparisons were derived using "lsmeans" package (Lenth, 2016).

The relationship between leaf trait space and plot level NPP and its components (Q4) was tested using Pearson's correlations. Prior to the analysis we transformed the principal components to community (basal area)-weighted means (CWM; Violle *et al.* 2007) and standard errors following:

$$\mathrm{CWM}k = \sum \cdot i \cdot \mu_i \cdot f_i$$

where k is a study plot, i is species, and μ_i and f_i are the average principal component value and abundance of the species based on basal area, respectively.

7.4.Results

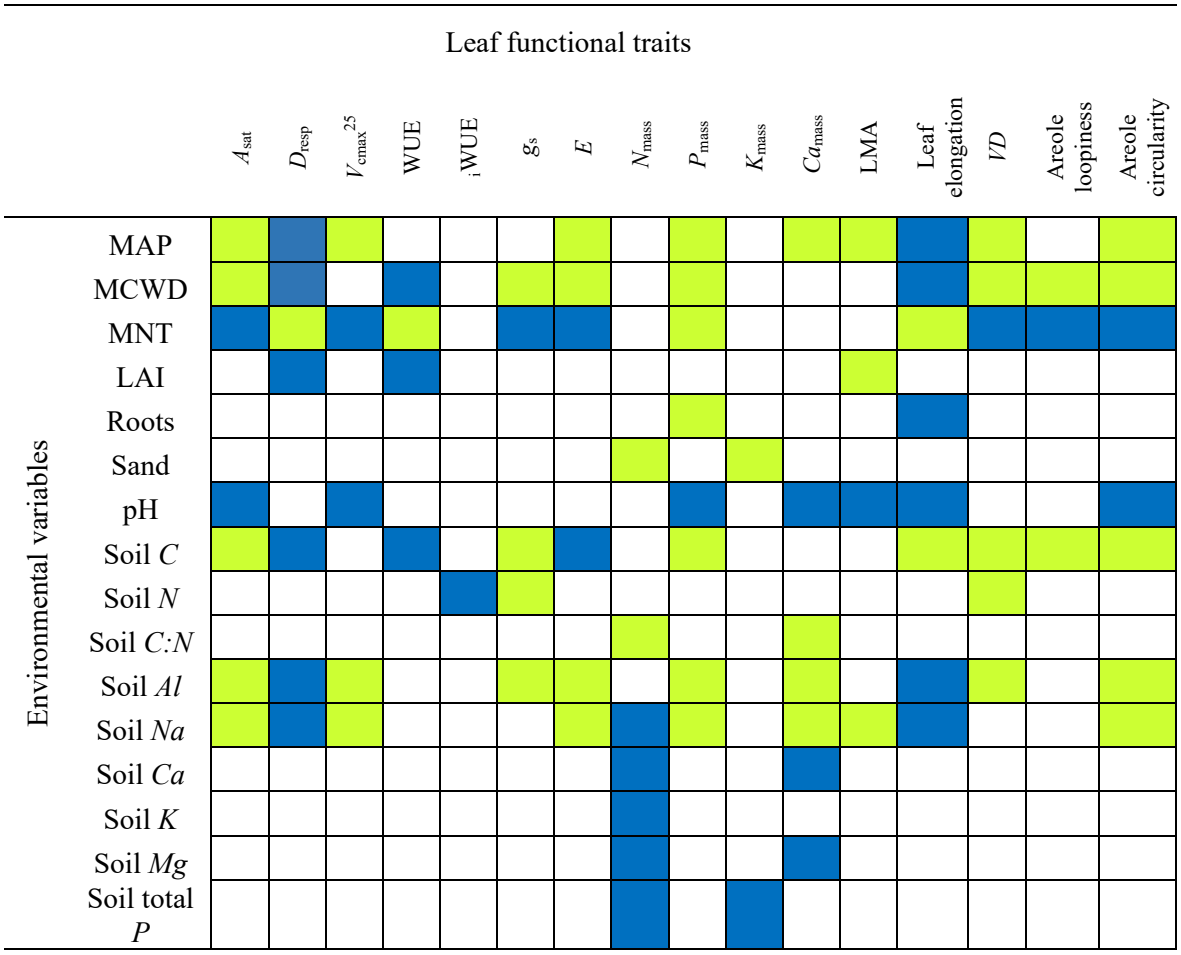
Selection of most responsive traits to the environmental gradient

Out of 48 leaf traits, fourth-corner analysis identified 16 that were significantly responsive to the specific environmental conditions and explained species abundance along the transect (Table 7.2).

In total there were 87 significant correlations between species traits and environmental variables. Leaf elongation and P_{mass} were the most responsive to the environmental gradient.

Out of climatic variables, Mean Noon Temperature (MNT) and MAP had the strongest effect on trait distributions, while the most important edaphic parameters were soil *C*, *Al* and *Na* (Table 7.2). A few traits, specifically, $_{i}$WUE, N_{mass} and K_{mass} showed no relation to climate.

Table 7.2 Results of the significant fourth-corner tests between leaf traits and environmental variables based on 49999 repetitions. Significant ($P < 0.05$) negative associations are represented by yellow cells, and significant positive associations correspond to blue cells. Relationships that were not significant are left blank.



The study plots were situated along two ordination axes of environmental conditions which explained 90% of total variation (Fig. 7.1; **Table S7.1**). The first axis was mostly defined by climate, pH, *Al*

and *Na* cation concentrations, while the second axis was explained by soil fertility, specifically, by soil sand content, soil *N*, *K* and *P* cation concentrations. The wet evergreen forest study sites (ANK01 and ANK03) and savanna woodland plot (KOG01) were located at the opposite ends of the first environmental axis. The rest of the forest-savanna ecotone plots (KOG03 and KOG04) and moist semideciduous forest plots (BOB01 and BOB02) were situated at the opposite ends of the second axis.

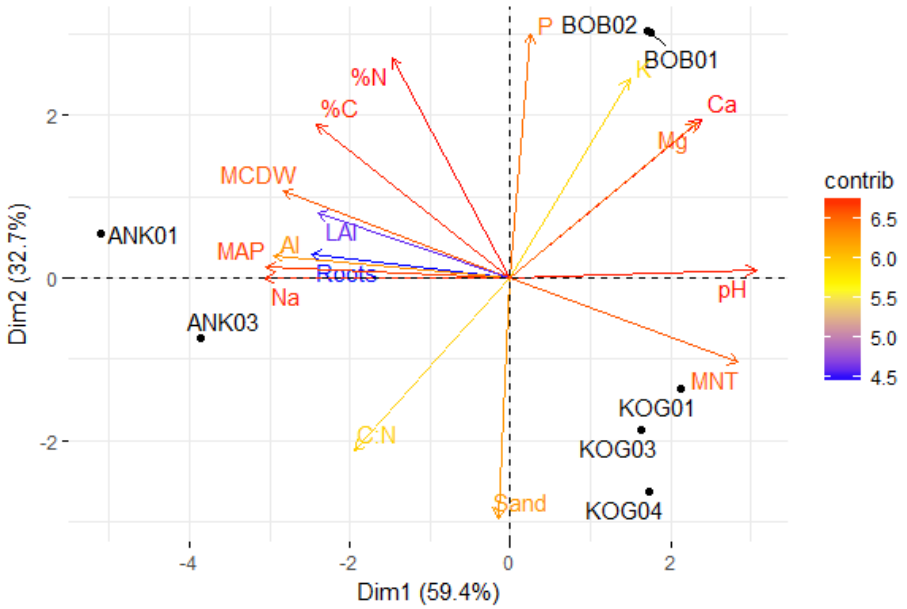


Fig. 7.1 Biplot of principal component analysis performed on the most relevant (for functional trait distribution) environmental variables. The scale represents the contribution of each environmental variable towards the axes, with high values suggesting greater contribution.

Leaf trait space

The first three significant axis of the PCA (Fig. S7.1) on leaf traits selected by the fourth-corner analysis accounted for 66.5% of the total variance and yielded three main ecophysiological dimensions (Fig. 7.2). The first axis of trait space (PC1; 36% of variance) was mostly defined by photosynthetic traits, with lesser correlations evident in P_{mass}, Ca_{mass} and leaf shape, and hereafter referred to as Photosynthesis axis. The second dimension (PC2; 22% of variance) related to $_{i}$WUE,

N_{mass}, K_{mass} and LMA and further referred to as Structure axis. The third axis of trait space (PC3; 9% of variance) was significantly correlated with minor venation metrics, specifically, *VD*, areole loopiness and circularity and further called venation axis.

(a)

	PC1	PC2	PC3
Variance	36.1%	21.8%	8.5%
LMA	-0.18	**0.29**	0.17
N_{mass}	-0.04	**-0.44**	-0.08
P_{mass}	**-0.32**	-0.17	0.15
K_{mass}	-0.01	**-0.41**	-0.21
Ca_{mass}	**-0.30**	0.05	-0.03
Leaf elongation	**0.18**	-0.07	0.06
WUE	0.18	**-0.35**	0.10
$_{i}$WUE	0.10	**-0.46**	-0.02
A_{sat}	**-0.36**	-0.21	0.28
g_s	**-0.38**	0.06	0.26
D_{resp}	**0.35**	-0.05	-0.11
V_{cmax}^{25}	**-0.32**	-0.28	0.26
VD	-0.29	0.16	**-0.41**
Areole circularity	-0.20	-0.17	**-0.43**
Areole loopiness	-0.29	0.02	**-0.55**

(b)

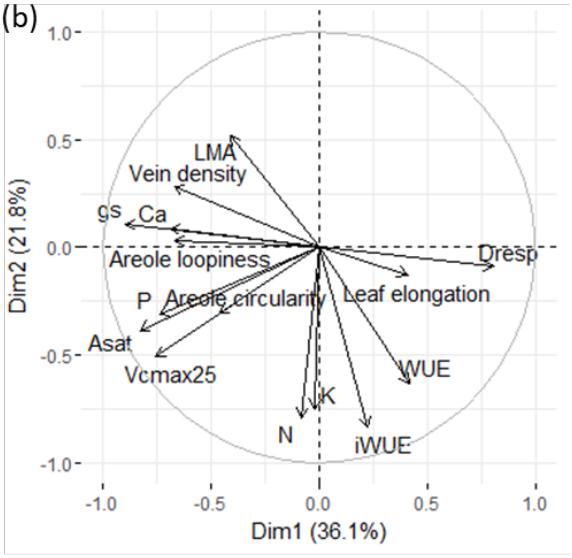


Fig. 7.2 Results of the principal component analysis performed on the selected leaf traits. a) shows the contribution of each trait to the first three axis of variation. The strongest correlations with each axis are indicated in bold. b) is the biplot of scaled functional traits along the first two PCA axes.

Species with high PC1 scores were mostly found in wet evergreen study sites and had low gas exchange rates and low nutrient concentrations (Fig. 7.3). Indeed, average plot level PC1 values were significantly higher at the wettest end of the gradient ($F_{6,81}$=35.4, P<0.001; Fig. S7.2a). Species exhibiting high PC2 scores had high LMA but low N_{mass}, K_{mass} and WUE. This trait space axis was significantly different among the study sites ($F_{6,81}$=5.4, P<0.001; Fig. S7.2b), but it was not because of the rainfall gradient as the lowest values of PC2 were observed in the moist semideciduous forests. Species with high PC3 scores had dense and reticulated vein networks but did not differ significantly along the transect ($F_{6,81}$=1.2, P=0.3; Fig. S7.2c).

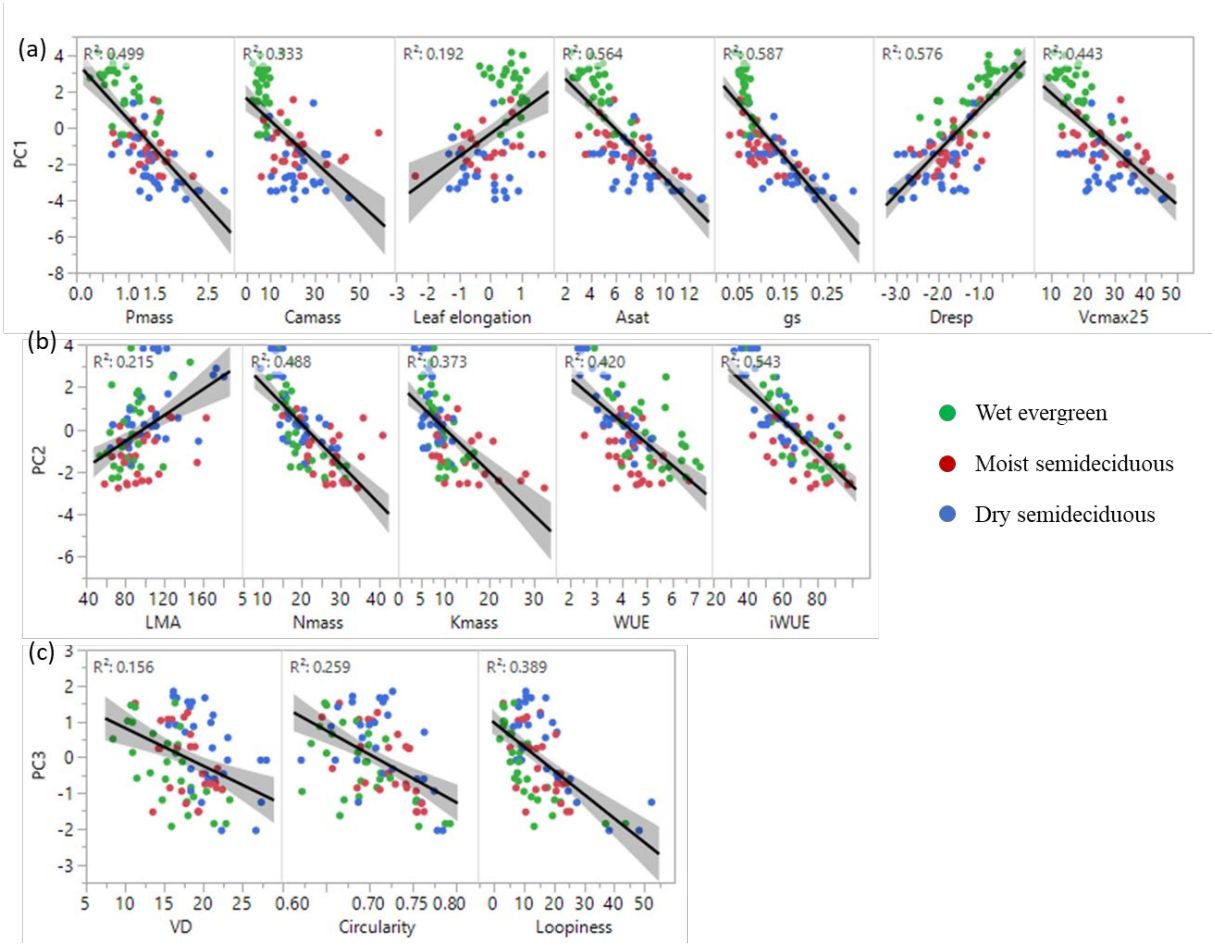


Fig. 7.3 Links between functional trait principal components, PC1 (a), PC2 (b), PC3(C) and individual traits that had the greatest contribution towards the axes variations.

Links between trait space and species ecological strategies

Shade-bearing species had significantly higher values of PC1 and PC2 than pioneers and non-pioneer light demanders ($F_{75.67}$=10.1 and $F_{77.84}$=4.37, respectively, P<0.05; Fig. 7.4). PC3 was not significantly different among the guilds (P=0.6). PC1 was significantly greater in evergreen species than in deciduous species ($F_{80.39}$=12.87, P<0.01). PC2 and PC3 were not different among species of different leaf habit.

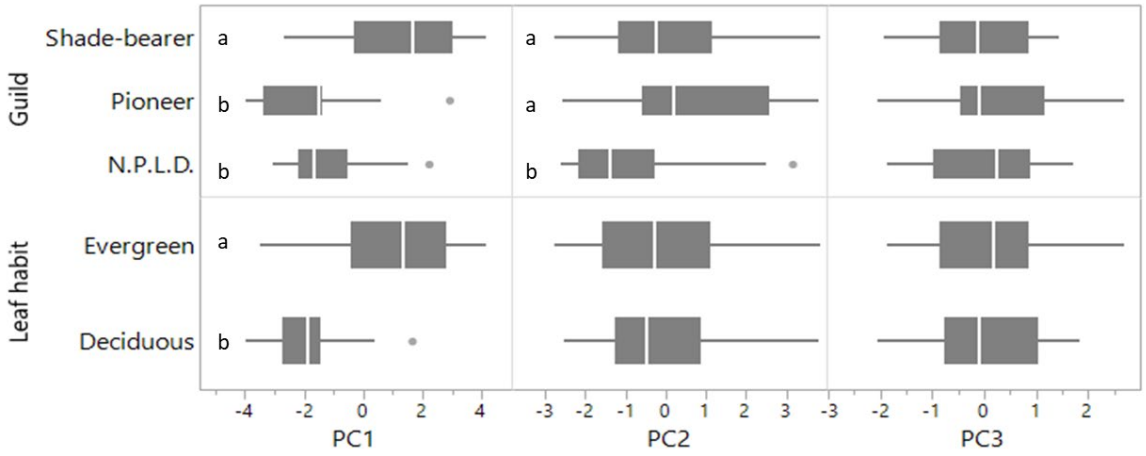


Fig. 7.4 Distribution of functional trait space components (PC1, PC2 and PC3) among species of different guilds and leaf habit. Different letters (or) a and b denote significant Tukey test results.

Wood density showed significant positive relationship with PC1 ($F_{29.18}$=6.35, P<0.05, r^2=0.12) and PC3 ($F_{84.33}$=8.41, P<0.001, r^2=0.10), but not with PC2 (P=0.27) (Fig. 7.5). The relationship between PC1 and *WD* was driven by wet evergreen forest plots, and by dry semideciduous plots between PC3 and *WD*.

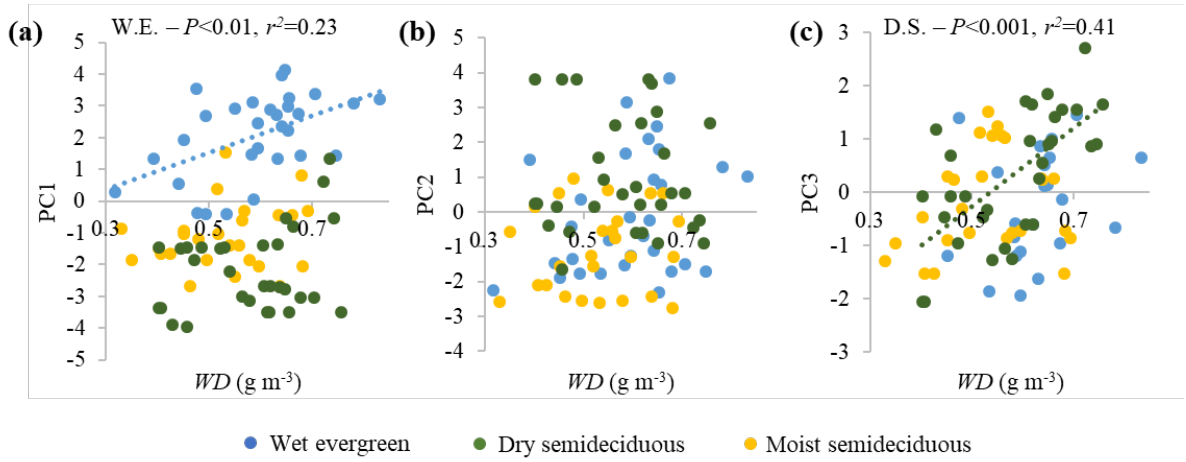


Fig. 7.5 Relationships between wood density (*WD*) and leaf trait space a) PC1, b) PC2 and c)PC3 in each forest type. *P* and r^2 values are for significant linear regressions: W.E. – wet evergreen, D.S. – dry semideciduous.

Net primary productivity, biomass and leaf trait space

There were strong positive correlations between NPP_{branch}, canopy biomass and PC1, while PC2 showed the strongest relationship with aboveground NPP and total aboveground biomass (Table 7.3). PC3 was also correlated with aboveground biomass but to a lesser extent. Interestingly, none of the principal components showed statistically significant correlation with NPP_{leaf}.

Table 7.3 Pearson correlations (*r*) between functional trait space and net primary productivity (NPP) and its' components. Only significant relationships are shown. Significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. = $P > 0.05$.

	PC1		PC2		PC3	
	P	*r*	*P*	*r*	*P*	*r*
NPP_{AG}	n.s.	0.33	*	-0.75	n.s.	-0.41
NPP_{branch}	**	0.94	n.s.	-0.59	n.s.	-0.29
NPP_{leaf}	n.s.	0.17	n.s.	-0.63	n.s.	-0.36
Canopy biomass	*	0.85	*	-0.77	n.s.	-0.27
Aboveground biomass	*	0.76	***	-0.97	*	-0.76

7.5. Discussion

Leaf traits and environmental filtering

Our findings are in agreement with previous studies demonstrating a significant effect of MAP and soil edaphic conditions on the distribution of species and functional traits in Ghana (Swaine & Becker, 1999; Maharjan *et al.*, 2011b). Maximum climatological water deficit (MCWD), in contrast to MAP, also explained trait variability related to species-specific leaf water use, i.e. WUE, g_s, and vein areole loopiness. It has been observed that MCWD explains the distribution of humid tropical forests better than annual rainfall (Zelazowski *et al.*, 2011) which might explain our findings too.

Out of measured edaphic conditions, *Na* and *Al* had the greatest effect on leaf trait dynamics, as changes in these cations along the transect were particularly strong, with the highest values observed in the wet evergreen forests (sites with highest MAP). Low concentrations of other measured cations and macronutrients here in comparison with semideciduous forest sites (Table 7.1) suggest nutrient leaching out of the soil due to high rainfall and potentially lesser nutrient deposition through Saharan dust (Washington *et al.*, 2009). Despite this, *Na* concentrations were ten times higher in the evergreen forest site than in other study sites. High soil salinity is most likely a result of *Na* deposition from oceanic aerosols (McDowell *et al.*, 1990), as these forests are situated less than 50 km away from the Atlantic Ocean. Sodium at such high concentrations can be toxic to plants and effect ecosystem functioning and productivity (Doughty *et al.*, 2016). High levels of *Al* can be explained by strongly acidic soils, which are characteristic of sites with high rainfall and leaching (Kricher, 2011), as even small changes in soil acidity increase the solubility of *Al*. In tropical forests, high levels of *Al* in the soil can contribute to low biomass production (Rehmus *et al.*, 2017), decrease in gas exchange rates (Yang *et al.*, 2015) and low uptake of other nutrients such as phosphorus and nitrogen.

Leaf traits that define trait space

Species-specific traits that varied significantly in response to the environmental gradient, suggested three primary ecophysiological dimensions of leaf trait variation: photosynthetic (PC1), structural (PC2) and venation (PC3). In the LES (Wright *et al.*, 2004), the most studied dimension of leaf economic function, traits are remarkably one-dimensional. If we included gas exchange values expressed per mass basis (not significant in the fourth-corner analysis) in the present study, these would conform to the LES (data not shown). This was also confirmed by Thomson *et al.* (2018) in a remote sensing study of leaf economic traits in some plots along the gradient.

The photosynthetic axis of the leaf trait space (PC1) was mostly defined by stomatal conductance. This is not unexpected as g_s is a functional trait particularly sensitive to environmental conditions in time and space (Flexas *et al.*, 2012). Interestingly, N_{mass} was not linked with the photosynthetic axis

as would be expected from the photosynthesis-nitrogen paradigm (Flexas *et al.*, 2012). Our earlier study (Gvozdevaite *et al.*, 2018) also observed *N* being a poor explanatory variable for photosynthetic capacity in the dry semideciduous forests. This is not to say that *N* did not have any effect on photosynthetic capacity along the gradient, as V_{cmax}^{25} was also a significant variable on all three trait space dimensions.

On the photosynthetic axis, A_{sat}, D_{resp} and V_{cmax}^{25} were also linked with leaf elongation (a leaf shape parameter). In our previous study (Gvozdevaite *et al.*, in prep.) we discuss the importance of narrow and elongated leaves and resulting reduction in boundary layers in environments of high evaporative demand. While it is possible that environmental filtering favours a more narrow leaf shape in hot, dry conditions, which are the same conditions that favour high gas exchange rates (Wright *et al.*, 2001) along the studied gradient, the link between gas exchange and leaf shape was non-coincidental in dry semideciduous forests.

The second leaf trait space axis was strongly linked with leaf water use efficiency. High LMA species are often characterised by higher modulus of elasticity, more negative osmotic potentials and lower hydraulic vulnerability (Scoffoni *et al.*, 2011a) which is beneficial in environments susceptible to drought and drought-induced mortality (Greenwood *et al.*, 2017). However, Bartlett *et al.* (2012) suggested that LMA does not play a role in drought adaptation, therefore, the mechanistic link between LMA and WUE remains to be fully understood.

Additionally, correlation between K_{mass} and $_{i}$WUE on the second leaf traits space dimension was not unexpected as *K* is essential for plant osmotic processes in the leaves, has a direct positive effect on stomatal and chloroplast functioning and plays an important role in long-distance water transport (Wang *et al.*, 2013b; Anschütz *et al.*, 2014; Tränkner *et al.*, 2018). Negative correlation between N_{mass} and LMA should also be anticipated (Wright *et al.*, 2004). Higher leaf density implies higher proportion of cell wall mass and therefore higher proportion of cellulose and lignin and lower *N* (Burton *et al.*, 2010).

Our findings of the third leaf trait axis support the previously suggested importance of minor venation traits to water transport and plant functioning (Sack & Scoffoni, 2013; Blonder *et al.*, 2018). Some studies have also suggested strong influences of leaf vein architecture on LES (Blonder *et al.*, 2011, 2013; Sack *et al.*, 2013). This is seen in strong (but to a lesser extent) manifestation of venation traits on the photosynthetic leaf trait dimension and strong correlations between *VD*, loopiness and g_s. Indeed, vein density is known to have a strong effect on gas exchange due to parallel evolutionary development of these traits and more efficient water supply and distribution across the leaf (Sack & Holbrook, 2006; Boyce *et al.*, 2009; McElwain *et al.*, 2016).

Links between trait space and species ecological strategies

Across the gradient, evergreen species had significantly higher PC1 values than deciduous species with the greatest differences observed in the moist semideciduous forests. This is in accordance established ecological differences between deciduous and evergreen species; the latter having lower gas exchange rates throughout the seasons and species with deciduous habit maximising carbon gain during the wet season (Eamus, 1999). However, while this pattern is evident along the gradient, it is possible that at smaller scales this ecological strategy has more important roles (for example, hydraulic) than maximising photosynthetic rates (Gvozdevaite *et al.*, in press). Despite other leaf trait space dimensions not differing among species of different leaf habit, it is evident that this ecological strategy has an effect on all individual traits across the gradient as indicated by a clustering analysis (Appendix 7.1)

Wood density and trait space

Photosynthetic functional trait space dimension observed here can be related to the "fast-slow" species spectrum (Reich, 2014). Species with low *WD* had low PC1 scores (high photosynthetic rates,

P_{mass} and Ca_{mass}) which is indicative of "fast" species. This group of species were N.P.L.D. and pioneers. "Slow" species were mostly shade-bearing evergreen species. When all species along the gradient were considered, "fast" species were concentrated in the dry semideciduous forests. This distribution of slow-fast spectrum was, however, not due to variability of *WD* along the rainfall gradient as *it* was not significantly different among wet evergreen and dry semideciduous forests, but lower in moist semideciduous forests ($F_{2,107}$=7.5, P<0.001; data not shown).

The relationships between *WD* and leaf trait space were idiosyncratic in each forest type. In dry semideciduous forests, PC3 showed a significant positive relationship with *WD*, indicating that species with low *WD*, which are more sensitive to drought, have higher *VD* and reticulation which can alleviate drought effects. This relationship, observed in sites of the greatest water stress, indicates a trade-off between investment in the leaves and wood. Venation traits have previously been shown to be important in hydraulic supply and maintenance (Sack & Scoffoni, 2013) as well as drought conditions. The relationship between these variables was the most prominent in the studied forest-savanna ecotone. High *WD* can expected at these study sites as they experience intermittent water availability and, potentially, drought conditions. These plots also have significantly higher *VD* and loopiness also potentially to avoid drought induced embolism in the leaves (Sack *et al.*, 2015). Surprisingly, the relationship between *WD* and venation parameters was negative. Potentially, there is a trade-off between resource allocation in wood or vein traits to avoid detrimental drought effects.

NPP and leaf trait space

Finegan *et al.* (2015) found that "fast" traits, specifically, LMA and leaf *P*, were significant predictors of aboveground biomass increments; sites with low LMA and high leaf *P* had greater biomass production. This is in agreement with our finding, as there was a negative relationship between biomass, NPP_{ABG} and PC2 (axis with LMA on it) and positive relationship between biomass, NPP_{branch} and PC1 (axis with P_{mass} on it).

Correlations between canopy, stand aboveground biomass, branch biomass increments and PC1 are expected as these variables vary in response to the rainfall gradient- the greatest factor affecting species and ecosystem diversity in this region. While PC3 did not vary significantly among the study plots, CWM PC3 was significantly greater in the dry semideciduous forests due to high *VD* and reticulation in these sites, suggesting linear-like (but to a lesser extent than PC1) changes of PC3 along the rainfall gradient. Hence, a significant lesser relationship between biomass and CWM PC3.

The strongest correlation was observed between PC2 and AGB driven by the high values in the moist semideciduous forests. It seems that particularly high soil fertility and low sand content in these study sites explains the lowest PC2 (low LMA, nutrient rich and high ¡WUE leaves) and carbon stocks significantly greater than in other forests types (Moore *et al.*, 2017).

Concluding remarks

Our findings demonstrated important and location specific links between leaf functional trait space and the environment. Additionally, the results demonstrated that quantifying trait space of multiple species requires the measurement of multiple variables. Priority should be given to sampling traits from different functional dimensions from multiple organs to maximize the species' functional niche represented by the traits measured. These observations are important to developing more accurate mechanistic understanding of climate driven changes in tropical forest ecosystem functioning.

7.6. Supplementary materials

The following Supplementary Information is available for this article:

Table S7.1 Contribution of environmental variables towards the PCA.

Fig. S7.1 Broken-stick analysis of trait PCA

Fig. S7.2 Differences in trait space across the study plots

Fig. S7.3 Correlations among selected leaf traits.

Appendix 1 Clustering analysis of functional traits

Table S7.1 Contribution of environmental variables towards the two significant axes of environmental PCA.

	PC1	PC2
Variance	59.4%	32.7%
MAP	**-0.32**	0.02
MCWD	**-0.30**	0.15
MNT	**0.30**	-0.15
LAI	**-0.25**	0.11
Roots	**-0.26**	0.04
Sand	-0.02	**-0.42**
pH	**0.32**	0.01
% *N*	-0.16	**0.38**
% *C*	-0.25	**0.27**
C:N	-0.20	**-0.30**
P	0.03	**0.43**
Al	**-0.31**	0.04
Ca	0.25	**0.28**
K	0.16	**0.35**
Mg	0.25	**0.27**
Na	**-0.32**	0.00

Fig. S7.1 According to broken stick criterion (b) only the first two axes explain more variation than the null expectation, but the other criteria identified the first three as significant, therefore, we kept 3 axes for further analyses.

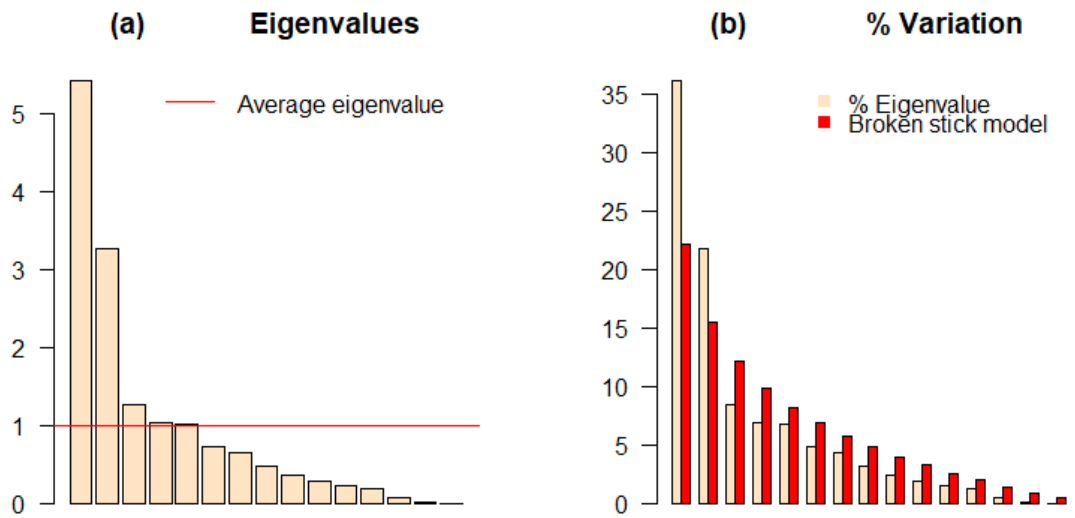


Fig. S7.2 Median values of first three trait principal component scores in each study plot. Letters represent significance.

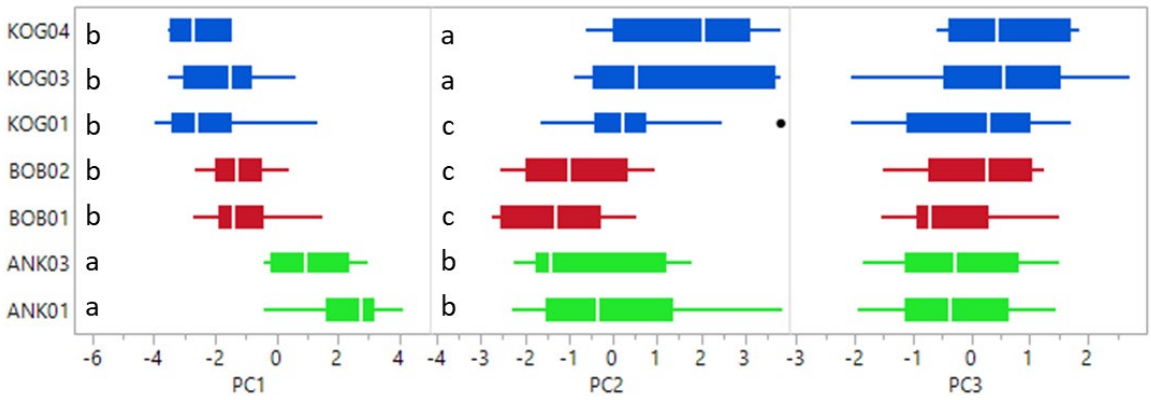
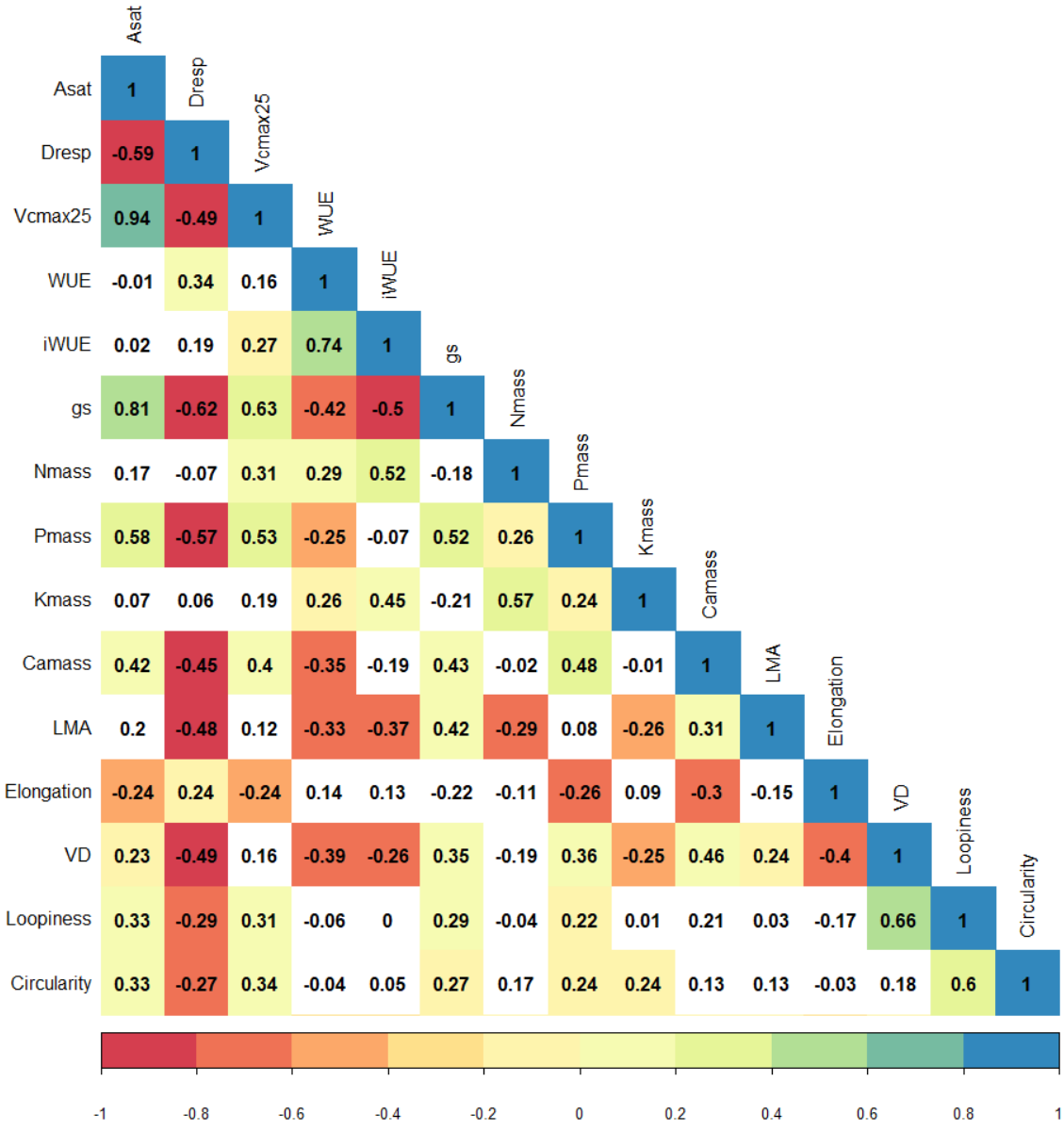


Fig. S7.3 Correlations between selected leaf traits. Number indicate correlation coefficient and are matched with a colour scale. Cells without colour were not significant.



Appendix 7.1 Clustering analysis of functional traits

To identify if leaf traits are reflected in other life strategies not already discussed we performed clustering analysis. Clustering of selected leaf traits was performed using hierarchical cluster analysis on a euclidean distance matrix of the selected leaf traits. The best number of clusters was informed by calculating the Calinski–Harabasz index which evaluates the cluster validity based on the average between and within-cluster sum of squares.

The Calinsky-Harabasz criteria indicated two distinct data clusters as the best division of trait data, hereafter referred to as Cluster 1 and Cluster 2 (Fig. S7.4). Cluster 1 had 41 species and Cluster 2 - 40 species.

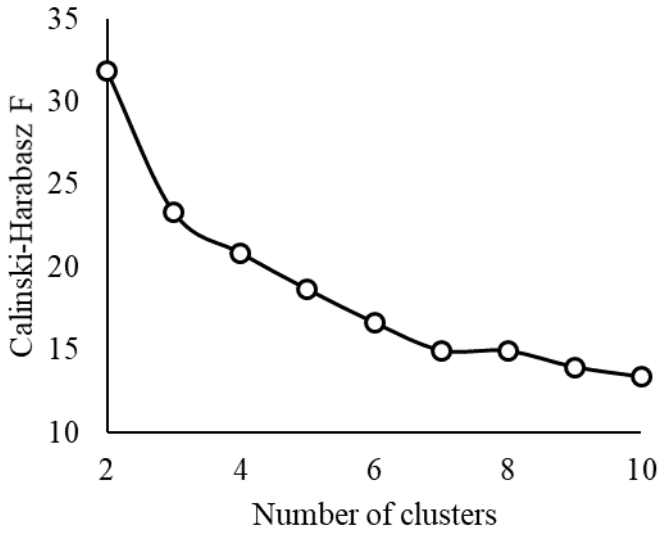


Fig. S7.4 Calisnky–Harabasz index values for a number of clusters ranging from 2 to 10 using the average species selected trait values.

All leaf traits were significantly different between the two different clusters (Fig. S7.5). Cluster 1 had species with high leaf nitrogen, WUE, areole roughness and strong leaf elongation. PC1 was significantly higher in Cluster 1 than in Cluster 2 ($t\text{-}test_{75.0}$=-14.0, P<0.001). PC2 and PC3 were not significantly different among the clusters.

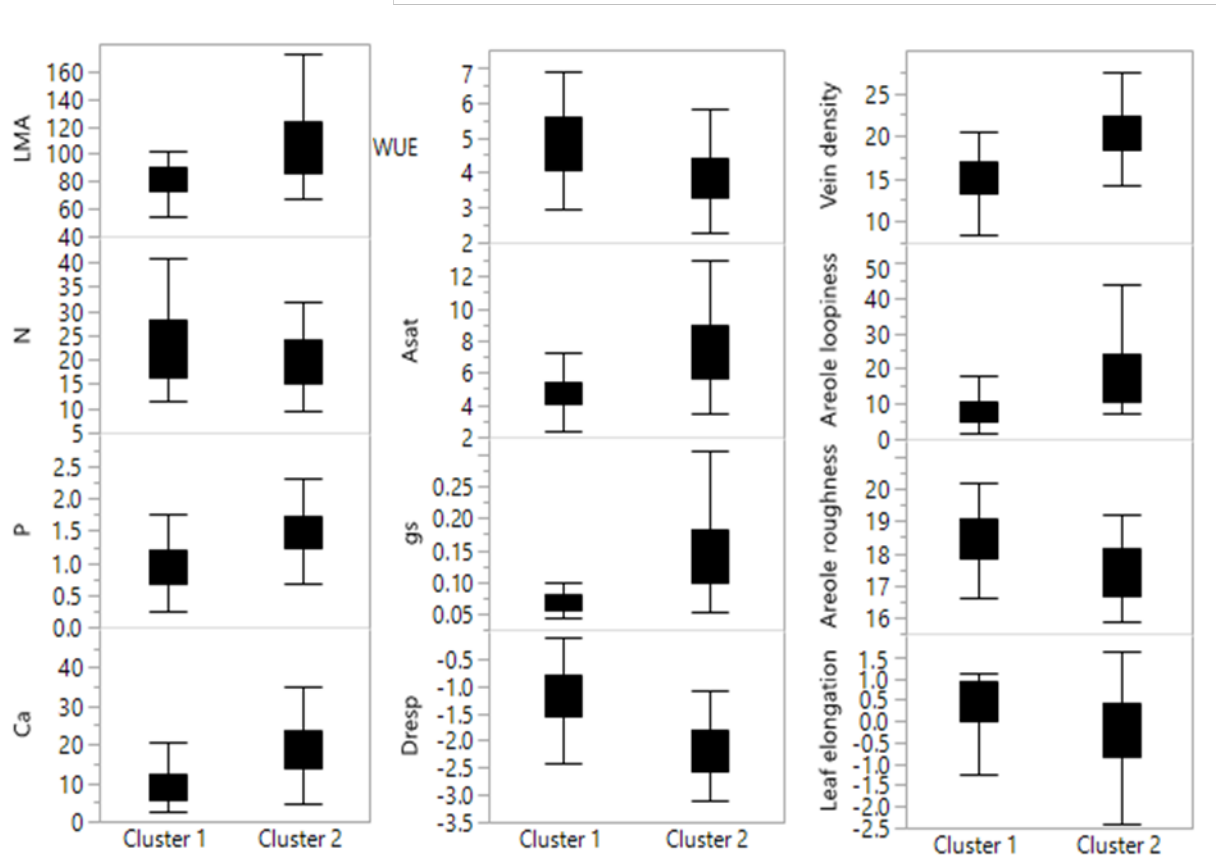


Fig. S7.5 Boxplots summarising the distribution of selected leaf trait values in each cluster.

Chi-squared test revieled that the two clusters were significantly related to species leaf habit. More than 80% of species in Cluster 1 were evergreen and 76% of species in Cluster 2 were deciduous (*Chisq*=31.88, *P*<0.001) (Fig. S6).

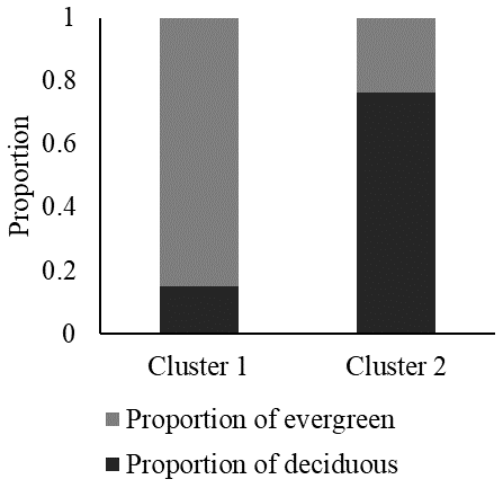


Fig. S7.6 Proportion of evergreen and deciduous species in each cluster.

Chapter 8

Synthesis and conclusions

8.1. Summary and implications of key findings

Changes in climate due to increases in atmospheric greenhouse gas concentrations are already having a significant impact on the functioning of tropical forest ecosystems. Environmental changes are particularly strong in West Africa, where reductions in rainfall have been consistent over the last few decades. Here, tropical forest-savanna transitions are expected to be amongst the most vulnerable vegetation types to climate and land-use change in the future as the stability of the boundaries between the forest and savanna biomes is usually weak (Hirota *et al.*, 2011; Staver *et al.*, 2011a; Oliveras & Malhi, 2016). With further predicted changes in temperature and precipitation patterns, understanding the sensitivity of trees and forests to changing environmental conditions is, therefore, of particular importance. Due to the lack of data (especially in Africa) and often inadequate mechanistic understanding of functional traits, the greatest uncertainties in predicting tropical forest sensitivity to climate change are the physiological responses of trees to a changing environment. Therefore, the overall aim of the thesis was to better understand the dynamics and functional role of key economic, morphological and venation traits in the tropical forests of West Africa. I achieved this by exploring the large collection of trait data in relation to abiotic and biotic factors considered to be the main driving forces of species distributions, abundance and diversity, namely, rainfall, temperature, light availability, soil fertility and leaf nutrient availability.

In summary, the findings of the thesis point towards multidimensional and multifunctional patterns of leaf trait variability. Strong environmental filtering along the studied gradient significantly affected different dimensions of leaf trait variation which, together, linked with different aspects of plant and ecosystem functioning. Some trait-trait and trait-environment relationships were strong in response to specific limiting environmental conditions but became weaker at larger scales due to their multifunctional role. Additionally, the thesis showed that phylogenetic considerations are important in functional trait studies as some relationships among traits, environment and function were strongly influenced by species evolutionary history and relatedness. These findings have strong implications for our understanding of functional trait significance and dynamics observed in the tropics, and highlights the need for further research.

The four key research questions of the thesis listed in **Section 1.4** were explored through four data chapters, the main findings, implications and future research recommendations of which are summarized below.

8.1.1. Photosynthetic capacity in forest-savanna ecotones

Question 1: *Are photosynthetic capacity, key leaf and soil nutrients linked along forest-savanna boundaries?*

This question was addressed in **Chapter 4**, through the research paper entitled "*Leaf-level photosynthetic capacity dynamics in relation to soil and foliar nutrients along forest-savanna boundaries in Ghana and Brazil*".

The key finding of this study was that key leaf nutrient concentrations (N_{area}, P_{area} and K_{area}) were poor predictors of photosynthetic capacity (V_{cmax}^{25}) along the two transects in forest-savanna ecotones (Fig. 4.3). From a statistical perspective, this was the result of a large scatter of nutrient values when compared to corresponding V_{cmax}^{25} values which did not vary significantly among the

study plots. Ecophysiologically, the findings suggest that species present in these forest-savanna ecotones do not maximise V_{cmax}^{25} for given key leaf nutrients; rather, these are utilized for other functions required to cope with irregular availability of water, long periods of drought, high temperatures and irradiance.

The lack of observed $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship has strong implications for the nitrogen-photosynthesis paradigm employed in many biosphere models. Often, when V_{cmax}^{25} data is not available, it is estimated from the available *N* values assuming constant relationship between these traits. This suggests that modelled GPP values for these ecotones are inaccurately estimated, which negatively impact our understanding of tropical carbon balances and cycling. The importance of this oversight is further exacerbated by the fact that tropical forest-savanna boundaries are widespread and extend across large parts of South America, Africa, Australia and Asia (Torello-Raventos *et al.*, 2013; Veenendaal *et al.*, 2014; Lloyd *et al.*, 2015).

Another finding with strong implications for our understanding of tropical forest functioning is the lack of difference in V_{cmax}^{25} as well as foliar nutrient between deciduous and evergreen species. It is generally assumed that one of the key differences between species of different leaf habit is to maximise carbon gain during the wet season in the case of deciduous species and to maintain photosynthesis during the dry season in evergreen species. The findings insinuate a more important role of leaf deciduousness than maximizing photosynthetic rates, at least in these ecotones and which may be related to hydraulic function (also indicated in **Chapter 6**).

Understanding the drivers of photosynthesis is a major avenue for future research towards better predictions of tropical carbon cycling and sensitivity of forest-savanna boundaries to environmental change. A few specific areas that require particular research attention are *N* allocation among photosynthetic machinery and leaf structure, links between soil and leaf nutrients and nutrient interactions within leaves. Additionally, it is important to further investigate the role of leaf habit in environments of high evaporative demand. It is possible that evergreen species present in these

regions function in a different way to evergreen trees in wetter environments with less water stress. From a methodological point of view, this study shows that photosynthesis is a highly variable trait even within a single branch and species level measurements might not provide full insights. While V_{cmax}^{25} data collection is time consuming, as shown in this study, it can be collected at species level and precisely estimated from more simple gas exchange metrics such as A_{sat} (Fig. S4.1). Additionally, the majority of studies, including this, exclude photosynthetic measurements with low stomatal conductance which can limit our understanding of the true drivers of gas exchange.

8.1.2. Significance of leaf shape

Question 2: *How and why does leaf shape vary along the studied environmental gradient?*

This question was addressed in **Chapter 5**, through the research paper entitled "*Significance of leaf shape dynamics along light and temperature gradients in the tropics*".

This study highlighted the importance of leaf shape, specifically, leaf elongation and effective width, for plant thermoregulation and adaptation to light availability. While elongated and narrow leaves were present at sites at both ends of the environmental gradient (Fig. 5.2), the interpretation of the results suggests that this is because of the different roles that leaf shape plays in environments of different stresses. Narrow leaves potentially enable significantly lower leaf-to-air temperature differences, thermal time constants and greater maximum photosynthetic rates in environments of high evaporative demand where stomatal conductance is supressed. Larger but also elongated leaves can improve coping mechanisms to limited light environments. The role of leaf shape in increasing efficient light acquisition was also confirmed by observed significant differences in shape traits among species with different light requirements for germination and establishment as well as sunlit and shaded leaves.

The dataset for leaf shape traits (almost 2,000 leaves from 72 species) was among the largest ever used in tropical leaf form studies, and the first one from West Africa. In contrast to other studies in the tropics, this was the first to use geometric morphometrics methodology, which offers quantitative description of leaf shape metrics at a leaf rather than species only level. This study also benefits from estimations of leaf-to-air temperature differences and thermal time constants according to the leaf energy budget theory. The study did not measure leaf temperatures directly, however, parameters used to estimate these variables, specifically, midday air temperatures, were conservative. This implies that the values we present were not overestimated but rather, conservative estimates. In reality (from personal observations), midday air temperatures can be significantly higher, especially at the end of the rainy season going into the dry season, further emphasizing the importance of thermoregulation and therefore, leaf shape role.

The findings of this study demonstrate that including leaf shape metrics in plant performance modelling can improve the understanding of ecosystem response to environmental change. The methodology this study utilized to quantify leaf shape as well as thermoregulation metrics can be easily adopted in other studies and existing datasets of leaf scans. Even when exact leaf temperatures are not available, the estimates of thermoregulation based on leaf shape can be useful in showing important patterns of plant adaptations to environmental conditions. The findings of this study highlighted areas that require further research. Specifically, it is important to understand the links between stomatal conductance and leaf shape in environments of high evaporative demand throughout the day, even when stomatal limitations occur. Additionally, in studies investigating leaf shape significance, it is imperative to include species that develop and exist in the tropical understory with limited light availability.

8.1.3. Venation and water availability

Question 3: *Does venation architecture play an important role in plant's adaptation to intermittent water availability?*

This question was addressed in **Chapter 6**, through the research paper entitled "*Water availability drives venation architecture along a tropical rainfall gradient in West Africa*".

The results of this study suggest that leaf venation varies in response to water availability and, potentially, is an import feature of a whole plant strategy in response to drought. This conclusion is based on the observations of denser and more reticulated networks at sites of the highest evaporative demand and most prone to water deficits (Fig. 6.1**)**. Additionally, venation traits were significantly different among species of different leaf habit and showed the strongest links with leaf potassium and leaf area, traits known to be important for osmotic adjustment and thermoregulation, especially in the driest study sites.

While the importance of venation for efficient water transport in drought conditions has been suggested before, this was the first study to demonstrate clear links between water availability and vein traits along a strong precipitation gradient. Findings of this study, together with previously highlighted structural and defensive roles of leaf venation along a strong elevation (Andes-Amazon) gradient (Blonder *et al.*, 2018) suggest multidimensionality and structural importance of vein architecture. This was also highlighted by the varying influence of species evolutionary history on the trait-trait relationships which was uncovered by using phylogenetic comparative methods.

Dynamics of vein traits still remain unmeasured for the vast majority of plant taxa, despite their importance in regulating gas exchange between leaves and atmosphere. This negatively impacts our understanding of vein functional significance, further highlighted in this study. Research areas that require particular attention are mechanistic understanding of vein and wood trait relationships and links between leaf deciduousness and vein architecture. Veins have high carbon costs and can displace photosynthetic tissue, but according to this study, are significantly higher in deciduous species, which raises many questions around the role of leaf habit in maximising carbon gain in

seasonal environments. Additionally, this study further suggests the importance of incorporating values of soil and leaf potassium concentrations into functional trait studies.

One factor hampering the advance in our understanding of the role that veins play in plant and ecosystem functioning is very time consuming and expensive data collection. Leaves have to be chemically cleared, photographed using a microscope and hand-traced which can take weeks to months, per batch. Numerous pieces of software that are able to characterise vein networks in a semi-automated way do exist, however, these do not produce acceptable results for many tropical species with complex vein networks, thick cuticles and/or trichomes. Therefore, there is a significant avenue for improving computational approaches to leaf vein analysis.

8.1.4. Leaf trait space and ecosystem function

Question 4: *How does multivariate leaf trait space affect plant and ecosystem performance?*

This question was addressed in **Chapter 7**, through the research paper entitled "*Leaf functional trait space reflects species ecological strategies and ecosystem productivity*".

The main purpose of this chapter was to bring together the aforementioned leaf traits without previous assumptions of their functional importance, identify the most significant ones for community assembly along the environmental gradient and accordingly, formulate a functional trait space which can further be linked to ecosystem functioning. Fourth-corner analysis, currently the most integrated method to test for relationships between multiple traits and environment while including species abundance information, identified 16 leaf traits with the greatest variability. These traits formed a trait space of three significant dimensions: photosynthetic, structural and venation which linked with species ecological strategies (deciduousness and light guilds, wood density), net primary productivity (NPP) components and canopy biomass.

As seen in this study (and previous chapters) environmental filtering was evident throughout the gradient which resulted in differentiation of trait space. The photosynthetic axis varied along the climatic gradient (greatest gas exchange at the driest end of the gradient), while the structure axis values were the lowest (high leaf mass per area but low nutrients and water use efficiency) in the most fertile sites. Intermittent water availability and high midday temperatures were mediating trait and trait space at one end of the studied gradient, poor soil fertility and low light environments at the other end (**Chapter 6** and **Chapter 7**). High macronutrient and cation concentrations in the moist semideciduous forests might explain low structural axis values and high NPP (See Moore *et al.*, 2017). Species here produce cheap (low LMA), nutrient rich leaves biannually, which is reflected in high $NPP_{herbivory}$ and high photosynthesis. Additionally, strong phylogenetic coordination between wood anatomy and leaf venation here is significantly lower than in other sites (**Chapter 6**) suggesting weaker environmental filtering.

This study shows that leaf traits alone have a strong predictive power of plant and ecosystem function when sampled from different functional dimensions including water transport traits, in addition to economic and morphological traits. It is evident that identification of changes in multivariate plant strategies is needed to further advance our understanding of ecosystem function. This study highlights many research gaps in our understanding of tropical forest functioning, especially in trait-trait trade-offs in multidimensional settings.

8.2. Concluding remarks

With further projections of global change, improved understanding of the causes and consequences of tropical forest ecosystem functioning and dynamics has never been more important. Based on one of the largest and wide-ranging datasets of leaf functional traits, the thesis provides evidence that integrating information about functional trait variability into terrestrial biosphere models will improve our ability to predict forest sensitivity and forest response to anthropogenic climate change. Additionally, while highly dynamic environmental gradients are a multidimensional problem and often challenging to interpret, they provide some of the most holistic opportunities to better understand functioning of tropical forest ecosystems.

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