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PAULO ROBERTO DE LIMA BITTENCOURT

ECOPHYSIOLOGY AND ECOHYDROLOGY OF A  
TROPICAL RAINFOREST AND A TROPICAL CLOUD  
FOREST

ECOFISIOLOGIA E ECOHIDROLOGIA DE UMA  
FLORESTA TROPICAL CHUVOSA E UMA FLORESTA  
TROPICAL NEBULAR

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RAINFOREST AND A TROPICAL CLOUD FOREST**

**ECOFISIOLOGIA E ECOHIDROLOGIA DE UMA FLORESTA  
TROPICAL CHUVOSA E UMA FLORESTA TROPICAL NEBULAR**

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*Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.*

“Ensinar a pesquisar: essa é uma das grandes alegrias do professor, somente comparável à do pai que vê o filho partindo sozinho, como pássaro jovem que, pela primeira vez, se lança sobre o vazio com suas próprias asas. O professor vê o discípulo partindo para o desconhecido, para voltar com os mapas que ele mesmo irá fazer, de um mar onde ninguém mais esteve. É isso que deve ser uma pesquisa e uma tese: uma aventura por um mar que ninguém mais conhece.”

Rubem Alves - Do universo à jabuticaba

Há 10 anos comecei minha caminhada como pesquisador. Não é uma tese que forma um pesquisador. Pesquisador é o curioso que quer preencher com mais uma peça um quebra-cabeças que lhe cativou. Mestre, doutor, professor, a diferença é meramente quantitativa, de habilidade, do tempo que se passou jogando o jogo e das técnicas que aprendeu para jogar. Pesquisador é aquele que tem diante de si um quebra-cabeças para resolver. A grande diferença do pesquisador profissional e amador é apenas a sistematicidade com que pesquisa. Mas que sejamos todos pesquisadores profissionalmente amadores.

Em um mundo perfeito, não precisamos de engenheiros, técnicos, médicos carpinteiros... Todas estas são profissões necessárias, meios, mas não fins. Em um mundo perfeito não há problemas. No mundo perfeito restam os artistas, os amantes e os pesquisadores. Pois é isto que move a alma humana, a criação, o belo e a curiosidade pelo mistério da existência, do universo e da consciência que somos. Estas são as profissões realmente necessárias à humanidade e à alma na essência do homem, quando a não-idealidade do universo é resolvida: o artista, o amante, o engenheiro artista, o técnico artista, o médico artista, o carpinteiro artista e o pesquisador. Tenho orgulho de, junto aos artistas, que criam pelo prazer de criar, e os amantes, que amam pelo prazer de amar, fazer parte de uma das três únicas ocupações que continuariam existindo em no mundo ideal, o pesquisador, que descobre pelo prazer de descobrir.

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# Resumo

A água é um dos principais fatores afetando quase todos os processos bióticos e abióticos que ocorrem na superfície do planeta. Sobre a superfície do planeta, plantas modulam fortemente a dinâmica da água. Plantas conectam o solo com a atmosfera através de suas raízes até as folhas, através do seu sistema de transporte hídrico, formando o contínuo solo-planta-atmosfera. Entretanto, temos muitas lacunas no nosso entendimento do transporte de água de plantas, desde os mecanismos atmosféricos determinando o transporte de água, disponibilidade de água no solo até a fisiologia do sistema de transporte hídrico de plantas e seus custos e demandas conflitantes. Para preencher essas lacunas, nesta tese eu uso novas abordagens para entender a demanda atmosférica de água, o transporte de água da atmosfera para o solo e os custos nutricionais do sistema de transporte de água de plantas em uma Floresta Tropical Nebular e uma Floresta Tropical Chovosa. No primeiro capítulo, eu uso um método inovador para estudar com alta resolução temporal os efeitos da neblina na água do solo e da atmosfera. No segundo capítulo, eu estudo um processo frequentemente ignorado, o transporte de água da atmosfera para o solo. No terceiro capítulo, eu estudo e proponho novas perspectivas para entender os custos e as eficiências do sistema de transporte hídrico de plantas. No quarto capítulo, eu estudo os custos nutricionais da madeira e suas relações com o sistema de transporte hídrico de plantas. Por fim, eu discuto os principais resultados e suas implicações para a ecohidrologia e ecofisiologia de plantas.

# Abstract

Water is one of the key drivers of almost all biotic and abiotic process that occurs in the surface of the planet. Over the terrestrial surface of the planet, plants modulate most of water dynamics. Plants connect water in the soil with the atmosphere through their roots and up to the leaves passing through its water transport system, forming the soil-plant-atmosphere continuum. However we have many gaps in the understanding of plant water transport, from the understanding of the atmospheric drivers of water transport, soil water availability up to the plant water transport system physiology and its costs and tradeoffs. To adress those gaps, in this Thesis I use novel approaches to understand atmospheric water demand, water transport from the atmosphere to the soil and the nutritional costs of the water transport system of plants in a Tropical Cloud Forest and in a Tropical Rainforest. In the first chapter of this work, I use an innovative method to study with high temporal resolution fog effects on atmospheric and soil water inputs. In the second chapter, I study an often overlooked process, water flow from the atmosphere to the soil. In the third chapter, I study and propose new perspectives to understand the costs and efficiencies of a plant is water transport system. In the forth chapter, I study the nutritional cost of wood and its relations with plant is water transport system. Finally, I discuss the key results and its implications to plant ecohydrology and ecophysiology.

# Sumário

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# Introduction

Tropical rainforests (TRs) store 67% of terrestrial biomass (Pan *et al.*, 2013), control local and global rainfall (Lawrence & Vandecar, 2014) and house much of the world's biological and genetic diversity (Slik *et al.*, 2015). However temperature and drought induced changes to TR's ecological structure and function are predicted to pose a major threat to the Earth System in the coming century (Lenton *et al.*, 2008). Drought mortality of trees is already reported worldwide (Allen *et al.*, 2010) and data suggests different plant types have different vulnerabilities to future climates, with larger trees more vulnerable than smaller trees (Phillips *et al.*, 2010; Rowland *et al.*, 2015) and plants with less robust water transport system more vulnerable to drought (Anderegg *et al.*, 2016; Greenwood *et al.*, 2017). However, we lack basic knowledge on plant traits and function to accurately predict consequences of climate change to ecosystems. For some ecosystems with peculiar climates, such as ecosystems with frequent fog, we even lack basic information on plant functioning and even on hydrology (Oliveira *et al.*, 2014). To complicate this scenario, plant response or adaptation to climate change may be limited by nutrient availability (Wieder *et al.*, 2015; Gessler *et al.*, 2017)..

Ecosystems with frequent fog, such as tropical montane cloud forest (TMCF), have a peculiar climate with low radiation, reduced evapotranspiration, surfaces frequently wet and additional water inputs from fog drip (Bruijnzeel *et al.*, 2011a; Jarvis & Mulligan, 2011). Due to low evapotranspiration and additional water inputs from fog drip they have a disproportional contribution to their basin is water discharge (Bruijnzeel *et al.*, 2001). While we some basic knowledge of their hydrological importance, we have very limited data on plant functioning in those ecosystems. We know they have a distinct assemblage of plants (Bertoncello *et al.*, 2011) and studies suggest fog occurrence may have an important role in alleviating drought stress of TMCF plants (Eller *et al.*, 2013, 2016). However, fog effects on plants are complex and may depend on fog timing and intensity (Berry *et al.*, 2014a; Oliveira *et al.*, 2014). Part of our lack of understanding of plant functioning in TMCFs is because we lack basic data on fog regime and effects on the abiotic environment. Part of this gap is because traditional meteorological instrumentation is not adequate to monitor fog and part of it is because traditional climatological analysis focus on environmental traits that are not the most relevant to plant functions. To

address this basic gap in knowledge, in my chapter 1 – **“High temporal resolution of fog occurrence and its effects on water and light availability in a tropical montane cloud forest in Brazil”**, I report the results of our work in a TMCF in the Mantiqueira Range in the Atlantic Forest, where we used, to our knowledge for the first time in ecological work, a visibilimeter to monitor fog occurrence and intensity with high resolution. We use this data to evaluate fog effects on light and water availability to plants, using plant-relevant environmental analysis, and discuss consequences of fog patterns to plant function.

Another of the elements that make fog prone ecosystems unique is the amount of time leaves stay wet. Tropical forests trees usually stay more than 25% of their lifetime with wet leaves just due to dew (Alvares *et al.*, 2015). In ecosystems with frequent rain or fog plants may stay more than half their lifetimes with wet leaves (Bruijnzeel *et al.* 2001). It is known for a long time that plants can absorb water through their leaves (Slatyer, 1956) and recent studies indicate foliar uptake of water may be important for plant functioning (Burns *et al.*, 2009; Berry *et al.*, 2014a; Eller *et al.*, 2016). However, we also lack basic knowledge of foliar water uptake patterns. We do not know in which climatic conditions it occurs, how it varies between individuals and species and what are the plant traits modulating foliar water uptake. I address this gap in my chapter 2 – **(TITLE HERE)** - by measuring reverse sap flow in 38 TMCFs trees of 11 species. Reverse sap flow occurs when plants are absorbing water through their leaves due to the atmosphere being wetter than the soil. In this work, I evaluate if reverse sap flow occurs, and how intense it is, in different climatic conditions – dry and wet season, daytime and nighttime, and during dew, fog, rain and fog-rain events. Additionally, I analyze the abiotic environment in which reverse sap flow occurs and if tree hydraulic traits modulate reverse sap flow occurrence.

After addressing gaps in knowledge of abiotic drivers of tree water transport in fog prone ecosystems and exploring in depth, for the first time, foliar water uptake patterns, I address the water transport system of plants. Plants transport water from roots to leaves through their xylem. Water in the xylem of plants is transported under tension (Cruziat *et al.*, 2002; Tyree, 2003). Liquid water inside leaves is transpired to a drier atmosphere, pulling water up the transporting tissues (the xylem) from the soil. Water transport in a plant therefore occurs under tension, if however this tension becomes too high, the water column can rupture causing hydraulic failure and impaired function or mortality. Understanding how trees control xylem

tension and how resistant they are to hydraulic failure is fundamental to understand tree function and predict what will happen to ecosystems exposed to future drier climates (Sperry & Love, 2015). One of the most theoretically expected tradeoffs in plant water transport system is that plants that can transport water under high tension (i.e. more safely) have a low water transport efficiency due to conduit traits determining safety and efficiency being antagonists (Cruziat *et al.*, 2002). However, a recent global dataset only found very weak evidence for this highly expected relationship (Gleason *et al.*, 2015). To address this theoretical problem, I problematize in my chapter 3 – **“On xylem hydraulic efficiencies, wood space-use and the safety-efficiency tradeoff”** – the theoretical framework of xylem water transport efficiency. We argue that there are multiple xylem hydraulic efficiencies and that the one currently used, xylem specific conductance, is actually a measure of xylem space-use efficiency. We propose new measures of xylem efficiency and propose hypothesis that this new approach on plant water transport system raise.

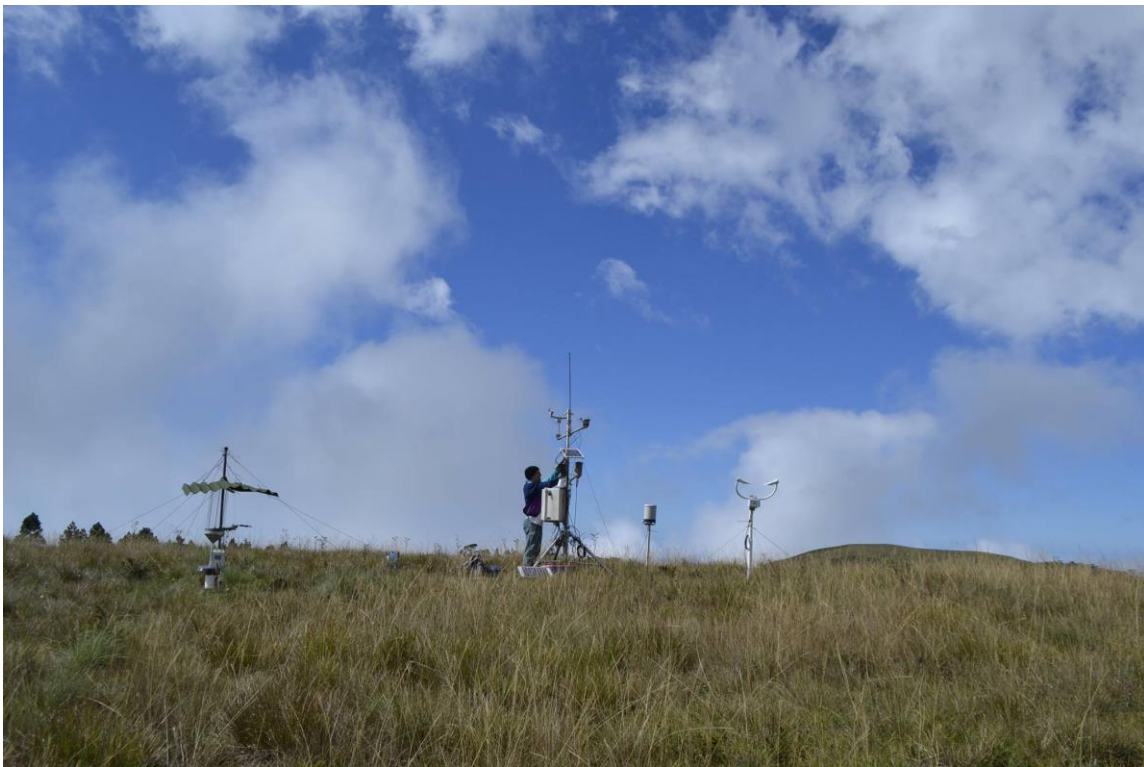
One of the xylem efficiency traits proposed in chapter 3 is that water transport system has a nutritional cost and maximization of water transport per unit nutrient may be a factor under selection. In effect, most of a tree is wood and almost 90% of its nitrogen and phosphorus is in wood (Johnson *et al.*, 2001). Also, 20 to 40% of sap wood is also a living tissue (Morris *et al.*, 2015), which requires nutrients for its metabolism. If different wood requires different amount of nutrients, wood nutrient use may an important trait affecting tree fitness. Furthermore, if wood hydraulic traits, by requiring different anatomical structures, constrain wood nutritional costs, there may be tradeoffs relating wood costs and function. Despite most of a tree is nutritional costs being in wood, relationships between wood nutrition and wood function are almost inexistent. I address this gap in knowledge in my chapter 4 – **“Branch nutrients may be more important than previously thought: costs, relationships and possible coordination with wood hydraulics in an Eastern Amazon Forest”**. In this chapter, I analyze the branch wood nitrogen and phosphorus concentration of 100 Amazon trees and the hydraulic traits of their branch wood. My results provide new insights in the cost of wood and highlights the need to consider the nutritional axis of wood.

Finally, I finish this thesis by linking how the results of my research presented in the 4 chapters increase our current knowledge of tree function: starting from chapter 1, addressing the

abiotic environment affecting water transport; proceeding to chapter 2, addressing an often disconsidered water transport phenomena in trees; following, to chapter 3 proposing a new theoretical framework to study water transport which I start evaluating in chapter 4. I finish highlighting new interesting research questions raised by my work.

# Chapter 1

## High temporal resolution of fog occurrence and its effects on water and light availability in a tropical montane cloud forest in Brazil



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## Abstract

Fog is a frequent phenomenon in tropical montane cloud forests (TMCFs). Those ecosystems are known to provide high quantities of quality water to the lowlands strongly influenced by water inputs from fog water and reduced transpiration due to fog occurrence. Understanding fog regimes is key to predict plant functioning and effects of global changes in TMCFs, as well as consequence to ecosystem services they provide. Here, we combine a novel suite of micrometeorological and hydrological sensors with a visibiliter, a reliable sensor of fog occurrence, to gather fine-grained information on fog frequency, duration and timing and its contribution to water inputs and light availability in a TMCF. Despite occurring 64% of days, fog was highly variable at intra-day, inter-day and seasonal scales, occurring mostly at night and much reduced in the dry season. Approximately 1200 liters of fog were intercepted per tree per year (10.7% of total net precipitation) and fog also increased net precipitation generated by concomitant fog-rain events. Monthly net precipitation to precipitation ratio was 0.964, much higher than the 0.72 typical of lowland rainforest, and was determined by fog occurrence. Contrary to expectations, cloud, and not fog, dominated light availability and inter-day microclimatic variability. Fog was heterogeneous in time, space and coordination with rainfall, clouds and microclimate, many times not alleviating drought and reducing light availability when plants were well hydrated. High fog variability indicates understanding of net plant responses to fog requires integration of plant function on fine-grained data of fog and cloud occurrence. Our results suggest fog uplift due to climate change in the Mantiqueira Range will decrease water supplied by TMCFs to a densely populated areas in Southeastern Brazil but its effect on TMCFs will also depend on whether climate change will change cloud regimes.

## Introduction

Above a certain altitude, fog becomes a frequent event in tropical mountains (Brujinzeel, 2001). At these locations, two peculiar vegetation types start dominating the landscape: tropical montane cloud forests (TMCFs) and high altitude grasslands (Brujinzeel *et al.*,

2011a). These environments have a unique floristic composition with high endemism and contribute disproportionately to lowland hydrology (Bruijnzeel *et al.*, 2011b) due to its peculiar climatic and ecological characteristics (Oliveira *et al.* 2014; Martin & Bellingham 2016). High altitude grasslands and TMCFs are understudied and currently threatened by land use changes (Martínez *et al.*, 2009; Martin & Bellingham, 2016). At the same time climate changes may increase the cloud base height and, consequently, decrease fog occurrence in certain areas where it was frequent (Still *et al.*, 1999; Bruijnzeel, 2001). In Southeastern Brazil, a hotspot of cloud forests and high altitude grasslands occur as part of the Atlantic Forest biome in the mountain tops of the Mantiqueira Range (Bruijnzeel, 2001; Bertonecello *et al.*, 2011). These ecosystems have an important role on the water supply to the Paraíba Valley, one of the most important technological and agricultural areas of Brazil with a population size over 2.4 million. Despite their importance, detailed studies on microclimatic conditions, fog regimes and hydrology are virtually inexistent.

Fog in tropical mountains starts occurring above a particular elevation, depending on the mountain proximity to sea and on its latitude (Bruijnzeel, 2001). Above this elevation, cloud immersion occurs with frequency as high as 70% of the time (Bruijnzeel *et al.*, 2011b). Cloud immersion events, combined with low temperature, high wind speed, eventual frost events in winter, high variability of incoming radiation, and sometimes strong seasonality create a very unique environment (Leuschner, 2000; Jarvis & Mulligan, 2011; Oliveira *et al.*, 2014). This leads to plants in those regions having a particular suite of traits (Gotsch *et al.*, 2016), such as small stature, small, sclerophyllous, and angled leaves (Bruijnzeel, 2001) with lower photosynthetic capacity (van de Weg *et al.*, 2011), and often, high leaf water absorption capacity (Goldsmith *et al.*, 2013; Eller *et al.*, 2016).

All these forest and climate characteristics contribute to a reduction on the evaporative demand (Bruijnzeel & Veneklaas, 1998), and additional water inputs through fog water interception and, possible, through leaf water absorption (Bruijnzeel, 2001; Eller *et al.*, 2013), making tropical mountainous regions “water towers” for the lowlands (Viviroli *et al.*, 2007). Thus, understanding fog dynamics on tropical mountain cloud forests is of primordial importance to predict ecohydrological changes caused by global changes, preserving important ecosystem services.

Fog is a complex phenomenon that affects ecosystems water and light availability, microclimate and, consequently, plant functioning. Hydrologically, fog water reduces

evapotranspiration (Bruijnzeel *et al.*, 2011a) and can be intercepted by canopy, depending on wind speed, fog liquid water content, droplet size and forest canopy architecture (Martínez *et al.*, 2009; Roman *et al.*, 2013), and drip to soil affecting plant water availability with consequences to plant mortality, species distribution and stream dynamics (Gutiérrez *et al.*, 2007; Bruijnzeel *et al.*, 2011b; Bittencourt, 2014). Net precipitation to precipitation ratio (NP/P), a measure of how much water arrives at soil and how much evaporates from the canopy is approximately 0.72 in lowland rain forest however raises to almost or more than 1.0 in cloud forests due to fog interception and reduced canopy evaporation (Bruijnzeel *et al.*, 2011a). In terms of light environment, fog affects light availability, light quality, diffuse fraction and, possibly, nighttime frost formation through radiative cooling (Jordan & Smith, 1994; Mercado *et al.*, 2009; Reinhardt *et al.*, 2010; Zhang *et al.*, 2014). Fog also affects the microclimate and may input considerable amounts of nutrients to ecosystems (Eugster, 2007; Bittencourt, 2014; Vandecar *et al.*, 2015). Effects on plant function will further depend on fog frequency, duration, intensity and timing of occurrence. Daytime fog decreases plant water stress and gas exchanges at the same time its effect in productivity are uncertain (Bruijnzeel & Veneklaas, 1998; Ritter *et al.*, 2009). It may reduce or increase productivity depending on whether light availability reduction or reduced water stress effects dominates. This is probably dependent on the hour it occurs, reducing relative light availability more at early morning and late afternoon than at midday and reducing water stress more at midday (Berry *et al.*, 2014b). Night-time fog occurrence reduces night-time transpiration and increases leaf wet time, driving leaf water absorption and rehydration of plant tissues which may have positive effects on plant function in the following day (Eller *et al.*, 2013; Alvarado-Barrientos *et al.*, 2015). However, wet leaves may favour occurrence of leaf diseases and reduce leaf carbon intake through stomata (Rosado & Holder, 2013; Fernández *et al.*, 2014) and nighttime transpiration may increase plant nutrient uptake (Matimati *et al.*, 2014). The intensity of fog effects on plant water stress and directionality of effect on productivity will further depend on season, with a stronger water stress reduction and consequent productivity increase in the dry season while in the wet season it may reduce productivity due to reduced light availability (Oliveira *et al.*, 2014). As fog effects on vegetation are complex, to understand the overall effect of fog in vegetation we need to know how intense, how often and when fog occurs.

A major problem to study fog is fog detection. Fog detection is usually done through one of the fog effects on climate or hydrology using a myriad of methods: measuring fog water input

as throughfall (Liu, 2004); soil recharge (Brown *et al.*, 2008); and leaf weight increases (Chang *et al.*, 2006) in the absence of rain; air liquid water content with active or passive fog collectors (Michna *et al.*, 2007; Baguskas *et al.*, 2016), usually when there is no rain or rain is excluded using a rain shelter; leaf wetting with leaf mimicking wetness sensors (Eller *et al.*, 2013); and ground level cloud cover from satellite sensors and eddy-covariance estimates of liquid water flow (Eugster *et al.*, 2000; Gultepe *et al.*, 2007). However all the above methods have problems and limitations and the only reliable and accurate method to detect fog is by measuring horizontal visibility (Carrillo *et al.*, 2008), which is greatly reduced during fog events. Visilimeters allow real time detection of fog and, with proper care, they can even be used to detect fog during rain. Without precise and real time fog detection many fog effects on vegetation cannot be determined. Surprisingly, use of visibilimeters in ecological and hydrological studies is rare.

In this study, we use a visibilimeter to accurately detect fog events and use additional instrumentation to analyze multiple dimensions of fog in a TMCF ecosystem in the Mantiqueira Range in Southeastern Brazil. With this combination we quantify fog duration, frequency, timing, water inputs and effects on light availability. We address the following questions: 1) What is the fog regime in a TMCF? What is the relative contribution of fog to soil water inputs? How does fog affect light availability? Fine -grained information on fog regimes represents an important knowledge gap in TMCF climatology. This information is essential to model the impact of climatic variability on TMCF productivity, ecohydrology and provision of key ecosystems services.

## **Methods**

### *Study site*

We studied for one year (April 2015 to April 2016) a tropical montane ecosystem in the Mantiqueira Range, near the town of Guaratinguetá (22°41'52"S 45°25'16"W). The study site comprises cloud forest and grasslands vegetation types, with some anthropic influence in some areas (i.e. pasture). The site is 2000 m a.s.l., approximately 120 km from the Atlantic Ocean, at the border of the Campos do Jordão Plateau. Mean annual air temperature is  $15.8 \pm 3.0$  °C with

the monthly mean ranging from  $12.0 \pm 3.9$  °C in July to  $18.8 \pm 2.9$  °C in February. A dry season occurs between July and September and two or three weeks drought spells may occur during the wet season (Bittencourt, 2014). Frost forms frequently in the grasslands in winter and minimum temperature can sometimes be lower than 0°C. Annual rainfall during the studied year was 2517 mm (Fig. S1.1). Mean incident solar radiation on site is  $363.8 \pm 257.2$  W m<sup>-2</sup> (maximum of 1141.2 W m<sup>-2</sup>). Winds are almost always northward but sometimes southward wind blows; mean wind velocity is high ( $4.3 \pm 2.7$  m s<sup>-1</sup>) and peak velocities of more than 15 m s<sup>-1</sup> can occur during storms. Soils in the region, except in valleys, are mostly organic matter up to 0.6-0.8 m, followed by a lateritic layer and then 0.3-1m of clay (unpublished data).

#### *Micrometeorological measurements*

We installed micrometeorological instruments in a pasture on a approximately 50m height hilltop at the site. We measured the following data using sensors from Campbell Scientific installed in a tripod (CM110) at 2 m height: air temperature and relative humidity (HMP155A probe protected with a 14-plate radiation shield), incident solar radiation (LI200X pyranometer), incident photosynthetic active radiation (LI190SB), air pressure (CS106 barometer), wind horizontal velocity and direction (MetOne 034B windset), rain fall (TB4 rain gauge) and atmospheric horizontal visibility (CS120 visibilimeter). Sensors were scanned every minute and data was logged every 15 minutes with a CR1000 data logger (Campbell Scientific, UK).

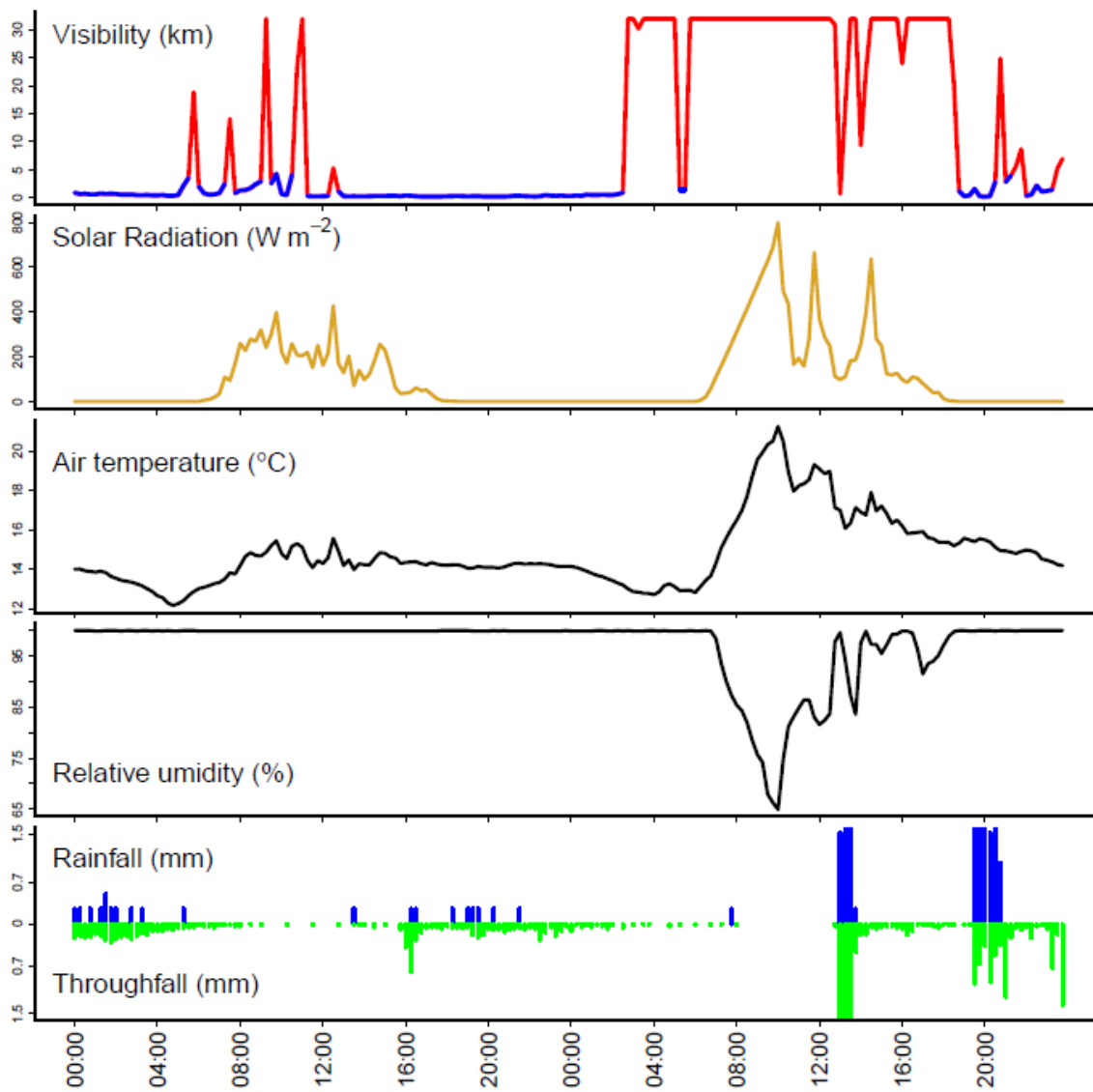
#### *Fog detection*

Fog events were detected using a visibilimeter (CS120, Campbell Scientific). The visibilimeter measures horizontal ground level visibility by quantifying infrared backscatter from air. As it produces the infrared signal it senses, it can detect visibility both at day and night. Its response ranges from visibilities of 0 m to a maximum saturating value of 32000 m. Visibility data was logged to the same CR1000 used in micrometeorological measurements.

Fog or mist definitions based on visibility follow arbitrary criterion. According to the National Oceanic and Atmospheric Administration (1995), meteorological fog occurs when visibility is reduced below 1000 m, mist is considered to happen between 1000 m and 11000 m.

Values higher than 11000 m are uncommon and usually happen during transition from fog events to clear sky. Here, we consider fog events as all visibility events lower than 10000 m and make no distinction between fog and mist as both phenomena have ecological significance to plants. This approach is supported by visibility data when no rain was happening, presenting two clear, distinct, modes (Fig. S1.2a): low visibility and high visibility. Visibility events lower than 10000 m represent 15.7% of data, while events between 10000 m and 3000 m represent only 4.3% of data. Visibility events lower than 5000 m represent 14.0% of total. Thus, increasing or decreasing the fog limit criterion would make a small difference when rain was not happening.

Visibility data during rain events presented the same clear bimodal pattern (Fig. S1.2b) but with more data between the two modes. From the no-rain visibility data, we can safely assume that all visibility values above 10000 m do not represent fog events. The question then is if the visibility values below 10000 m can occur due to rain events without concomitant fog. The effects of rain with fog on visibility are not well studied and parameterized. Gultepe & Milbrandt (2010) analysis of visibility response to rain indicates that rain events lower than  $10 \text{ mm h}^{-1}$  rarely present visibility lower than 2000 m. An example of visibility event and its microclimate properties is presented in Fig. 1.1.



**Figure 1.1.** Example of a two day period (13 and 14 March 2016) with and without fog and its visibility (blue lines mark visibility periods considered as fog and red as non-fog periods); incident solar radiation; air temperature; relative humidity ; and rainfall (blue) and throughfall depth (green). Note i) almost constant night time fog, ii) fog alternating with non-fog in first daytime, iii) second daytime almost without fog except for quick fog events in early morning and afternoon.

We analyzed the relationship between visibility and rainfall intensity (fig. S1.2c) and found that high intensity rain events can happen in all visibility values including during clear

days. Rainfall in the visibility range of 10000-20000 m was even more intense than rainfall with low visibility values (median of 1.0 mm hour<sup>-1</sup> in low visibility and 2.0 mm hour<sup>-1</sup> in 10000-20000 visibility; Mann-Whitney test  $p < 0.0001$ ; Fig. S1.2d). This suggest that using a threshold visibility value of less than 2000 m as criterion for fog events during rain events is a robust criterion and that the amount of false positives should be very low, although there may be some false negatives.

### *Fog light attenuation and cloudness*

We used incident solar radiation at ground level ( $S_r$ ; W m<sup>-2</sup>) as an indicator of fog, rain and cloud optical properties affecting radiation. We used as a measurement of light attenuation, the percentage of incident solar radiation above the clouds that reach the ground ( $S_{rmax}$ ). We calculated  $S_{rmax}$  as the highest hourly  $S_r$  value for each hour in each month, which corresponds to  $S_r$  in clear and cloudless days. Light attenuation capacity ( $L_{cap}$ ; % hourly maximum) was calculated for each daytime data “i” as:

$$(1) L_{cap[i]} = 100(1 - S_{r[i]}/S_{rmax[i]})$$

Total light attenuated ( $L_{tot}$ ; % period total) was calculated in the same way, but with data aggregated over a time period “p”:

$$(2) L_{tot[p]} = 100 * (1 - \text{sum}(S_{r[p]})/\text{sum}(S_{rmax[p]}))$$

$L_{cap}$  is relative to  $S_{rmax}$  of the particular hour, thus it is an index of attenuation capacity.  $L_{tot}$ , although it still is relative to the time period, is an absolute index of total light attenuation as most of  $S_{rmax}$  variability happens at the intra-daily scale. We considered  $L_{tot}$  in normal conditions (rainless and fogless conditions) as an index of cloudness, since no other element would reduce incident radiation.



### *Stemflow and throughfall measurements*

We measured stemflow and throughfall in a TMCF 300 m distant from the hilltop where micrometeorological and fog instrumentation were installed. The TMCF covers both north and south facing slopes and a hilltop. According to a survey of all trees with more than 10 cm of perimeter at breast height we did in 15 plots of 225 m<sup>2</sup> (15 x 15 m), tree height is  $8.4 \pm 3.5$  m (mean  $\pm$  SD), tree density is 275.8 individuals per hectare and tree basal area is  $0.022 \pm 0.039$  m<sup>2</sup>. We measure throughfall depth using rain gauges (ECRN-100, Decagon, 0.027m<sup>2</sup> collecting area) in 13 locations at least 50 meters apart from each other in the understory of the TMCF. Rain gauge data was logged every 30 minutes with EM50 dataloggers (Decagon). The throughfall rain gauge was frequently cleaned to avoid clogging from falling debris. We calculate throughfall per tree by converting throughfall depth to cubic meters of water per surface area and dividing it by tree density.

We measured stemflow in 5 plots. Stemflow was collected by channeling water from five trees to a rain gauge (ECRN-100, Decagon). Stemflow water from each tree was channeled by tightly fixing a ring of silicon tube cut in half longitudinally around the tree and directing the water to the rain gauge. The silicon tube was periodically cleaned. Our observations showed that all stemflow water was channeled to the rain gauge, there was no or very little leakage from the silicon tube ring. Data was logged on the same loggers we used for the rain gauges.

We sampled a total of 30 trees for stemflow, 6 in each location. The mean basal stem area of sampled trees is  $0.032 \pm 0.065$  m<sup>2</sup>. According to our survey, tree basal stem area coverage in the forest is 0.0048 m<sup>2</sup> of stem by square meter of soil surface. Thus, the total surface area sampled for stemflow was 201.6 m<sup>2</sup>. We used tree basal area of each sampled tree to estimate the canopy surface area it was covering and summed it in each location to get  $S_a$  (m<sup>2</sup>), the surface area sampled in each location. We then used  $S_a$  to calculate stemflow depth ( $S_f$ ; mm) as:

$$(3) S_f = 1000 * V / S_a$$

Where  $V$  is the volume of stemflow channeled to each rain gauge ( $V$ , mm<sup>3</sup>), calculated as:

$$(4) V = (R_f/1000) * C_a$$

Where  $C_a$  is rain gauge collecting area ( $0.027 \text{ m}^2$ ) and  $R_f$  (mm) is the logged data, i.e. rainfall depth if the rain gauge was measuring rainfall. Both, throughfall and stemflow data from the five locations was then averaged to get mean throughfall and stemflow of the TMCF. Net precipitation (i.e., total amount of water arriving at forest soil; NP) was calculated as throughfall plus stemflow.

### *Data processing and analysis*

We used R version 3.1.2 (R Core Team, 2014) with base packages for all data processing and analysis. To find out the relative importance of monthly hours of fog or rain in determining NP/P, as both were correlated, we used an analysis of residuals with the residuals of fog hours as a function of rain hours ( $r(\text{fog} \sim \text{rain})$ ) and the inverse ( $r(\text{rain} \sim \text{fog})$ ) as non correlated variables of fog and rain effects on NP/P. For estimating cloudiness or fog effects on microclimate we used a linear mixed model with month as random factor. We used “lme” function from “nlme” package for linear mixed effect models (Pinheiro *et al.* 2014) calculated marginal and conditional pseudo- $R^2$  using “r.squaredGLMM” function from “MuMIn” package (Barton 2016). When daily means were used as statistics, nighttime of each day was considered the full night period, starting at sunset and ending at sunrise, so that each daily data comprised one continuous night event and not two halves. Weather conditions were divided in the following groups:

- Fog: fog events when there was no rainfall;
- Rain: rain events without fog;
- Fog-rain: fog events when there was rainfall;
- Day fog: fog events during daytime when there was no rainfall;
- Night fog: fog events during nighttime when there was no rainfall;
- Normal conditions: events without rain or fog;
- Clear-sky conditions: daytime events without clouds, when incident solar radiation was maximum for that hour of the day (see section above for cloudiness estimation).

Conditions considered as fog are described in the section *Fog Detection*; daytime was defined as all time where maximum incident solar for each hour of each month ( $S_{\text{rmax}}$  see *Fog Light Attenuation* section for details) was higher than  $30 \text{ Wm}^{-2}$  (2.5% of maximum value

observed). Subsets consisting of all data exclusive to a given weather event were used in the analysis.

We used a moving window procedure to summarize data from the beginning to the end of a continuous fog or fog-rain event. The window started when fog events started and stopped when there was no fog event in the following 30 minutes. If no rain was happening when the continuous fog or fog-rain event ended, stemflow, throughfall and fog collection that happened in the 30 min after the end of the fog event was considered to be due to the fog event that just happened and were included in the continuous fog or fog-rain event summary.

#### *Canopy drainage lag*

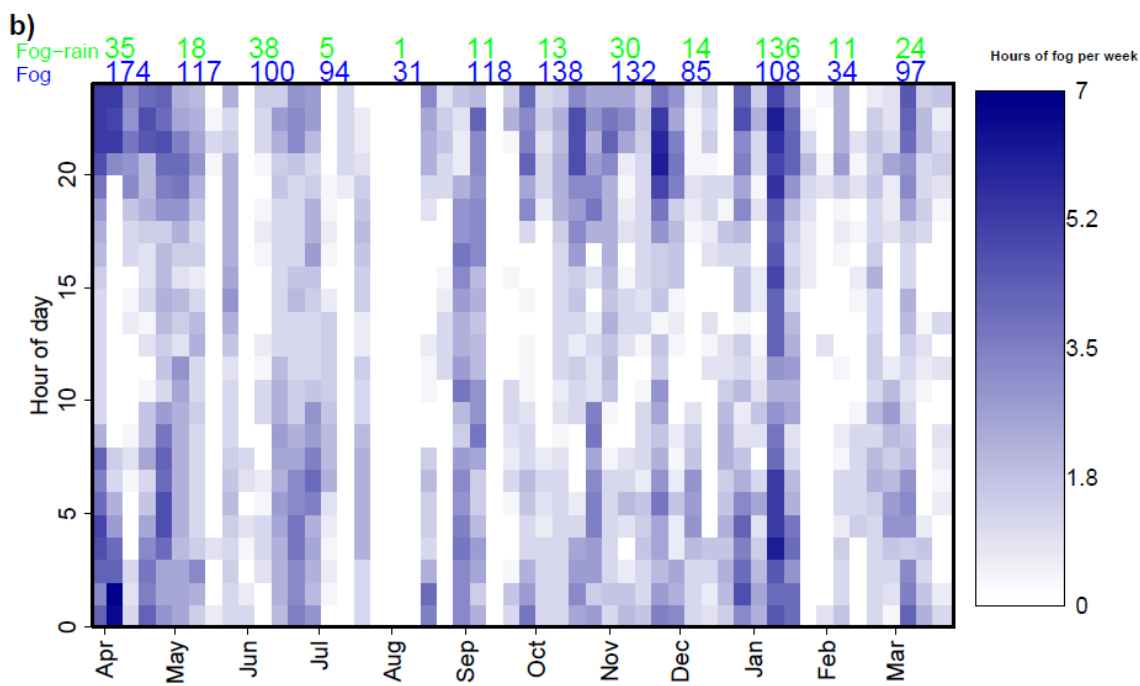
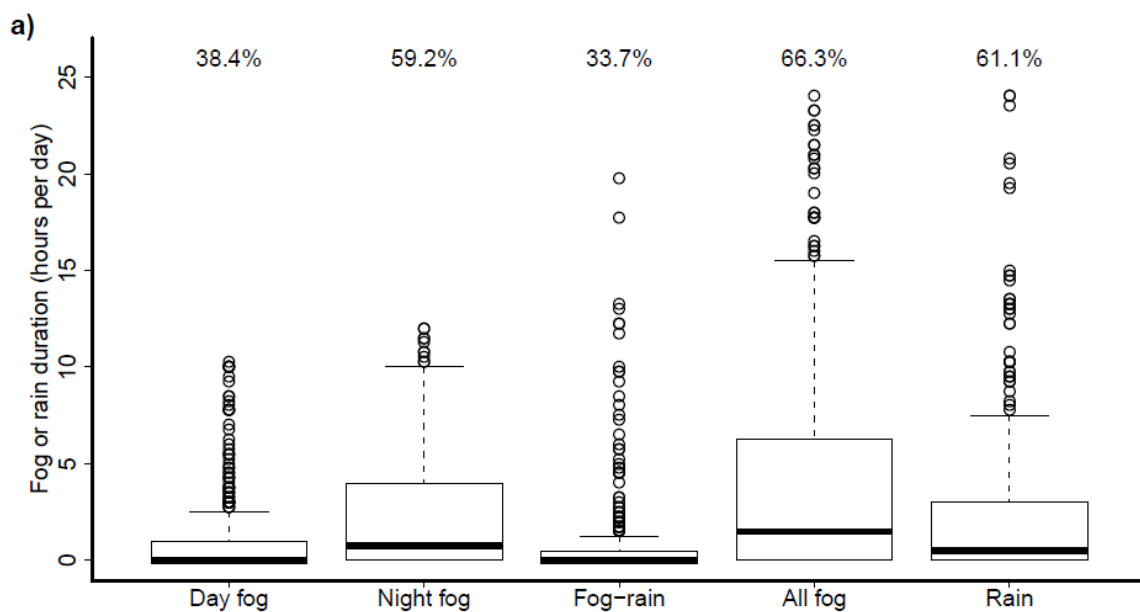
Drainage of canopy water to the understory can take some time, creating a lag in throughfall generation. This can create a lag between NP generation and the fog, rain or fog-rain event that caused it. When fog, rain and fog-rain are occurring in close time windows, NP lag can make an incorrect assignment of rain generated NP to a fog event, or vice-versa. To account for this effect, we quantified NP lag using another version of the moving window described above. We identified all continuous rain, with or without fog, strong enough to saturate the canopy (>4 mm) that when ended had no fog or recurring rain in the next hour. We quantified NP 15 and 30 minutes after the rain event as lagged NP and compared it to the rainfall in the last 15 minutes of rain event.

## **Results**

#### *Frequency, timing and duration of fog*

Fog occurrence was highly variable on daily and seasonal basis and was dominated by nighttime fog. On average, fog occurred during  $3.4 \pm 4.4$  (mean  $\pm$  SD; Fig. 1.2a) hours per day, with day fog less frequent than night fog ( $1.1 \pm 2.1$  against  $2.3 \pm 3.0$  hours per day, respectively). However fog occurrence is highly variable, occurring most as continuous events (Fig. 1.2 and Fig S1.3), which leads to a median fog occurrence of 0.0 hours per day and 0.75 hours per night.

Fog-rain occurred  $0.9 \pm 2.5$  hours per day, represent 21% of all fog events and occurred 72% at nighttime. All fog events amounted to  $4.3 \pm 5.7$  hours per day. Fog (fog-rain included) was more frequent in the morning (0600-1000 hours) and afternoon (1400-1800 hours) than during midday (1000-1400 hours) ( $0.74 \pm 1.40$ ,  $0.55 \pm 1.15$  and  $0.41 \pm 1.00$  hours per day, respectively; Fig. S1.3).



**Figure 1.2.** Fog occurrence. a) Hours per day of different types of fog and rain. Numbers above outliers are the percentage of days in the year each type of event occurred. Boxplot indicates median and upper and lower quartiles, whiskers indicate 10% and 90% percentiles. b) Weekly fog occurrence (hours per week) in different weeks of the year (x-axis) and in each hour of the day of each week (y-axis). White indicates 0 hours of fog and deep blue 7 hours of fog in the respective hour and week, according to bar on right side of plot. Blue numbers above are total hours of fog in each month and green numbers total hours of fog-rain in each month. Another version of this figure is presented in Fig. S1.3 showing each individual fog, fog-rain and rain event at 15 minutes resolution.

The occurrence of fog presented a marked seasonality and short dry spells. Fog-rain was higher than fog in January, but it was much lower than fog during the rest of the year. Fog events happened  $64.3 \pm 18.9\%$  of days in each month. Fog did not occur on 61.6% of days and 41.1% of nights. A total of 124 fogless day+night occurred in a total of 48 fogless periods, 28 of which lasted one day, 41 lasted 3 or less days, 44 lasted less than 7 days and the other ones lasted 7, 9, 11 and 24 days. A period with low fog occurrence (fog-rain included) happened in the dry season in July and August with 13% and 4% of total month with fog compared to the average of 18% in the whole study period. A shorter low fog occurrence period happened in the wet season in February with 5% total month with fog. In July and August low fog occurrence happened with low rainfall (43 mm and 37 mm) while in February rainfall was high (171 mm).

Our moving window analysis for continuous fog events showed both short duration fogs with high frequency as well as long duration fog with smaller frequency mixed with rain are important phenomena. Continuous fog with no rain lasted for  $1.51 \pm 2.1$  hours (66.4% of all continuous fog) while fog events that occurred entirely during rains lasted for  $1.10 \pm 2.71$  hours (10.2% of all continuous fog) and fog events with and without rain lasted for  $6.50 \pm 7.56$  hours (23.4% of all continuous fog). However, when the total hours of fog in each event are computed, due to continuous fog-rain events having longer duration, they accounted to 930 hours per year, while continuous fog without rain amounted to 612.5 hours per year and fog entirely during rain amounted to 68.5 hours per year.

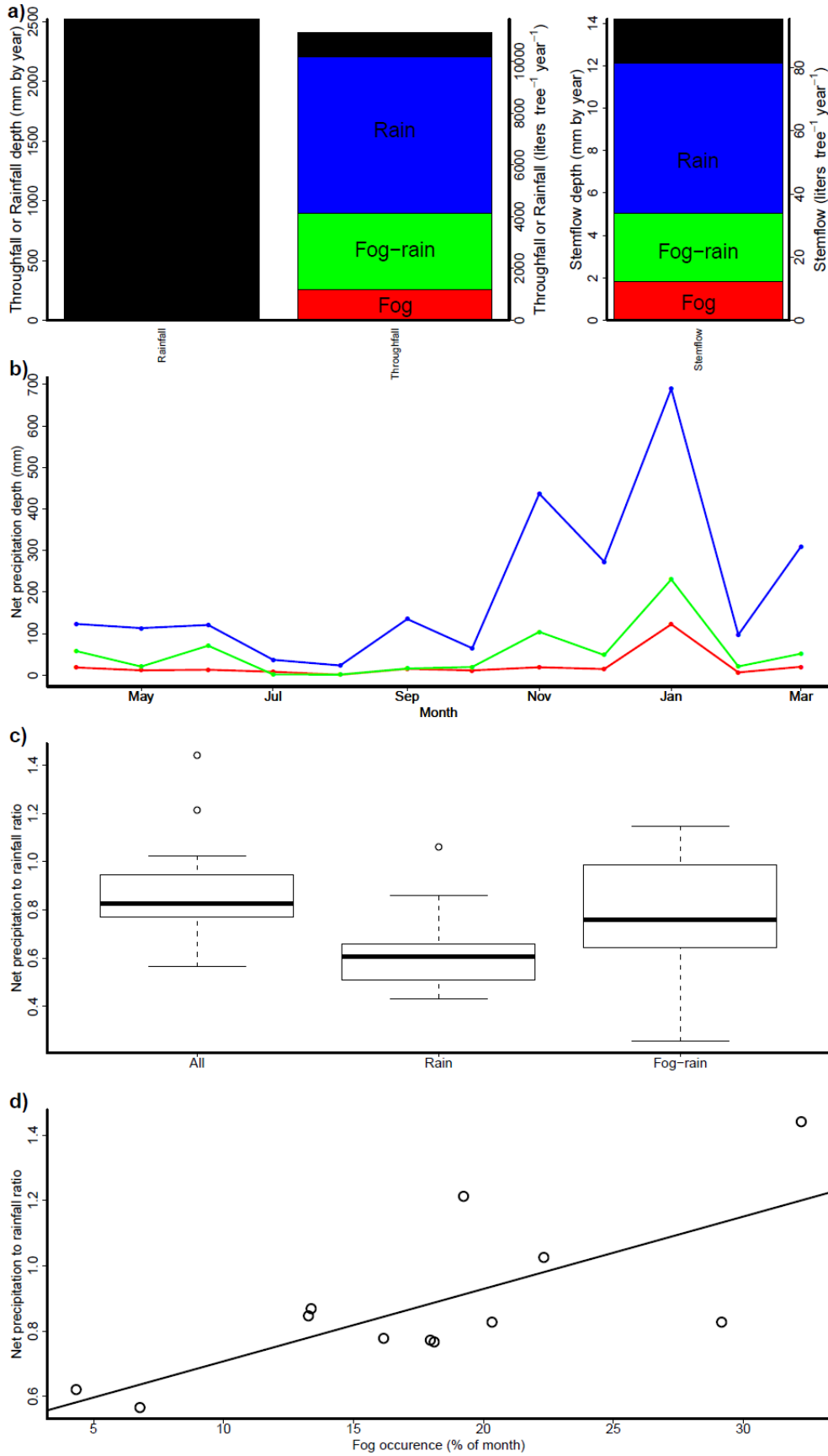
### *Lagged canopy drainage*

Lag in canopy drainage can lead to incorrect assignment of fog as the cause of throughfall actually generated by rainfall, however this bias should be small. We found a total of 33 rain events that had no rain or fog one hour after its end and produced rainfall enough to saturate the canopy ( $> 4\text{mm}$ ). Throughfall 15 minutes after the end of the rain event was  $27 \pm 30\%$  of the rainfall in the last 15 minutes of the rain event. Throughfall in the 15-30 minutes after the rain events was  $10 \pm 16\%$  of the rainfall in the last 15 minutes of the rain event. Thus, as much as 37% of fog event throughfall can be due to immediately preceding rain events. Total events where fog was immediately preceded by rain were 11% of all fog events. Fog preceded by rain had a higher throughfall than fog unpreceded by rain ( $0.20 \pm 0.80\text{ mm}$  and  $0.03 \pm 0.19\text{ mm}$ , respectively), although part of this difference may be due to fog events that occur temporally near rain events having different properties than fog events temporally isolated from rain events. Annual throughfall during fog events is 241.2 mm, of which 114.1 mm is from fog events immediately preceded by rain. As approximately 40% of this value can be throughfall lag from the preceding rain event, annual throughfall that can be safely attributed to fog is 195.6 mm, a 19% difference from the estimate not considering throughfall time lags. This difference is probably overestimated because fog throughfall also should have a time lag, and some fog throughfall may be counted as rain throughfall due to this lag (15% of rain events were preceded by fog). Additionally, not all rain events preceding fog events were strong enough to generate throughfall. Thus, not considering throughfall lag in our analysis should lead to no or small bias in our analysis of fog throughfall contribution.

### *Fog contribution to water inputs*

Both fog and fog during rain had a significant contribution to water inputs. Total throughfall depth in the year was 2408 mm (Fig. 1.3a), of which 54.3% occurred during rain events, 26.5% during fog-rain events and 10.7% during fog events. The remaining 8.5% happened when there was neither rain nor fog and the throughfall is canopy drainage preceded mostly by rain events. Annual throughfall in the 13 different collectors was highly variable, with a range of 630.6 to 3171.2 mm and a standard deviation of 806.2 mm. Total stemflow depth in

the year was much lower than throughfall, 14.2 mm, 13% of which occurred during fog events. Total NP generated during fog events was 1198.2 liters per tree and varied during the year (Fig. 1.3b).





**Figure 1.3.** Water inputs to the region. a) Rainfall, throughfall and stemflow depths and volume per tree partitioned with different colors according to contribution of different events. Black bar above throughfall and stemflow are amounts that occurred when no fog or rain was occurring due to lag between water input and water reaching ground level and is mostly due to rainfall. b) Total net precipitation depth (mm) in the different months of fog (red), fog-rain (green) and rain (blue) events. c) Boxplots of monthly net precipitation to precipitation ratio of all events, only rain events and only fog-rain events. d) Monthly net precipitation to precipitation ratio as a function of monthly fog occurrence (% of month). Black line is the best linear fit.

Annual net precipitation to precipitation ratio (NP/P), a measure of how much rainfall is intercepted by canopy and does not reach the ground, and, in fog prone ecosystems, how much fog is intercepted by canopy, was 0.962. Monthly NP/P was  $0.879 \pm 0.244$ . Monthly NP/P of fog-rain events was 21% higher than rain events (Paired t-test = 1.95; d.f. = 11; one sided  $p = 0.039$ ; fig. 1.3c). Total NP/P of fog-rain was 0.942. Total NP/P of rain events was 0.715 and of rain and fog-rain together was 0.776, indicating that NP/P was increased by 8.5% because of fog during rain events. Total NP/P without fog events is 0.859, indicating fog water interception increases total NP/P by 10.7%.

Monthly NP/P was positively related to amount of fog hours in the month ( $F_{(1,10)} = 11.5$ ;  $r^2 = 0.53$ ;  $p = 0.007$ ; Fig. 1.3d; Table 1.1) and also to rain hours in the month ( $F_{(1,10)} = 13.31$ ;  $r^2 = 0.57$ ;  $p = 0.004$ ) but not to total monthly rainfall ( $F_{(1,10)} = 3.4$ ;  $p = 0.096$ ). As monthly hours of fog and rain also were correlated ( $F_{(1,10)} = 6.7$ ;  $r^2 = 0.40$ ;  $p = 0.027$ ). We found that NP/P was not related neither to  $r(\text{fog} \sim \text{rain})$  ( $F_{(1,10)} = 1.2$ ;  $p = 0.30$ ) nor  $r(\text{rain} \sim \text{fog})$  ( $F_{(1,10)} = 1.7$ ;  $p = 0.23$ ). However  $r(\text{fog} \sim \text{rain})$  and  $r(\text{rain} \sim \text{fog})$  together strongly predicted monthly NP/P ( $F_{(1,9)} = 9.45$ ;  $r^2 = 0.68$ ;  $p = 0.006$ ) and when the interaction between the two predictors is considered the relation is stronger ( $F_{(1,8)} = 8.46$ ;  $r^2 = 0.76$ ;  $p = 0.007$ ), indicating both, rain and fog occurrence in the month co-determine NP/P. When considering monthly nighttime NP/P only, the same pattern was found, but rain occurrence independent of fog occurrence ( $r(\text{rain} \sim \text{fog})$ ) was related to NP/P ( $F_{(1,8)} = 7.11$ ;  $r^2 = 0.42$ ;  $p = 0.024$ ), indicating a stronger rain determination of NP/P. Interestingly, considering daytime NP/P, neither rain nor fog occurrence were related to it. The same patterns were found when weekly data was considered (data not shown), although with more noise.

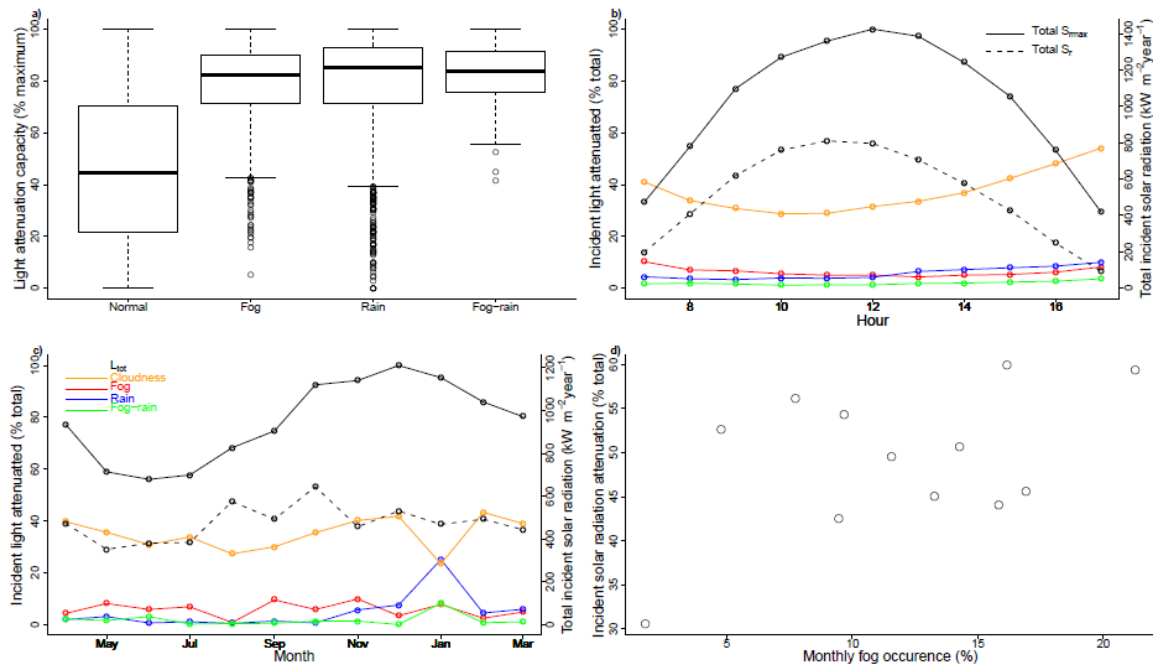
**Table 1.1.** Linear regression statistics of response variable as a function of predictor variable of monthly data of all day, daytime only and nighttime only data.  $r(\text{fog} \sim \text{rain})$ , for example, is the residuals of fog as predicted by rain linear regression (i. e. rain independent fog occurrence).

Response	Predictor	Day+Night			Day			Night		
		p	r <sup>2</sup>	F	p	r <sup>2</sup>	F	p	r <sup>2</sup>	F
Rain (%)	Fog (%)	0.027	0.40	6.70	0.039	0.36	5.64	0.035	0.37	5.94
NP/P	Rainfall (mm)	0.127	0.22	2.77	0.176	0.18	2.12	0.179	0.17	2.09
	Fog (%)	0.007	0.53	11.48	0.153	0.19	2.39	0.016	0.45	8.30
	Rain (%)	0.004	0.57	13.31	0.790	0.01	0.07	0.000	0.85	56.44
	Rainfall (mm)	0.096	0.25	3.38	0.448	0.06	0.62	0.019	0.44	7.72
	$r(\text{fog} \sim \text{rain})$	0.301	0.11	1.19	0.110	0.24	3.08	0.664	0.02	0.20
	$r(\text{rain} \sim \text{fog})$	0.225	0.14	1.67	0.487	0.05	0.52	0.024	0.42	7.11
	$r(\text{fog} \sim \text{rain}) + r(\text{rain} \sim \text{fog})$	0.006	0.68	9.45	0.286	0.24	1.44	0.000	0.87	29.87
$r(\text{fog} \sim \text{rain}) * r(\text{rain} \sim \text{fog})$	0.007	0.76	8.46	0.311	0.34	1.40	0.000	0.91	25.83	

### *Fog effects on light availability*

Fog did not have a strong impact on overall light availability. Incident solar radiation attenuation capacity ( $L_{\text{cap}}$ ) of fog, fog-rain and rain events were similar ( $76.1 \pm 14.3 \%$ ,  $80.3 \pm 10.0 \%$  and  $74.3 \pm 21.1 \%$ , respectively) while  $L_{\text{cap}}$  during normal conditions (cloudness) was much lower ( $44.3 \pm 26.2 \%$ ; Fig. 1.4a). Total incident radiation above cloud level (i.e. in clear days), estimated as the sum of  $S_{\text{rmax}}$ , was  $11041.9 \text{ kW m}^{-2} \text{ year}^{-1}$ , of which 35.2% was attenuated in normal conditions, 5.6% during fog events, 5.1% during rain events and 1.7% during fog-rain events. Total incident radiation arriving at ground was thus  $5792.8 \text{ kW m}^{-2} \text{ year}^{-1}$  (or 52.5% of above cloud incident radiation). Total incident solar radiation attenuation ( $L_{\text{tot}}$ ) by fog was higher in the early morning while  $L_{\text{tot}}$  by rain was higher in the afternoon and by cloudness was lowest in the midday (Fig. 1.4b).  $L_{\text{tot}}$  varied during the year and was lowest from August to October,

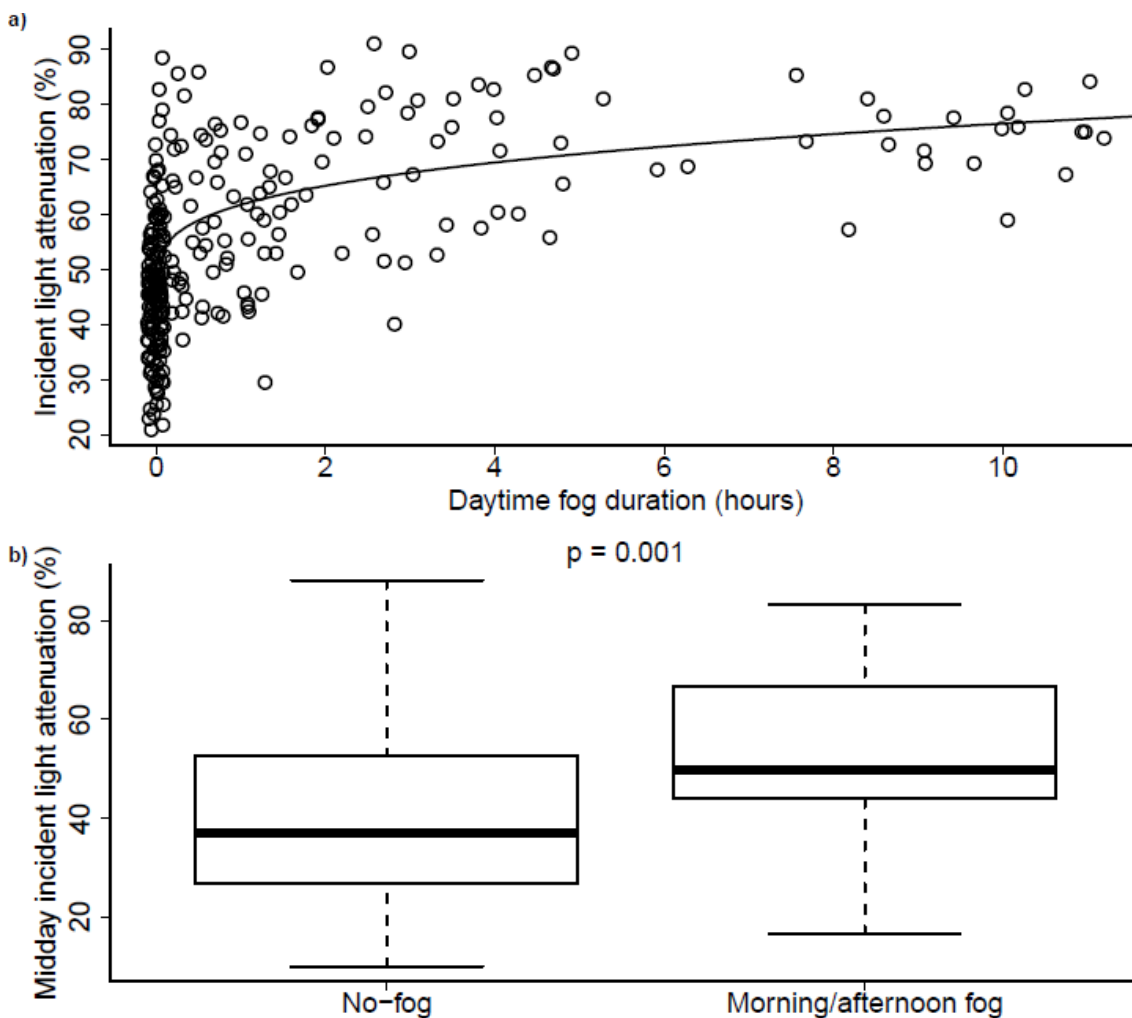
which made those months the ones with highest light availability despite not being at the peak of  $S_{rmax}$  (fig. 1.4c). Monthly  $L_{tot}$  was only marginally related to monthly fog occurrence ( $F_{(1,10)} = 4.1$ ;  $r^2 = 0.29$ ;  $p = 0.07$ , 4d). Monthly cloudness explained almost entirely  $L_{tot}$  ( $F_{(1,10)} = 104.9$ ;  $r^2 = 0.91$ ;  $p < 0.001$ ).



**Figure 1.4.** Drivers of light availability in the studied tropical montane cloud forest. a) Incident solar radiation attenuation capacity (% of maximum incident solar radiation) for different normal condition and fog, fog-rain and rain events. b) and c) Mean incident solar radiation attenuation (% of above cloud incident solar radiation reaching ground in the time period;  $L_{tot}$ ) of each hour (b) or month (c) for fog (red), fog-rain (green) and rain (blue); and total incident solar radiation above clouds ( $\text{kW m}^{-2} \text{ year}^{-1}$ ;  $S_{rmax}$ ; continuous black line) or at ground level ( $\text{kW m}^{-2} \text{ year}^{-1}$ ;  $S_r$ ; dashed line) for each hour or month. d) Relationship between monthly incident solar light attenuation (% total) and monthly fog occurrence (% of month;  $p = 0.12$ ). Legend in b) and c) apply for both graphs.

Fog and cloud occurrence were related. Fog days had higher  $L_{tot}$  than fogless days even though fogless days had more variable cloudness and as high as highly foggy days (July to September excluded from analysis to not bias data for months with low fog; Fig. 1.5a). Fog and

$L_{tot}$  relationship was non-linear and an exponential fit explained 64% of data variability (correlation between predicted and actual data). Days with morning or afternoon fog, but without midday fog, had midday  $L_{tot}$  12.6% higher than fogless days ( $t = -3.5$ ;  $df = 34.7$ ;  $p < 0.001$ ; Fig. 1.5b), confirming days with fog are also cloudier days. Despite fog and cloud being associated, cloudiness alone explained most of microclimatic variability.  $L_{tot}$  in days without fog or rain explained 74%, 55% and 56% of mean daytime air temperature, relative humidity and VPD, with slopes of -0.08, 0.67 and -0.016, respectively ( $df = 141$ ; Fig. S1.4). When all days, including fog and rain, are analyzed, the explanatory power of  $L_{tot}$  for air temperature, relative humidity and VPD is 76%, 68% and 62%, with slopes of -0.087, 0.58 and -0.013 ( $df = 353$ ), respectively, which is similar to values when only fogless and rainless days are considered. Days with fog did not decrease the predictive power and effect of  $L_{tot}$  on microclimatic variables, indicating cloudiness, rather than fog, drives mostly of daytime microclimate variability. Considering daytime fog occurrence as a fixed effect together with  $L_{tot}$  almost did not increase its predictive power (Table S1.1).



**Figure 1.5.** Relationship between fog and cloud occurrence. a) Daytime fog occurrence (hours) and incident light attenuation (% above cloud incident solar radiation reaching ground), and index of cloudiness. b) Midday incident light attenuation (cloudiness) of days when there was no fog during midday but had fog during morning or afternoon. Black line in a) is the best exponential fit ( $y = 1.0 + 15.4 \cdot x^{0.3}$ ; correlation between predicted and actual data is 0.64). A small jitter was applied to x-axis for better data visualization.

## Discussion

Our results show that fog in the tropical montane site studied is frequent but highly variable with significant seasonal, intra and inter-day variation. Fog occurrence is dominated by

night fog and almost does not occur during the driest period of the day. Fog is important to hydrology and contributes to approximately 10.7% of water inputs and fog-rain produces more throughfall than average fog. However fog water inputs are low during low rain periods and possibly do not alleviate soil water stress in the dry season. Fog and cloud occurrence are not independent and light availability and microclimate are mostly driven by overall cloudiness and not fog events. Our results are key to understand fog effects on different fog-prone ecosystems, on plant functioning and to predict effects of fog uplift on tropical mountains.

*Fog occurrence is extremely variable in time*

Fog events are extremely frequent on the studied site, however fog is heterogeneous in time and in its association with rain (Fig. 1.2 and Fig. S1.3). We identified different possibilities of fog: high frequency fog with short duration and without rain, low frequency fog with high duration mixed with rain, frequent nighttime fog without daytime fog and all day fog. However, completely fogless periods and, particularly, daytime fogless periods are also frequent and fog during the driest period of the day is uncommon. Fog occurrence was usually associated with rain and was lowest when rainfall was also low – in the dry season in July and August. This suggests that fog, most of the time, does not alleviate atmospheric water stress in the hours when it is highest and in the season when soil is more water depleted and air humidity is lowest. While we cannot ascertain the seasonal pattern occurs all years, fog in the studied region is associated to clouds and to rains, which is different from fog formed from uplifted sea moisture in coastal regions of California or Frey Jorge in Chile (Gutiérrez *et al.*, 2007; Baguskas *et al.*, 2016) and may occur independently of overall cloud occurrence. Thus, fog effects on alleviating daytime water stress is occasional and TMCF plants have to deal with days with both high atmospheric and soil water deficits.

Nighttime fog was the most common fog occurring 65% of the year with different durations (Fig. 1.2). Nighttime fog can cause foliar water absorption when the soil is dry with fog water recharging plant tissues and sometimes even being redistributed to soil (Eller *et al.* 2013). Foliar water absorption impacts on soil hydrology are supposed to be small (Oliveira *et al.*, 2014), but between 10 to 50% of tree transpiration comes from stored water that also buffers plant water potentials during the day (Scholz *et al.*, 2011). Nighttime fog, together with early

morning fog, may have an important carryover effect to daytime tree and ecosystem water relations (Eller *et al.*, 2013). However nighttime fog was also reduced in the dry season and long fogless periods (up to 21 days) occur. Night fog effects on increasing plant water status by rehydrating plants is thus quite complex and does not always happens. Overall, fog occurrence is not a reliable and predictably event in the studied region and does not always protect plants from the full combination of high luminosity, low air humidity and shallow soils of tropical mountains.

#### *Fog contribution to water inputs*

We assessed the contribution of fog throughfall and stemflow to the yearly water budget. Stemflow was negligible, probably due to the high epiphyte load in lateral branches of trees, which reroute water away from branches and to the soil as throughfall (Van Stan & Pypker, 2015). Fog had a significant contribution to annual NP of 10.7%, which is in the range of cloud forests (Bruijnzeel, 2001; Fig. 1.3). A wet season was present with NP from November to January with 58% of annual NP, while from April to October NP was only 25% of annual NP (88.0 mm per month). Although evapotranspiration data from upper montane cloud forest is scarce, according to (Bruijnzeel *et al.*, 2011a) it is approximately 60 mm per month. This value is higher than NP of July and August and approximately the same as October, and very near the mean from April to October. Those months either have soil water deficit or have very little water excess and fog NP, although low, together with any fog-driven reduction in transpiration, may be important to avoid or delay soil water deficit.

Our data shows that fog occurrence is key to increase NP/P from 0.72, typical of lowland rain forest, to 0.962, typical of upper montane cloud forest (Bruijnzeel *et al.*, 2011a). Fog water interception almost nullifies rainfall canopy interception. Despite NP lag difficulting attribution of NP to fog, our analysis suggests that the overall error should be small. NP/P during fog-rain events was higher than during pure rain indicating fog-rain events should not be neglected in hydrological studies. As a gross estimate, assuming fog together with fog-rain increases NP 19.2% (8.5% from fog-rain and 10.7% from fog NP), NP for the site in the absence of fog would be 1956 mm instead of the measured 2421mm. Thus, fog contributes approximately 465 mm of water per year to TMCF water budget, which suggest that cloud water interception could be as

high as 2147 liters of water per tree per year. Assuming that, in the absence of fog, transpiration would remain as 703 mm per year (Bruijnzeel *et al.*, 2011a) and not considering leaf water absorption, absence of fog in cloud forests would reduce annual water surplus from 1718 mm to 1253 mm, a decrease of 27% in water surplus drained to rivers. While this is a very rough estimate that needs to be better constrained, it indicates fog has an important role in stream flow generated in TMCFs and fog uplift may have important consequences to the lowland stream water availability.

### *Fog and light availability*

Fog, rain and fog-rain can reduce incident light ( $L_{\text{cap}}$ ) by almost 80% (Fig. 1.4), which is much higher than the approximately 40%  $L_{\text{cap}}$  during normal conditions. This indicates cloud during fog and rain events are deeper and/or have less patches of clear sky than it usually has in normal conditions. Days with fog also have more clouds even when fog is not occurring (Fig. 1.5). Although fog has a great light reducing capacity, the total light reduction by fog ( $L_{\text{tot}}$ ) is small - 5.5% of the total 46% of above cloud incident solar radiation not reaching the ground. This is probably due to low daytime fog occurrence, which is even lower at midday. Clouds thus dominates the site energy balance, which is the reason why cloudiness explains most of the temperature, air humidity and VPD variability on the site, with fog adding having a small effect. Light attenuation in the studied site (47.5%) is higher than in other nearby sites where  $L_{\text{tot}}$  ranges from 25 to 40% (Table S1.2). The high  $L_{\text{tot}}$  of the site makes its mean solar radiation lower than tropical regions while its maximum is not much different (Table S1.2). This may be one of the reasons TMCFs have genera and traits typical of both tropical rainforests and temperate rainforests (Barros *et al. in prep.*). Cloudiness, and not fog, dominating the energy balance of TMCFs suggests fog uplift would not change the energy balance of the site much as long as the clouds are still there, albeit not touching the ground. Further studies are needed to understand what will happen, not only with fog, but also with overall clouds, over tropical mountains in the future.

### *Complex effects of fog and clouds to plant function*



Our results shows fog effects on TMCFs are complex and highly variable. Fog has an ameliorating effect on plants water status and allows plants to keep stomata to open for longer and increase water use efficiency (Ritter *et al.*, 2009; Eller *et al.*, 2013; Oliveira *et al.*, 2014). However low daytime fog occurrence, particularly during the most stressing part of the day, suggests this effect may not be so important, unless nighttime fog and early morning fog improvement of plant status can ameliorate overall plant water status for the all day. To our knowledge, nighttime fog effect on plant fitness is only known for three species and in greenhouse but may be considerable (Eller *et al.*, 2013, 2016). However, during the driest part of the year fog did not occur, indicating, at least on the year we measured fog occurrence, fog is not buffering plants from soil and atmospheric stress. Fog water inputs were also low in the dry season and were highest on the seasons when just rainfall was enough to ensure no soil water deficit. Overall, positive effect of fog on plant fitness probably depends on whether, during the wet season, nighttime fog increases the amount of time plants can keep their stomata open.

Fog effect on productivity of TMCFs depends on the balance of its ameliorating effect on plant water status at the same time it reduces light availability. Most of daytime fog occurred in early morning, when water is less limiting and light is possibly the most limiting factor. During midday, when excess light can cause drought and light stress and fog could have a particularly beneficial effect to plants it was uncommon. However days with fog in the morning had more clouds at midday. Possibly, the clouds producing fog were still there albeit not touching the ground. Fog effects on plant-light availability must consider the full impact of clouds whether it is at ground level or not. Those effects will be further modulated by how much fog increases diffuse radiation, which is known to ameliorate photosynthesis by better reaching all the canopy, but which data is a major knowledge gap (Mercado *et al.*, 2009).

We found cloudness dominating light availability and microclimatic variability of the studied TMCF. This suggests that cloudness - and not fog - dominates plant gas exchanges and productivity. High cloud occurrence did not always prevent high light incidence and was also low during the dry season. Fog-affected ecosystems probably function different when fog and cloud occurrence are not coupled. The complexity of TMCFs environment leads us to believe that to understand plant functioning in TMCFs we need to consider the degree of plant responses to different cloud regimes, from a clear day to a cloudy day and to a foggy day, and the results in

larger time scales will depend on how many of each of those days happened and in which season (Goldsmith *et al.* 2013).

### *Conclusion*

Our study shows fog occurrence is extremely variable at the intra-day, inter-day and seasonal time scales. Fog occurrence can ameliorate plant functioning when it occurs, but fogless periods are also frequent and TMCF plants have to deal with full stress of cloudless, fogless and dry days. Fog was mostly frequent during night and fog effects on trees will depend on what is the nocturnal effect of fog during wet season, and how it affects plant functioning during the following daytime. Fog inputs between 1200 to 2150 liters of water per tree per year to soil (292 to 466mm) and fog during rain produce more throughfall than rain without fog. Most of fog water inputs occur when rainfall is enough to recharge the soil so fog water inputs should mostly affect stream water supply. Cloud regime, and not fog regime, determined incident solar radiation and VPD of the studied TMCF. Fog days are, in effect, peculiar days with low water stress and low light availability, but cloudiness effectively dominates the light availability and microclimate of TMCFs. However, as fog and clouds are coupled in this ecosystem, the fog cloud still affects the light environment at midday when it is often not touching the ground. While fog uplift due to climate change will reduce water inputs to TMCFs and increase transpiration, a large part of its effects on TMCFs will be mediated by nighttime effects on fog, which are poorly known, and by how much cloudiness will change if fog starts occurring as clouds. High fog variability at all scales indicates net plant responses to fog and ecosystem functioning in fog-prone ecosystems require integrating plant responses along time in fine-grained, intra-daily fog and cloudiness data, which is only possible through the use of visibilimeters to detect fog. While it is too soon to estimate fog uplift effects on Mantiqueira Range TMCFs, it will certainly diminish water supplied to streams in the Paraíba Valley, with its more than 2.4 million inhabitants, and better understanding of the total effect is urgent.

## Supplementary Tables

**Table S1.1.** Statistical summaries for linear mixed models with months as random factor affecting intercept.  $L_{tot}$  is incident light attenuation (% above cloud incident radiation reaching ground).  $R^2C$  is conditional pseudo- $R^2$ . VPD is vapour pressure deficit. In the multiple regression, first slope is for  $L_{tot}$ .

Predictor	Response	Slope	$R^2C$	d.f.
	Air temp.	-0.08	0.74	
$L_{tot}$ (rainless and fogless days)	Relative humidity	0.67	0.55	141
	VPD	-0.016	0.56	
	Air temp.	-0.087	0.76	
$L_{tot}$	Relative humidity	0.58	0.68	353
	VPD	-0.013	0.62	
	Air temp.	-0.07 / -0.24	0.77	
$L_{tot}$ + Daytime fog hours	Relative humidity	-0.52 / -0.66	0.68	352
	VPD	-0.011 / -0.012	0.61	
	Air temp.	-0.48	0.64	
Daytime fog hours	Relative humidity	2.6	0.45	353
	VPD	-0.05	0.39	

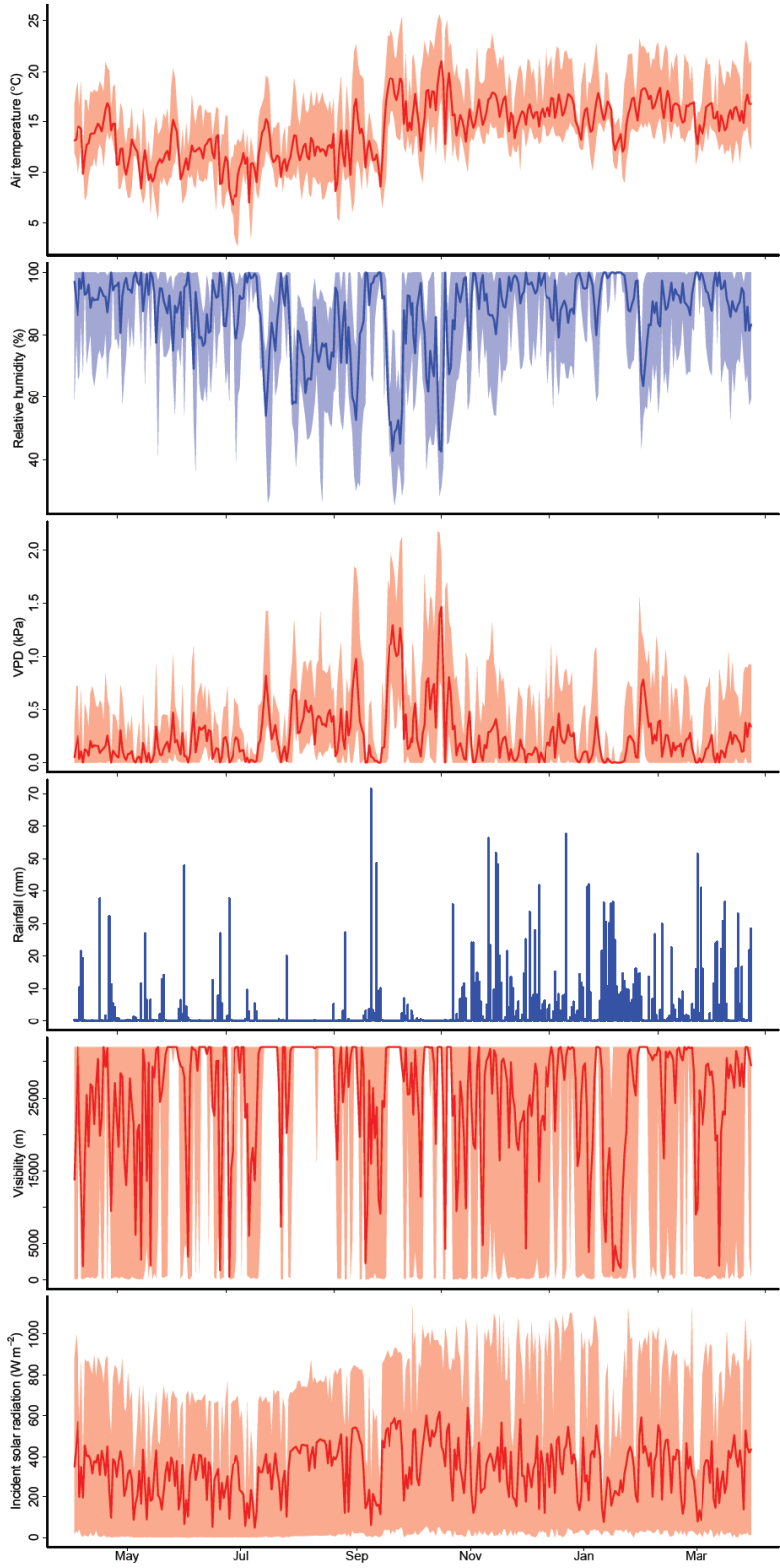
**Table S1.2.** Incident light attenuation ( $L_{tot}$ ; % above sky incident solar radiation reaching ground; i.e. a cloudness index) for different sites. As  $L_{tot}$  is not a commonly measured variable values presented here are to allow for comparison with results of this study.  $S_r$  (incident solar radiation;  $W m^{-2}$ );  $S_{rmax}$  (maximum incident solar radiation, 0.99 quantile;  $W m^{-2}$ );  $A_t$  (air temperature  $^{\circ}C$ ); VPD (vapour pressure deficit; kPa). Height is height a.s.l. Places with \* are locations nearby the studied site. Data not from our site is either from Instituto de Meteorologia Nacional or from Centro de Estudios Avanzados en Zonas Áridas databases.

Place	$L_{tot}$	$S_r$	$S_{rmax}$	$A_t$	VPD	Height	Details
El Tololo	23.96	413.3	981.4	14.8	1.18	2154	Temperate desert
Santarém	28.52	428.4	994.9	28.4	1.22	137	Seasonal Amazon rainforest
Querência	28.88	448.4	1065.1	29.1	1.72	361	Savanah-Amazon forest transition
Santa Rita do Passa Quatro*	32.75	362.1	1027.1	20.8	1.02	1017	Savanah-Atlantic forest transition
Copacabana*	35.57	384.5	1096.4	24.8	0.85	26	Atlantic rainforest
São Luis do Paraitinga*	35.59	397.6	1089.5	21.4	0.80	862	Montane Atlantic forest
Manaus	35.61	376.7	949.6	29.9	1.55	49	Low-seasonality Amazon rainforest
Marambaia*	35.68	358.9	1099.2	24.8	0.91	9	Atlantic rainforest
Seropédica*	35.68	397.3	1068.0	28.1	1.38	35	Atlantic rainforest
Belém	35.70	328.9	889.5	29.0	1.19	21	Low-seasonality Amazon rainforest
Taubaté*	36.51	375.3	1059.9	23.6	1.17	582	Montane Atlantic forest
Maria da Fé*	37.56	387.0	1079.7	20.2	0.77	1281	Upper-montane Atlantic forest
São Joaquim	37.56	401.1	1146.6	15.5	0.54	1400	Sub-tropical montane cloud forest

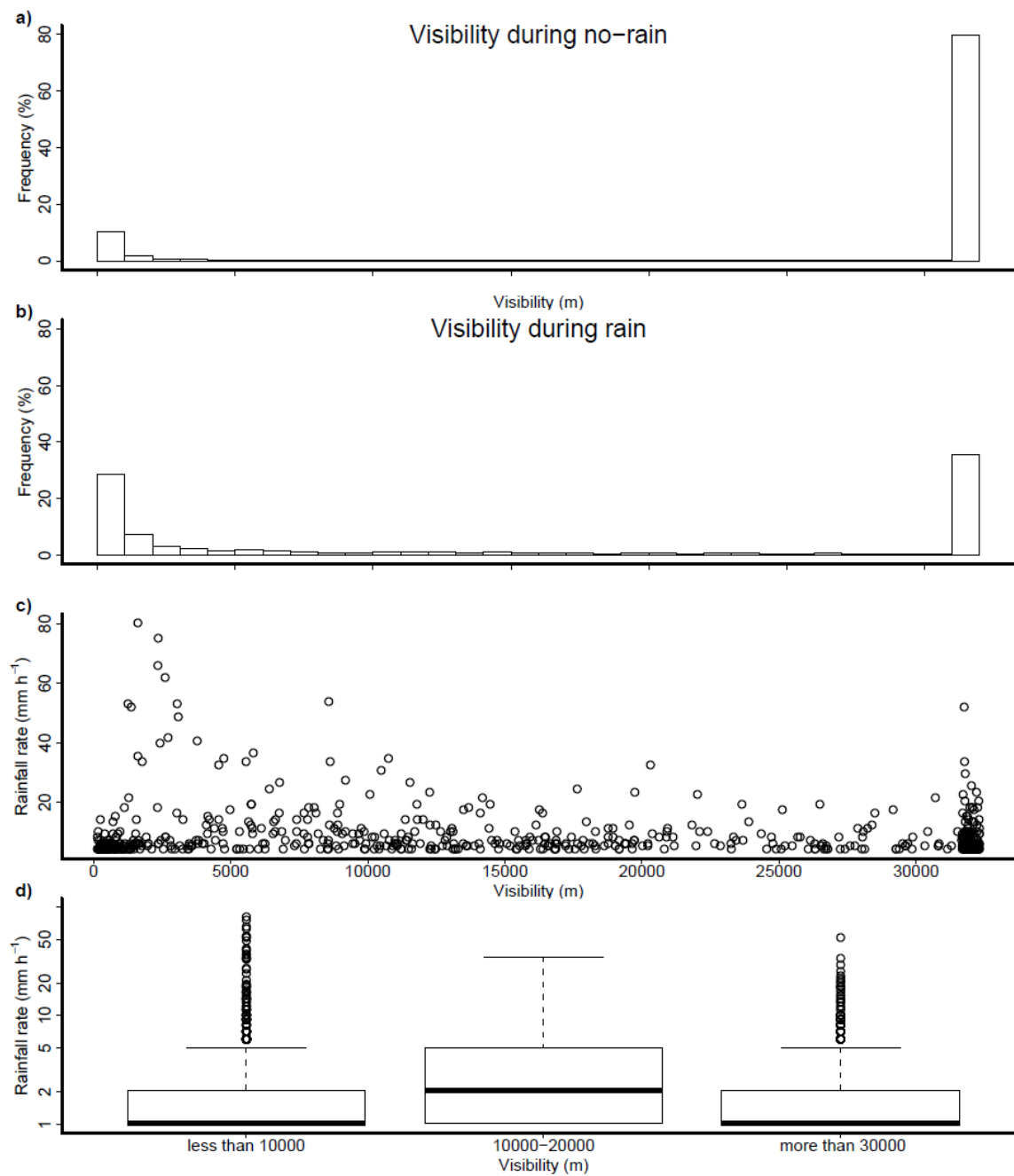
Passa Três*	37.67	350.8	1029.9	21.1	0.98	516	Atlantic rainforest
Monte Verde*	37.77	382.3	1044.6	18.1	0.62	1545	Tropical montane cloud forest
Resende*	38.57	375.1	1088.9	24.2	1.19	439	Atlantic rainforest
Paraty*	40.73	347.2	1070.2	25.0	0.94	3	Atlantic rainforest
Morro da Igreja	42.90	344.2	1122.8	11.9	0.59	1822	Sub-tropical montane cloud forest
Ancud	42.99	261.2	826.5	12.8	0.39	30	Temperate rainforest
Campos do Jordão*	45.99	297.4	1037.6	17.7	0.65	1663	Araucaria forest
Studied site	47.50	318.0	958.8	15.5	0.33	2000	Tropical montane cloud forest

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### Supplementary Figures

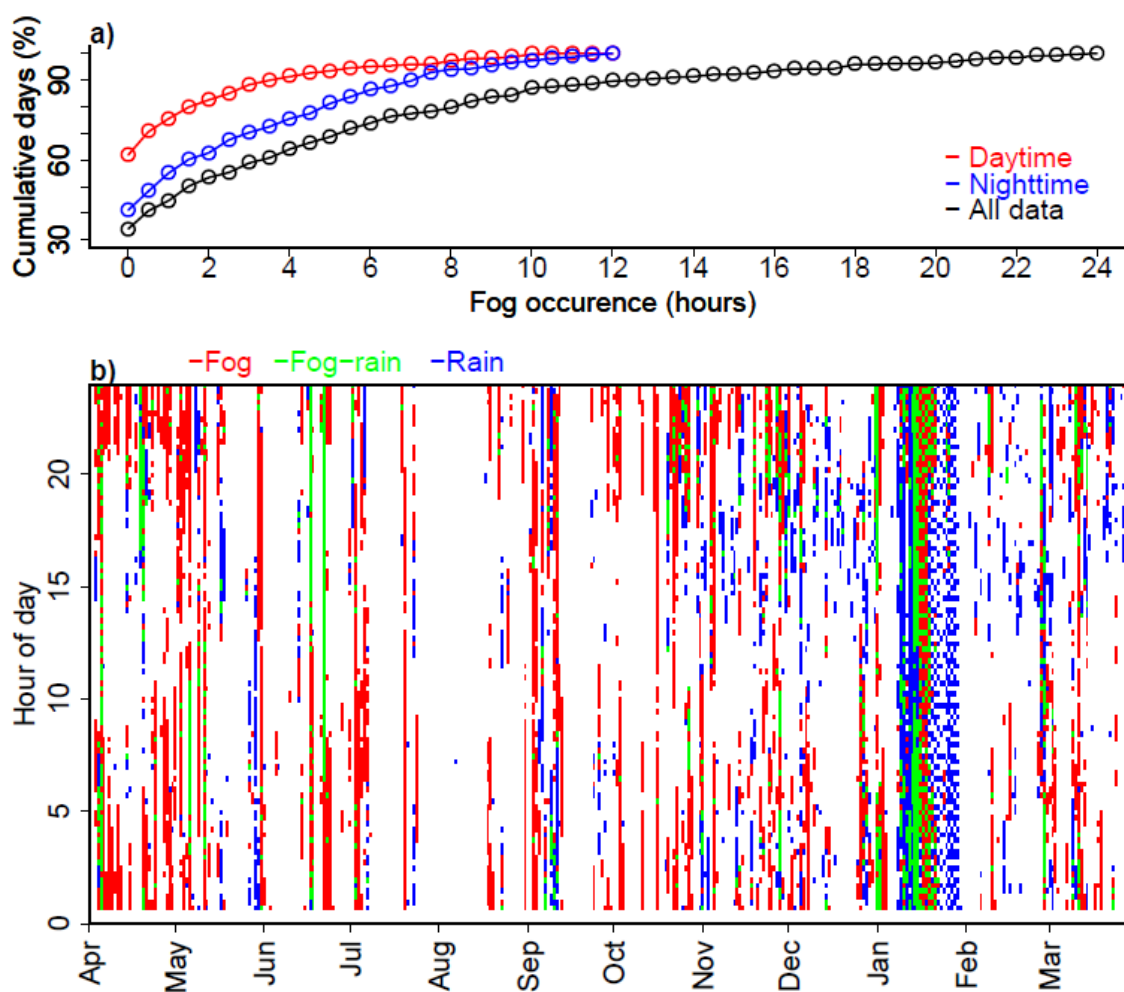


**Figure S1.1.** Air temperature ( $^{\circ}\text{C}$ ; a), air relative humidity (%; b), vapour pressure deficit (kPa; c), rainfall (mm; d); visibility (m; e) and incident solar radiation ( $\text{W m}^{-2}$ ; f) from april 2015 to april 2016. Thick line is daily mean, shades are daily maximum and minimum.



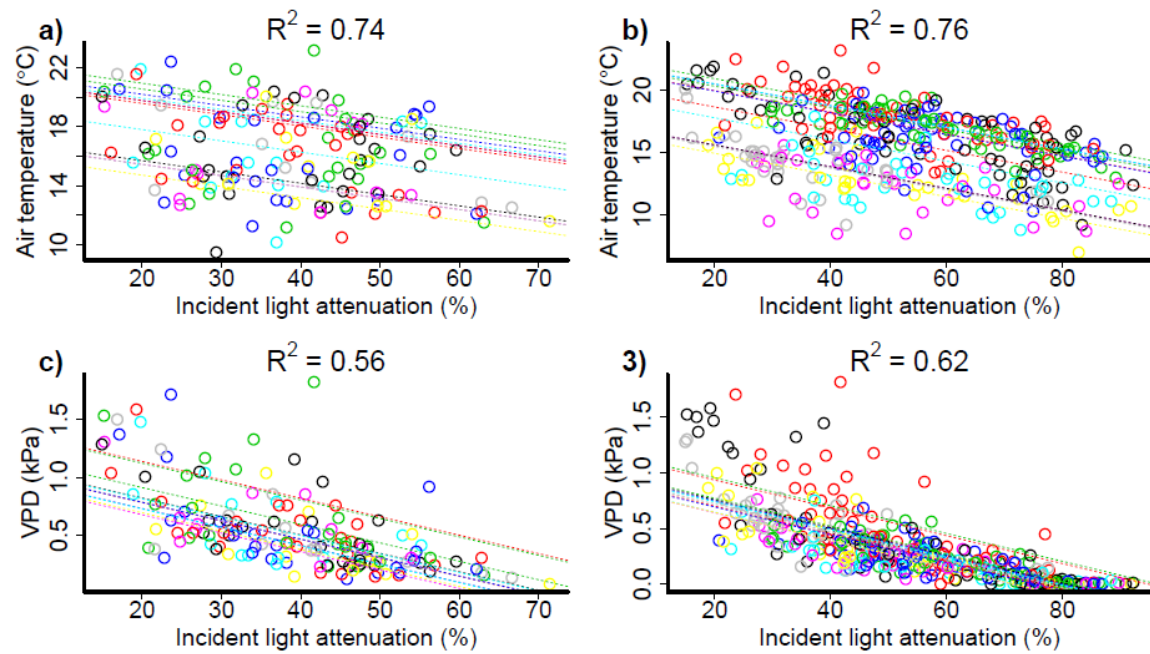
**Figure S1.2.** Relationship between fog, rain and horizontal visibility. a-b) Histogram of visibility (m) values when there is no rain (a) and when there is rain (b). c) Relationship between visibility

(m) and rainfall rate ( $\text{mm hour}^{-1}$ ) during rain and fog-rain events. d) Rainfall rate for three ranges of visibility values. Rainfall can occur at any visibility and intermediary visibilities are uncommon, indicating low visibility can be safely attributed to fog during rain also. Note y-axis on d) is on logarithmic scale. A small jitter was applied to x-axis = 32000 on c) for better visualization.



**Figure S1.3.** Fog occurrence. a) Cumulative percentage of days with a given amount of daytime fog (red), nighttime fog (blue) or both (black). b) fog (red), fog-rain (green) and rain (blue) occurrence in the different hours of the different months at a 15min resolution. The 15min resolution in b) allows to notice additional complexity and variability of fog occurrence not discernible in Fig. 3 is weekle averages. Note, for example, in January days with rain followed by fog-rain, fog with some fog-rain and then rain mixed with non-foggy and non-rain conditions.





**Figure S1.4.** Mean daily incident light attenuation (cloudiness; % incident light above clouds reaching ground) effects mean daytime air temperature (a-b) and mean daytime vapour pressure deficit (VPD; c-d). a) and c) are days without fog or rain during daytime, b) and d) are all days. Different colors are different months and dashed lines are the linear fit of mixed model with months as random factor affecting intercept.

## Chapter 2

### Diversity of foliar water uptake due to dew, rain and fog and its biotic and abiotic drivers in a Tropical Montane Cloud Forest



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## Introduction

Trees stay a considerable amount of their lifetime with their leaves wet. In Brazilian tropical forests, dry forests excluded, plants stay 6 to 12 hours per day with wet leaves just due to dew (Alvares *et al.*, 2015). This amount can be much higher in environments with frequent rain or fog events. In tropical cloud forests, fog occurrence alone wet leaves for 20 to 70% of their lifetime (Bruijnzeel *et al.*, 2011). Despite plants spending a considerable fraction of their lifetime with wet leaves, wet leaf physiology is poorly studied and trees water transport and photosynthesis models simply considered leaves to “shutdown” when wet. However, in certain conditions, when leaves are wet, foliar uptake (FU) of water may occur, inverting plant water flow from the atmosphere to the soil forming the atmosphere-plant-soil continuum (Goldsmith, 2013). FU may alter plant and soil water balance and has consequences to plant functioning by alleviating drought (Burns *et al.*, 2009; Eller *et al.*, 2013; Eller *et al.*, 2016). Despite FU possibly being an important process to plant and ecosystem functioning, we know very little of how much it varies between species and individuals and what are its biotic and abiotic drivers. Understanding its occurrence and importance to plant functioning may be crucial as climate change, such as cloud lift, which will reduce fog occurrence (Still *et al.*, 1999), may reduce the time leaves stay wet.

Foliar water uptake is known to occur for decades. Slatyer (1956) is classical work of water diffusion from leaves in a wet atmosphere to roots in a drier atmosphere, or even from leaves in a wet atmosphere to leaves in a drier atmosphere through the plant stem, highlights plant water flow is bidirectional and depends on the water potential gradients on plant parts. Since then, FU and positive consequences of FU to plants has been demonstrated in many different plant lineages, plant functional types and biomes: subtropical rainforest tree (Yates & Hutley, 1995), dry desert plants (Martin & von Willert, 2000; Hill *et al.*, 2015), coniferous arid and tropical semiarid shrubs (Breshears *et al.*, 2008; Pina *et al.*, 2016), redwoods and redwood plants more generally (Burns *et al.*, 2009; Emery, 2016), temperate semi-humid plants (Liu *et al.*, 2011), temperate and tropical conifers (Laur & Hacke, 2014; Cassana *et al.*, 2016), temperate and tropical cloud forests trees (Eller *et al.*, 2013; Berry *et al.*, 2014; Cassana *et al.*, 2016) and tropical rainforest trees (Binks *et al.* submitted). Above examples include FU from all leaf-wetting events – rain, dew and fog – and additionally (Slatyer, 1956) example shows direct water vapor absorption.

Water may enter leaves through multiple pathways as cuticles (Becker *et al.*, 1986; Eller *et al.*, 2013), hydathodes (Martin & von Willert, 2000), trichomes (Pina *et al.*, 2016) and maybe even stomata depending on stomata morphology, hydrophobicity and the surface tension of the water over the stomata (Schonherr & Bukovak 1971). Water may even enter plants by shoots and branches (Oliveira *et al.*, 2005; Mayr *et al.*, 2014). The efficiency of FU probably differs depending on the structure water enters through and its traits. Consequences of FU to plants range from plant rehydration (Yates & Hutley, 1995), increased CO<sub>2</sub> uptake (Martin & von Willert, 2000) to increased survival during drought (Cassana *et al.*, 2016). Some studies even show FU to be redistributed to other plant organs and reach the soil in certain conditions (Slatyer, 1956; Eller *et al.*, 2013; Cassana *et al.*, 2016).

While we know FU is common, we do not know when it happens neither how variable or important it is between individuals and species. We also do not know exactly the limiting abiotic conditions allowing it to occur and whether they modulate its intensity. Finally, we do not know if and how FU is modulated by plant traits. To fill those gaps in knowledge, we measured reverse sap flow (RSF) of 38 tropical cloud forest trees from 11 species. RSF is an indicator of FU as it is the actual transport of water from the canopy to the soil, which only occurs if water is being absorbed in the canopy through FU. With this dataset, we test the following hypothesis: 1) RSF intensity differs between species and individuals; 2) RSF occurs whenever leaves are wet (dew, fog and rain) but only when there is a water potential gradient from leaves to soil (i.e. in the dry season); 3) tree architectural and hydraulic traits modulate RSF. Finally, with our dataset we estimate the climatic envelope (vapour pressure deficit, fog occurrence, leaf wetness, rainfall and soil water content) allowing RSF. Our data set is the first one to go beyond point measurements of FU and to study its drivers and variability.

## Methods

### *Study site and tree species*

We studied a tropical montane cloud forest in the Atlantic Forest, at 2000m a.s.l. in the Mantiqueira Range (22°41'52"S 45°25'16"W), near the town of Guaratingueta. Mean annual air

temperature is  $15.8 \pm 3.0$  °C. Total annual rainfall from 2015 to 2016 was 2517 mm. . A dry season occurs between July and September and two or three weeks drought spells may occur during the wet season. Detailed information on site microclimate can be found on (Bittencourt *et al.* in prep). We inventoried all trees with more than 10 cm perimeter at breast height in 15 permanent plots (15 X 15 m) and measured diameter, height and identified to species level. We found a total of 55 species, 36 genera and 24 families in the inventory. Tree height is  $8.4 \pm 3.5$ m (mean  $\pm$  SD) and tree basal area is  $0.022 \pm 0.039$  m<sup>2</sup>. Detailed information on inventory and species can be found in Barros *et al.* (in prep). We studied eleven tree species: *Araucaria angustifolia* (Araucariaceae), *Croton piptocalix* (Euphorbiaceae), *Drimys brasiliensis* (Winteraceae), *Macropetalus dentatus* (Monimiaceae), *Myrceugenia cuculata*, *Myrceugenia ovalifolia* and *Myrceugenia ovata* (Myrtaceae), *Psychotria velloziana* (Rubiaceae), *Symplocos falcata* (Symplocaceae), *Tabebuia vellosi* (Bignoniaceae) and *Weinmannia organensis* (Cunoniaceae). *Drimys brasiliensis* is a basal angiosperm with tracheids. *Tabebuia vellosi* is deciduous in the dry season. All species are dominant in the site and represent 59% of forest basal stem area.

#### *Micrometeorological and soil water measurements*

We used meteorological data collected by Bittencourt *et al.* (in prep). A meteorological station near the site was used to monitor temperature, relative humidity, rainfall and atmospheric horizontal visibility (CS120 visibilimeter, Campbell). Visibility lower than 10km indicates fog occurrence as discussed in Bittencourt *et al.* (in prep). Additionally, we used a leaf wetness sensor (S-LWA-M003, Onset), coupled to a HOBO RX3000 logger (Onset), installed at 1.5m height near the weather station to infer wet leaf events, particularly dew, which is considered to occur whenever the leaf wetness sensor was wet without rain or fog conditions occurring at the same time or in a few hours. We monitored soil water content in 10 of the permanent plots at a 10cm depth with a time domain reflectometer (ECH<sub>2</sub>O EC-5, Decagon) coupled to a ECH<sub>2</sub>O Em50 data logger (Decagon). We used as indicator of the forest soil water conditions the average of the 10 sensors. Soil water content was standardized dividing each value by the 95% percentile and multiplying by 100 to obtain a percentage of the maximum soil water content. We used the 95% percentile instead of the maximum to avoid extreme values caused by strong rainfall supersaturating the soil and producing extreme values. Soil supersaturation

only lasts for one or two hours and quickly goes back to field capacity after draining so the 95% percentile is enough to avoid standardizing data by soil supersaturation water content.

### *Sap flow data*

We used sap flow data collected by Eller *et al.* (2018). Sap flow meters (SFM1, ICT International Pty Ltd.) installed at breast height were used to estimate tree sap flow using the heat-ratio method (Burgess *et al.*, 2001), which is sensitive to low and reverse sap flow velocities. Heat pulse velocity was calculated using the difference in needle temperatures after heat pulse in the upper and lower thermocouples and sapwood thermal diffusivity. Heat pulse velocity was corrected for needle wound effects and needle misalignment and sap velocity was calculated using sapwood density and heat capacity. Total tree sap flow ( $\text{g h}^{-1}$ ) was then calculated integrating sap velocity along the sap wood depth and active xylem area. Active xylem area was estimated by measuring xylem active area for trees with different diameter dye insertion (Goldstein *et al.*, 1998) and calculated for each individual tree based on its diameter. See (B. Eller *et al.*, 2018).

As sap flow calibration period was in March 2016, sap flow zero may be slightly wrong in months far away from calibration. While the error is negligible for analysis of regular sap flow, reverse sap flow magnitudes are usually small and wrong zeros may significantly affect the data. To account for this, we corrected zero sap flow for each month by selecting a nighttime period with no fog and rain and with zero VPD (see events in Fig. S1). Minimum sap flow in these conditions should be close to zero or slightly negative. We subtracted this value from that month's sap flow data to correct the month's zero sap flow. As dew usually occurred during the selected zero periods, the corrected sap flow data may actually be underestimating reverse sap flow.

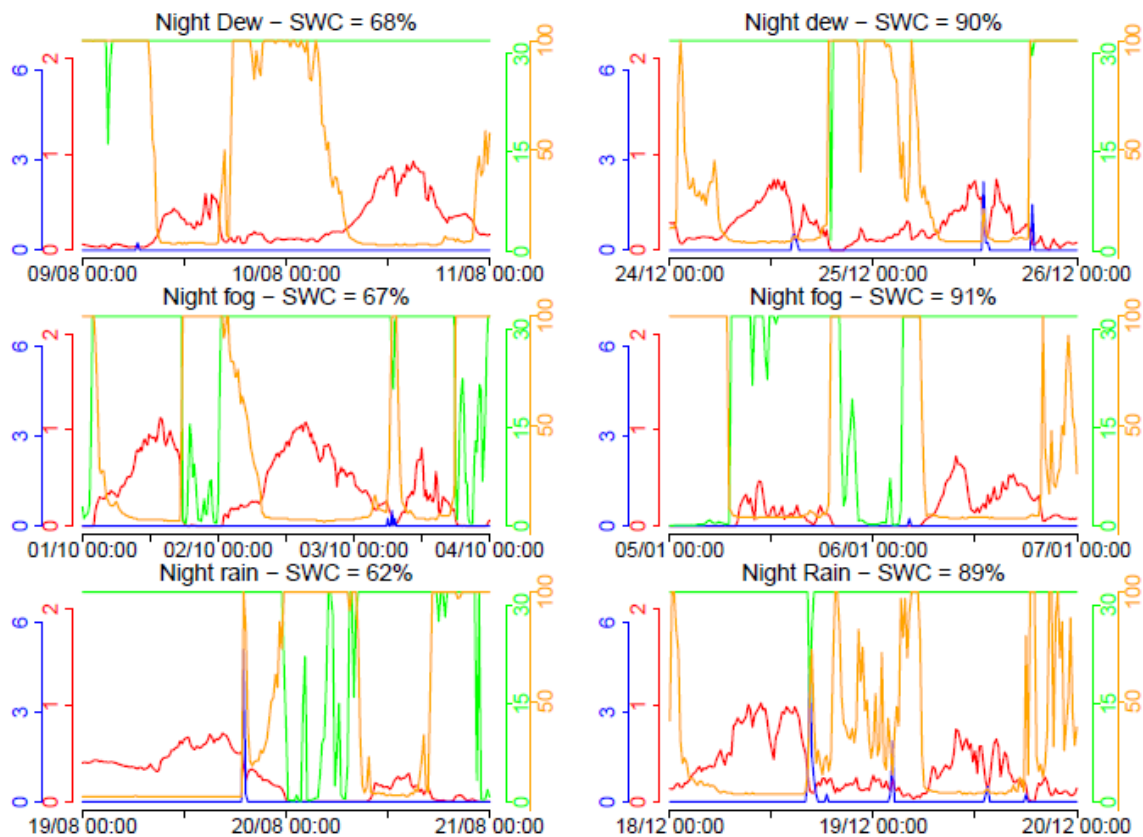
### *Hydraulic traits*

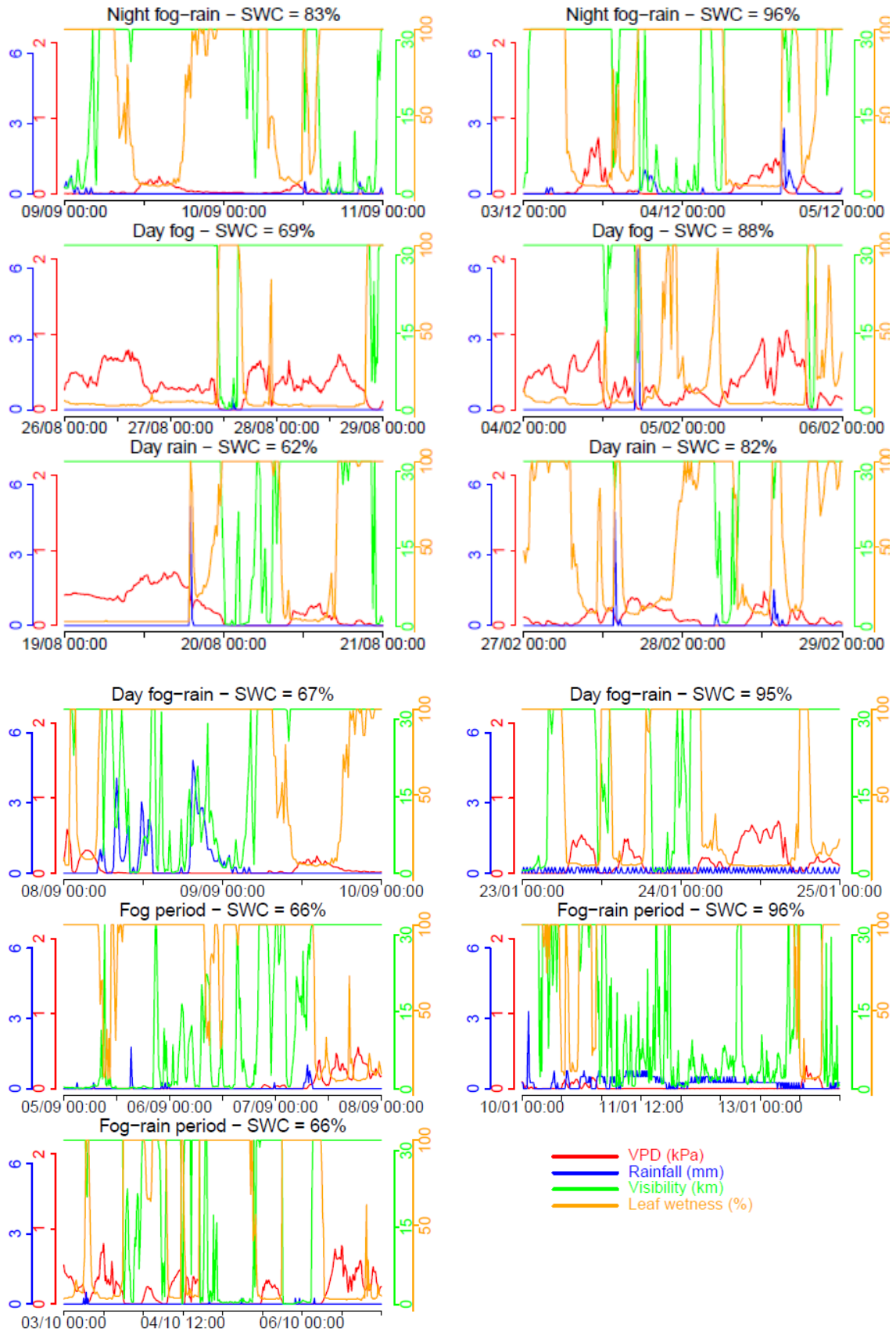
We used as hydraulic traits the traits dataset used by Barros *et al.* (in prep), the active xylem area of each individual tree (described in the above section) and the maximum sap flow per xylem area for each individual. Barros *et al.* (in prep) measured leaf predawn and midday water potential repeatedly in different months for two years in the studied trees and used the slope ( $\sigma$ ) of predawn and midday water potentials as an index of transpiration and conductance sensitivity to changes in water availability, following Martínez-Vilalta *et al.* (2014).  $\sigma$  was calculated only at species level. We used the minimum predawn and midday water potential

measured during the two years for each tree as an index of the driest soil conditions and strongest drought stress conditions each tree experienced, respectively.

### *Selected dew, fog and rain events*

We selected a total of 17 dew, fog and rain events in the dry season (August, September and October) and wet season (December, January and February) to evaluate sap flow conditions. We selected dew events (no rain, no fog and leaf wetness sensor wet), fog events (no rain with fog), rain events (rain with no fog) and fog-rain event (concomitant fog and rain) during nighttime and daytime, except for dew which never occurred during daytime. Additionally, we selected one prolonged (spanning more than one day) fog period in the dry season and one prolonged fog-rain period in the dry and one in the wet season to analyze sap flow conditions during it. We also calculated the absolute minimum reverse sap flow for each individual in the dry and wet season as a measure of reverse flow capacity. Selected climatic events and its conditions are presented in Fig. 2.1.







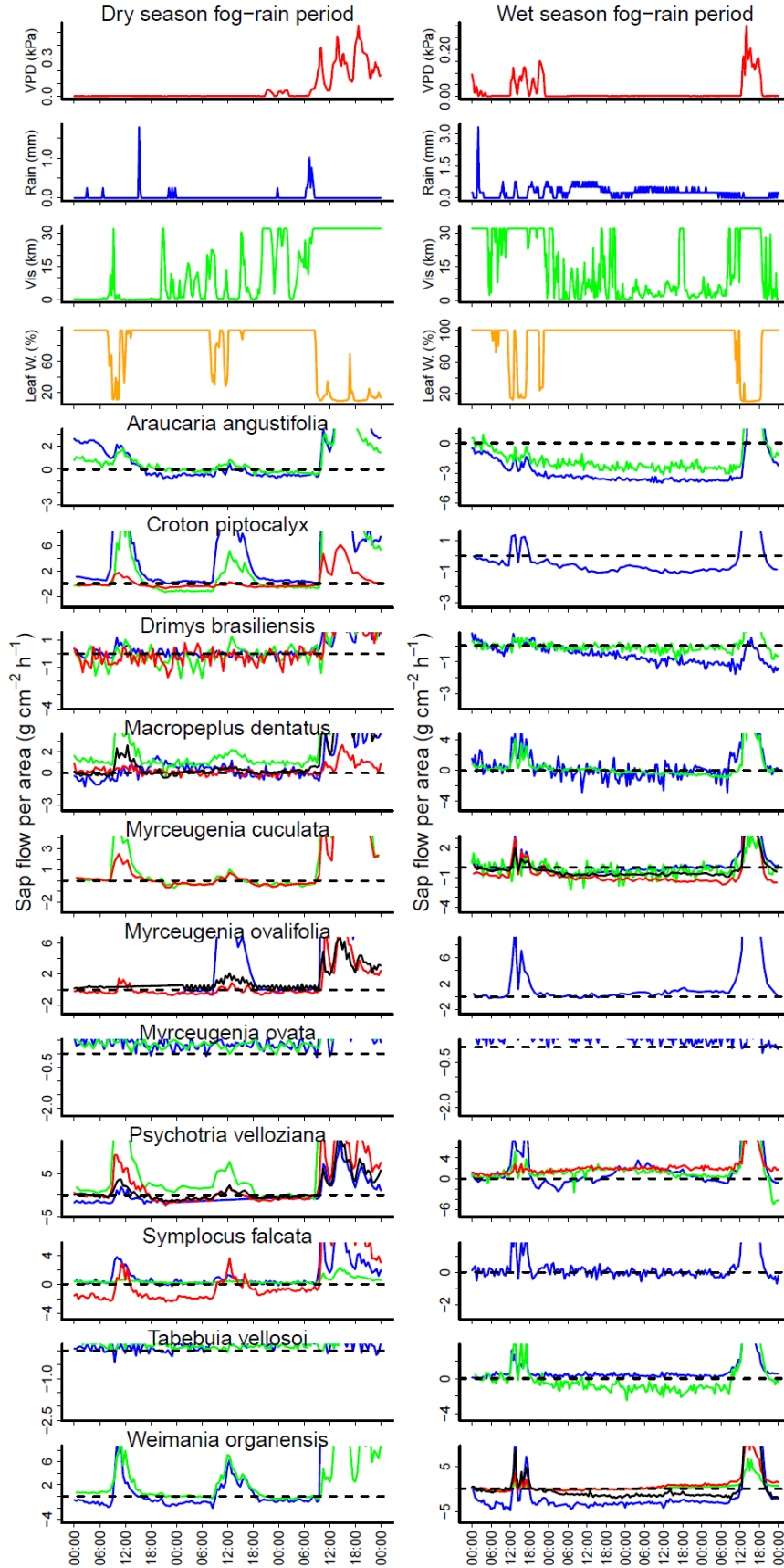
**Figure 2.1.** Vapour pressure deficit (VPD, kPa; red), rainfall depth (mm; blue), visibility (km; green) and leaf wetness (%; orange) of the climatic events selected to evaluate sap flow conditions. Event type and soil conditions are described in the top of each panel. Left panels are dry season and right panels wet season. Visibility less than 10km indicates fog and leaf wetness above 95% indicates wet leaves.

### *Data processing and analysis*

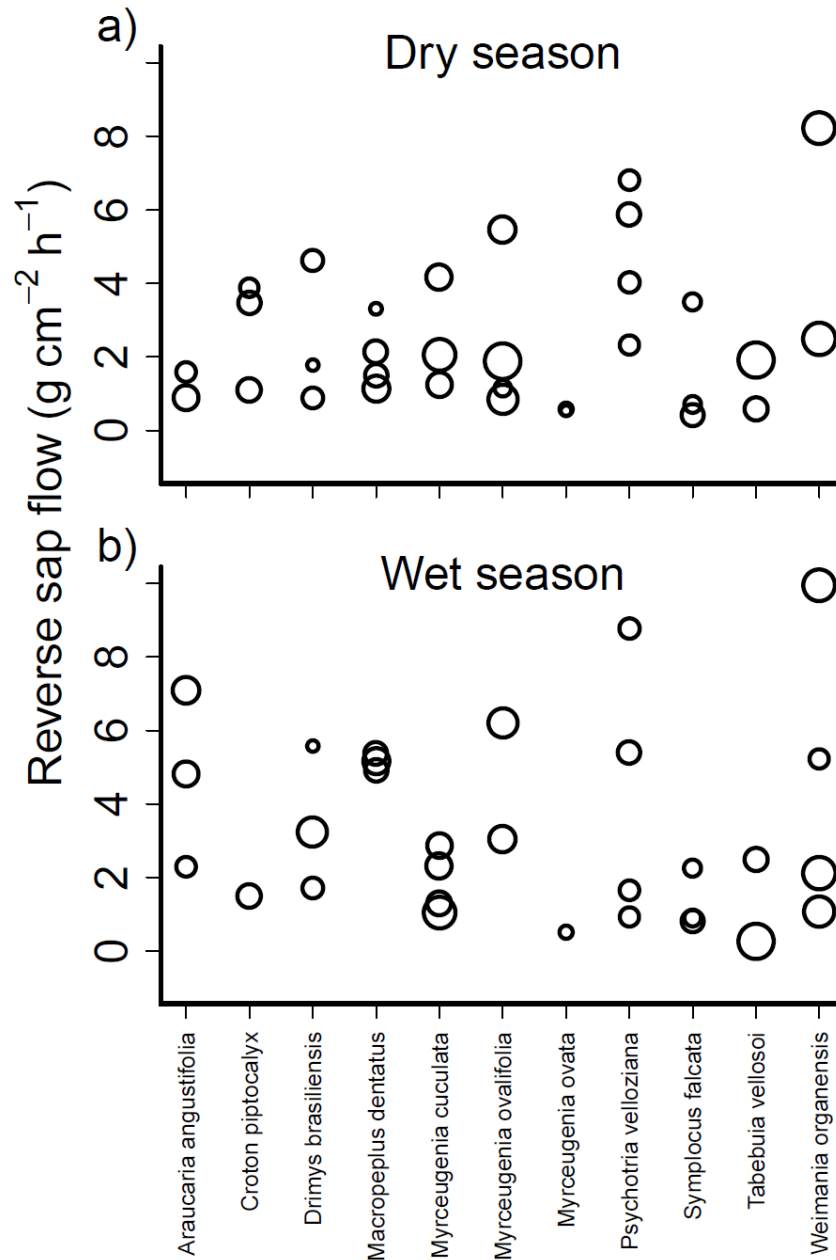
We used minimum nighttime or daytime sap flow velocity during each event as a measure of reverse sap flow intensity (RSF), which we present as positive values whenever sap reverse sap flow is occurring. We use RSF per xylem area ( $RSF_a$ ) instead of tree total RSF ( $RSF_t$ ) so comparisons between individuals with different sizes could be made. Deciduous trees were dry season sap flow was not used in the analysis. We used analysis of variance to compare differences between events and between species in  $RSF_a$ . We used Pearson's correlation to test for correlation between  $RSF_a$  of different events and between hydraulic traits and different events  $RSF_a$ . Additionally, we used linear discriminant analysis (LDA) to identify abiotic conditions separating non-reverse sap flow from reverse sap flow occurrence. The LDA calculates a composite axis of predictor variables that best groups the categorical response variables. We used vapour pressure deficit, rainfall, soil water content, visibility and leaf wetness as predictors of non-reverse sap flow, daytime reverse sap flow and nighttime reverse sap flow. LDA thus estimates the overall abiotic conditions in the forest separating reverse sap flow from non-reverse flow occurrence. We used R version 3.1.2 (R Core Team, 2014) with base packages for all data processing and analysis. In the LDA analysis we pooled all sap flow and climatic data together and used "lda" and "predict.lda" function from package "MASS" (Venables & Ripley 2002) after applying Box-Cox transform, centralizing and scaling the data with the "preProcess" function of the "Caret" package (Kuhn *et al.* 2016). Quality of the LDA was assessed by comparing model prediction, using "predict.lda", of reverse or non-reverse sap flow with the real data to assess false positives and false negatives of the model.

## **Results**

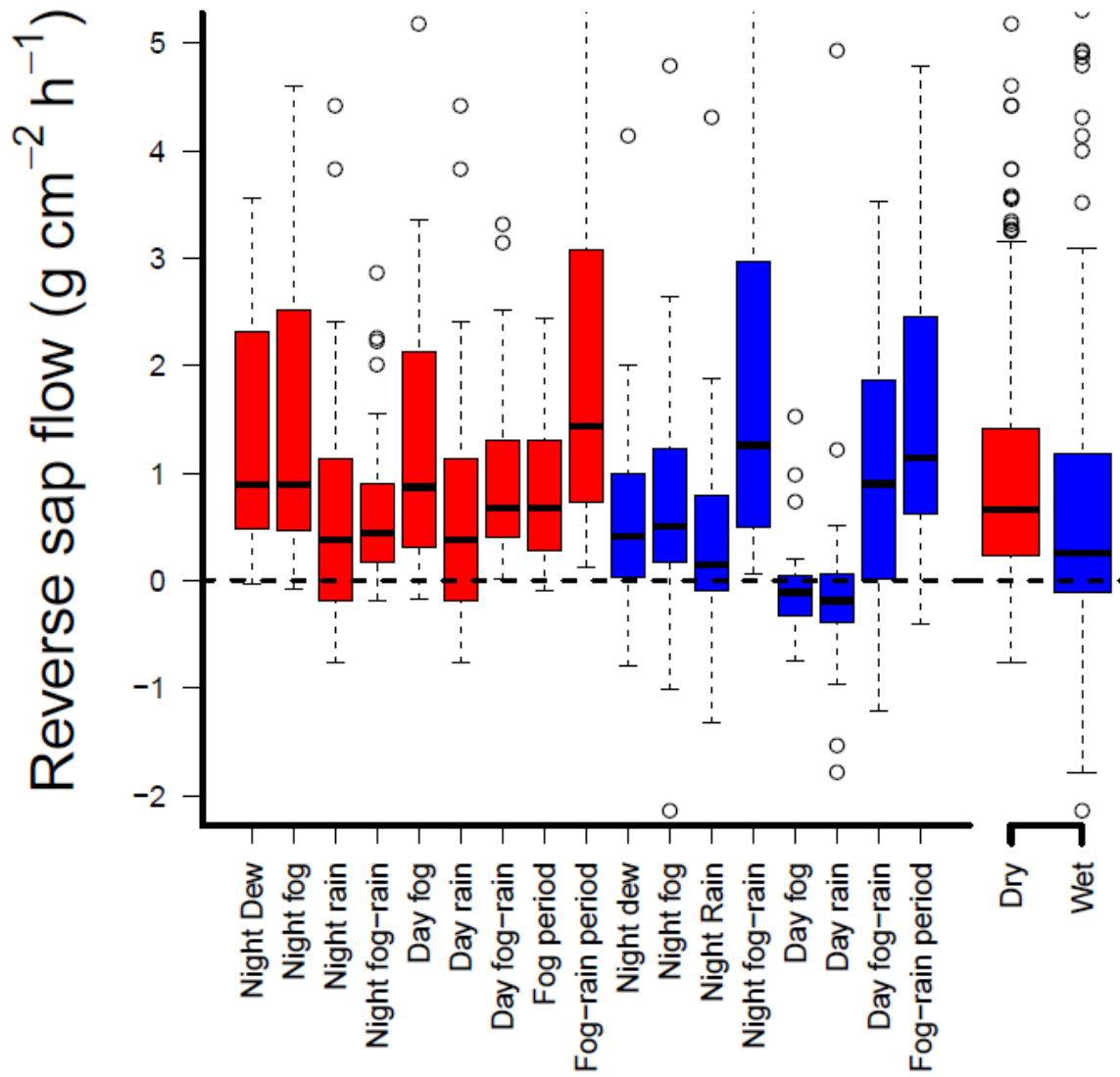
All trees presented reverse sap flow (RSF) both in the wet and dry season. RSF per xylem area ( $RSF_a$ ) varied almost 10 times between species and individuals (Fig. 2.2 and Fig. 2.3) while total tree RSF ( $RSF_a$  times tree xylem area,  $RSF_t$ ) varied from 16.6 to 4770.7 g h<sup>-1</sup>. Reverse flow capacity (i.e. the highest reverse flow in the dry or wet season,  $RSF_c$ ) for the analyzed individuals in the dry season was  $2.5 \pm 2.0$  g cm<sup>-2</sup> h<sup>-1</sup> (range of 0.4 to 8.2 g cm<sup>-2</sup> h<sup>-1</sup>) and in the wet season was  $3.36 \pm 2.5$  g cm<sup>-2</sup> h<sup>-1</sup> (range of 0.3 to 10. g cm<sup>-2</sup> h<sup>-1</sup>). Dry and wet season  $RSF_c$  were not significantly different (paired T-test  $t = 1.61$ ; d.f. = 24,  $p = 0.12$ ).  $RSF_c$  of individuals in the dry season was  $9.9 \pm 9.3$  % of maximum sap flow and  $RSF_c$  of individuals in the wet season was  $12.2 \pm 11.2$  % of maximum sap flow. While  $RSF_c$  was not significantly different between dry and wet season, median  $RSF_a$  of the selected climatic conditions in the dry season was 2.5 times higher than in the wet season (Wilcoxon Paired Rank Test  $V = 2964$ ;  $p = 0.03$ ; Fig. 2.4). We could not detect any inter-specific difference in  $RSF_a$ .



**Figure 2.2.** A continuous fog-rain period in the dry season (right panels) and wet season (left panels) showing the period climate (vapour pressure deficit, VPD, kPa; rainfall, mm; visibility, km; and leaf wetness, %) and the studied individuals sap flow per xylem area ( $\text{g cm}^{-2} \text{s}^{-1}$ ). Visibility less than 10km indicates fog periods and left wetness higher than 95% indicates wet leaves. Note *Tabebuia vellosi* has no sap flow in the right panel even when VPD increases due to it being deciduous.

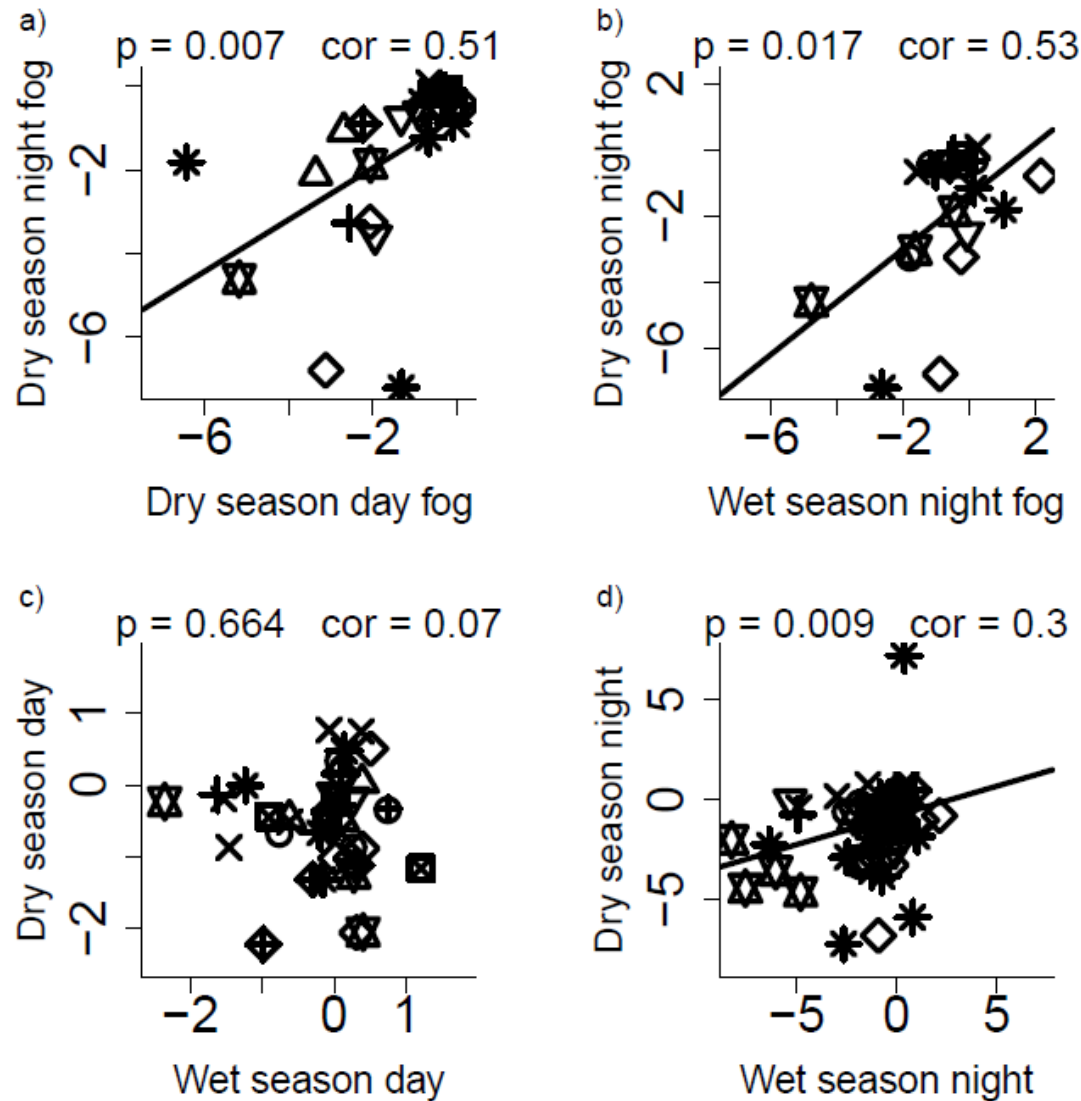


**Figure 2.3.** Reverse sap flow capacity per xylem area ( $RSF_c$ ;  $g\ cm^{-2}\ h^{-1}$ ) of the studied individuals in each species. Data is the highest  $RSF_a$  (lowest negative sap flow per xylem area) in the entire dry season (a) or wet season (b). Point size is proportional to tree size with largest and smallest points being trees with, respectively, 1.13 m and 0.34 m of diameter at breast height.



**Figure 2.4.** Reverse sap flow per xylem area ( $RSF_a$ ;  $g\ cm^{-2}\ h^{-1}$ ) of the studied trees during different types of climatic conditions in the dry season (red) and wet season (blue). “Dry” and “wet” boxplots in the right side are all dry season or wet season data together, except for fog-period and fog-rain period. Dashed horizontal line marks 0  $RSF_a$  and negative values indicate reverse sap flow was not occurring (i.e. sap flow was towards the canopy).

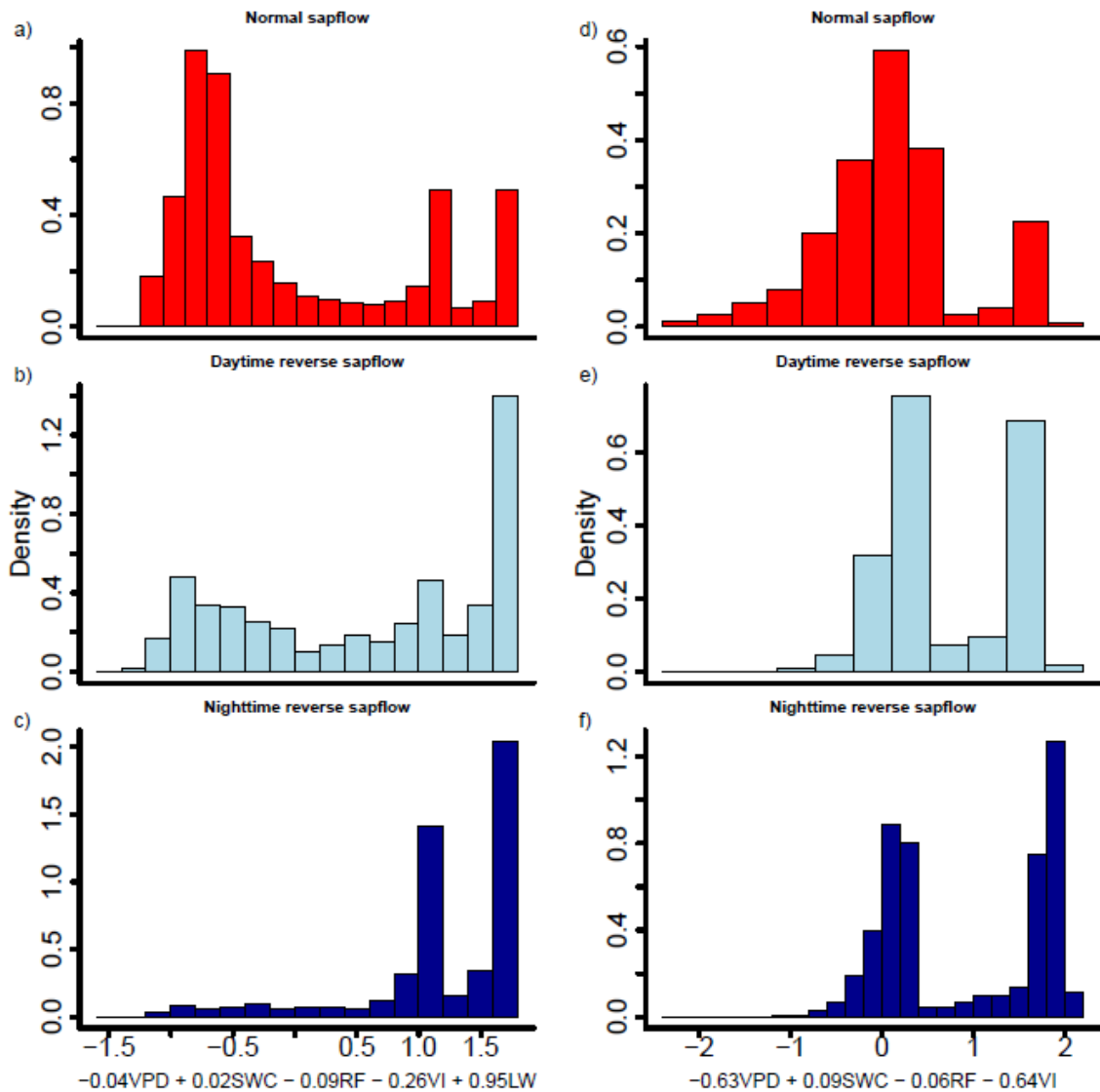
RSF<sub>a</sub> was highest in continuous fog-rain periods in both the dry and wet season and was lowest during the daytime and rain events (Fig. 4). In most events some individuals did not present reverse flow (and Table S1). RSF<sub>a</sub> of dry season night fog had a correlation of 0.51 with RSF<sub>a</sub> of dry season day fog and 0.53 with RSF<sub>a</sub> of wet season night fog ( $p = 0.007$  and  $p = 0.017$ ; Fig. 4). Dry season and wet season nighttime RSF<sub>a</sub> of all events were also related, albeit the relation was weaker ( $cor = 0.3$ ,  $p = 0.09$ ). However dry season and night season RSF<sub>a</sub> were not related ( $p = 0.66$ ). RSF<sub>a</sub> of different events were generally correlated in the same season but generally not between seasons (Fig. 2.5 and Table S2.1), indicating plants that have higher RSF<sub>a</sub> in one climatic condition usually also have higher RSF<sub>a</sub> in other events in the same season.



**Figure 2.5.** Correlation between reverse sap flow per xylem area ( $RSF_a$ , both axis;  $g\ cm^{-2}\ h^{-1}$ ) during a) dry season day and night fog; b) dry season night fog and wet season night fog; c) all dry season and wet season daytime events; and d) all dry season and wet season nighttime events. Negative values are non-reverse sap flow. Correlations for all climatic conditions are presented in Table S2.1.

The linear discriminant analysis shows reverse sap flow during day or night occurs similarly and the most important variable in separating the groups is leaf wetness, followed by visibility (fog occurrence) (Fig. 2.6 and Table 2.1). When leaf wetness is removed from the analysis (Fig. 2.6 right panels), VPD, rainfall and visibility dominates the axis separating reverse flow occurrence, while soil water content had a small effect in separating the groups. This

results highlight that whenever leaves are wet reverse flow may occur, mostly independent of soil conditions and depending solely on atmospheric conditions allowing leaves to get wet (rain, fog and low VPD). In effect, both the model with leaf wetness and without leaf wetness correctly predicted sap flow direction 79% of the time, with 13% of false positives (reverse sap flow attributed when it was not) and 7.1% of false negatives (normal sap flow attributed when it was not).





**Figure 2.6.** Linear discriminant analysis (LDA) of abiotic conditions when reverse sap flows does not occurs (a and d), when daytime reverse flow occurs (b and e) and when nighttime reverse flow occurs (c and f). Histograms are distributions of the composite LDA axis of climatic data when reverse flow occurs or not. Reverse flow data occurs in the climatic conditions in the right side of the LDA axis. X-axis label indicate the coefficients of the linear discriminants loading the axis. VPD is vapour pressure deficit, SWC is soil water content, RF is rainfall, VI is atmospheric horizontal visibility and LW is leaf wetness. All values are Box-Cox transformed, centered and scaled so they can be directly compared. Left panels include leaf wetness, which dominates the LDA axis (0.95) while in right panels leaf wetness was removed so the discriminating power of the other variables can be accessed. Only the first LDA axis is presented as it explained at least 95% or more of the between group variance. LDA summaries are presented in Table 1.

**Table 2.1.** Climatic variable group means for daytime and nighttime reverse flow and non-reverse flow (normal flow) and the linear discriminant axis coefficients for two different models, with and without leaf wetness data. VPD – vapour pressure deficit; SWC – soil water content; RF – rainfall; VI – visibility; LW – leaf wetness. Data used is Box-Cox transformed, centered and scaled. White values are Box-Cox, centered and scaled values and gray values are non-transformed values.

Group means					
Group	VPD	SW	RF	VI	LWE
Normal flow	0.17	-0.08	-0.02	0.17	-0.22
	0.37	79.31	0.07	27.9	34.4
Daytime reverse flow	-0.66	0.37	0.05	-0.49	0.47
	0.05	85.36	0.1	20.29	60.34
Nighttime reverse flow	-0.69	0.32	0.1	-0.73	1.04
	0.04	84.71	0.13	17.31	89

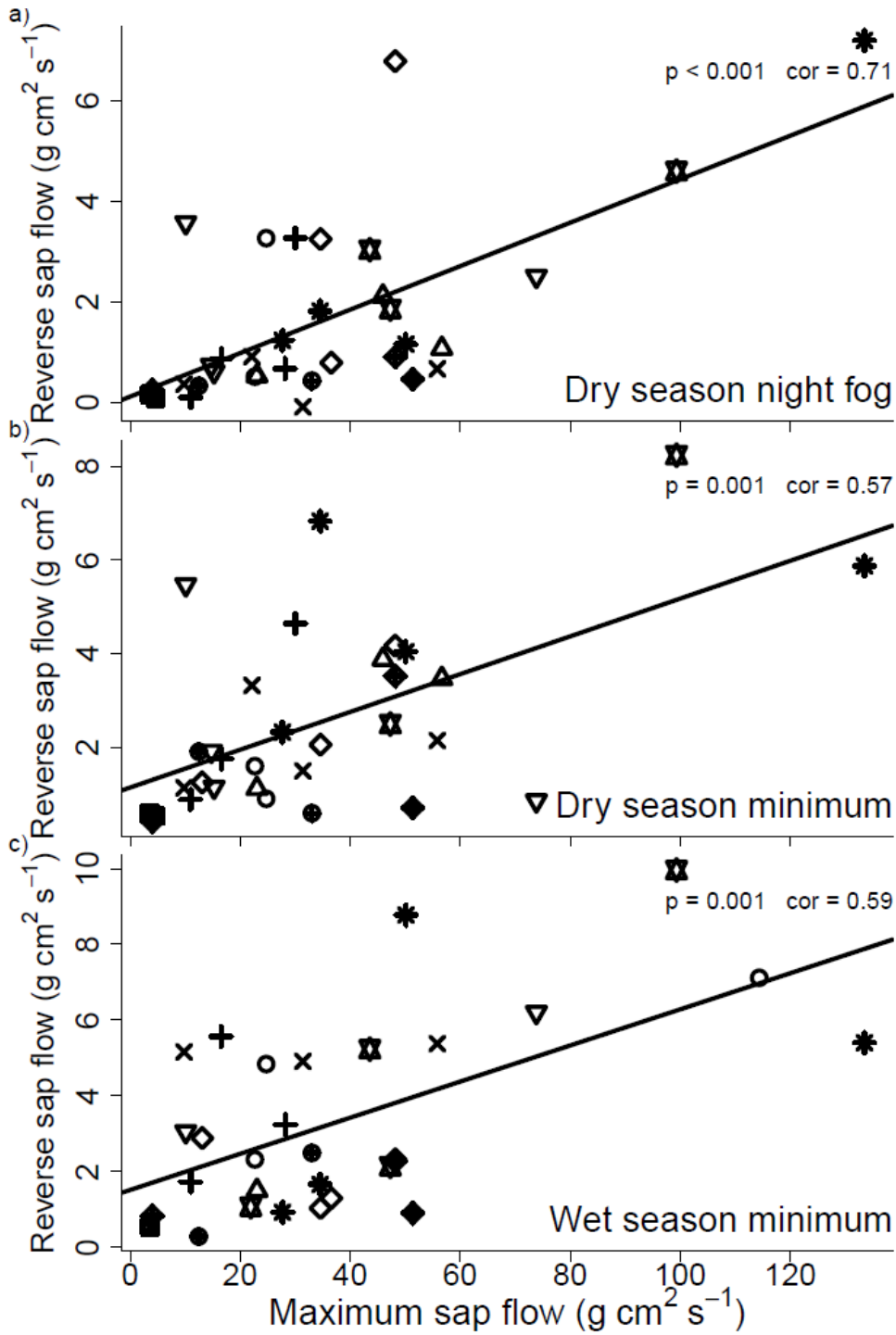
  

Model	Axis 1 linear discriminants coefficients				
VPD + SWC + RF + VI + LWE	-0.04	-0.02	-0.09	-0.3	0.95
	-0.42	0.001	-0.19	-0.02	0.02
VPD + SWC + RF + VI	-0.63	0.1	-0.06	-0.65	

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-1.62	0.007	-0.11	-0.05
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RSF<sub>c</sub> was not explained by tree height, xylem area, predawn, midday water potential or the transpiration and stomatal sensitivity index ( $\sigma$ ) ( $p > 0.05$ ; Table 2.2). Similar results were found when comparing those traits with RSF<sub>a</sub> of different climatic conditions and only weak and marginally significant relations were found with tree height and xylem area, which are correlated, and  $\sigma$ . Those results would not be significant if a penalty for multiple correlation tests were applied to p values. However, maximum sap flow per xylem area was strongly and highly significantly related to RSF<sub>c</sub> and RSF<sub>a</sub> of most climatic conditions (correlations of 0.4 to 0.85 and  $p < 0.001$  for most of the results; Fig. 2.7 and Table 2.2). However RSF<sub>a</sub> of some climatic conditions was not related to maximum sap flow per xylem area, suggesting different climatic conditions may have different drivers of RSF.



**Figure 2.7.** Relationship between maximum sap flow per xylem area ( $\text{g cm}^{-2} \text{h}^{-1}$ ) of each individual and reverse sap flow per xylem area ( $\text{RSF}_a$ ;  $\text{g cm}^{-2} \text{h}^{-1}$ ) in a foggy night in the dry season (a), reverse sap flow capacity per xylem area ( $\text{RSF}_c$ ) during the dry season (b) and  $\text{RSF}_c$  during the wet season (c).

**Table 2.2.** Correlation between reverse sap flow of different events and tree height, total xylem area, maximum sap flow, predawn and midday water potential ( $\Psi_{pd}$  and  $\Psi_{md}$ ) and the transpiration and stomatal sensitivity index ( $\sigma$ ). Dry and wet season maximum indicate reverse sap flow capacity. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

Reverse sap flow	Height	Xylem area	Max Sap Flow	$\Psi_{pd}$	$\Psi_{md}$	$\sigma$		
Dry season maximum	0.17	0.18	0.57***	0.35	0.36	0.3		
Wet season maximum	0.19	0.26	0.59***	0.25	0.28	0.42*		
Night	Dew	0.24	0.29	0.85***	0.33	0.31	0.26	
	Fog	0.3	0.25	0.65***	0.23	0.2	0.07	
	Rain	0.23	0.19	-0.26	-0.02	0.03	-0.12	
	Fog-rain	-0.12	0.04	0.59***	0.23	0.29	0.35	
Dry season	Fog	0.39*	0.36	0.4*	0.15	0.24	0.06	
	Day	Rain	0.23	0.19	-0.26	-0.02	0.03	-0.12
	Fog-rain	-0.17	-0.03	0.55**	0.25	0.3	0.41*	
Period	Fog	0.32	0.26	0.71***	0.23	0.19	0.14	
	Fog-rain	-0.17	-0.06	0.25	0.14	0.33	0.24	
Wet season	Dew	0.52**	0.42*	0.34	0.03	0.16	0.16	
	Night	Fog	0.26	0.26	0.53**	0.22	0.26	0.27
	Rain	0.45*	0.44*	0.27	0.15	0.17	0.26	

	Fog-rain	0.2	0.26	0.44*	0.28	0.33	0.38
	Fog	-0.47*	-0.57**	-0.32	-0.17	-0.25	-0.08
Day	Rain	0.05	0.16	0.62**	0.32	0.17	0.23
	Fog-rain	0.23	0.05	0.34	0.02	0.24	0.14
Period	Fog	0.08	0.22	0.48*	0.12	0.21	0.2

**Table 3.** Sap flow and tree traits measured. Tree height (m), tree diameter (m), tree xylem area (cm<sup>2</sup>), dry and wet season reverse sap flow capacity per xylem area (RSF<sub>c</sub>; g cm<sup>-2</sup> h<sup>-1</sup>), maximum sap flow per xylem area (g cm<sup>-2</sup> h<sup>-1</sup>), midday and predawn water potential ( $\Psi_{md}$  and  $\Psi_{pd}$ ; MPa) and transpiration and stomatal sensitivity index ( $\sigma$ ).

Species	Individual	Height	Diameter	Xylem area	Dry RSF <sub>c</sub>	Wet RSF <sub>c</sub>	Max sap flow	$\Psi_{md}$	$\Psi_{pd}$	$\sigma$
<i>Araucaria angustifolia</i>	a14	15.20	0.78	236.23	-0.91	-4.82	24.76	-1.79	-0.76	
	c29	17.00	0.84	258.74		-7.10	114.37	-1.18	-0.96	
	b12	11.80	0.62	125.94	-1.59	-2.31	22.68	-0.73	-0.80	0.88
<i>Croton piptocalyx</i>	h46	14.60	0.71	176.37	-3.47		56.67	-1.15	-0.33	
	c42	10.30	0.59	130.28	-3.88		45.95	-1.37	-1.02	
	l17	13.70	0.75	166.24	-1.12	-1.50	23.05			0.50
<i>Drimys brasiliensis</i>	i10	13.70	0.92	146.38		-3.24	28.18	-0.97	-0.50	
	i27	9.30	0.65	106.25	-0.88	-1.72	10.98	-1.01	-1.25	
	e38	8.90	0.67	103.80	-4.64		30.07	-0.91	-0.59	
	c22	4.40	0.36	23.93	-1.76	-5.57	16.56	-0.77	-0.45	0.63
<i>Macropheplus dentatus</i>	c21	5.70	0.38	13.69	-3.32		22.12	-1.45	-0.58	
	i21	12.20	0.71	139.63	-2.15	-5.38	55.82	-1.35	-0.41	
	e9	13.40	0.83	243.91	-1.14	-5.16	9.80	-0.64	-0.22	1.45

	o26	10.90	0.71	183.09	-1.50	-4.91	31.39	-1.24	-0.61	
	e6	10.90	0.75	182.62		-1.29	36.55			
Myrceugenia cuculata	j14	12.20	0.77	196.74	-1.25	-2.88	13.09	-1.42	-1.70	
	l35	16.50	0.80	213.08	-4.17	-2.32	48.20			
	m30	15.00	1.00	365.39	-2.05	-1.03	34.60			0.54
	i8	22.00	0.92	343.10	-0.86	-6.20	73.85			
Myrceugenia ovalifolia	h18	16.20	0.84	185.88	-5.47	-3.05	10.12			
	j26	13.70	0.86	270.69				-3.10	-2.00	
	c28	7.30	0.51	79.51	-1.15		15.25	-2.93	-1.36	
	e41	25.00	1.13	527.04	-1.92		14.77	-1.82	-0.41	0.71
Myrceugenia ovata	a32		0.42	30.71	-0.59	-0.54	3.56	-2.80	-1.63	
	o24	5.60	0.34	30.92	-0.54		4.58	-3.00	-2.03	0.75
Psychotria velloziana	a43	12.20	0.61	111.20	-2.32	-0.93	27.69	-1.33	-0.80	
	o18	10.90	0.71	193.48	-5.87	-5.40	133.49	-1.10	-0.41	
	i30	11.30	0.64	192.04	-4.04	-8.77	50.12	-1.45	-0.69	
	f3	11.70	0.62	89.57	-6.83	-1.66	34.54			1.30
Symplocus falcata	o28	12.50	0.53	56.23	-0.71	-0.90	51.39	-1.39	-0.56	
	i20	9.50	0.70	116.87	-0.41	-0.83	4.04	-1.49	-0.63	0.81

	c17	10.70	0.54	89.99	-3.52	-2.27	48.21	-1.38	-1.02	
Tabebuia vellosi	a24	10.60	1.11	468.38	-1.91	-0.29	12.47	-0.50	-0.17	
	i39	10.50	0.74	180.48	-0.59	-2.49	33.03	-1.45	-0.65	0.59
	f21	20.00	1.00	478.82	-8.23	-9.96	99.32	-0.83	-0.42	
Weimania organensis	m36	19.00	0.96	283.50		-1.07	21.93	-1.68	-0.35	
	i13	20.00	1.02	260.07	-2.50	-2.14	47.30	-1.74	-1.10	
	c5	9.70	0.61	60.07		-5.23	43.57			1.30



## Discussion

Our results show that foliar water uptake (FU), as measured from the reverse sap flow per xylem area (RSF) at stem breast height, occurs in all measured individuals but not in all climatic events. RSF was variable both within species and between species, suggesting abiotic or biotic traits can be strong drivers of this process (Burgess *et al.*, 2001). Different climatic events caused different amounts of RSF and dry season RSF was higher than wet season, albeit not much. Surprisingly, RSF occurred in the wet season, in a period spanning some days with fog-rain period, where soil and atmosphere were fully water saturated. This implies another mechanism, not necessarily soil driven water potential gradient, is causing RSF. We did not find tree height, minimum leaf water potential and minimum soil water potential to explain RSF capacity in the dry season, which should occur as higher trees should intercept more fog and trees with more negative water potential should have a stronger water potential gradient driving RSF. However, maximum sap flow was related to RSF capacity in the dry and wet season and RSF intensity in different events. Our results suggest FU may be more important than previously thought.

### *Foliar water uptake diversity and occurrence in the wet season*

RSF capacity was approximately 10% of maximum sap flow, which is in the range of what was found by Eller *et al.* (2013). RSF differed across climatic conditions but was produced almost whenever leaves were wet, with wet leaves being the best predictor of RSF, followed by VPD and fog occurrence (low visibility; Figure 2.6). RSF was higher during prolonged fog and fog-rain periods. This is not unexpected as different events wet the canopy differently and different trees have different expositions to the atmosphere. More exposed canopies intercept more fog and are the first to receive rainfall, but also are more coupled to the atmosphere and should form less dew. Less exposed canopy should form more dew while shaded leaves should take longer to lose heat by radiation and form dew, but should stay wet longer. In effect, canopy wetting is complex and this may explain why RSF did not occur in all individuals in all events, although it did occur in all individuals in the prolonged periods of fog and fog-rain. It is interesting that in the dry and wet season during prolonged events when all the canopy should be wet RSF was

similar, which further suggests differential wetting of tree canopies may explain part of RSF diversity between events.

Our results show FU occurs during the wet season, even after more than one day of continuous rain and fog, when both atmosphere and the soil were completely wet and there should be no driving force for water to enter leaves (see Fig. 2.2 right panels). We believe the driving force for FU in this situation is the gravity potential of water in the leaves. Measured trees had between 8 to 20 m height, which creates a gravitational potential of -0.08 to -0.2 MPa. Even when the water potential in the atmosphere and in the soil is 0 MPa, leaves should be at this water potential and be able to uptake water from its surfaces. Water entry into the leaves when soil and atmosphere are water saturated should increase leaf water potential above its expected gravity-generated water potential maximum. This should cause an increase in the water potential gradient of the whole tree with a consequent positive pressure in the lowest parts of the tree, the root-soil interface, pulling water outside of roots and into the soil. We believe this mechanism is the cause of RSF during the wet season. Occurrence of leaf predawn water potentials higher than theoretical maximum due to tree height, as we frequently observe in field measurements and as Binks *et al.* (submitted) shows, to our knowledge could only be caused by FU.

Wet season RSF capacity was similar to dry season RSF capacity. This is unexpected as dry season RSF has a drier soil creating a stronger driving force to RSF. We believe part of this similarity is because in the dry season part of the water absorbed by leaves recharges non-saturated plant tissues. In the wet season, as tissues are well hydrated FU, despite having a smaller driving force – the gravitational water potential, is mostly directed to soils and not to recharge plant tissues, creating a relatively higher RSF. This is a limitation of measuring RSF to estimate FU, as not all FU becomes RSF at breast height. In effect, the larger a tree is the more water storage it has and the less RSF should be produced in the dry season as FU has to recharge a larger amount of biomass before producing high rates of RSF. This may partially explain why dry and wet season RSF capacities were not different.

We should caution that a potential problem with RSF data is whether it is an artifact derived from calibration or zeroing of the sap flow baseline. The heat-ratio method has enough

resolution to allow accurate measurement of RSF (Burgess *et al.*, 2001) and we took all recommended and necessary steps to calibrate the sap flow sensor data. Furthermore, we corrected zero sap flow for each month using a low VPD period hours after sunset, with some dew formation. This procedure should ensure no transpiration was occurring and in fact should even underestimate RSF, as it could be occurring during each month is zero. Finally, RSF is an underestimate of FU, as FU is not entirely transported to stem but also refills tree tissues capacitance, as noted by Binks *et al.* (submitted). Considering we have no reason to suspect wrong calibration of the sensors, our zero may underestimate RSF and RSF probably underestimates FU, we believe our conclusions regarding FU process are corrected or even underestimated.

#### *Biotic drivers of foliar water uptake*

RSF differed between species and even within the same species, indicating tree traits may be driving different FU capabilities or causing different patterns of FU transport to the lower parts of the tree as RSF. The first, and theoretically larger, barrier to FU is the leaf cuticle (Becker *et al.*, 1986), which is diverse between species (Burghardt & Riederer, 2008) and even within the same species (Geyer & Schönherr, 1990). However water can also enter plants through trichomes and maybe stomata (Schönherr & Bukovak 1971; Pina *et al.*, 2016), probably with different efficiencies than through the cuticle. Leaf lamina conductance to liquid water may partly explain the variability found in RSF. While height was expected to drive differences in RSF by affecting leaf gravitational water potential, it was not, possible due to height also affecting the mechanisms of canopy wetting. It would be interesting to use a larger height gradient within a controlled environment to remove this confusing effect. Finally, predawn and minimum water potential of leaves were also not good predictors of RSF as it would be expected due to them affecting the driving forces for FU. However, lower predawn and minimum water potential also affect plant water storage, which can at the same time increase FU and reduce RSF due to FU water being routed to recharge tissues upper in the canopy and not in the stem at breast height.

Interestingly, RSF was related to maximum sap flow per xylem area. Two mechanisms may explain this. First, higher maximum sap flow rates occur either because leaves transpire more or because there is more leaf area being supplied per xylem area. If higher maximum flow

rater in our data is due to higher leaf area then the correlation is due to a larger leaf area absorbing water. However, if they are the same, then some leaf traits related to higher transpiration rates may also be causing higher FU. This view is supported by Berry *et al.* (2014) results showing higher FU is related to higher leaf vapour conductance and by Eller *et al.* (2016) showing plants with lower FU have higher stomatal control. In effect, both differences in leaf traits and in leaf area per xylem area may be concomitantly explaining the relationship between RSF and maximum sap flow per xylem area. Secondly, if leaf lamina conductance to liquid water (i.e. leaf FU efficiency), is in the same order of the xylem conductance to redistribute FU to plant tissues, redistribution of FU to lower parts of the tree to lower parts of the may be slower. Leaf predawn water potential higher than theoretical gravitational maximum (Binks *et al.* submitted) should only occur in this situation, else FU would readily be redistributed to soil and leaves would be keep at their height predicted water potential. If plants with higher maximum sap flow also have higher xylem conductance to liquid water, FU would more readily reach the stem at breast height causing higher RSF rates. However, all proposed mechanisms are speculative and need testing as physiology of FU is virtually an inexistent area.

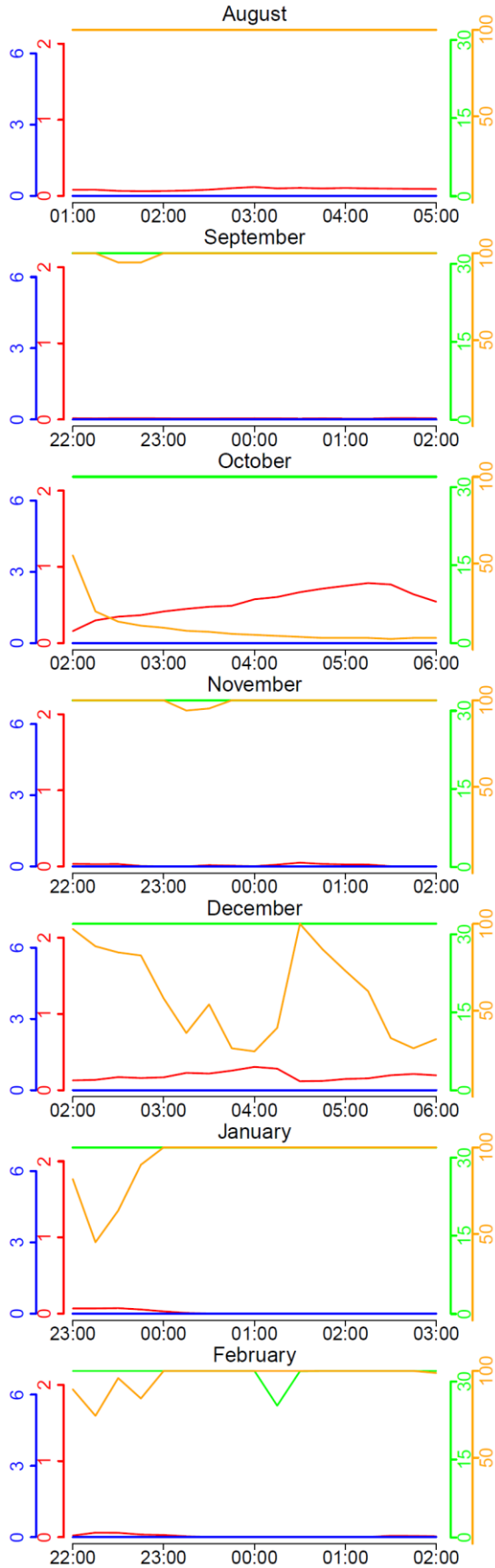
### *Conclusion*

Our results shows foliar water uptake (FU) is a much more frequent process than frequently considered and can occurs most of the times when leaves are wet, during the dry season or in the wet season, night or day and due to dew, fog or rain, as long as leaves are wet. Interestingly, FU occurs during prolonged rain and fog periods in the wet season, when neither the soil or the atmosphere should be creating a gradient for water flow. We hypothesize this occurs due to gravitational potential of leaf water forcing water inside the leaves and then being redistributed to the soil. If gravitational water potential is part of the driving force for FU, tall trees should have much higher FU, which, however, would be harder to detect as reverse flow at ground level, as they have higher capacitance. Reverse sap flow per xylem area, our measure to estimate FU, was highly diverse between individuals of the same species and in different climatic conditions. We believe both biotic and abiotic factors combine to create this variability: differential canopy wetting and wetting duration due to tree position in different leaf wetting events (dew, fog or rain); differential leaf traits facilitating or making it difficult for water entry in leaves; different leaf areas per xylem area; or even different xylem conductance affecting FU

redistribution to stem at breast height, where we measured reverse sap flow. FU is known to alleviate drought stress and affect plant functioning (Martin & von Willert, 2000; Berry *et al.*, 2014; Eller *et al.*, 2016). Our work points FU uptake may be much more frequent than previously thought and its importance may be underestimated. Future works should focus on mechanisms driving FU and on estimating FU importance to plant functioning.

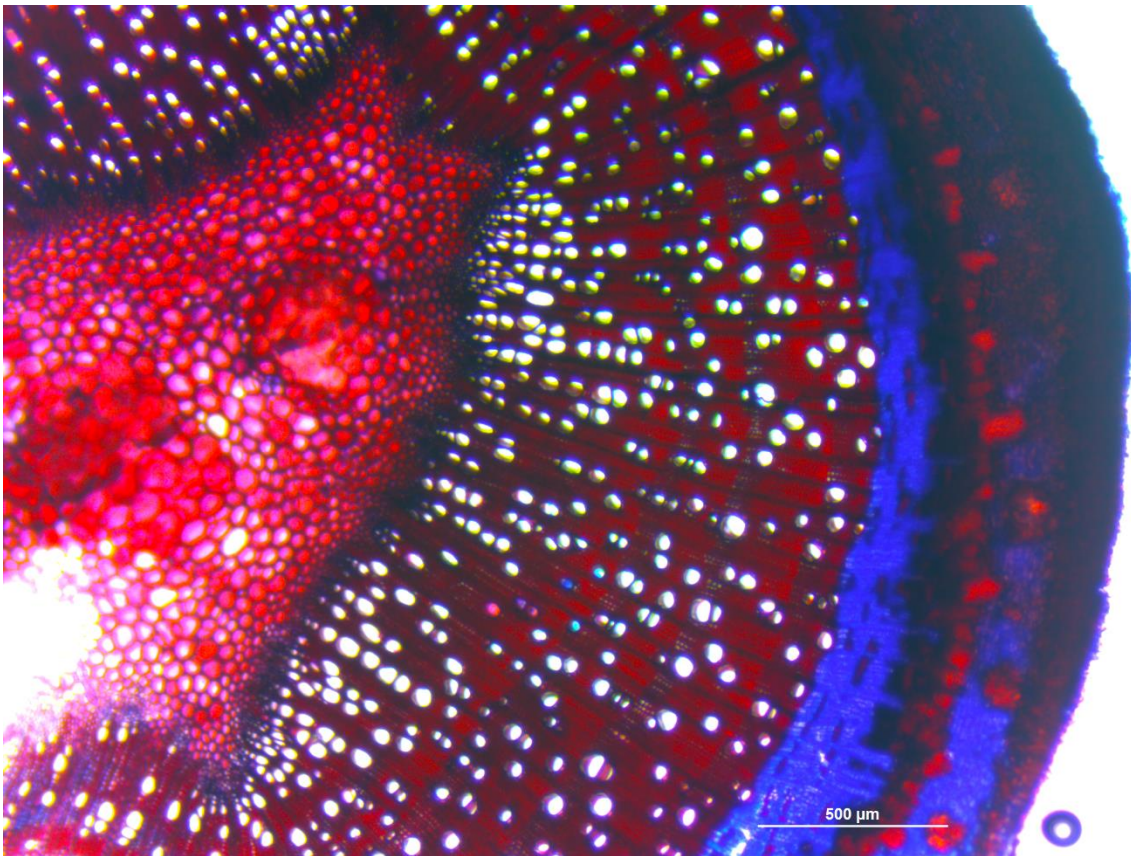
### Supplementary Figures

**Figure S1.** Periods used to correct zero sap flow in the studied months and their climatic conditions. Red line is vapor pressure deficit (kPa), blue is rainfall depth (mm), green is horizontal visibility (km) and orange is leaf wetness (%). Each sap flow sensors was zeroed in each month using the minimum sap flow values of the periods above.



## Chapter 3

### On xylem hydraulic efficiencies, wood space-use and the safety-efficiency tradeoff



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## Introduction

In a recent issue of *New Phytologist*, Gleason *et al.* (2016) compiled a remarkable data set of wood traits to investigate the tradeoffs between xylem hydraulic safety and xylem hydraulic efficiency, measure as xylem specific hydraulic conductivity ( $K_s$ ;  $\text{mol}_{\text{H}_2\text{O}} \text{m}_{\text{length}}^{-1} \text{s}^{-1} \text{m}_{\text{transversal}}^{-2} \text{Pa}^{-1}$ ). This tradeoff is expected and somewhat studied at the conduit level, but how this should propagate to other plant levels is not known. In their paper, Gleason *et al.* compared the relationship between two tissue level traits emerging from conduit level traits, hydraulic safety, and  $K_s$ , to test for this tradeoff. The authors show conclusively that it is not possible to have high efficiency and high safety in plants hydraulic systems. Nevertheless, many species present low efficiency and low safety, which suggests the existence of other axes of variation affecting hydraulic efficiency and safety. In a comment on the same issue, Brodersen (2016) highlights the complexity of traits and contexts that may be affecting the hydraulic and safety tradeoff. Brodersen (2016) also proposes that there may actually be other traits that can better represent hydraulic safety. Here we look at other xylem hydraulic efficiencies that may help explain Gleason *et al.* results and propose new questions.

Efficiency may be defined as achieving maximum productivity with minimum wasted effort or expense (Oxford Dictionaries, 2015). What kind of efficiency is measured by  $K_s$ ? What “waste” is reduced by increasing this efficiency?  $K_s$  is flow per unit area and unit pressure gradient. Unit pressure gradient can be considered as longitudinal xylem efficiency, as higher longitudinal efficiency leads to smaller pressure gradients and higher  $K_s$ .  $K_s$  then integrates transversal and longitudinal water supply efficiency. This can be clearly seen in the following sentence of Gleason *et al.* (2016; p10) “efficient xylem can transport the same volume of water as inefficient xylem, but does so with a smaller cross-section of living wood”, or, we add, can transport the same volume of water as inefficient xylem through longer wood section, or, yet, can supply a greater leaf area with the same wood diameter and height.  $K_s$  is thus a measure of space-use efficiency and possible tradeoffs or relations should be analyzed from the viewpoint of wood space use.

Many other xylem hydraulic efficiencies could be considered:

- Hydraulic energy efficiency: the energy invested (production and maintenance) in the hydraulic system. It could be measured as total plant conductance divided by the



photoassimilates or the calories used to produce and/or maintain it. A rough metric for it, that does not differentiate the energetic costs of different molecule building blocks (i.e. lignin is more energetic costly than cellulose), would be the grams of dry mass invested to obtain a given hydraulic specific conductivity (with Gleason *et al.* data it could be calculated dividing hydraulic specific efficiency by wood density; or whole plant conductance by plant biomass, as in Mencuccini (2003)).

- Hydraulic nutrient efficiency: the nutrient investment (production and maintenance) of the hydraulic system - hydraulic conductance by unit nutrient invested.
- Leaf specific hydraulic efficiency: already widely used, the xylem water transport conductivity invested to supply water to each unit transpiration area.

By redefining what efficiency we are actually measuring many new questions arise from Gleason *et al.* (2016). First, acknowledging that the authors compared hydraulic space-use efficiency, we can rather say that there is weak ( $r^2 < 0.09$ ) tradeoff between hydraulic space use of 0.4-1.0 cm branches (data selection criterion used) and hydraulic safety. Either space of branches of this size class is not under selection (they can increase or decrease branch length/diameter freely or with small consequences) or other tradeoffs are acting. For example, branches of wider diameter have a longer diffusive pathway to O<sub>2</sub> which may limit O<sub>2</sub> supply to living cells (Hook 1972) while smaller diameter branches have a larger area to volume ratio with possible consequences related to stem photosynthesis (Schmitz *et al.* 2012; Wittmann & Pfanz H. 2014).

An implicit assumption of Gleason *et al.* and many other studies is that higher K<sub>s</sub> should be selected because it has a lower energetic cost. Considering that hydraulic space-use efficiency is different from energy-use efficiency, should higher K<sub>s</sub> imply higher energy efficiency? At a first glance this is true as higher K<sub>s</sub> can allow for a plant to reduce its volume of wood and this should lead to a relationship between K<sub>s</sub> and stem and branch diameters. But the real picture may be more complex and, to our knowledge, the energy cost of plant water transport system has never been measured (but see the analysis of mass-specific hydraulic conductance and the cost of being higher in Mencuccini (2003)). If the assumption that higher K<sub>s</sub> also leads to lower energetic costs is not always true, higher K<sub>s</sub> would be free to vary in the boundaries set by hydraulic safety, as seen on Fig. 2 of Gleason *et al.* (2016).

### **Wood space use**

As  $K_s$  is a space-use efficiency and is not necessarily related to energetic efficiency, would there be situations where wood space is under selection that are not related to energetic costs? We believe that to understand  $K_s$  variability we have to look at factors that (1) limit available wood space or (2) affect wood space use. Considering point (1), we ask when is wood space limiting to plants? When can plants afford to avoid regulating hydraulic conductance by either having larger cross sectional area and/or smaller lengths? While space limitations on 0.4-1.0 cm branches, as on Gleason *et al.* (2016) data, may not seem to be directly limiting to plants, root space is probably limiting because smaller roots can better penetrate the soil and increase surface area (Chimungu *et al.* 2015). At the same time, due to allometric scaling, space limitations or constraints on stem size may be projected to branch size, or vice-versa, and space limitations may actually be indirectly affecting small branches (Sperry *et al.* 2008; Savage *et al.* 2010). Also, as Larjavaara & Muller-Landau (2010) notice, changing stem and branch diameter would allometrically change bark surface and thus bark functioning may be constraining wood space.

Regarding point 2,  $K_s$  depends both on fractional space allocated to conduits and on the efficiency of the conduits present in this space. Space allocation to conduit thus necessarily conflicts with space allocation to parenchyma, fibers and cell walls. Any situation that constrains fiber and parenchyma allocation must tradeoff with space available for conduits. Changes in conduit number and efficiency may accommodate for increases or decreases in conduit-available space to regulate  $K_s$  and this is actually an interesting pathway for future research. Maybe the crucial question regarding hydraulic space-use efficiency would be what other functions are related to wood space?

One way to approach this question is to partition  $K_s$  between the different tissues that are invested to sustain hydraulic conductance (Fig. 3.1).  $K_s$  as a measure of total space invested to conductance integrates fiber, parenchyma and conduit volume invested to conductance. By analyzing its individual constituents new insights may be possible. Lumen space invested to conductance is key to understand overall conduit hydraulic efficiency. Fiber and conduit wall space allocated to conductance may be important to understand implosion-efficiency tradeoffs. Parenchyma space allocated to conductance may be related to refilling efficiency or to water supply by capacitance. How different wood space allocation setups relate to hydraulic safety and efficiency and to different environmental and phylogenetic contexts may be the next steps to

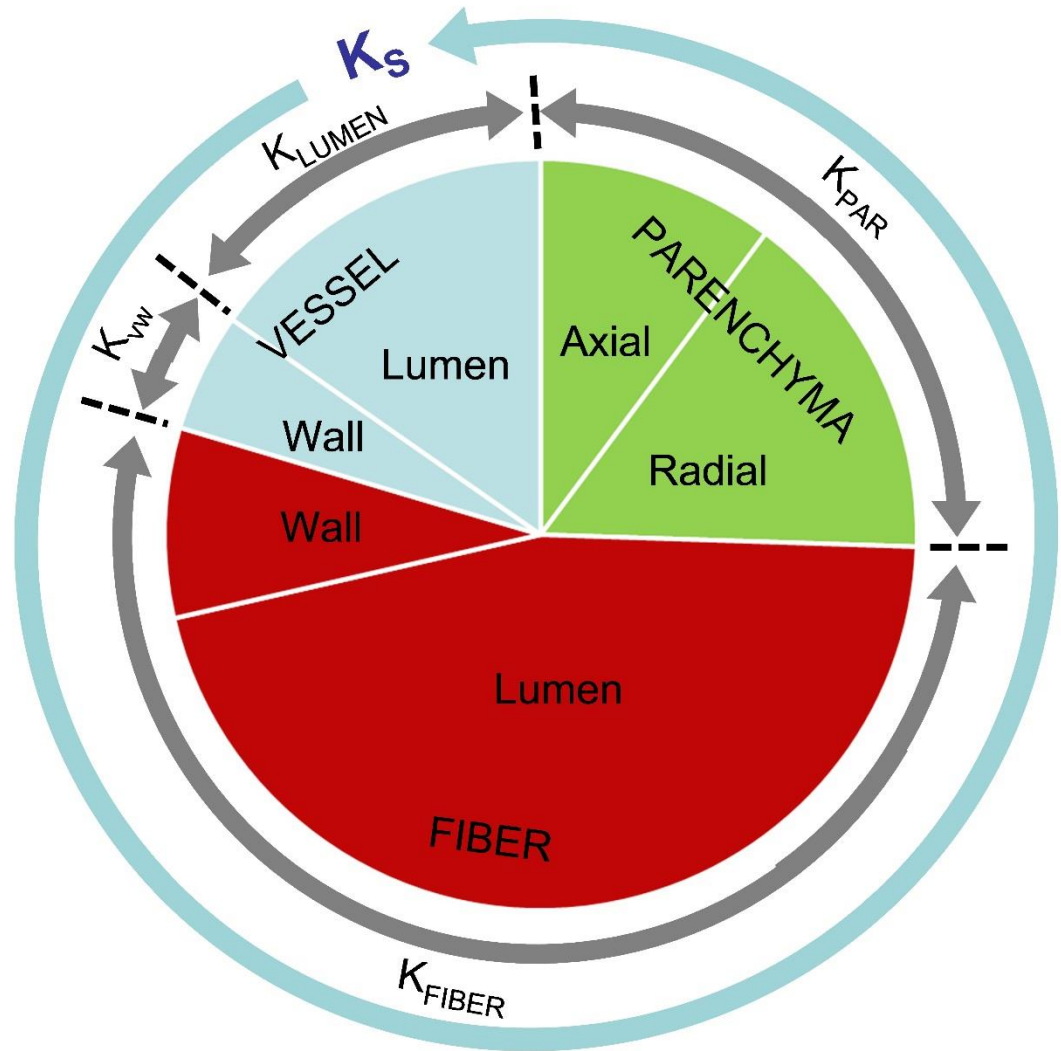
build upon Gleason *et al.* findings. Particularly interesting will be to compare belowground and aboveground wood space use as they are subject to different mechanical constraints on space use (Sperry *et al.* 2008; Larjavaara & Muller-Landau 2010). We believe future investigations on some of the hydraulic efficiencies traits proposed here (summarized in table 3.1) will contribute in those next steps.

**Table 3.1** Possible xylem hydraulic efficiencies traits, their definition and functions they potentially integrate.

<b>Hydraulic Efficiency</b>	<b>Definition</b>	<b>Description</b>	<b>Possible importance</b>
K – conductance*	Water flow per pressure difference	Total water transport capacity of a sample	Needed to calculate other efficiencies
KI – conductivity*	Water flow per unit pressure gradient	Axial water transport efficiency of a sample	Needed to calculate other efficiencies
Ks - space-use efficiency	KI by unit xylem area**	Integrates axial and transversal xylem water transport efficiency	Space use information
Ks[fiber] - fiber-use efficiency	KI by fiber area (or amount)	Fiber related to the water transport system	Inner and outer mechanical resistance
Ks[par] - parenchyma-use efficiency	KI by parenchyma area (or amount)	Parenchyma related to the water transport system	Storage (water, nutrients, carbon) and living xylem functions
Ks[wall] - conduit-wall-use efficiency	KI by conduit wall area (or amount)	Conduit wall invested to sustain water transport system	Implosion resistance and conduit cost
Ks[conduit] - conduit specific efficiency	KI by conduit number	Mean conduit water transport efficiency	Conduit efficiency
Ks[lumen] - lumen-use efficiency	KI by lumen area	Lumen space invested in water transport	Lumen space-use efficiency
Ks[bark] – bark-use efficiency	KI by bark area or mass	Bark investment related to the water transport system	Bark-xylem coupling and tradeoffs

Ks[energy] – energy-use efficiency	KI by unit energy cost of transversal area	Energy use efficiency of water transport system	Energetic cost of water transport system
Ks[mass] – mass-use efficiency	KI by unit dry weight in transversal area	Mass specific hydraulic conductance	Structural investment of water transport system
Ks[nutrient] – nutrient-use efficiency	KI by unit nutrient in transversal area	Nutrient use efficiency of water transport system	Nutrient investment in the water transport system
KUE*** – water transport system use efficiency	KI by unit photosynthetic rate	Water transport efficiency by carbon gain rate	Photosynthetic gain by water transport system invested

\* Not efficiencies, but listed on the table as they are necessary to calculate the efficiencies.  $K$  = water flow by pressure difference ( $\text{mol s}^{-1} \text{Pa}^{-1}$ );  $KI$  = water flow by pressure difference times sample length ( $\text{mol m s}^{-1} \text{Pa}^{-1}$ ). \*\* Unless stated otherwise, transversal area. \*\*\* Analogous to WUE/PUE/NUE – water/phosphorus/nitrogen use efficiency of photosynthesis.



**Fig. 3.1** Space allocation setup of a branch to different tissues and examples of different hydraulic efficiencies that can be analyzed.  $K_{s[\text{fiber}]}$  and  $K_{s[\text{par}]}$  are, respectively, the amount of fiber and parenchyma related to water transport capacity (see Table 3.1).  $K_{s[\text{vw}]}$  and  $K_{s[\text{lumen}]}$  are, respectively, the amount of conduit wall and conduit lumen area invested in water transport capacity.  $K_s$  integrates all the tissues invested in water transport capacity. The area of each slice represents the mean percent space allocation to that tissue in branches of 24 angiosperm species (Ziemińska *et al.* 2013).

### ***A limit relationship?***

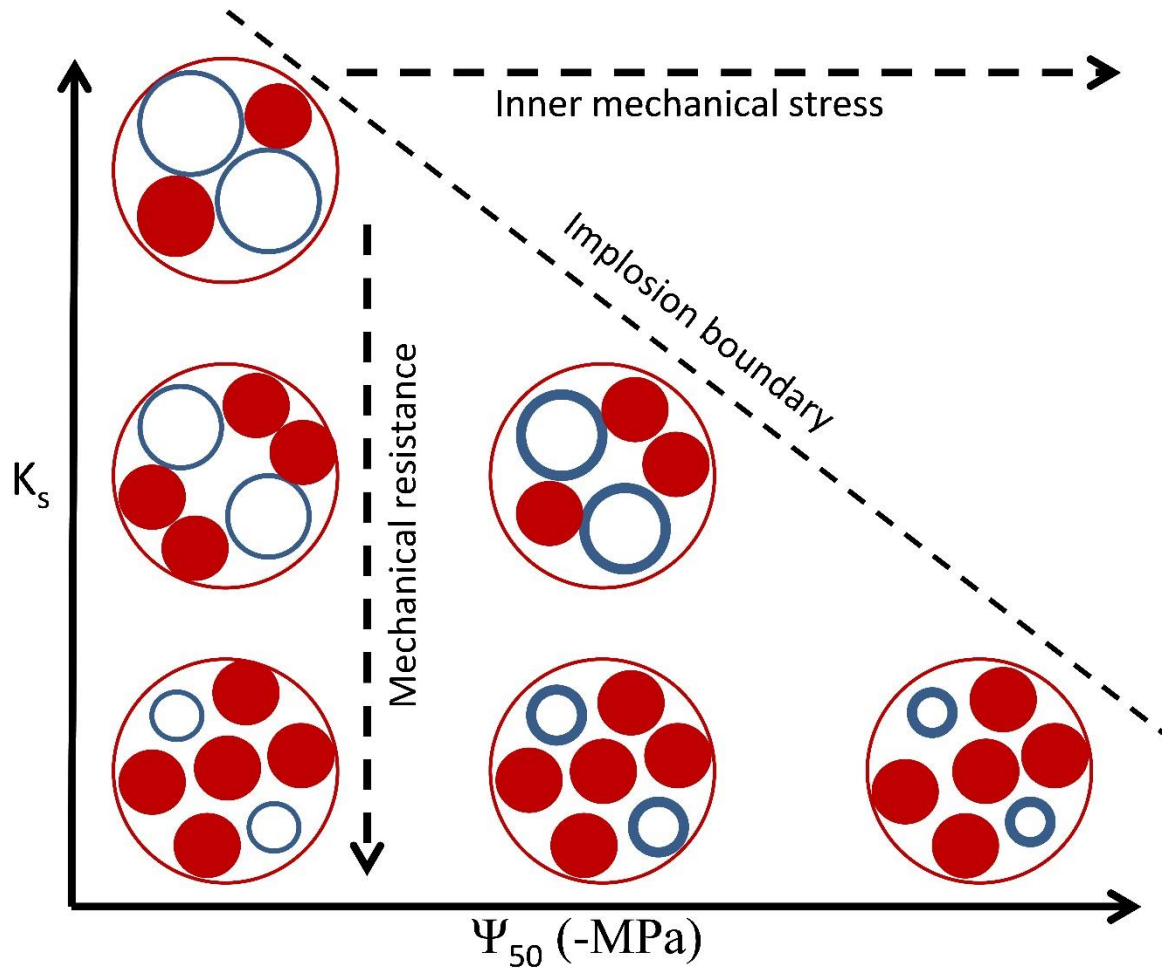
We propose that xylem space-use does coordinate with hydraulic safety in some species, but not in all, and this generates a limit, and not a direct relationship between hydraulic safety and hydraulic space-use efficiency. This line can be roughly imagined as a quantile linear fit on Fig. 2 of Gleason *et al.* (2016) with hydraulic safety setting the boundaries of  $K_s$  variability. Or inversely, with  $K_s$  setting boundaries in which hydraulic safety can vary. In this case a different mechanism than the one explored by Gleason *et al.* would be required, for example, assume that  $K_s$  directly correlates with leaf water supply capacity, higher  $K_s$  would allow plants to operate in higher water potentials and, consequently, allow it to survive with lower hydraulic safety. In this mechanism, the causal order is inversed and  $K_s$  determines hydraulic safety.

As Brodersen (2016) highlights, an important aspect of wood space use is space allocation to mechanical support of the plant body. The fibers (or tracheid walls in gymnosperms), which are related to mechanical support, compose about 70% of the wood (Ziemińska *et al.*, 2013) and the variability in mechanical resistance explains up to 77% of the variability in wood density (Chave *et al.*, 2009). Another aspect of plant mechanical resistance supporting this view can come from the perspective of plant inner mechanic stress, i.e. the stress generated inside its tissues due to water transport. Tension in the water transport system of plants generates implosive forces (Hacke *et al.* 2001). Besides implosive forces, other forces occur due to water tension, particularly if gas space with positive pressures occur inside the wood (Pereira *et al.* 2016). For example, a non-embolized conduit in contact with an embolized one will cause moment force in the embolized one. The implosive force acting on the tissue that connects both vessels will force the embolized one to rotate longitudinally and this rotation force would need to be counteracted by other plant tissues. The interplay between negative and positive pressures inside the stem leads to a complex mechanical system whose resulting forces should increase with the degree of embolism and with the decrease of water potential inside the wood. Formation of cracks larger than tracheids and vessels do occur during the drying process of wood material (Hanhijarvi 2003) and their occurrence decreases with wood density (Ilic 1999). Cracks are often observed in cut dehydrating branches, indicating propagation of stress from conduits to other tissues. Although we do not know of any work that studied inner mechanical stresses other than implosive ones in living

plants, plants that operate in lower water potentials should have a higher inner mechanical stress over all its tissues, and not only the vessels.

We believe inner mechanical stress may play an important role in the hydraulic safety-efficiency limit relationship. Plants that operate under more negative water potentials due to higher hydraulic safety have to deal with stronger implosive and moment forces in the xylem. For this, they would need reinforced conduits with high thickness to span ratio (Hacke et al. 2001) and/or thicker fibers (Jacobsen et al. 2005) to resist both forces (Fig. 3.2). Higher thickness to span ratio can be achieved either with thicker walls or reduced cell diameter, which would, respectively, reduce space available to conduit and conduit efficiency. The limit relationship between  $K_s$  and hydraulic safety would thus be mediated by the tradeoff between  $K_s$  and mechanical resistance and the synergy between inner mechanical stress and hydraulic safety (Fig. 3.2). This would allow a high variance in  $K_s$  in plants with low hydraulic safety, as possible xylem space setups are less inner-mechanically constrained, and a small variance in  $K_s$  of plants with high hydraulic safety due to high inner mechanical constrain of space use.





**Fig. 3.2** The limit relationship between hydraulic safety ( $\Psi_{50}$ ) and hydraulic specific efficiency ( $K_s$ ) and its possible explanation due to mechanical constraints of space use. Circles represent a transverse section of an angiosperm branch with fibers (red), vessel lumen and vessel wall (blue circles) and available space to other tissues (green). Available space is the space not constrained to a specific function related to either  $K_s$  or  $\Psi_{50}$  constraints. Lower water potentials increase wood inner mechanical stress, i.e. mechanical stress generated by water transport under tension causing implosion and moment forces on wood tissues. To resist inner mechanical stress, more space has to be allocated to fibers and walls while vessels have to be smaller to increase implosion resistance. Plants with higher  $\Psi_{50}$ , operate at higher water potentials and, thus, smaller inner mechanical stress. At higher  $\Psi_{50}$  plant space use is less constrained, less tissue needs to be allocated to mechanical support and more space is available. At lower  $K_s$ , assuming vessel length and pit traits stay the same, less space is allocated to conduits, more space is available to other tissues. A greater variety of xylem space use setups, with different  $K_s$ , is thus possible. Plants with

lower  $\Psi_{50}$  operate in lower water potentials and higher inner mechanical stress. This may constrain its space use to greater fiber and wall areas, with, consequently, less available space to vessels, smaller vessels with reduced efficiency and available space to tissues not related to maintaining  $K_s$  or  $\Psi_{50}$ . A limit relationship is thus established where plants with lower hydraulic safety have more possible xylem space allocation with different possible  $K_s$ . Although the example here considers angiosperms, the same relationship would be expected in gymnosperms, with increases in tracheid wall in response to lower water potentials having the same function as vessel wall and fibers increases in angiosperms.

Another way to look at the limit relationship between  $K_s$  and hydraulic safety is by considering parenchyma space. Water storage in parenchyma makes the water transport system operate in non-steady state, effectively buffering the plant from high xylem tensions and uncoupling diurnal xylem water potential from plant conductance (Sperry *et al.* 2008). Water storage in wood gives plants access to a water source that is closer to leaves and thus, accessed with a higher efficiency than the soil. Thus, low  $K_s$  with high parenchyma could also be associated with low hydraulic safety because water storage is increasing water transport efficiency under non-steady state conditions. Although water storage can buffer plants against water stress due to air water deficits, it cannot buffer against soil water deficits. How xylem space-use and the hydraulic efficiency *versus* safety relationship changes in situations where soil or air water deficits drives most of xylem water potential variation is certainly an important next step to investigation.

Why then Gleason *et al.* found a tradeoff between  $K_s$  and hydraulic safety for *Acer*? As Brodersen (2016) asks, “why *Acer*”? Is it just a false positive? One possible explanation lies in its xylem space setup. If this genus has a more conserved xylem space setup, space allocation variability not related to water transport would be reduced and the safety-efficiency tradeoff signal may be visible. Either way, we highlight the need to further study wood space allocation setups, how they are related to water transport and the traits of plants that are in the boundary of the  $K_s$ -hydraulic safety limit relationship.

## Acknowledgements

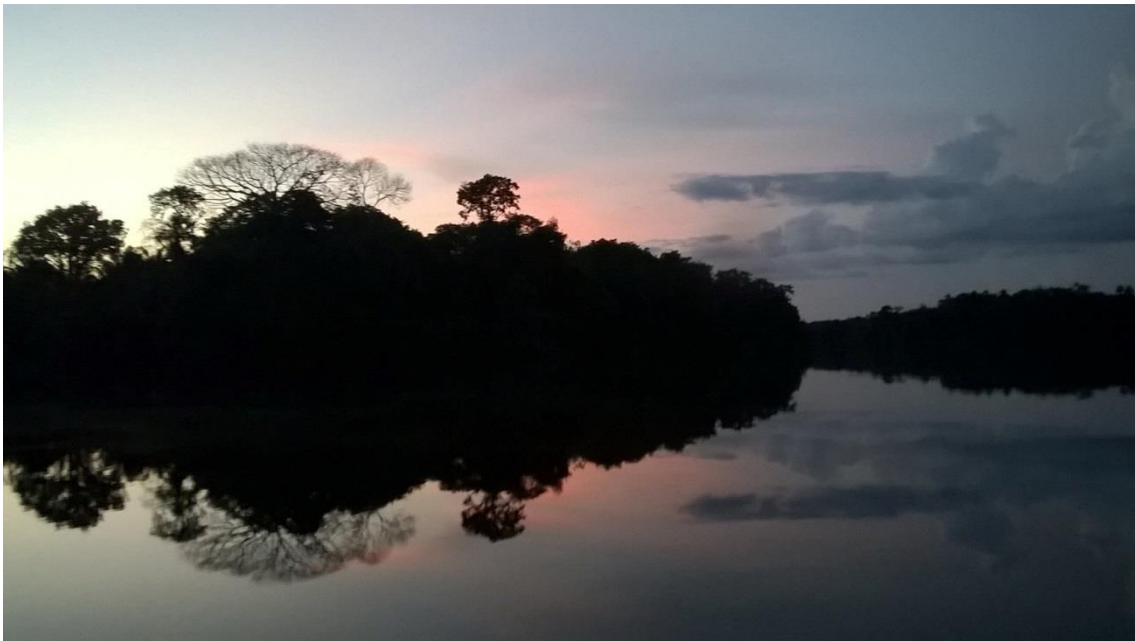
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### **Author contributions**

P.R.L.B., L.P. and R.S.O. designed and wrote the manuscript.

## Chapter 4

### **Branch nutrients may be more important than previously thought: costs, relationships and possible coordination with wood hydraulics in an Eastern Amazon Forest**



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## **Abstract**

Plant mineral nutrition is a major axis of plant function determining fitness and species distribution. As most of a tree's biomass is in wood and 80-90% of a tree's nutrients are in wood, it is expected that tree mineral nutrition is dominated by wood nutrient requirements. However, wood nutrients are much less studied than leaf nutrients. Branch wood, the wood most subject to water stress, richer in nutrients and, which comprises a considerable part of trees' living wood, is even less studied. Wood is a living tissue possibly requiring nutrients for its functions. Different wood structures, anatomies and tissue fractions may also lead to different nutrient requirements, possibly creating relationships between wood nutrient content and function. In this work, we study branch wood nitrogen (N) and phosphorus (P) in an Eastern Amazon forest and analyze how it relates to leaf nutrients, tree ontogeny and wood hydraulic traits. Wood nutrient content was determined by its concentration; leaf and branch-wood nutrients were unrelated. Wood N content changes along tree ontogeny. Wood N was related to midday water potential and wood P was related to xylem embolism resistance. We discuss

consequences of our findings to tree function, ecosystem nutrient stocks and possible mechanisms relating wood function to wood nutrients.

## Introduction

Plant mineral nutrition represents one of the major axis of plant functioning and nutrient availability is one of the principal determinants of species distribution (John *et al.*, 2007; Cleveland *et al.*, 2011; Lambers *et al.*, 2008; Condit *et al.*, 2013). However, plant mineral nutrition is mostly studied in leaves but when plants get taller there is a major shift in plant biomass allocation with most of a tree being comprised of wood and leaves accounting for only a small portion of its mass (Poorter *et al.*, 2015). Despite a tree being mostly wood, wood physiology is much less studied and understood than leaf physiology (Heineman *et al.*, 2016). Wood mineral nutrition studies are rare and consist mostly of studies quantifying wood nutrients stocks in different organs to estimate ecosystem-level nutrient balance, forest management practices and scaling relationships (e.g. Hagen-Thorn *et al.*, 2004, p.; Heineman *et al.*, 2016). However, as most of a tree's nutrients are located in its wood (e.g. 84-88% of aboveground N and P in Amazon primary forest is in wood; Johnsona *et al.*, 2001), it would be expected that tree mineral nutrition is dominated by wood nutrients requirements. Given the potentially large amounts of nutrients required to support woody tissue it has even been proposed that forest occurrence may be dependent on soils having sufficient nutrients to support wood biomass (Bond, 2010). Given that soil-nutrient availability correlates with wood productivity across the Amazon (Quesada *et al.*, 2010, 2012), it is possible that such relationships could be driven by nutrient demands of woody tissues. However, very little is known about woody tissue nutrient demands.

Wood nutrient concentration can vary more than 30 times (Heineman *et al.*, 2016) and although the exact uses for these nutrients within woody tissues are poorly studied, there are numerous potential processes they are likely to require nutrients. Firstly, wood has large and variable respiration rates associated to wood maintenance (Teskey *et al.*, 2007), probably generated by its living cells (20-40% of wood is living parenchyma cells; Morris *et al.*, 2015), requiring nutrients for enzyme and cell structure and function. Secondly, use of water stored in the xylem may also depend on activation of parenchyma aquaporins (Pfautsch *et al.*, 2015) and

recent evidence suggests conduit refilling is dependent on the activity of parenchyma surrounding these conduits (Secchi *et al.*, 2017). Thirdly, tree sap most likely contains ions, surfactants, sugars, proteins and phospholipids to promote water transport efficiency and, possibly, embolism resistance (Iwai *et al.*, 2003; Buhtz *et al.*, 2004; Nardini *et al.*, 2011; Schenk *et al.*, 2017). The production of these substances requires nitrogen (N) and phosphorus (P). As a consequence of being an active tissue, parenchyma requires nutrients for its functions. Fourthly, vessel walls and pit membranes are also not constituted entirely of carbon and contain phospholipids and proteins (Schenk *et al.*, 2017; Pereira *et al.* submitted), which require nutrients. Finally, even fiber walls which represent 45% of angiosperm xylem, require nitrogen and phosphorus for their synthesis (Whitehead & Quicke, 1960; Vanholme *et al.*, 2010; Zieminska *et al.*, 2013) and in some cases fibers may retain their protoplast and therefore remain metabolically active (Yamada *et al.*, 2011). In summary, wood is an active tissue and requires not only energy, but nutrients, particularly phosphorus and nitrogen, to both construct and allow for DNA, RNA, energy transfer, membranes and enzyme functioning.

Given the likely important role wood nutrients play in the function of plant woody tissues it is possible nutrients are coordinated with wood anatomical structure and potentially the wood hydraulic processes that these structures are likely to control. Wood water-supply efficiency is determined by vessel number and diameter with more efficient wood having less parenchyma tissue (Zanne *et al.*, 2010) and wood vessel diameter is related to wood axial parenchyma fraction (Morris *et al.*, 2017). Wood embolism resistance is determined by pit membrane traits and tradeoffs, albeit weakly, with wood water supply efficiency and, possibly, the amount of fibers in the xylem, suggesting different embolism resistance reflects different xylem tissue fractions (Jacobsen, 2005; Gleason *et al.*, 2015). If both wood hydraulic functions and wood structure are linked to parenchyma fraction and conduit composition (Schenk *et al.*, 2017) then it is possible that wood nutrient content may provide information on plant hydraulic traits. Indeed, fertilization experiments have shown that wood hydraulic traits changes in response to nutrient availability (Harvey & Van Den Driessche, 1997; Bucci *et al.*, 2006; Samuelson *et al.*, 2008; Goldstein *et al.*, 2013). However whether those changes are mechanistic changes due to more nutrients being available for woody tissue functioning or are a product of wood anatomical structure and plant function changing in response to added nutrients remains highly uncertain (Goldstein *et al.*, 2013). Other studies report that higher nutrient availability leads to higher vessel diameters (Coomes *et al.*, 2007); relationships between wood density and

wood P or N and growth rate (Heineman *et al.*, 2016; Martin *et al.*, 2014); wood N content and wood water content (Becker *et al.*, 2012); and transpiration rates and P acquisition and storage (Cernusak *et al.* 2011). Those studies suggest wood hydraulic function is not completely independent from wood nutrients. Further suggestive of possible relationships is that wood of small diameter branches (<1cm), the ones closer to leaves and the most tensioned during water stress (Cruziat *et al.*, 2002) have 4-5 times more nutrients than higher diameter branches and stems. (André *et al.*, 2010; Albaugh *et al.*, 2017). Part of this difference may be due changes in tissues fractions and vessel diameters with changes in stem diameter (Schuldt *et al.*, 2013) but it may also be related to physiological functions of wood as small branches need higher embolism resistance (Johnson *et al.*, 2016) or higher conduit refilling capacity than larger diameter branches to avoid or repair hydraulic failure . If refilling depends on parenchyma activity (Secchi *et al.*, 2017) or embolism resistance depends on conduit content and composition (Schenk *et al.*, 2017) then a mechanistical, and not only structural, relationship between wood nutrients and wood function is also possible. Nutrient storage is also required for producing new tissues after stress and higher wood nutrient content may be one strategy to recover from drought stress (Gessler *et al.*, 2017). Surprisingly, to our knowledge, no one has tested if wood nutrients and wood functions are related or whether wood nutrients may be a proxy for wood hydraulic traits.

Also, to our knowledge, no one has tested whether wood nutritional costs and plant fitness are related. The exception is Sardans & Peñuelas (2013) who shows that tree growth of three different forest types is higher for trees that maximize leaf to wood P content, i.e., that minimize their relative wood P cost. It is known that wood nutrients are removed from sapwood when it becomes heartwood, (Meerts, 2002) despite remobilization costs, indicating wood nutrients are valuable for trees. Wood nutrient concentrations may differ more than 30 times between species (Heineman *et al.*, 2016) but if wood could function independently of nutrient concentrations, it would be expected that all wood would converge to minimum values of nutrient concentration. Nutrient acquisition is one of the most energetically expensive processes in plants, particularly if soils are poor and plants require special adaptations (Tuomi *et al.* 2001; Lynch *et al.*, 2005). A tree whose wood requires fewer nutrients to function – i.e. a wood with higher wood nutrient use efficiency (WNUE) – would spend much less energy in nutrient acquisition and be able to grow faster and invest more in reproduction. The benefits of higher WNUE would increase for large trees with a higher wood fraction. WNUE has not been estimated for any of the major wood functions – storage, mechanical support and water



transport. Regarding water transport, wood with higher hydraulic space-use efficiency (Bittencourt *et al.*, 2016), that is, wood that transport water more efficiently per unit volume (i.e. wood specific conductivity -  $K_s$ ), would allow for a plant to invest less in wood tissue and could be by itself a measure of WNUE in the water transport axis (HNUE, for hydraulic nutrient-use efficiency, one of the possible WNUE). However, if wood with different  $K_s$  have different nutrient costs, then  $K_s$  would not be a good estimator of HNUE. A more direct way to estimate HNUE is to estimate how much nutrients it costs for each unit of hydraulic conductivity (Bittencourt *et al.*, 2016). While there is the complication of wood nutrients being related to other wood functions, this approach may be informative as water transport in conduits is not decoupled of its accompanying parenchyma and fibers. However caution should be used in interpretation of indexes of HNUE until a better understanding of wood metabolism and wood structural, non-structural and metabolic nutrients are acquired. As knowledge of wood physiology, apart from anatomy and structure, is an almost unexplored land, initial inquiries in HNUE may provide valuable insights.

In this work, we explore basic questions regarding wood N and P, two of the most limiting nutrients for tropical trees (Wright *et al.*, 2011), and its relations on a tropical rainforest in Eastern Amazon with nutrient poor soils (Quesada *et al.*, 2010). We focus on branch wood as they are richer in nutrients than stem wood (André *et al.*, 2010; Albaugh *et al.*, 2017), subject to higher water stress than stems and, comprise a smaller volume of tree wood and, as they are recently produced, may have a smaller function as nutrient storage. Branch wood is also less studied than stem wood, which is known to correlate with leaf nutrients (Kerkhoff *et al.*, 2006; Heineman *et al.*, 2016). First, we analyze the relationships between wood N and P content and concentration and wood density. If wood N and P content and concentration are not related wood nutrient concentration will be a poor indicator of overall wood nutrient allocation. We then test the following hypothesis: 1) leaf nutrients correlate with branch wood nutrient, if they are uncoupled it would suggest leaf and wood nutrients are being driven by different factors; 2) trees change their wood P and N with ontogeny. Taller trees receiving more light would be able to invest more in nutrient acquisition however they also have more wood, which would require higher nutrient investment in wood; 3) wood space-use nutrient-use efficiencies are related. If they are not, or poorly related, it would suggest optimizing space-use would not necessarily imply optimizing nutrient-use in hydraulic terms. Finally, we look for evidence of a nutritional axis of wood hydraulic function by 4) testing whether wood hydraulic traits ( $K_s$ , embolism

resistance) and water status (dry season midday water potential) are related to wood nutrients and whether there is a tradeoff between hydraulic safety and HNUE, as proposed by (Bittencourt *et al.*, 2016).

To address those questions we measured wood hydraulic traits ( $K_s$ , embolism resistance and hydraulic safety margin) and wood nutrients of 100 Amazon trees from 14 different genera. Our work is the first one to explore wood function and nutrients, which dominates a tree's energy and nutrient allocation. We explore intra-specific variability of wood nutrients, which is often neglected with wood nutrients usually studied at species level. We also measure, for the first time, the HNUE of water transport system of trees. Our results provide valuable insight towards a virtually unexplored area of tree functioning.

## Methods

### *Site and species*

We carried the study in an Eastern Amazon lowland tropical rainforest in Caxiua National Forest reserve (1°43' S. 51°27' W). The forest is at 15m above sealevel and the soil is yellow oxisol (Ruivo & Cunha, 2003). Site is mean annual precipitation is 2000-2500mm with a dry season between June and November. We sampled 14 of the most common genus in this forest (*Vouacapoua*, *Inga*, *Aspidosperma*, *Pouteria*, *Protium*, *Swarztia*, *Licania*, *Eschweilera*, *Minquartia*, *Syzygiopsis*, *Micropholis*, *Virola*, *Guatteria* and *Tetragastris*; Table S4.1), totaling 100 trees. We sampled *Guatteria* and *Tetragastris* from the throughfall experiment plot as they did not occur in the control plot (see Rowland *et al.*, 2015 for details on the experiment) and special care is taken when analyzing their data, as well as the genus *Vouacapoua*, *Swarztia* and *Inga*, which are leguminous and probably have root nodules. We sampled all other genus from the control plot. For all genus we sampled 4-12 trees, except for *Virola*, which we could only sample 2 trees. We selected trees to represent different sizes with diameter at breast height ranging from 10.3 – 88.8 cm (Table S4.1). A tree climber collected sun exposed branches 1 to 2m long from each tree in September 2016, during the dry season, and we used them for measurements

of hydraulic traits, wood and leaf N and P concentration. Branches were bagged immediately after collection and transported to laboratory (~20min distance) for measurements.

#### *Wood and leaf nitrogen and phosphorus measurement*

We collected wood and leaf samples from the same branches used for hydraulic traits measurements. We used for wood nutrient analysis the same samples that we used for  $K_s$  measurements after carefully removing bark and cambium. We dried wood and leaf samples at 60°C for 48h, powdered it using a 2010 Geno/Grinder tissue homogenizer (SPEX Sample Prep.) and sent them to Universidade Estadual Paulista is Plant Mineral Nutrition Laboratory (municipality of Botucatu, Brazil) for nutrient concentration analysis. Nitrogen concentration measurement was made after sulfuric digestion of plant material using the semi-microKjeldahl method and phosphorus concentration measurement was made after nitro-perchloric digestion by coloring samples with ammonium metavanadate and measuring with ultraviolet-visible spectrophotometer (model 600 Plus, Femto, São Paulo, Brazil) (Malavolta *et al.* 1997; Raji *et al.*, 2001). We calculated wood N and P content (g nutrient cm<sup>-3</sup> wood) by multiplying wood N and P concentration (mg nutrient g<sup>-1</sup> dry weight) by wood density (g wood cm<sup>-3</sup> wood).

#### *Hydraulic space-use and nutrient-use efficiency*

We used branches 1.5 to 2m long collected at midday from each tree for measurement of wood space-use efficiency ( $K_s$ ; specific conductivity). We cut 10-15cm from each branch base underwater and let then rehydrate for 15min to release tension and avoid artifacts (Venturas *et al.*, 2015). Subsequently, we cut underwater 1-1.5m of branch from base to leaves, in steps of ~15cm, and used the distal end of the branch for hydraulic measurements, to ensure no artificially embolized vessels were present on the measured sample. Maximum vessel length measured on a subsample of 17 branches was  $32.7 \pm 15.2$ cm (55.5cm maximum), confirming 2m long branches were enough to avoid artifacts. All samples were first or second order branches (counting from leaves), had between 30-55mm length and 3-5mm diameter and were recut with a sharp razor before connecting to apparatus to ensure all vessels were open. We measured maximum hydraulic conductance connecting samples to a hydraulic apparatus, flushing it for

two minutes to remove any emboli (Martin-StPaul *et al.*, 2014) and then applying 3 to 6 kPa water pressure to the sample and measuring water flow (Sperry *et al.*, 1988). We measured flow using the pressure drop over a capillary method (Pereira & Mazzafera, 2012). Basically, a capillary of known conductance is connected in series with the sample and flow through it, which equals flow through the sample, is calculated as pressure drop over the capillary times its conductance. All pressures in the system were measured using a pressure transducer (PX26-005GV, Omega). After measurement, we measured diameter and length of samples with a precision caliper and calculated  $K_s$  by multiplying conductance by sample length and dividing by sample transversal area. We used as solution for the apparatus distilled, deionized, filtered (0.21 $\mu$ m membrane), degassed water with no chemical addition, as suggested by Espino & Schenk (2011). For each branch, we measured conductance of two replicates in series one with the other (i.e. collected a ~8cm segment and cut in half, ensuring they had same transversal area and supplied the same leaves) and whenever their conductivity differed we discarded their data. We calculated N and P nutrient-use efficiency ( $HNUE_N$  and  $HNUE_P$ ) as  $K_s$  divided by sample N or P content.

#### *Xylem embolism resistance to drought*

We used 1 to 1.5m long branches collected from each tree before sunrise for embolism resistance measurement. We rehydrated branches for 8 hours and cut 2 or 3 smaller branches of approximately 40-70cm from each branch. We measured xylem embolism resistance of each branch using the pneumatic method, as described by Pereira *et al.* (2016). In the pneumatic method, the loss of hydraulic conductance is estimated from the increase in air volume inside the wood caused by embolism formation, which is measured repeatedly during branch dehydration. Air volume is estimated from the air discharge from the cut end of the branch to a vacuum reservoir (~50kPa absolute pressure) of known volume during a given amount of time (2.5 minutes). Initial and final pressure inside the vacuum reservoir is measured (PX140 pressure transducer, Omega) and the volume of air discharged is calculated using the ideal gas law. Percentage loss of conductance for each branch is estimated from percentage air discharged (PAD) from it in the course of its dehydration. PAD is calculated by standardizing air discharge for each branch by its minimum (fully hydrated) and maximum (most dehydrated). We dehydrated branches using the bench dehydration method. Before each air discharge

measurement, we bagged branches for one hour for leaf and wood xylem water potential ( $\Psi_x$ ) to equilibrate. After the air discharge we measured  $\Psi_x$  as the leaf water potential of one or two leaves with a pressure chamber (Model 1505, PMS). Drought embolism resistance is then given by the increase in PAD with decreasing  $\Psi_x$  for each tree. We used as index of embolism resistance the P50, the  $\Psi_x$  where 50% of hydraulic conductance is lost. To calculate P50, we pooled together the data for the 2-3 branch replicates from the same tree and fitted a logistic curve to the paired PAD where P50 is one of the fitted parameters (Pammenter & Vander Willigen, 1998).

#### *Midday water potential and wood density*

We measured leaf midday water potential ( $\Psi_{md}$ ) in between 1200 and 1400 hours in the dry season using a pressure chamber (Model 1505, PMS). We measured all leaves in the field in a maximum of three minutes after its collection. For each tree we measured two leaves and if they differed much in  $\Psi_{md}$  we measured a third leaf.

We measured wood density on the same branches used for pneumatic measurements. We debarked samples, immersed them in water for 24 hours to rehydrate, measured their saturated volume using the water displacement method, oven dried them at 60°C for 48 hours and measured their dry weight with a precision scale. Branch samples had slightly larger diameters (6.5mm) than the ones used for  $K_s$  and wood nutrients measurements (4mm).

#### *Data analysis*

We used linear mixed models to test relationship between individual trees variables using genus with diameter at breast height classes (in 10m steps) nested in as random factor affecting intercept. To choose whether to include random effect we used log likelihood test between the model with and without (using “gls” base function) the random effect. Not all measurements are available for all trees as we had either limitation on branch collection, to discard some hydraulic data or not enough plant material for nutrient analysis (see sampling details in Table S4.1). We also analyzed hydraulic traits ( $K_s$ , P50 and  $\Psi_{md}$ ) relationships to wood nutrients at genus level by calculating genus means of traits and using simple linear regressions.

Three outliers were removed in the analysis of difference and correlations between leaf and wood P concentration, two outliers were removed from the  $\Psi_{md}$  – wood [N] analysis and one outlier was removed from the P50 – wood [P] analysis. We processed and analyzed all data in R programming environment using base statistical packages (version 3.1.2; R Core Team 2014) and “lme” function from “nlme” package for linear mixed effect models (Pinheiro *et al.* 2014). We calculated marginal and conditional pseudo- $R^2$  for the mixed models using “r.squaredGLMM” function from “MuMIn” package (Barton 2016).

## Results

Wood nutrient content ( $\text{g cm}^{-3}$  wood) was strongly determined by wood nutrient concentrations ( $\text{g g}^{-1}$  dry weight) for both N ( $p < 0.0001$ ;  $r^2 = 0.84$ ; Fig. 4.1 and Table S4.2 for statistical summaries) and P ( $p < 0.0001$ ;  $r^2 = 0.95$ ). However, wood nutrient content variation was only weakly determined by wood density for N ( $p = 0.021$ ;  $r^2 = 0.08$ ) and not significantly determined by wood density for P ( $p = 0.96$ ). Wood density also could not explain variation in wood N and P concentration ( $p = 0.43$  and  $p = 0.15$ ). As wood nutrient content and concentration are strongly related, wood nutrient concentration (wood [N] or wood [P]) is used throughout the analysis. Wood [N] ranged from 2 to 12  $\text{mg g}^{-1}$  while wood [P] ranged from 0.1 to 1.4  $\text{mg g}^{-1}$ . Wood [N] and [P] had higher intra-genus than inter-genus variability (Table 4.1). Intra-genus SD of wood [N] for studied genus was  $1.36 \pm 0.75$  while inter-genus SD was 0.71. Similarly, wood [P] had higher intra-genus variability ( $0.23 \pm 0.15$  SD) than inter-genus variability (0.12 SD). Due to high intra-specific variation we do not detect significant differences in wood [N] and wood [P] between genera ( $p > 0.36$  and  $p > 0.21$ ).

**Table 4.1.** Mean and standard deviation of measured traits for each genus: wood and leaf nitrogen and phosphorus concentration ( $\text{mg g}^{-1}$ ), wood density (WD;  $\text{g cm}^{-3}$ ); hydraulic space-use efficiency ( $K_s$ ;  $\text{kg m m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ), hydraulic nitrogen-use and phosphorus-use efficiencies (HNUE<sub>N</sub> and HNUE<sub>P</sub>;  $\text{kg m m}^{-2} \text{s}^{-1} \text{MPa}^{-1} \text{mg}^{-1}$ ), midday leaf water potential ( $\Psi_{\text{md}}$ ; MPa) and xylem water potential at 50% loss of conductance (P50; MPa).

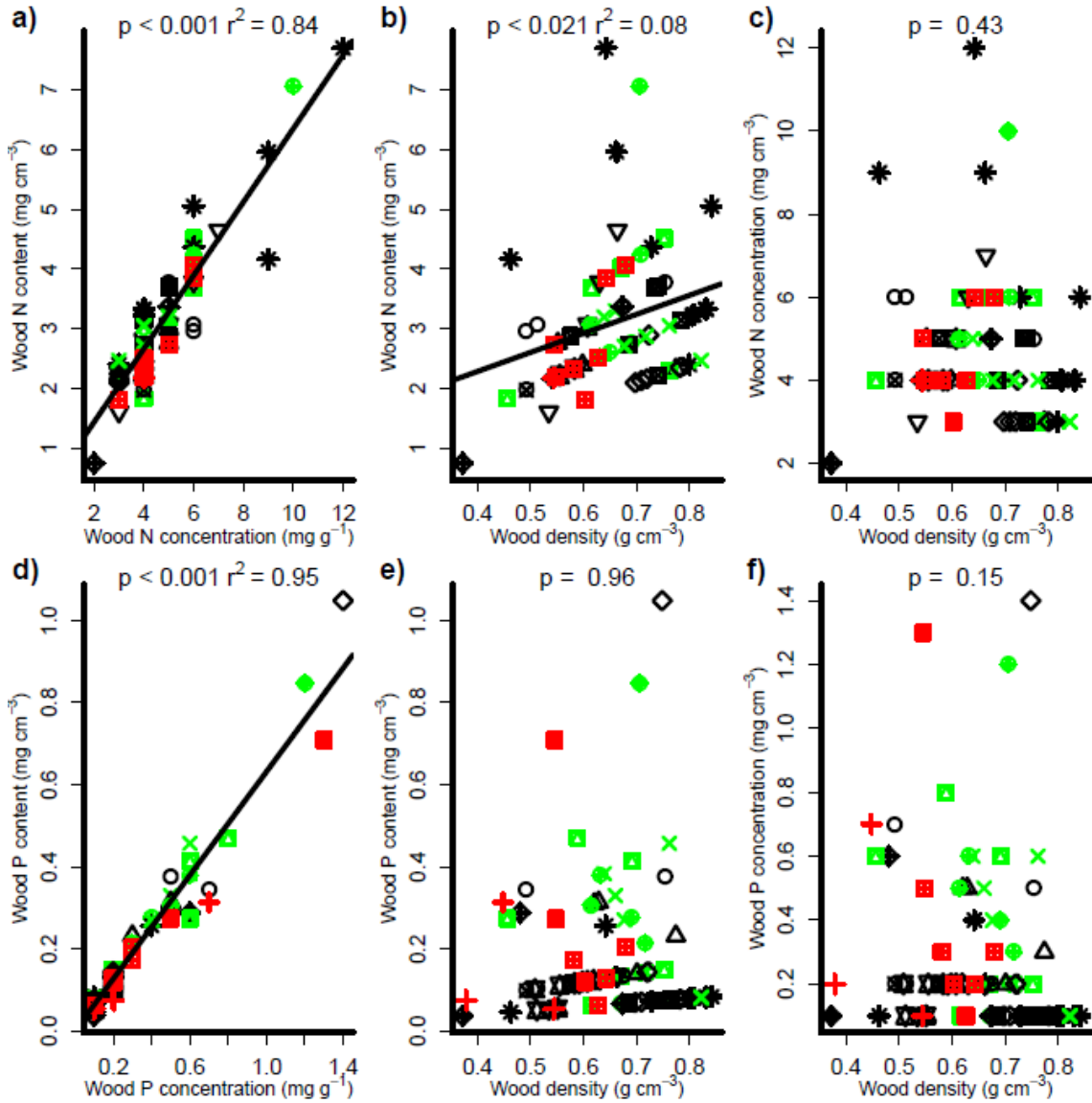
Genus	Wood		Leaf		Hydraulic traits					
	[N]	[P]	[N]	[P]	WD	$K_s$	HNUE <sub>N</sub>	HNUE <sub>P</sub>	$\Psi_{\text{md}}$	P50
Aspidosperma	5.20 ± 0.84	0.34 ± 0.25	16.67 ± 3.79	0.35 ± 0.17	0.61 ± 0.13	2.27 ± 2.08	0.66 ± 0.60	12.48 ± 15.75	-2.16 ± 0.74	-1.86 ± 0.38
Eschweilera	4.00 ± 1.00	0.29 ± 0.16	18.33 ± 0.82	0.40 ± 0.08	0.64 ± 0.07	3.46 ± 2.51	1.86 ± 1.62	22.79 ± 22.20	-1.67 ± 0.39	-2.31 ± 1.08
Guatteria	4.00	0.42 ± 0.32	17.33 ± 0.58	0.43 ± 0.05	0.46 ± 0.08	0.53 ± NA	0.24 ± NA	9.75	-1.44 ± 0.23	-2.56 ± 1.22
Inga	4.14 ± 0.69	0.36 ± 0.22	26.00 ± 2.92	0.49 ± 0.20	0.71 ± 0.07	6.09 ± 2.17	2.01 ± 0.75	24.47 ± 9.44	-1.88 ± 0.37	-2.56 ± 0.98
Licania	3.33 ± 0.52	0.33 ± 0.48	13.50 ± 2.12	0.34 ± 0.11	0.73 ± 0.03	0.72 ± 0.48	0.27 ± 0.11	6.50 ± 4.37	-1.26 ± 0.27	-2.04 ± 0.73
Micropholis	5.00 ± 1.83	0.18 ± 0.04	16.00 ± 2.00	0.33 ± 0.14	0.60 ± 0.04	3.22 ± 0.30	1.18 ± 0.44	31.77 ± 10.67	-2.38 ± 0.88	-1.23 ± 0.63
Minquartia	4.29 ± 0.76	0.12 ± 0.05	16.33 ± 1.87	0.47 ± 0.11	0.71 ± 0.07	2.65 ± 1.00	0.91 ± 0.42	34.35 ± 10.43	-1.54 ± 0.21	-1.99 ± 0.89
Pouteria	5.91 ± 2.88	0.17 ± 0.14	15.62 ± 5.55	0.24 ± 0.09	0.75 ± 0.12	2.41 ± 0.92	0.68 ± 0.33	29.16 ± 13.08	-2.20 ± 0.48	-2.15 ± 1.19
Protium	4.40 ± 1.52	0.28 ± 0.22	16.60 ± 2.19	0.42 ± 0.15	0.52 ± 0.13	3.02 ± 1.42	0.94 ± 0.66	39.24 ± 44.22	-1.54 ± 0.44	-2.29 ± 0.72
Swartzia	5.50 ± 2.35	0.44 ± 0.35	23.50 ± 3.42	0.54 ± 0.13	0.67 ± 0.04	4.46 ± 3.03	0.83 ± 0.31	36.86 ± 59.77	-2.08 ± 0.52	-3.08 ± 1.55
Syzygiopsis	4.33 ± 0.58	0.17 ± 0.05	13.80 ± 1.10	0.36 ± 0.09	0.55 ± 0.04	2.34 ± 0.83	1.00 ± 0.46	29.27 ± 18.80	-1.79 ± 0.19	-1.41 ± 0.75
Tetragastris	4.62 ± 1.06	0.52 ± 0.49	12.57 ± 1.13	0.47 ± 0.11	0.60 ± 0.04	1.79 ± 1.93	0.44 ± 0.19	8.38 ± 8.80	-1.87 ± 0.43	-3.22 ± 1.00
Virola	5.50 ± 2.12	0.30 ± 0.14	16.50 ± 0.71	0.35 ± 0.21	0.49	3.37	NA	NA	-2.35 ± 0.21	-1.93 ± 1.38

Vouacapoua  $4.67 \pm 1.51$   $0.34 \pm 0.28$   $20.38 \pm 1.92$   $0.43 \pm 0.16$   $0.65 \pm 0.09$   $1.97 \pm 1.71$   $0.56 \pm 0.50$   $17.08 \pm 27.06$   $-2.10 \pm 0.73$   $-3.00 \pm 0.59$

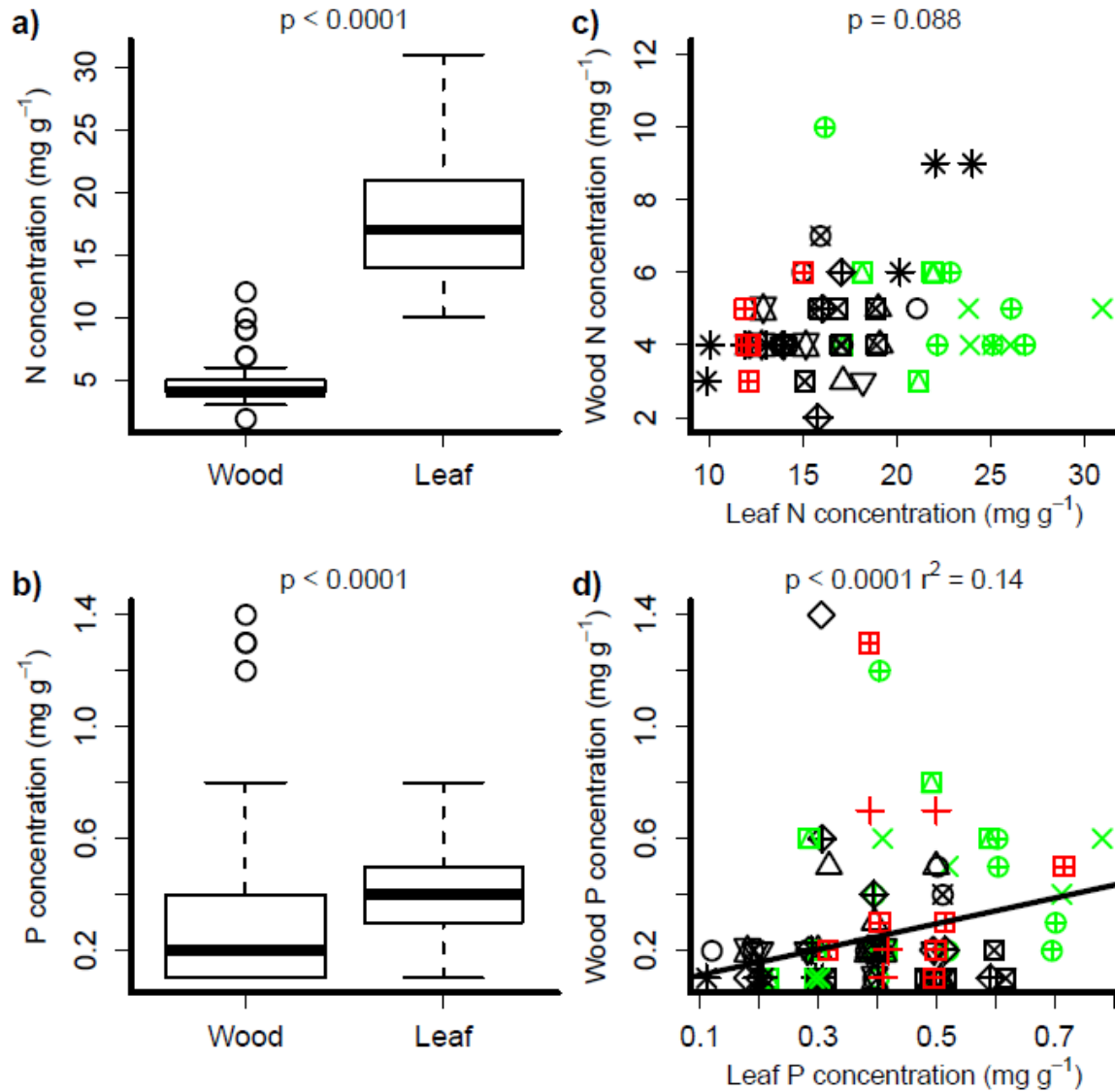
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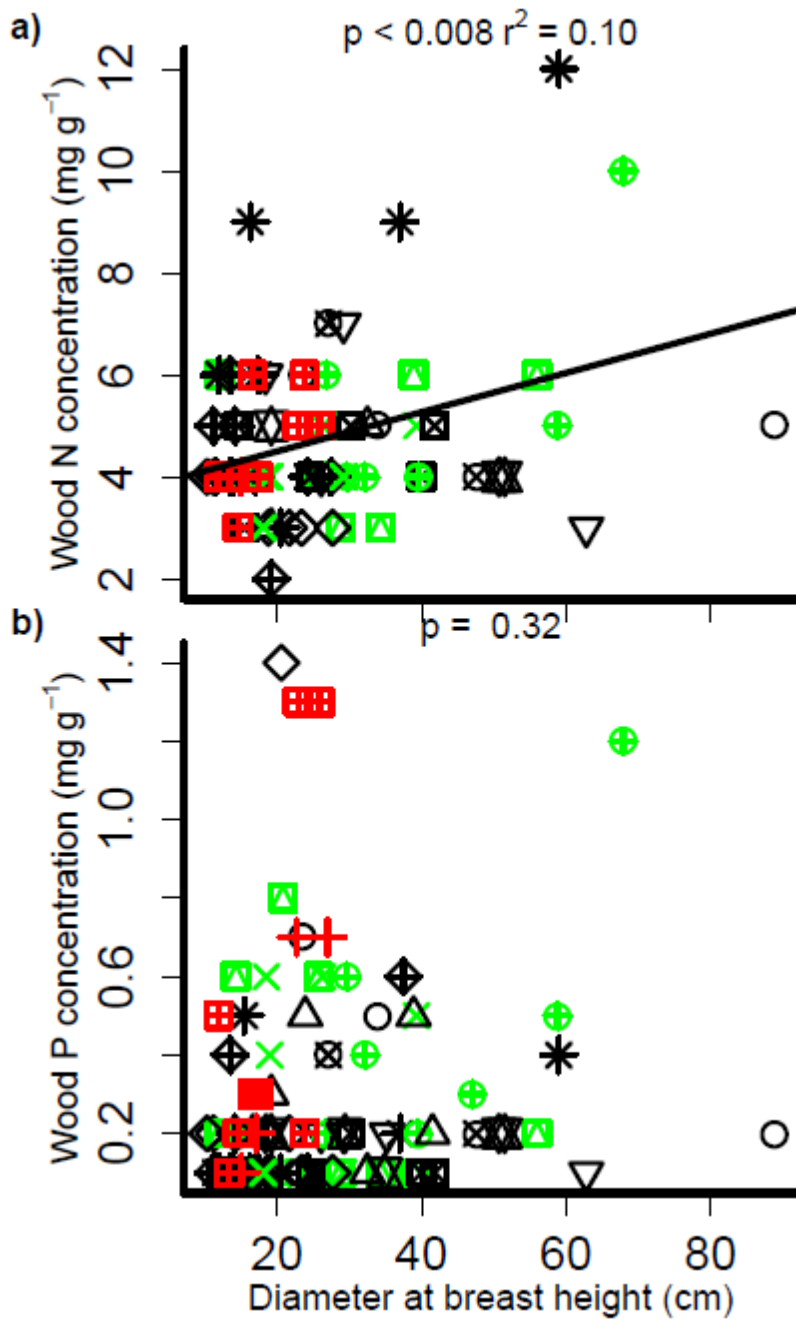
Wood nutrient concentrations were lower than leaf nutrient concentrations (Fig. 4.2). Wood [N] represented 27% of leaf [N] ( $4.73 \pm 1.68 \text{ mg g}^{-1}$  and  $17.74 \pm 4.54 \text{ mg g}^{-1}$ ) while wood [P] represented 73% of leaf [P] ( $0.30 \pm 0.28 \text{ mg g}^{-1}$  and  $0.41 \pm 0.14 \text{ mg g}^{-1}$ ). Leaf [N] was more variable than wood [N] (4.5 and 1.6 of SD, respectively;  $p < 0.0001$ ) while wood [P] was more variable than leaf [P] (0.28 and 0.14 of SD, respectively;  $p < 0.0001$ ). Leaf [N] did not explain wood [N] variability ( $p = 0.088$ ; Fig. 4.2) and leaf [P] only weakly explained wood [P] variability ( $p < 0.0001$ ;  $r^2 = 0.14$ ). Wood [N] and wood [P] were only weakly correlated ( $p = 0.013$ ;  $r^2 = 0.08$ ). Wood [N] content was weakly but significantly affected by tree diameter at breast height (DBH) ( $p = 0.008$ ;  $r^2 = 0.10$ ; Fig. 4.3) while wood [P] was not affected by DBH ( $p = 0.14$ ). Furthermore in our mixed effect model analysis we find genus is not a significant random effect.



**Figure 4.1.** Relationship between branch wood N and P content, concentration and wood density for a-c) wood nitrogen and d-f) wood phosphorus. Red points are trees from the throughfall exclusion experiment, green points are Fabaceae. Black line is the linear fit when the relationship is significant.

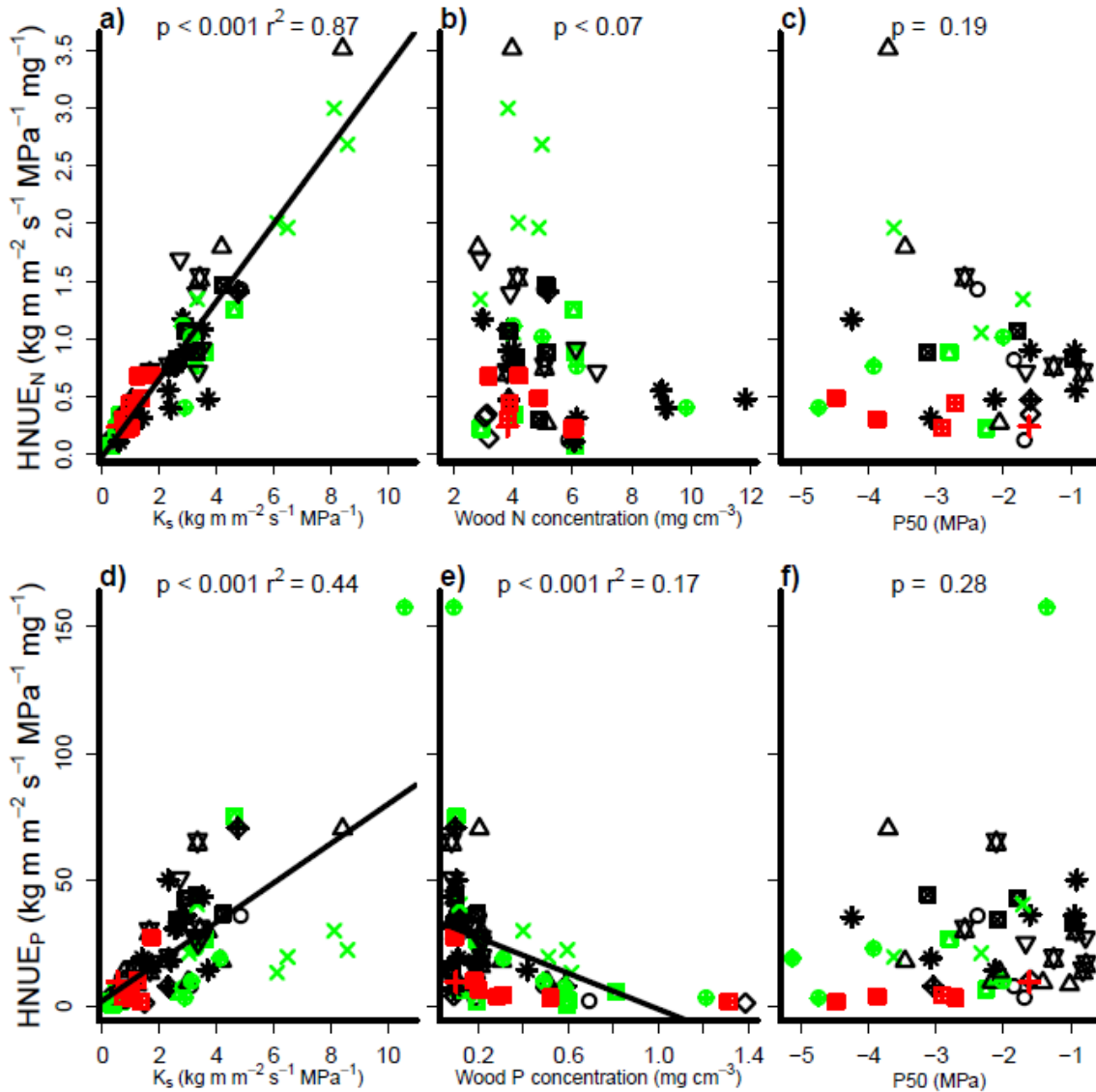


**Figure 4.2.** Branch wood and leaf nitrogen and phosphorus concentrations distributions (a-b). Relationship between branch wood and leaf nitrogen and phosphorus concentrations (c-d). Red points are trees from the throughfall exclusion experiment, green points are Fabaceae. Black line is the linear fit when the relationship is significant. A small jitter was applied to x-axis in c) and d) for better data visualization.



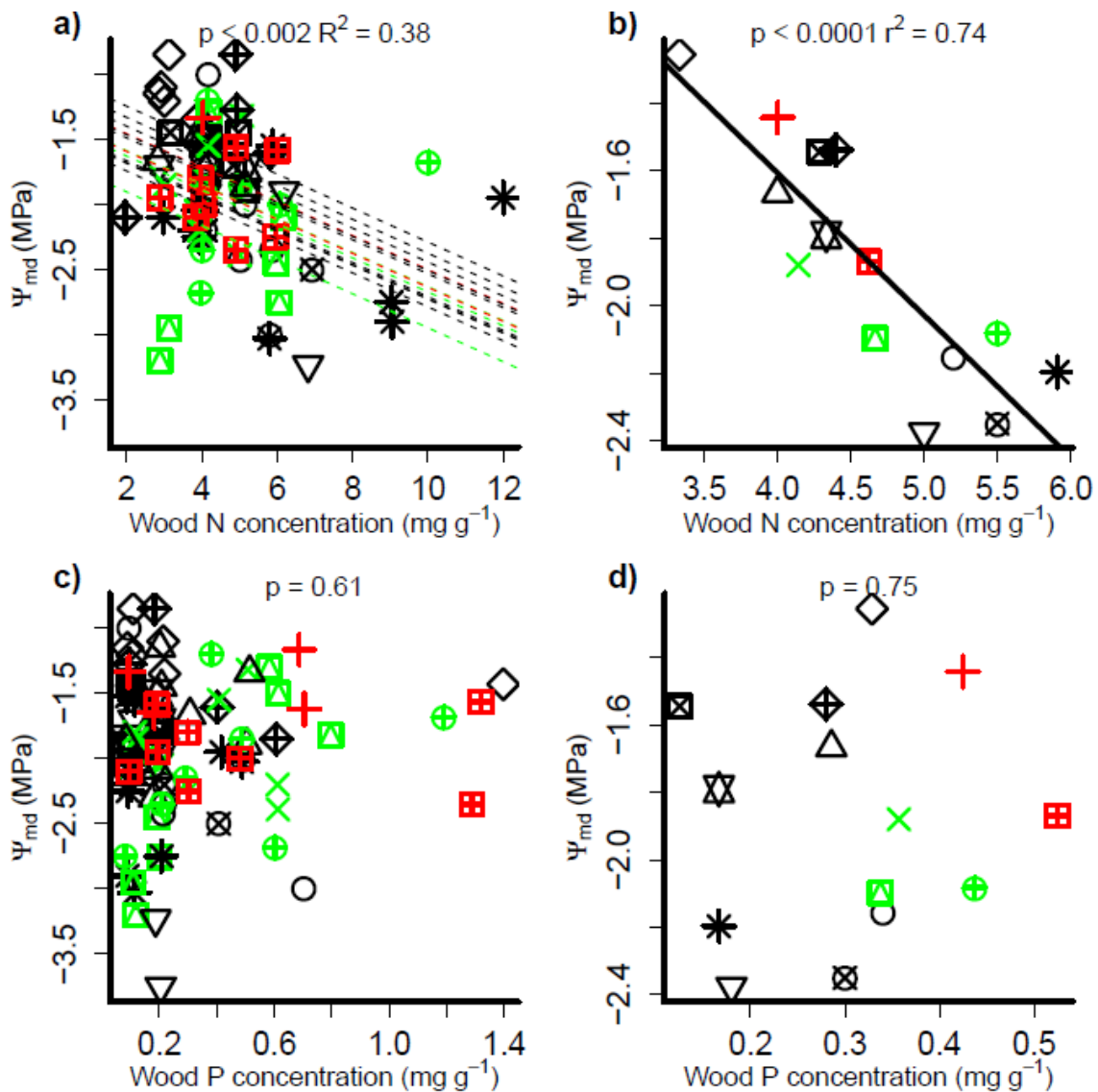
**Figure 4.3.** Relationship between branch wood nitrogen (a) and phosphorus (b) concentration and tree diameter at breast height. Red points are trees from the throughfall exclusion experiment, green points are Fabaceae. Black line is the linear fit when the relationship is significant.

Hydraulic nitrogen use-efficiency ( $\text{HNUE}_N$ ) was strongly determined by hydraulic space-use efficiency ( $K_s$ ) ( $p < 0.0001$ ;  $r^2 = 0.87$ ; Fig. 4.4) and was only marginally determined by wood [N] ( $p = 0.066$ ;  $r^2 = 0.06$ ).  $\text{HNUE}_P$  was also significantly correlated with  $K_s$  ( $p < 0.0001$ ;  $r^2 = 0.44$ ) and was more determined by wood [P] ( $p = 0.0003$ ;  $r^2 = 0.17$ ). While it is expected that  $K_s$  is related to HNUE as  $K_s$  equals HNUE divided by wood nutrient content, this division has the potential to weaken or remove the relationship between  $K_s$  and HNUE. However, the above results shows that this almost did not happen for  $\text{HNUE}_N$  ( $r^2 = 0.87$ ) and happened partially for  $\text{HNUE}_P$  ( $r^2 = 0.44$ ), implying  $K_s$  is the main driver of  $\text{HNUE}_N$  but for  $\text{HNUE}_P$ . There was no tradeoff between  $K_s$ ,  $\text{HNUE}_N$  or  $\text{HNUE}_P$  and embolism resistance (P50) ( $p = 0.38$ ,  $p = 0.19$  and  $p = 0.28$ , respectively; Fig. 4.4).

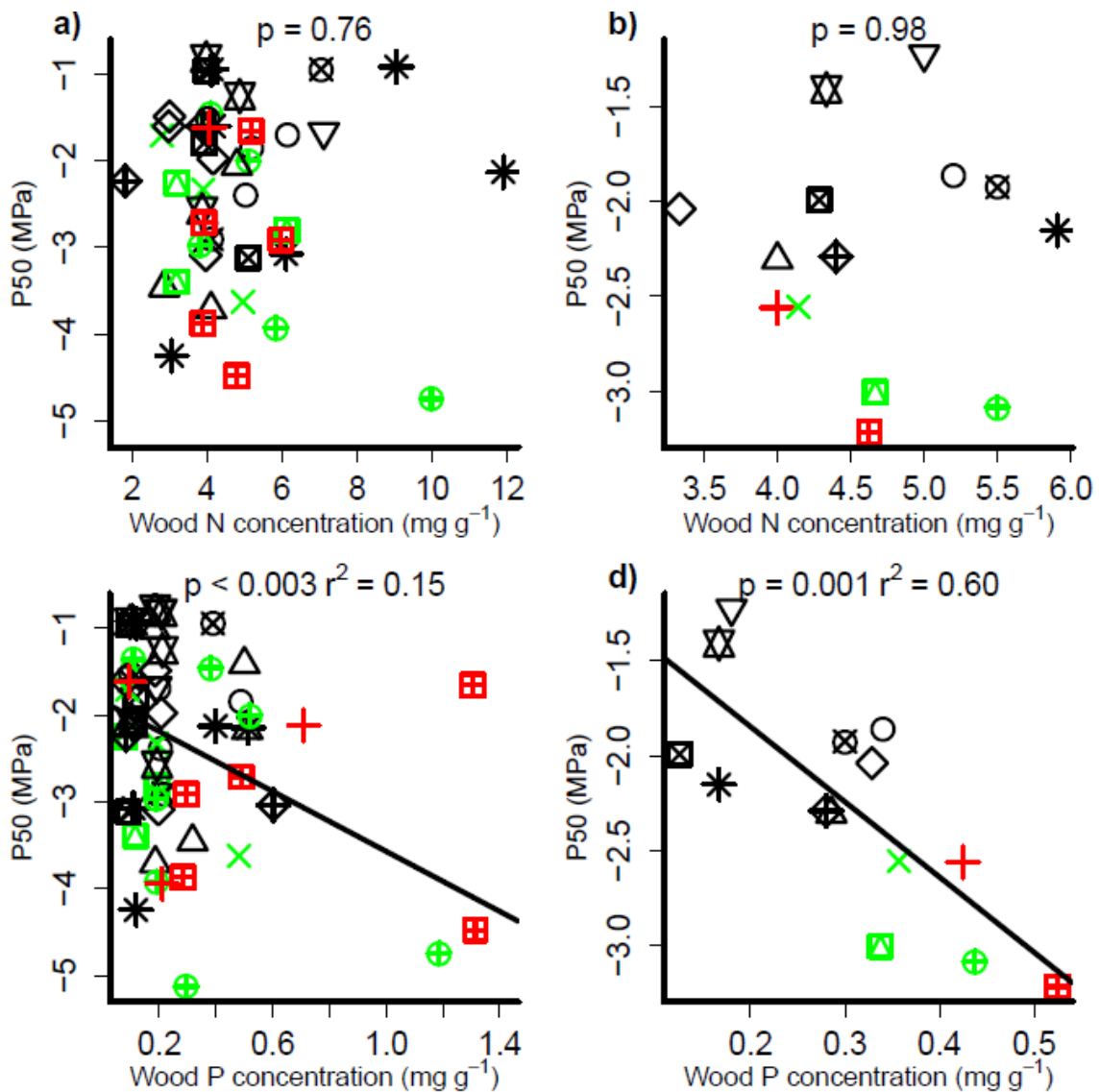


**Figure 4.4.** Relationship between branch wood hydraulic nitrogen-use efficiency ( $\text{HNUE}_N$ ) and space-use efficiency ( $K_s$ ; a), wood nitrogen concentration (b) and P50 (c); and relationship between branch wood hydraulic phosphorus-use efficiency ( $\text{HNUE}_P$ ) and space-use efficiency ( $K_s$ ; d), wood phosphorus concentration (e) and P50 (f). Red points are trees from the throughfall exclusion experiment, green points are Fabaceae. Black line is the linear fit when the relationship is significant. A small jitter was applied to x-axis in b) and e) for better data visualization.

$K_s$  variation was not explained by wood [N] or wood [P] ( $p = 0.77$  and  $p = 0.84$ ; no significant random factor; Fig. S4.1). Wood [P] was not related to  $\Psi_{md}$  ( $p = 0.57$ ; Fig. 4.5) however wood [N] was related to  $\Psi_{md}$  ( $R^2 = 0.38$ ;  $p = 0.0023$ ), with genus with DBH nested as a marginally significant random factor –  $p = 0.06$ ). Wood [N] and  $\Psi_{md}$  relationship became much stronger when data was analyzed at genus level ( $p < 0.0001$ ;  $r^2 = 0.74$ , Fig. 4.5), but did not change when wood [P] and  $\Psi_{md}$  were considered at genus level ( $p = 0.75$ ). Xylem embolism resistance (P50) was not related to wood [N] content ( $p = 0.78$ ; Fig. 4.6), however it was related to wood [P] ( $p = 0.003$ ;  $r^2 = 0.15$ ), but without a significant effect of genus. This relationship became substantially stronger when analyzed at genus level ( $p = 0.0011$ ;  $r^2 = 0.60$ ) while P50 and wood [N] remained unrelated ( $p = 0.98$ ).



**Figure 4.5.** Relationship between midday leaf water potential ( $\Psi_{md}$ ) and branch wood nitrogen concentration (a and b) or phosphorus concentration (c and d). Left panels are data at individual level (a and c) and right panels are genus means (b and d). Red points are trees from the throughfall exclusion experiment, green points are Fabaceae. Black line is the linear fit when the relationship is significant. Dashed lines in a) are the linear fit for each genus in the linear mixed model. A small jitter was applied to x-axis in a) and b) for better data visualization.



**Figure 4.16.** Relationship between branch xylem embolism resistance (P50) and branch wood nitrogen concentration (a and b) or phosphorus concentration (c and d). Left panels are data at



individual level (a and c) and right panels are genus means (b and d). Red points are trees from the throughfall exclusion experiment, green points are Fabaceae. Black line is the linear fit when the relationship is significant. A small jitter was applied to x-axis in a) and b) for better data visualization.

## Discussion

Our results show unexpected relationships between branch wood N and P concentration, content and wood density. Wood nutrient content was almost entirely determined by nutrient concentration, which means the quality of the dry mass used in wood, and not the amount of dry mass per volume, is determining wood nutrient content. Wood density changes then occurs without increasing or decreasing wood nutrient requirements. Wood nutrient concentration is also not related to wood density, a major trait determining plant life strategy (Chave *et al.*, 2009). Branch wood was 80% and 172% richer in [N] and [P], respectively, than stem wood in Central America tropical rainforests (Heineman *et al.*, 2016). Leaf and branch wood N and P content are not related, indicating there is differential control on branch or leaf nutrient content making a leaf rich in nutrients to not have a branch wood rich in nutrient. We found only a weak evidence, and only for N, of trees adjusting their wood nutrient concentration with ontogeny despite major changes in plant wood biomass, light availability and root traits with ontogeny. Hydraulic space-use efficiency ( $K_s$ ) determines almost entirely hydraulic nitrogen-use efficiency ( $HNUE_N$ ) but  $K_s$  determines much weaker  $HNUE_P$ . We find no evidence of hydraulic safety trading off with hydraulic efficiency either in terms of  $HNUE$  and  $K_s$ . However our results shows midday water potential ( $\Psi_{md}$ ) is related to wood [N] and embolism resistance (P50) is related to wood [P], indicating wood function is somehow related or reflected in wood nutrients.

### *Wood nutrients*

We found branch wood to be 2-3 times more enriched in nutrients than stem nutrients in the literature (Johnson *et al.*, 2001; Heineman *et al.*, 2016), similarly to what was found in other studies (André *et al.*, 2010; Albaugh *et al.*, 2017). Branch wood comprises a significant portion of a tree is biomass (~20% for a tree with 40cm DBH; Ketterings *et al.*, 2001). However,

most of a tree's wood is heartwood with low nutrient amounts. In the studied site, 34% of trees basal area is sap wood (Rowland *et al.* 2017), which makes branch sap wood biomass a much higher proportion of trees living biomass. Not considering higher nutrient content of small branches may lead to large underestimation of forest nutrient storages. Johnsona *et al.* (2001) estimates Amazon primary forest wood N and P, based on stem cores, as having ~85% of aboveground nutrients is probably an underestimate. Our results also show leaf cannot be used as a predictor of branch wood nutrients, which, together with high intra-specific variability of branch wood nutrients may further complicate assessment of total tree nutrient stocks, indicating need for focus on tree level and different organs instead of species level analysis. Furthermore, it highlights the need of studies to understand drivers of wood nutrient variability. Branch wood [N] was 27% of leaf [N] and wood [P] was 73% of leaf [P], which is a relatively high nutrient requirement in relation to leaves, which are highly active metabolically. Leaf [N] and [P] was not related to branch wood [N] and [P], indicating richer leaves does not couple with richer branches, as seems to occur in stems (Kerkhoff *et al.*, 2006; Heineman *et al.*, 2016). This suggests something is driving the high nutrient requirement, variability and decoupling of branch wood nutrients from leaf nutrients. As branch nutrient costs may be high and are variable between different trees (i.e. trees with different canopy architecture and, particularly, different stem specific length), understanding drivers of wood nutrient requirements may also be important to understand whole tree functioning.

Branch wood content, i.e. the nutrients in a volume of wood, were determined by the concentration of nutrients in dry mass and only wood N was slightly determined ( $r^2 = 0.08$ ) by the amount of dry mass per volume (wood density). This implies that increasing wood density of branches does lead to higher nutrient requirements and further implies that the mechanisms driving wood-density increase are basically increasing the amount of carbon-based structures (cellulose and lignin possible) and not of enzymes, membranes, and other N and P containing molecules. Finally, it also suggests that nutrient containing molecules are more diluted in the volume of branch wood with smaller wood densities. This diluting effect of wood density on wood chemistry may have important implications for wood decomposition and may be related to the complex relationships between wood structural and chemical traits with wood decomposition rates (Zanne *et al.*, 2015). Wood density affects plant biomechanics, hydraulic traits, water storage and, possible, whole plant wood volume (i.e. the same amount of structural material may be used for more, less dense wood or less, more dense wood, with different

consequences; Chave *et al.*, 2009; Poorter *et al.*, 2010; Schuldt *et al.*, 2013; Ziemska *et al.*, 2013; Larjavaara & Muller-Landau, 2010). Our data suggests different wood density does not have differential nutrient requirements and, in the nutrient axis, wood density may be relatively free to vary.

#### *Ontogeny and plant water status effect*

When a tree gets taller, it has more energy available, a more developed root system and a higher amount of wood to sustain. This may allow taller trees to have access and invest more energy in acquiring nutrients at the same time it increases wood nutrient requirements. We found only a weak increase in branch wood [N] content with ontogeny, and no change in branch wood [P]. Possible, higher nutrient availability with ontogeny is being compensated by higher nutrient requirements or, all additional nutrient inputs are directly being used for growth and reproduction, instead of producing more nutrient rich wood. The fact that we found a relationship between branch wood [N] and tree size, but not wood [P] is interesting and suggests the requirement for those two nutrients have different drivers. Our data shows that plants with more negative water status in the driest hour of the driest season ( $\Psi_{md}$ ) are also richer in wood [N]. Larger plants are also more coupled to atmosphere and receive more light, which may cause them to have more negative  $\Psi_{md}$ . Thus, it is possible that changes in wood [N] with ontogeny are mediated by changes in  $\Psi_{md}$ . However, the fact that wood [N] is changing with either  $\Psi_{md}$  or ontogeny is interesting in itself. Whether this is a consequence of metabolic requirements of wood operating in lower water potentials or a change in wood structure leading to changes in wood [N] is unknown, but suggests we need to look deeper in where in the wood N is being allocated and why P, which did not relate to  $\Psi_{md}$ , is being differentially allocated than N.

It is also puzzling that the relationship between wood [N] and  $\Psi_{md}$  is much stronger at genus level than at individual level. We do not know why this occurs, but two possibilities we can think of are that wood [N] and/or  $\Psi_{md}$  have low measurement precision or part of this intra-specific variability is dependent on some process operating at individual or species (and not genus) scale which does affect both variables at the equally. In both cases averaging would lead to a reduced variability and closer to true mean values, which can be making wood [N] and  $\Psi_{md}$ ,

and also wood [P] and P50, relationships stronger at genus level. The problem with this is that it suggests there may be false negatives in our results. A power analysis (R base function “power.anova.test”) with the intra-genus and inter-genus variability found here indicates that to detect difference in genus means, with a significance level of 0.05 and a statistical power of 0.95, requires a minimum sampling of 11.8 trees per genus, which is higher than we measured. While this does not invalidate our findings, (particularly wood [N] with  $\Psi_{md}$  and wood [P] with P50, whose probability to occur by chance were lower than 1 in 1000), studies replicating the analysis done here are necessary to sort out these questions.

#### *Hydraulic space-use and nutrient-use efficiency and safety relationship*

We present, for the first time, the amount of N and P required per volume for each unit conductivity. While this N and P may not be directly associated to water transport and are not the perfect measure of HNUE, they are required in the wood transporting water and its analysis may bring new knowledge of plant function. Our data shows that the water transported capacity per unit N and P can vary approximately 10 times for P and 3 times for N. We found that  $K_s$  and  $HNUE_N$  are almost perfectly coupled while  $K_s$  and  $HNUE_P$  are not so strongly coupled. Increasing hydraulic space-use efficiency produces a correspondent decrease in the amount of N and P associated with wood water transport however not necessarily for P as it has a higher variability. This implies that increasing space-use efficiency decreases P requirement, but not necessarily, or, rather, more unpredictably. We also did not find support for a HNUE and hydraulic safety relationship (Bittencourt *et al.*, 2016), however as HNUE varies by one order of magnitude and, for P is somewhat uncoupled from  $K_s$ , it is possible multiple hydraulic efficiencies may be simultaneously affecting the efficiency and safety relationship, making no single efficiency correlate with safety..

#### *Embolism resistance and wood phosphorus*

Our data shows that wood richer in P is also more embolism resistant. This is an interesting and hard to explain finding and we can only speculate on whether drivers of this relationship are wood structure, metabolism or whole plant strategy. In terms of structure,

embolism resistance may be increasing with wood [P] due to changes in the xylem that do not produce proportional changes in wood [N], else wood [N] would also be related to P50. Pit membrane traits are known to affect embolism resistance (Cruziat *et al.*, 2002), but whether and how changes in pit membrane traits could lead to changes in wood [P] is unknown. In terms of metabolically, P50 may be determined by phospholipids in conduits, as proposed by (Schenk *et al.*, 2017). Although the vessel content of the samples were replaced with distilled water during hydraulic measurements and prior to wood nutrient analysis, phospholipids in pit membranes, conduit surfaces and yet to be secreted by parenchyma cells should not have been removed and may be causing this relation but analysis of P location in wood are required. In terms of whole plant strategies, Cernusak *et al.* (2011) found tropical trees which transpire more acquire more P. If total transpiration is related embolism resistance, it is possible that more embolism resistant plants are more enriched in P. Eller *et al.* (2018) recently found a trade-off between wood growth and safety and proposes less safe wood has lower longevity, but also lower costs. Similarly, it is possible P richer wood increases its longevity by being safer (lower P50). All above mechanisms are speculative and require testing. However, that P50 coordinates with wood [P] introduces a new axis of possible tradeoffs affecting safety. If P50 trades off with other variables besides hydraulic efficiency, it is entirely possible lack of safety-efficiency relationships is due to tradeoffs affecting P50. Independently, this is an intriguing result and further highlights the need of better understanding wood functioning besides anatomy and structure.

### *Conclusion*

Our study analyses tree-branch wood N and P concentrations and their relation to branch-wood nutrient content, leaf nutrients, ontogeny and wood density. It highlights possible biases in wood nutrient stocks estimates due to not considering intra-specific, intra-individual wood nutrient contents and the weak relation between branch wood and leaf nutrient contents. Correct understanding of forest nutrient requirements and stocks is fundamental as it may be the bottle neck for increased forest productivity and CO<sub>2</sub> storage in response to increased atmospheric CO<sub>2</sub> (Wieder *et al.*, 2015; Jin *et al.*, 2015). We also quantify, for the first time, an index of hydraulic nutrient-use efficiency and find evidences that wood nutrients concentrations are connected to wood hydraulic functioning with wood N related to xylem water tension and

wood P related to embolism resistance. Mechanisms driving these relationships are unknown but could be correlations produced by differences in wood anatomy, tissue fraction or metabolism, which are somehow making wood hydraulic function dependent on wood nutrients. Our data shows wood N and P are not coupled and are probably being differentially allocated in branch wood, possible as consequences of different wood functions. Lowland Amazon forests are nutrient poor ecosystems (Quesada *et al.*, 2010) and nutrient acquisition and allocation are energy consuming and strategical points for whole tree function. It is known that soil nutrient availability, particularly phosphorus, affects tropical forest species distribution (Condit *et al.*, 2013) and Amazon productivity (Quesada *et al.*, 2012). As most of a tree is wood and most of its nutrients are in wood, the above patterns may be mediated by wood nutrient requirements and, maybe, its relations to wood function. It is surprisingly that wood chemistry and function, except for anatomy and structure, are understudied. Our work highlights importance of studies on wood chemistry and function and points to interesting new research pathways.

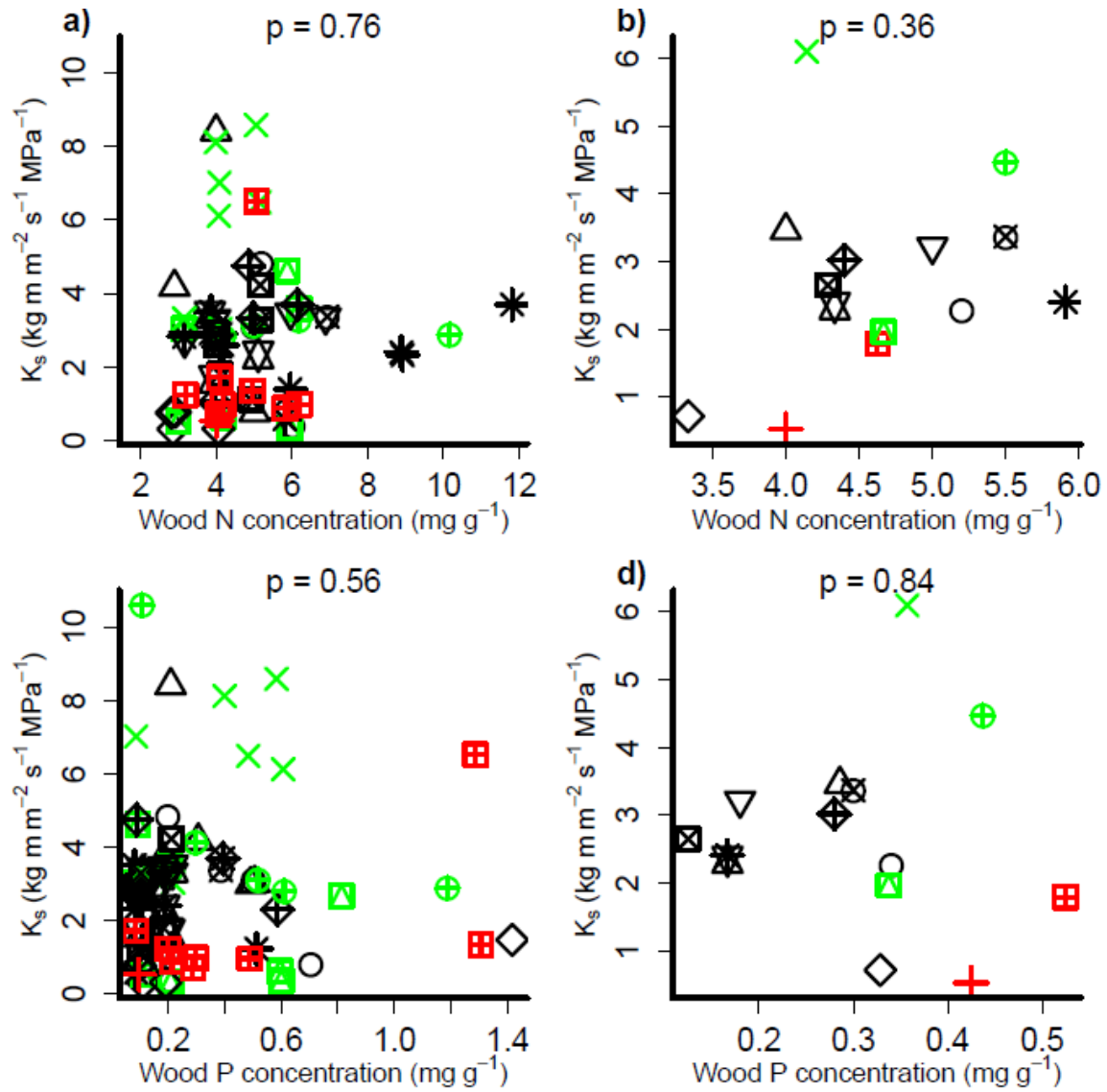
### Acknowledgments

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### Supporting Figures

**Figure S4.1.** Relationship between branch space-use efficiency ( $K_s$ ; specific conductivity) and branch wood nitrogen concentration (a and b) or phosphorus concentration (c and d). Left panels are data at individual level (a and c) and right panels are genus means (b and d). Red

points are trees from the throughfall exclusion experiment, green points are Fabaceae. Black line is the linear fit when the relationship is significant. A small jitter was applied to x-axis in a) and b) for better data visualization.



**Table S4.1.** Sampled data for each species and tree: diameter at breast height (DBH; cm), wood and leaf nitrogen and phosphorus concentration, wood density (WD), hydraulic space-use,





<i>Inga rubiginosa</i>	487	26	X	X	X	X	X	X	X	X	X	X
<i>Licania membranaceae</i>	327	23	X	X			X	X	X	X	X	
<i>Licania membranaceae</i>	499	28	X	X			X	X	X	X	X	X
<i>Licania membranaceae</i>	504	21		X	X	X	X	X		X	X	
<i>Licania octandra</i>	136	19	X	X		X	X	X	X	X	X	
<i>Licania octandra</i>	445	10	X	X		X		X			X	X
<i>Licania octandra</i>	525	22	X	X		X	X				X	X
<i>Licania octandra</i>	63	11	X	X	X	X	X				X	X
<i>Micropholis venulosa</i>	213	21			X	X	X				X	
<i>Micropholis venulosa</i>	264	26	X	X	X	X	X	X	X	X	X	
<i>Micropholis venulosa</i>	359	35		X		X	X	X		X	X	X
<i>Micropholis venulosa</i>	393	63	X	X	X	X	X	X	X	X	X	
<i>Micropholis venulosa</i>	440	18	X	X		X	X	X	X	X	X	
<i>Micropholis venulosa</i>	500	29	X	X		X	X	X	X	X	X	X
<i>Minquartia guianensis</i>	169	15	X	X	X	X	X	X	X	X	X	
<i>Minquartia guianensis</i>	223	17	X	X	X	X		X			X	
<i>Minquartia guianensis</i>	224	16	X	X	X	X	X				X	
<i>Minquartia guianensis</i>	400	12		X	X	X	X	X		X	X	X
<i>Minquartia guianensis</i>	416	20										
<i>Minquartia guianensis</i>	421	40	X	X	X	X	X	X	X	X	X	X
<i>Minquartia guianensis</i>	432	25	X	X	X	X	X	X	X	X	X	X
<i>Minquartia guianensis</i>	459	30	X	X	X	X	X	X	X	X	X	
<i>Minquartia guianensis</i>	491	17			X	X	X				X	
<i>Minquartia guianensis</i>	507	42	X	X	X	X	X	X	X	X	X	X
<i>Pouteria anomala</i>	161	17	X	X	X	X	X	X	X	X	X	X
<i>Pouteria anomala</i>	268	14	X	X		X	X	X	X	X	X	X
<i>Pouteria anomala</i>	363	59	X	X			X	X	X	X	X	X



<i>Syzygiopsis oppositifolia</i>	423	29		X	X	X	X	X		X	X	X
<i>Syzygiopsis oppositifolia</i>	427	35		X	X	X	X	X		X	X	X
<i>Tetragastris nitidum</i>	212	26	X	X				X			X	X
<i>Tetragastris nitidum</i>	213	26			X	X	X				X	
<i>Tetragastris panamensis</i>	122	18	X	X	X	X	X	X	X	X	X	X
<i>Tetragastris panamensis</i>	197	14			X	X	X				X	X
<i>Tetragastris panamensis</i>	234	24	X	X	X	X	X	X	X	X	X	
<i>Tetragastris panamensis</i>	291	23	X	X	X	X	X	X	X	X	X	X
<i>Tetragastris panamensis</i>	300	15	X	X	X	X	X	X	X	X	X	
<i>Tetragastris panamensis</i>	316	12	X	X		X	X	X	X	X	X	X
<i>Tetragastris panamensis</i>	322	17	X	X		X	X	X	X	X	X	X
<i>Tetragastris panamensis</i>	369	13	X	X	X	X	X	X	X	X	X	
<i>Virola micheli</i>	257	48	X	X	X	X	X				X	X
<i>Virola micheli</i>	434	27	X	X	X	X		X			X	X
<i>Vouacapoua americana</i>	216	34	X	X		X		X			X	X
<i>Vouacapoua americana</i>	118	26	X	X	X	X	X	X	X	X	X	
<i>Vouacapoua americana</i>	142	12	X	X	X	X	X	X	X	X	X	
<i>Vouacapoua americana</i>	180	56	X	X	X	X	X	X	X	X	X	X
<i>Vouacapoua americana</i>	222	29	X	X	X	X	X	X	X	X	X	X
<i>Vouacapoua americana</i>	29	21		X		X	X	X		X	X	
<i>Vouacapoua americana</i>	317	22			X	X	X				X	X
<i>Vouacapoua americana</i>	322	24			X	X	X				X	
<i>Vouacapoua americana</i>	35	39	X	X	X	X	X	X	X	X	X	
<i>Vouacapoua americana</i>	406	14		X	X	X	X	X		X	X	

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# Resumo leigo

A Terra é o Planeta Planta. Se você pudesse colocar todas as folhas de todas as plantas que estão sobre o planeta lado a lado elas cobririam toda a superfície do planeta! Desde o surgimento de organismos fotossintetizantes e das plantas na história evolutiva, tudo o que ocorre sobre a superfície do planeta é afetado pelas plantas. Os primeiros organismos fotossintetizantes reduziram a concentração de  $\text{CO}_2$  na atmosfera e aumentaram  $\text{O}_2$ , tanto modificando a temperatura do planeta como permitindo a evolução da respiração aeróbica com suas conseqüências para a diversificação das formas de vida. O surgimento de plantas sobre a terra reduziu a erosão causada pela chuva e ventos e permitiu a formação de solos. O surgimento do sistema radicular de plantas permitiu a conexão de solos mais profundos com a atmosfera, através do sistema de transporte de água das plantas, aumentando em muito a evapotranspiração. O aumento da evapotranspiração, isto é, da água evaporada dos oceanos, que chove sobre a terra e então é evapotranspirada e retornada para a atmosfera para novamente precipitar em outras regiões, fez com que mais água permanecesse por mais tempo sobre os ambientes terrestres e permitiu maior precipitação sobre locais onde a água evaporada do mar chegaria em menor quantidade. Com isso, as plantas modificaram ainda mais os padrões e dinâmica dos rios, a erosão e a distribuição de energia, na forma de calor latente, sobre o planeta. Não é um exagero dizer que as plantas modulam o funcionamento da superfície terrestre do planeta. Entretanto, as plantas não ocorrem em todas as formas, com a mesma abundância e nos mesmos locais. Quantidade de chuva e sua distribuição ao longo do ano e entre anos, quantidade e tipos de solo, temperatura, umidade atmosférica e outros fatores afetam os tipos de plantas que ocorrem em um local, e os tipos de plantas que ocorrem em um local por sua vez afetam diretamente o ambiente local em que vivem e, de forma complexa, o ambiente regional e o planeta como um todo.

As plantas, principalmente através da captura de  $\text{CO}_2$  na fotossíntese e da captação de água pelas raízes e transpiração pelas folhas, modulam quase todos os processos bióticos e abióticos que ocorrem sobre a superfície do planeta. A resposta de fotossíntese, transpiração, reprodução e crescimento de plantas a diferentes climas e solos, bem como a sobrevivência das

plantas, diferem muito dependendo de como elas constroem suas folhas, raízes e madeiras. Também a forma como diferentes plantas vão responder a mudanças climáticas vai depender dos atributos que elas possuem. Porém, temos grandes lacunas no conhecimento de atributos e funcionamento de plantas. Para vários atributos que conhecemos e entendemos, não sabemos quão diversos eles são, como variam entre diferentes vegetações, entre espécies e dentro de uma mesma espécie em diferentes situações ou estágios de vidas. Para outros aspectos do funcionamento de plantas, como o transporte de água, ainda temos um entendimento fisiológico limitado. Sabemos como a água é transportada nas plantas e os principais atributos das raízes, madeira e folhas que determinam a captação, transporte e transpiração de água. Sabemos os limites em que determinados tipos de plantas podem ocorrer e temos um bom entendimento das estratégias que essas plantas têm que ter para sobreviverem em locais secos, úmidos ou frios. Mas nós entendemos muito pouco dos atributos químicos, anatômicos, morfológicos e estruturais que determinam o transporte de água dentro das plantas. Particularmente, não sabemos qual o custo, em termos de energia e nutrientes, para uma planta de ter diferentes sistemas de transporte de água. Como o xilema das plantas realiza diversas funções ao mesmo tempo (transporte de água, suporte mecânico, estoque de água, energia e nutrientes, resistência a patógenos), diferentes formas como plantas podem construir seu xilema também requerem um compromisso entre maximizar uma função ao custo de outras. Não é possível, por exemplo, uma planta ter uma madeira muito resistente e que ao mesmo tempo pode estocar muita água. Outra dessas demandas conflitantes é que plantas que podem transportar água com muita eficiência não conseguem transportar com muita segurança e podem sofrer de falhas nos seus conduítes caso o ambiente seque. Nosso conhecimento dessas demandas conflitantes é muito limitado e geralmente restrito a alguns biomas e linhagens de plantas.

Se por um lado o funcionamento de plantas depende dos seus atributos, por outro ele depende do envelope climático e do solo em que vivem. Plantas com determinados atributos não conseguem sobreviver ou competir com outras plantas em determinados envelopes climáticos. Assim, o ambiente filtra os atributos que ocorrem em uma determinada vegetação. Apesar de quantificar atributos climáticos ser muito mais simples do que quantificar atributos da vegetação, aqui também nosso conhecimento é limitado e diferentes aspectos climáticos causam diferentes efeitos nas plantas. Uma atmosfera muito seca em combinação com solos úmidos tem efeitos diferentes do que em combinação com solo seco. Secas longas e de baixa

intensidade tem diferentes conseqüências que secas curtas e intensas. A quantia de nuvens durante o dia afeta a disponibilidade de luz e pode tanto ter efeitos positivos quanto negativos para plantas, particularmente dependendo do horário em que ocorre. O horário e freqüência com que chuva e orvalho ocorrem determinam o tempo que plantas permanecem com as folhas molhadas, o que afeta suas trocas gasosas e ocorrência de patógenos. Todas essas relações ficam muito mais complicadas em ambientes em que ocorre neblina. Também os aspectos ambientais analisados há décadas por climatólogos não necessariamente avaliam aspectos fundamentais para o funcionamento de plantas.

Se queremos entender como o planeta e seus ecossistemas funcionam, e as conseqüências de 7 milhões de pessoas que dependem do planeta e estão ao mesmo tempo modificando-o de uma forma e em uma velocidade que nunca ocorreu nos últimos 4 bilhões de anos, entender como plantas funcionam é um passo fundamental, quais atributos possuem, e como afetam e são afetadas pelo ambiente. Precisamos entender e integrar o funcionamento de plantas na diversidade de ambientes onde vivem. Neste trabalho, contribuo com a compreensão do funcionamento de plantas ao estudar duas formações vegetais distintas, uma Floresta Nebular Tropical Montana e uma Floresta Tropical Chuvosa, e ao propor novas formas de pensar o funcionamento do principal tecido de transporte de água, sustentação e armazenamento de água, energia e nutrientes de plantas, o xilema. Eu busco responder às perguntas: quão importante e diversa é a neblina em florestas tropicais nebulares? Árvores de florestas tropicais nebulares podem utilizar a água de chuva, neblina ou orvalho que acumula sobre sua superfície? Quais custos podem ter o transporte de água e como se relacionam com o funcionamento de plantas? Será que madeiras com diferentes atributos possuem diferentes custos nutricionais?



Resquícios de neblina pela manhã nos vales da Florestas Nebular Tropical Montana em Guaratinguetá, Serra da Mantiqueira.

Em meu primeiro capítulo - *“High temporal resolution of fog occurrence and its effects on water and light availability in a tropical montane cloud forest in Brazil”*<sup>1</sup>, eu estudo uma formação vegetal peculiar, a Floresta Nebular Tropical Montana (FNTM). Esta floresta ocorre em topos de montanhas, acima de 1200m nos trópicos. As plantas que a compõem são distintas das florestas tropicais que ocorrem na mesma região em altitudes mais baixas.

Surpreendentemente, as FNTMs têm uma baixa diversidade de espécies arbóreas, embora com alto endemismo, e composta tanto de linhagens de plantas tropicais como temperadas. Sua composição florística peculiar provavelmente se deve ao seu ambiente também peculiar: baixas temperaturas com geadas no inverno, alta pluviosidade e neblina freqüente, baixa intensidade luminosa média, porém alta radiação quando não há nuvens e neblina. Destes aspectos, a neblina tem um papel chave ao reduzir a transpiração e luminosidade e, ao ser interceptada pelas copas das árvores, precipitar ao solo e aumentar a entrada de água nesses ambientes. A

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<sup>1</sup> Alta resolução temporal de ocorrência de neblina e seu efeito na disponibilidade de água e luz em uma Floresta Nebular Tropical Montana no Brasil.

baixa transpiração e entrada adicional de água pela neblina faz desses ambientes “caixas d’água”, suprimindo água em grande quantidade para as regiões mais baixas. Também, estudos recentes indicam que a água de neblina pode ser diretamente transportada das folhas até o solo pelas plantas e que sua ocorrência ao rehidratar as plantas e reduzir a transpiração, é importante para a sobrevivência e funcionamento de FNTMs.

Porém, o que sabemos dos efeitos da neblina nas plantas se deve principalmente a estudos pontuais de respostas de plantas e ecossistemas a neblina. Mas o efeito total da neblina na hidrologia e funcionamento das plantas vai depender de quando, quanto e qual a intensidade com que a neblina ocorre. Neblinas noturnas tem um efeito nas plantas diferentes de neblinas diurnas; se a neblina vai diminuir o estresse hídrico de plantas vai depender em grande parte de se ela ocorre durante a estação seca ou chuvosa; se ela vai aumentar a capacidade das plantas de fazerem fotossíntese vai depender de se seu efeito em rehidratar e reduzir a transpiração das plantas durante o dia é maior ou menor que a diminuição na fotossíntese devido a redução na energia luminosa; sua contribuição para a rehidratação dos solos e para o suprimento de água dos rios vai depender da intensidade com que a neblina ocorre, se ocorre de noite ou de dia, com chuva, com ou sem vento e outros aspectos. Assim, sem entender o regime de neblinas de FNTMs não há como entender o funcionamento de suas plantas e sua hidrologia. Porém detectar a ocorrência de neblina não é trivial e, até onde sabemos, nenhum trabalho ecológico estudou com profundidade o regime de neblinas de FNTMs. Para lidar com esse problema, eu utilizei um método pouco explorado em ecohidrologia, um medidor de visibilidade horizontal, que detecta com precisão e alta resolução temporal a intensidade e ocorrência de neblinas. Com isso, eu respondo quanto, quando e com qual frequência e intensidade a neblina ocorre e qual seu efeito na disponibilidade hídrica e disponibilidade de luz de uma FNTM na Serra da Mantiqueira. Meus resultados permitem uma compreensão mais completa do funcionamento de plantas e hidrologia de FNTMs e do papel da neblina, levantando novas predições a serem testadas.





Copa das árvores e torre para acesso às copas em Florestas Nebular Tropical Montana em Guaratinguetá, Serra da Mantiqueira.

Em meu segundo capítulo, - (Título aqui), eu estudo um fenômeno pouco compreendido em plantas, a absorção de água pelas folhas. Normalmente, plantas captam água do solo e transportam para as folhas, onde ela é transpirada. Isso ocorre por que na maioria das vezes a atmosfera é mais seca que o solo, fazendo com que a água flua do local mais úmido para o mais seco. Porém, quando a atmosfera está mais úmida que o solo e as folhas molhadas, ao invés de transpirar as folhas podem absorver água. Alguns estudos mostram inclusive que essa água absorvida pode ter papel importante em sustentar a planta durante períodos de seca. Porém nós não sabemos quão variável é a absorção de água foliar entre diferentes indivíduos da mesma espécie ou de espécies diferentes. Também não sabemos em quais condições climáticas a absorção de água pela folha pode ocorrer e nem quais atributos das plantas determinam a absorção de água pelas folhas.

Para preencher as lacunas de conhecimento acima, medimos o fluxo de seiva de 38 árvores de uma Floresta Nebular Montana Tropical. Quando ocorre absorção de água pelas folhas, ela pode ser transportada pelo caule até o solo, invertendo o fluxo de seiva. Assim, o fluxo reverso de seiva é um indicador de absorção de água pelas folhas. Eu analiso quão diverso

é o fluxo reverso de seiva em condições climáticas diferentes na estação seca e chuvosa, durante o dia e a noite e em diferentes eventos em que folhas estão molhadas: orvalho, neblina, chuva e neblina com chuva. Além disso, eu analiso se atributos hidráulicos das plantas determinam a intensidade do fluxo reverso de seiva. Meus resultados indicam que a absorção de água pelas folhas ocorre em todas as árvores da floresta que estudamos, que diferentes condições climáticas levam a diferenças na absorção de água pelas folhas e que atributos hidráulicos modulam a intensidade de absorção foliar. Como plantas em florestas tropicais podem passar de 20 a 70% de suas vidas com as folhas molhadas e absorção foliar ocorre frequentemente, meus resultados apontam para a necessidade de estudarmos as conseqüências da absorção foliar para o funcionamento de plantas.



Espécie de árvore de floresta tropical chuvosa com madeira em formato não cilíndrico, talvez para minimizar os gastos com madeira? Floresta Nacional de Caxiunã, Pará.

Em meu terceiro capítulo – “*On xylem hydraulic efficiencies, wood space-use and the safety-efficiency tradeoff*”<sup>2</sup>, eu comento um trabalho de alta importância publicado recentemente<sup>3</sup> sobre relação entre segurança e eficiência hidráulica de plantas. A água em plantas é transportada das raízes para as folhas por conduítes no xilema pela força evaporativa da atmosfera atuando na água dentro das folhas. A força evaporativa da atmosfera faz a água dentro da planta ser transportada sob tensão, como se a atmosfera estivesse “chupando” a água, da mesma forma como chupamos a água de um copo com um canudo. Porém isso ocorre com uma força centenas de vezes mais forte e essa alta energia pode levar a coluna de água a se romper. Quando a coluna de água nos conduítes do xilema da planta se rompem, o conduíte enche de ar e não pode mais transportar água. Este processo se chama falha hidráulica e a consequência é que, quando ocorre falha hidráulica, as plantas conseguem transportar menos água para as folhas. Por receberem menos água, as folhas tem que também perder menos água mantendo os estômatos mais fechados e reduzindo a fotossíntese. Em casos extremos, a incapacidade de suprir água pode levar à mortalidade de folhas, galhos e da planta inteira.

Diferentes plantas tem diferentes capacidades de suportar tensão no xilema e essa propriedade é a segurança hidráulica da planta. Porém, diferentes plantas também tem diferentes capacidades de suprir água para suas folhas – diferentes eficiências hidráulicas. A segurança e a eficiência hidráulica são determinadas por atributos anatômicos, morfológicos e químicos dos conduítes e os estudos realizados nas últimas décadas indicam que vasos com alta eficiência têm baixa segurança, ou seja, há um conflito entre segurança e eficiência hidráulica. Isso levou à hipótese, amplamente aceita, de que plantas não podem ser eficientes e seguras hidraulicamente ao mesmo tempo. Porém, contrário as expectativas, o estudo que comento encontrou que a relação entre eficiência e segurança é fraca a nível global. Em meu comentário, eu discuto possibilidades inovadoras para explicar a baixa relação entre eficiência e segurança hidráulica encontrada pelos autores. Eu problematizo o que é eficiência hidráulica do xilema. Embora

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<sup>2</sup> Sobre as eficiências hidráulicas do xilema, uso do espaço da Madeira e a demanda conflitante entre segurança e eficiência.

<sup>3</sup> Gleason SM, Westoby M, Jansen S, Choat B, Hacke UG, Pratt RB, Bhaskar R, Brodribb TJ, Bucci SJ, Cao K-F, *et al.* 2015. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world’s woody plant species. *New Phytologist*.

extremamente estudada nas últimas décadas, apenas um tipo de eficiência do xilema tem sido estudado, e muitas vezes sem compreensão de seu significado maior para a planta, que é a eficiência de uso-do-espço do sistema hidráulico. Baseado nisso, eu proponho que diferentes eficiências hidráulicas de plantas existem e precisam ser estudadas para entender o funcionamento hidráulico de plantas.



Laboratório “de campo” para coleta de material e medidas hidráulicas na Floresta Nacional de Caxiuanã, Pará.

Finalmente, em meu quarto capítulo – *“Branch nutrients may be more important than previously thought: costs, relationships and possible coordination with wood hydraulics in an Eastern Amazon forest”*<sup>4</sup>, baseado em uma proposta feita no segundo capítulo, que a eficiência de uso dos nutrientes no transporte hidráulico de plantas pode ser importante para o funcionamento de plantas, eu estudo o nutriente da madeira de galhos de 100 árvores de 14 espécies de árvores da Amazônia Oriental de diferentes tamanhos. Em árvores, quase toda a biomassa e aproximadamente 90% dos nutrientes estão na madeira. Surpreendentemente, a maioria

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<sup>4</sup> Nutrientes de galhos podem ser mais importantes do que pensado anteriormente: custos, relações e possíveis coordenações entre hidráulica da madeira em uma floresta da Amazônia Oriental.

dos trabalhos existentes estudam os nutrientes das folhas e não da madeira, embora a madeira provavelmente domine o uso de nutrientes de árvores. Uma árvore que consegue construir o mesmo volume de madeira com menos nutrientes ou transportar mais água ou ser mais segura hidraulicamente com um custo menor de nutrientes pode investir mais em crescimento e reprodução. Devido a forma como o xilema é construído, madeiras com diferentes atributos possivelmente também tem custos nutricionais diferentes. Neste capítulo, eu quantifico a concentração e o conteúdo de fósforo e nitrogênio da madeira de galhos e folhas, a densidade da madeira, a segurança, e as eficiências de uso-do-espço e de uso de fósforo e nitrogênio, dois dos nutrientes mais importantes e limitantes para árvores em florestas tropicais. Eu testo hipóteses pioneiras sobre coordenação entre nutrientes da madeira e das folhas, relações entre concentração e conteúdo nutricional da madeira, mudanças nutricionais da madeira ao longo da ontogenia de árvores e se há relação entre atributos hidráulicos da madeira e seu conteúdo nutricional e a eficiência de uso de nutrientes no transporte de água.

## Discussion

While it is known that fog is a key phenomena in tropical montane cloud forests (TMCFs), my research in Chapter 1 shows that fog not only contributes to water inputs, but that its contributions occurs mostly at night. Fog occurs much less frequently during the day, however the clouds that were fog in the morning may stay in the site even if not touching the ground, modulating daytime climate and light availability. Fog effects on TMCFs plants are probably modulated by nighttime fog effects on plant rehydration, which are not only due to decreased VPD, but also to direct leaf water absorption (Eller *et al.*, 2013) , which I show to occurs almost whenever leaves are wet in Chapter 2. However, cloud occurrence during the day may be one of the most important traits modulating transpiration rates in TMCFs, as they strongly determine daytime climatic variability and light energy driving transpiration.

Cloudness is variable in all ecosystems, but plant responses to cloudness is seldom evaluated. In effect, the standard conditions to measure plant photosynthetic or water related traits is often when there is full sunlight and no clouds (see recommendations for some traits in Pérez-Harguindeguy *et al.*, 2013) . While clear days during the dry season may set climate extremes plants have to adjust their traits to, mean working conditions of plants are probably determined by cloud occurrence affecting mean climate and interday variability. As climate change will not only affect temperature and rainfall, but cloud patterns in general, plant water use must be modeled through a range of different cloud conditions if we want to understand how ecosystems will function in the future.

TMCFs plants stay with their leaves frequently wet. However, tropical forest trees in general, except dry forests, stay a large amount of their time wet. My results in Chapter 2 shows leaf water absorption occurs even in the wet season and in conditions when everything is wet for days, probably with the pressure gradient generated by tree height from tree canopy to the soil driving water flow. TMCFs trees are small trees, with less than 15m in height generally. Amazon trees, for example, can be as high as 50m and this difference in height implies that the driving force for leaf water absorption can be more than 3 times higher. Leaf water absorption, which occurs even in the dry season due to dew, may be an important water source for trees during drought and may be one explanation on how Amazon trees can flush new leaves during

the dry season (Lopes *et al.*, 2016). However, tall trees have a high water storage and it is most likely that foliar water absorbed does not reach the ground, contrary to small trees in TCMFs, as found by Binks *et al.* (submitted). Foliar water uptake in tall trees may then be an overlooked and hard to monitor process if it does not reach the ground, but important nevertheless as found for many plants (Martin & von Willert, 2000; Berry *et al.*, 2014b; Eller *et al.*, 2016).

Moreover, different trees have different leaf water uptake capabilities and this capacity is in part related to transpiration rates per unit xylem – plants which transpire more per unit sapwood also absorb more water through their leaves. This synergy, high leaf water absorption capacity due to hydraulic traits and height, suggests foliar water uptake of tall and high transpiration trees should be even higher. However emergent trees are more coupled to the atmosphere and dew is less likely to form on them as surface radiative cooling effects are diminished by coupling to atmospheric temperatures (Beysens *et al.*, 2006). Emergent trees also should stay less time wet as their higher coupling to atmosphere should evaporate leaf water faster. Tall, but not emergent, trees should be the ones to most benefit from leaf absorption then. While my results highlight foliar water uptake as a phenomena much more frequent than previously thought, we still need more information about leaf absorption mechanisms, effects on plant physiology and monitoring of climatic traits, such as dew and leaf wetness, which are seldom monitored traditionally. Whether climate change effects on leaf wetness may have consequences to plant functioning is an open question.

Finally, my last chapter, by studying part of the theoretical framework proposed on Chapter 3, open many new questions on plant physiology. Different wood have different costs. It is hypothesized that nutrient availability may limit forest formation due to nutrient costs required for producing the large amount of biomass of trees (Bond, 2010). Maybe not limit, but set different costs to trees having different heights. Our results further expand this idea by showing that different hydraulic traits are associated to different nutrient requirements. While mechanisms driving this relation are unclear and the relations must be further tested, our results indicate plants in drier places require a more phosphorus and nitrogen enriched wood to have more xylem embolism resistance and operate at lower water potentials. Our results corroborates that nutrient availability may be limiting tree adjustment to climate change, as the adjustment will cost nutrients (Gessler *et al.*, 2017). Furthermore, places with less drought stress as TCMFs or places with high cloudness may require less nutrients enriched wood. While much



of our conclusions on wood nutrients need further testing in other biomes, conditions and with different controls, our results strongly highlight the need to study the nutritional axis of wood function. It is amazing that this axis is almost unexplored, even though 80-90% of nutrients of trees are in wood (Johnson *et al.*, 2001).

In conclusion, in this thesis, I studied the soil-plant-atmosphere continuum, showing it is bidirectional much more frequently than usually considered. By analyzing the abiotic environment of a peculiar environment – the TMCF – I found that climatic traits often neglected, as nighttime fog effects, daytime cloudness and leaf wetting events in general, are particularly important to TMCFs and, possible, to other environments. In my last two chapters, by discussing a new theoretical framework on water transport and evaluating nutritional costs of wood, my work points to a new axis of water transport that may improve our models of plant functioning and explain unexpected results predicted by current theory. My work highlights water in the soil-plant-atmosphere continuum must be studied as a whole, from relevant atmosphere climatic traits in different ecosystems, considering its bidirectionality and the costs, particularly the nutritional ones, associated with plant water transport.

# Referências

## Introdução

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EHT, *et al.* 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660–684.

Alvares CA, de Mattos EM, Sentelhas PC, Miranda AC, Stape JL. 2015. Modeling temporal and spatial variability of leaf wetness duration in Brazil. *Theoretical and Applied Climatology* 120: 455–467.

Anderegg WR, Klein T, Bartlett M, Sack L, Pellegrini AF, Choat B, Jansen S. 2016. Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. *Proceedings of the National Academy of Sciences* 113: 5024–5029.

Berry ZC, White JC, Smith WK. 2014. Foliar uptake, carbon fluxes and water status are affected by the timing of daily fog in saplings from a threatened cloud forest. *Tree Physiology* 34: 459–470.

Bertoncello R, Yamamoto K, Meireles LD, Shepherd GJ. 2011. A phytogeographic analysis of cloud forests and other forest subtypes amidst the Atlantic forests in south and southeast Brazil. *Biodiversity and Conservation* 20: 3413–3433.

Bruijnzeel LA, Mulligan M, Scatena FN. 2011. Hydrometeorology of tropical montane cloud forests: emerging patterns. *Hydrological Processes* 25: 465–498.

Bruijnzeel LA, others. 2001. Hydrology of tropical montane cloud forests: a reassessment. *Land use and water resources research* 1: 1–1.

Burns EL, Simonin KA, Bothman AG, Dawson TE. 2009. Foliar water uptake: a common water acquisition strategy for plants of the redwood forest. *Oecologia* 161: 449–459.

CATHARINO EL, Blanc L, GUNATILLEKE N, LAURANCE WF, WITTMANN F, BOECKX P, FRANCO G, POWERS J, BUNYAVEJCHEWIN S, BELLINGHAM PJ, *et al.* 2015. An estimate of the number of tropical tree species.

Cruziat P, Cochard H, Améglio T. 2002. Hydraulic architecture of trees: main concepts and results. *Annals of forest science* 59: 723–752.

- Eller CB, Lima AL, Oliveira RS. 2013. Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *The New phytologist* 199: 151–161.
- Eller CB, Lima AL, Oliveira RS. 2016. Cloud forest trees with higher foliar water uptake capacity and anisohydric behavior are more vulnerable to drought and climate change. *New Phytologist* 211: 489–501.
- Gessler A, Schaub M, McDowell NG. 2017. The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* 214: 513–520.
- Gleason SM, Westoby M, Jansen S, Choat B, Hacke UG, Pratt RB, Bhaskar R, Brodribb TJ, Bucci SJ, Cao K-F, *et al.* 2015. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytologist*: n/a-n/a.
- Greenwood S, Ruiz-Benito P, Martínez-Vilalta J, Lloret F, Kitzberger T, Allen CD, Fensham R, Laughlin DC, Kattge J, Bönisch G, *et al.* 2017. Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area (J Chave, Ed.). *Ecology Letters* 20: 539–553.
- Jarvis A, Mulligan M. 2011. The climate of cloud forests. *Hydrological Processes* 25: 327–343.
- Johnson CM, Vieirab IC, Zarinc DJ, Frizano J, Johnson AH. 2001. Carbon and nutrient storage in primary and secondary forests in eastern Amazonia. *Forest Ecology and Management* 147: 252.
- Lawrence D, Vandecar K. 2014. Effects of tropical deforestation on climate and agriculture. *Nature Climate Change* 5: 27–36.
- Lenton TM, Held H, Kriegler E, Hall JW, Lucht W, Rahmstorf S, Schellnhuber HJ. 2008. Tipping elements in the Earth's climate system. *Proceedings of the national Academy of Sciences* 105: 1786–1793.
- Morris H, Plavcová L, Cvecko P, Fichtler E, Gillingham MAF, Martínez-Cabrera HI, McGlenn DJ, Wheeler E, Zheng J, Ziemińska K, *et al.* 2015. A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. *New Phytologist*: n/a-n/a.
- Oliveira RS, Eller CB, Bittencourt PRL, Mulligan M. 2014. The hydroclimatic and ecophysiological basis of cloud forest distributions under current and projected climates. *Annals of Botany* 113: 909–920.
- Pan Y, Birdsey RA, Phillips OL, Jackson RB. 2013. The Structure, Distribution, and Biomass of the World's Forests. *Annual Review of Ecology, Evolution, and Systematics* 44: 593–622.

- Phillips OL, Van Der Heijden G, Lewis SL, López-González G, Aragão LE, Lloyd J, Malhi Y, Monteagudo A, Almeida S, Dávila EA, *et al.* 2010. Drought–mortality relationships for tropical forests. *New Phytologist* 187: 631–646.
- Rowland L, da Costa ACL, Galbraith DR, Oliveira RS, Binks OJ, Oliveira AAR, Pullen AM, Doughty CE, Metcalfe DB, Vasconcelos SS, *et al.* 2015. Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature*.
- Slatyer RO. 1956. Absorption of water from atmospheres of different humidity and its transport through plants. *Australian Journal of Biological Sciences* 9: 552–558.
- Sperry JS, Love DM. 2015. What plant hydraulics can tell us about responses to climate-change droughts. *New Phytologist* 207: 14–27.
- Tyree MT. 2003. The ascent of water. *Nature* 423: 923–923.
- Wieder WR, Cleveland CC, Smith WK, Todd-Brown K. 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience* 8: 441–444.

### Capítulo 1

- Alvarado-Barrientos MS, Holwerda F, Geissert DR, Muñoz-Villers LE, Gotsch SG, Asbjornsen H, Dawson TE. 2015.** Nighttime transpiration in a seasonally dry tropical montane cloud forest environment. *Trees* 29: 259–274.
- Baguskas SA, Still CJ, Fischer DT, D’Antonio CM, King JY. 2016.** Coastal fog during summer drought improves the water status of sapling trees more than adult trees in a California pine forest. *Oecologia* 181: 137–148.
- Barros, FV, Bittencourt, PRL, Eller, CB, Muller, CS, Meireles, LD, Oliveira RS.** Phytogeographic origin and phylogenetic diversity explain community hydraulic diversity in a tropical montane cloud forest. In prep.
- Berry ZC, White JC, Smith WK. 2014.** Foliar uptake, carbon fluxes and water status are affected by the timing of daily fog in saplings from a threatened cloud forest. *Tree Physiology* 34: 459–470.
- Bertoncello R, Yamamoto K, Meireles LD, Shepherd GJ. 2011.** A phytogeographic analysis of cloud forests and other forest subtypes amidst the Atlantic forests in south and southeast Brazil. *Biodiversity and Conservation* 20: 3413–3433.

- Bittencourt, PRLB. 2014.** How does fog affects microclimatic conditions and leaf functioning in tropical montane cloud forests? Dissertation. 60p. Universidade Estadual de Campinas.
- Brown R, Mills AJ, Jack C. 2008.** Non-rainfall moisture inputs in the Knersvlakte : Methodology and preliminary findings. *Water SA* **34**: 275–278.
- Bruijnzeel LA. 2001.** Hydrology of tropical montane cloud forests : A Reassessment. *Water Use and Water Resources Research* **1**: 1–18.
- Bruijnzeel LA, Mulligan M, Scatena FN. 2011a.** Hydrometeorology of tropical montane cloud forests: emerging patterns. *Hydrological Processes* **25**: 465–498.
- Bruijnzeel LA, Scatena FN, Hamilton LS. 2011b.** *Tropical Montane Cloud Forests: Science for Conservation and Management*. Cambridge: Cambridge University Press.
- Bruijnzeel LA, Veneklaas EJ. 1998.** Climatic conditions and tropical montane forest productivity: the fog has not lifted yet. *Ecology* **79**: 3–9.
- Carrillo JH, Emert SE, Sherman DE, Herckes P, Collett JL. 2008.** An economical optical cloud/fog detector. *Atmospheric Research* **87**: 259–267.
- Chang S-C, Yeh C-F, Wu M-J, Hsia Y-J, Wu J-T. 2006.** Quantifying fog water deposition by in situ exposure experiments in a mountainous coniferous forest in Taiwan. *Forest Ecology and Management* **224**: 11–18.
- Eller CB, Lima AL, Oliveira RS. 2013.** Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *The New phytologist* **199**: 151–161.
- Eller CB, Lima AL, Oliveira RS. 2016.** Cloud forest trees with higher foliar water uptake capacity and anisohydric behavior are more vulnerable to drought and climate change. *New Phytologist* **211**: 489–501.
- Eugster W. 2007.** The Relevance of Fog for the Vegetation : is it the Water or the Nutrients That Matter ? In: Proceedings of the Fourth International Conference on Fog, Fog Collection and Dew. 359–362.
- Eugster W, Burkard R, Klemm O, Wrzesinsky T. 2000.** Fog deposition measurements with the eddy covariance method. *measurements* **6**: 7.
- Fernández V, Sancho-Knapik D, Guzmán P, Peguero-Pina JJ, Gil L, Karabourniotis G, Khayet M, Fasseas C, Heredia-Guerrero JA, Heredia A, et al. 2014.** Wettability, Polarity, and Water Absorption of Holm Oak Leaves: Effect of Leaf Side and Age. *Plant Physiology* **166**: 168–180.
- Goldsmith GR, Matzke NJ, Dawson TE. 2013.** The incidence and implications of clouds for cloud forest plant water relations (J Penuelas, Ed.). *Ecology Letters* **16**: 307–314.

- Gotsch SG, Asbjornsen H, Goldsmith GR. 2016.** Plant carbon and water fluxes in tropical montane cloud forests. *Journal of Tropical Ecology* **32**: 404–420.
- Gultepe I, Milbrandt JA. 2010.** Probabilistic Parameterizations of Visibility Using Observations of Rain Precipitation Rate, Relative Humidity, and Visibility. *Journal of Applied Meteorology and Climatology* **49**: 36–46.
- Gultepe I, Tardif R, Michaelides SC, Cermak J, Bott A, Bendix J, Müller MD, Pagowski M, Hansen B, Ellrod G, et al. 2007.** Fog Research: A Review of Past Achievements and Future Perspectives. *Pure and Applied Geophysics* **164**: 1121–1159.
- Gutiérrez AG, Barbosa O, Christie D a., Del-Val E, Ewing H a., Jones CG, Marquet P a., Weathers KC, Armesto JJ. 2007.** Regeneration patterns and persistence of the fog-dependent Fray Jorge forest in semiarid Chile during the past two centuries. *Global Change Biology*: 071121035853006–???
- Jarvis A, Mulligan M. 2011.** The climate of cloud forests. *Hydrological Processes* **25**: 327–343.
- Jordan DN, Smith WK. 1994.** Energy balance analysis of nighttime leaf temperatures and frost formation in a subalpine environment. *Agricultural and Forest Meteorology* **71**: 359–372.
- Kamil B. 2016.** MuMIn: Multi-Model Inference. R package version 1.15.6. <http://CRAN.R-project.org/package=MuMIn>
- Leuschner C. 2000.** Are high elevations in tropical mountains arid environments for plants? *Ecology* **81**: 1425–1436.
- de Lima Bittencourt PR, others. 2014.** How does fog affects microclimatic conditions and leaf functioning in tropical montane cloud forests?=: Como a neblina afeta as condições microclimáticas e o funcionamento foliar em florestas nebulares montanas nos trópicos?
- Liu W. M. 2004.** Water Input from Fog Drip in the Tropical Seasonal Rain Forest of Xishuangbanna, South-West China. *Journal of Tropical Ecology* **20**: 517–524.
- Martin PH, Bellingham PJ. 2016.** Towards integrated ecological research in tropical montane cloud forests. *Journal of Tropical Ecology* **32**: 345–354.
- Martínez ML, Pérez-Maqueo O, Vázquez G, Castillo-Campos G, García-Franco J, Mehlreter K, Equihua M, Landgrave R. 2009.** Effects of land use change on biodiversity and ecosystem services in tropical montane cloud forests of Mexico. *Forest Ecology and Management* **258**: 1856–1863.
- Matimati I, Anthony Verboom G, Cramer MD. 2014.** Do hydraulic redistribution and nocturnal transpiration facilitate nutrient acquisition in *Aspalathus linearis*? *Oecologia* **175**: 1129–1142.

- Mercado LM, Bellouin N, Sitch S, Boucher O, Huntingford C, Wild M, Cox PM. 2009.** Impact of changes in diffuse radiation on the global land carbon sink. *Nature* **458**: 1014–1017.
- Michna P, Schenk J, Wanner H, Eugster W. 2007.** MiniCASCC—A battery driven fog collector for ecological applications. In: Proceedings of the Fourth International Conference on Fog, Fog Collection and Dew, La Serena, Chile. 22–27.
- National Oceanic and Atmospheric Administration, 1995.** Surface weather observations and reports. Federal Meteorological Handbook, vol.1. 94 pp.
- Oliveira RS, Eller CB, Bittencourt PRL, Mulligan M. 2014.** The hydroclimatic and ecophysiological basis of cloud forest distributions under current and projected climates. *Annals of Botany* **113**: 909–920.
- Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team. 2014.** nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-118. <http://CRAN.R-project.org/package=nlme>
- R Core Team. 2014.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reinhardt, K, Smith WK, Carter GA. 2010.** Clouds and cloud immersion alter photosynthetic light quality in a temperate mountain cloud forest. *Botany* **88**:462-470.
- Ritter A, Regalado CM, Aschan G. 2009.** Fog reduces transpiration in tree species of the Canarian relict heath-laurel cloud forest (Garajonay National Park, Spain). *Tree physiology* **29**: 517–28.
- Roman P, Polkowska Ż, Namieśnik J. 2013.** Sampling Procedures in Studies of Cloud Water Composition: A Review. *Critical Reviews in Environmental Science and Technology* **43**: 1517–1555.
- Rosado BHP, Holder CD. 2013.** The significance of leaf water repellency in ecohydrological research: a review: LEAF WATER REPELLENCY IN ECOHYDROLOGICAL RESEARCH. *Ecohydrology* **6**: 150–161.
- Safford, HD. 1999.** Brazilian Paramos I. An introduction to the physical environment and vegetation of the campos de altitude. *Journal of Biogeography* **26**:693-712.
- Scholz FG, Phillips NG, Bucci SJ, Meinzer FC, Goldstein G. 2011.** Hydraulic Capacitance: Biophysics and Functional Significance of Internal Water Sources in Relation to Tree Size. In: Meinzer FC, Lachenbruch B, Dawson TE, eds. Size- and Age-Related Changes in Tree Structure and Function. Dordrecht: Springer Netherlands, 341–361.
- Still CJ, Foster PN, Schneider SH. 1999.** Simulating the effects of climate change on tropical montane cloud forests. *Nature* **398**: 608–610.

**Van Stan JT, Pypker TG. 2015.** A review and evaluation of forest canopy epiphyte roles in the partitioning and chemical alteration of precipitation. *Science of The Total Environment* **536**: 813–824.

**Vandekar KL, Runyan CW, D’Odorico P, Lawrence D, Schmook B, Das R. 2015.** Phosphorus input through fog deposition in a dry tropical forest: FOG PHOSPHORUS INPUTS. *Journal of Geophysical Research: Biogeosciences* **120**: 2493–2504.

**Viviroli D, Dürr HH, Messerli B, Meybeck M, Weingartner R. 2007.** Mountains of the world, water towers for humanity: Typology, mapping, and global significance. *Water Resources Research* **43**: 1–13.

**van de Weg MJ, Meir P, Grace J, Ramos GD. 2011.** Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest. *Oecologia*: 23–34.

**Zhang Y-J, Holbrook NM, Cao K-F. 2014.** Seasonal dynamics in photosynthesis of woody plants at the northern limit of Asian tropics: potential role of fog in maintaining tropical rainforests and agriculture in Southwest China. *Tree Physiology* **34**: 1069–1078.

## Capítulo 2

**Alvares CA, de Mattos EM, Sentelhas PC, Miranda AC, Stape JL. 2015.** Modeling temporal and spatial variability of leaf wetness duration in Brazil. *Theoretical and Applied Climatology* **120**: 455–467.

**Barros, FV, Bittencourt, PRL, Eller, CB, Muller, CS, Meireles, LD, Oliveira RS.** Phytogeographic origin and phylogenetic diversity explain community hydraulic diversity in a tropical montane cloud forest. In prep.

**Becker M, Kerstiens G, Schoenherr J. 1986.** Water permeability of plant cuticles: permeance, diffusion and partition coefficients. *Trees* **1**: 54–60.

**Berry ZC, White JC, Smith WK. 2014.** Foliar uptake, carbon fluxes and water status are affected by the timing of daily fog in saplings from a threatened cloud forest. *Tree Physiology* **34**: 459–470.

**Binks O., Meir P., Rowland L, Costa ACL, Bittencourt PRL, Eller C, Salles G, Soza A, Ferreira L, Vasconcelos SS, Oliveira R, Mencuccini M.** Foliar uptake in Amazon rainforest trees and its impact on hydraulic stress. Submitted to *New Phytologist*.

**Bittencourt, PRLB. 2014.** How does fog affects microclimatic conditions and leaf functioning in tropical montane cloud forests? Dissertation. 60p. Universidade Estadual de Campinas.



- Bittencourt PRLB, Barros FV, Eller CB, Oliveira RS.** High temporal resolution of fog occurrence and its effects on water and light in a tropical montane cloud forest in Brazil. In prep.
- Breshears DD, Mcdowell NG, Goddard KL, Dayem KE, Martens SN, Meyer CW, Brown KM, Mcdowell G, Goddard L, Martens N, et al.** 2008. Foliar Absorption of Intercepted Rainfall Improves Woody Plant Water Status Most during Drought. *Ecology* **89**: 41–47.
- Bruijnzeel LA, Scatena FN, Hamilton LS.** 2011. *Tropical Montane Cloud Forests: Science for Conservation and Management*. Cambridge: Cambridge University Press.
- Burgess SS, Adams MA, Turner NC, Beverly CR, Ong CK, Khan AA, Bleby TM.** 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. *Tree physiology* **21**: 589–598.
- Burghardt M, Riederer M.** 2008. 9 Cuticular transpiration. *Annual Plant Reviews, Biology of the Plant Cuticle* **23**: 292.
- Burns EL, Simonin KA, Bothman AG, Dawson TE.** 2009. Foliar water uptake: a common water acquisition strategy for plants of the redwood forest. *Oecologia* **161**: 449–459.
- Cassana FF, Eller CB, Oliveira RS, Dillenburg LR.** 2016. Effects of soil water availability on foliar water uptake of *Araucaria angustifolia*. *Plant and Soil* **399**: 147–157.
- Eller CB, Lima AL, Oliveira RS.** 2013. Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *The New phytologist* **199**: 151–161.
- Eller CB, Lima AL, Oliveira RS.** 2016. Cloud forest trees with higher foliar water uptake capacity and anisohydric behavior are more vulnerable to drought and climate change. *New Phytologist* **211**: 489–501.
- Eller C, Barros F, Bittencourt P, Rowland L, Mencuccini M, S. Oliveira R.** 2018. Xylem hydraulic safety and construction costs determine tropical tree growth: Tree growth vs hydraulic safety trade-off. *Plant, Cell & Environment*.
- Emery NC.** 2016. Foliar uptake of fog in coastal California shrub species. *Oecologia* **182**: 731–742.
- Geyer U, Schönherr J.** 1990. The effect of the environment on the permeability and composition of Citrus leaf cuticles. *Planta* **180**: 147–153.
- Goldsmith GR.** 2013. Changing directions: the atmosphere–plant–soil continuum. *New Phytologist* **199**: 4–6.

- Goldstein G., Andrade J.L., Meinzer F.C., Holbrook N.M., Cavelier J., Jackson P. & Celis A. 1998.** Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell and Environment* **21**:397-406.
- Hill AJ, Dawson TE, Shelef O, Rachmilevitch S. 2015.** The role of dew in Negev Desert plants. *Oecologia* **178**: 317–327.
- Laur J, Hacke UG. 2014.** Exploring *Picea glauca* aquaporins in the context of needle water uptake and xylem refilling. *New Phytologist*: n/a-n/a.
- Liu Y, Liang X, Su DR. 2011.** Processes of Water Absorption and Desorption for Intercepted Rainwater by the Leaf of Two Land Cover Plants. *Advanced Materials Research* **347–353**: 1953–1958.
- Martin CE, von Willert D. 2000.** Leaf Epidermal Hydathodes and the Ecophysiological Consequences of Foliar Water Uptake in Species of *Crassula* from the Namib Desert in Southern Africa. *Plant Biology* **2**: 229–242.
- Martínez-Vilalta J, Poyatos R, Aguadé D, Retana J, Mencuccini M. 2014.** A new look at water transport regulation in plants. *New Phytologist* **204**: 105–115.
- Max K et al. 2016.** caret: Classification and Regression Training. R package version 6.0-68. <http://CRAN.R-project.org/package=caret>
- Mayr S, Schmid P, Laur J, Rosner S, Charra-Vaskou K, Damon B, Hacke UG. 2014.** Uptake of Water via Branches Helps Timberline Conifers Refill Embolized Xylem in Late Winter. *PLANT PHYSIOLOGY* **164**: 1731–1740.
- Oliveira RS, Dawson TE, Burgess SSO. 2005.** Evidence for direct water absorption by the shoot of the desiccation-tolerant plant *Vellozia flavicans* in the savannas of central Brazil. *Journal of Tropical Ecology* **21**: 585–588.
- Pina ALCB, Zandavalli RB, Oliveira RS, Martins FR, Soares AA. 2016.** Dew absorption by the leaf trichomes of *Combretum leprosum* in the Brazilian semiarid region. *Functional Plant Biology*.
- R Core Team. 2014.** R: A language and environment for statistical computing. R. Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Schönherr J, Bukovak MJ. 1971.** Penetration of stomata by liquids. *Plant Physiology* **49**:813-819.
- Slatyer RO. 1956.** Absorption of water from atmospheres of different humidity and its transport through plants. *Australian Journal of Biological Sciences* **9**: 552–558.
- Still CJ, Foster PN, Schneider SH. 1999.** Simulating the effects of climate change on tropical montane cloud forests. *Nature* **398**: 608–610.

**Venables, W. N. & Ripley, B. D. 2002.** Modern Applied Statistics with S. Fourth Edition. Springer, New York.

**Yates D, Hutley L. 1995.** Foliar Uptake of Water by Wet Leaves of *Sloanea woollsii*, an Australian Subtropical Rainforest Tree. *Australian Journal of Botany* **43**: 157–157.

### Capítulo 3

**Brodersen CR. 2016.** Finding support for theoretical tradeoffs in xylem structure and function. *New Phytologist* **209**: 8–10.

**Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. 2009.** Towards a worldwide wood economics spectrum. *Ecology Letters* **12**: 351–366.

**Chimungu JG, Loades KW, Lynch JP. 2015.** Root anatomical phenes predict root penetration ability and biomechanical properties in maize (*Zea Mays*). *Journal of Experimental Botany* **66**: 3151–3162.

**Gleason SM, Westoby M, Jansen S, Choat B, Hacke UG, Pratt RB, Bhaskar R, Brodribb TJ, Bucci SJ, Cao K-F, et al. 2016.** Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytologist* **209**:123-136.

**Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh K a. 2001.** Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**: 457–461.

**Hanhijarvi H., Wahl P., Rasanen J. & Silvennoinen R. 2003.** Observation of Development of Microcracks on Wood Surface Caused by Drying Stresses. *Holzforschung* **57**:561-565.

**Hook DD, Brown CL, Wetmore RH. 1972.** Aeration in trees. *Botanical Gazette* **133**:443-454.

**Ilic J. 1999.** Shrinkage-related degrade and its association with some physical properties in *Eucalyptus regnans* F. Muell. *Wood science and technology* **33**: 425–437.

**Jacobsen AL, Ewers FW, Pratt RB, Paddock III WA, Davis SD. 2005.** Do xylem fibers affect vessel cavitation resistance? *Plant Physiology* **139**: 546–556.

**Larjavaara M, Muller-Landau HC. 2010.** Rethinking the value of high wood density: Rethinking the value of high wood density. *Functional Ecology* **24**: 701–705.

**Mencuccini M. 2003.** The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell & Environment* **26**: 163–182.

**Oxford Dictionaries. 2015.** [http://www.oxforddictionaries.com/us/definition/american\\_english](http://www.oxforddictionaries.com/us/definition/american_english). Accessed on 10/21/2015 at 10:00.

**Pereira L, Bittencourt PRL, Oliveira RS, Junior MBM, Barros FV, Ribeiro RV, Mazzafera P. 2016.** Plant pneumatics: stem air flow is related to embolism - new perspectives on methods in plant hydraulics. *New Phytologist* doi: 10.1111/nph.13905.

**Savage VM, Bentley LP, Enquist BJ, Sperry JS, Smith DD, Reich PB, von Allmen EI. 2010.** Hydraulic trade-offs and space filling enable better predictions of vascular structure and function in plants. *Proceedings of the National Academy of Sciences* **107**: 22722–22727.

**Schmitz N, Egerton JGG, Lovelock CE, Ball MC. 2012.** Light-dependent maintenance of hydraulic function in mangrove branches: do xylary chloroplasts play a role in embolism repair? *New Phytologist* **195**: 40–46.

**Sperry JS, Meinzer FC, McCulloh K a. 2008.** Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant, cell & environment* **31**: 632–45.

**Wittmann C, Pfanz H. 2014.** Bark and woody tissue photosynthesis: a means to avoid hypoxia or anoxia in developing stem tissues. *Functional Plant Biology* **41**: 940.

**Ziemińska K, Butler DW, Gleason SM, Wright IJ, Westoby M. 2013.** Fibre wall and lumen fractions drive wood density variation across 24 Australian angiosperms. *AoB Plants* **5**: 1–14.

**Key words:** hydraulics, efficiency, space-use, mechanical stress, tradeoff

#### Capítulo 4

- Albaugh TJ, Rubilar RA, Maier CA, Acuña EA, Cook RL. 2017. Biomass and nutrient mass of *Acacia dealbata* and *Eucalyptus globulus* bioenergy plantations. *Biomass and Bioenergy* 97: 162–171.
- André F, Jonard M, Ponette Q. 2010. Biomass and nutrient content of sessile oak (*Quercus petraea* (Matt.) Liebl.) and beech (*Fagus sylvatica* L.) stem and branches in a mixed stand in southern Belgium. *Science of The Total Environment* 408: 2285–2294.
- Becker GS, Braun D, Gliniars R, Dalitz H. 2012. Relations between wood variables and how they relate to tree size variables of tropical African tree species. *Trees* 26: 1101–1112.
- Bittencourt PR, Pereira L, Oliveira RS. 2016. On xylem hydraulic efficiencies, wood space-use and the safety–efficiency tradeoff. *New Phytologist* 211: 1152–1155.
- Bond WJ. 2010. Do nutrient-poor soils inhibit development of forests? A nutrient stock analysis. *Plant and Soil* 334: 47–60.
- Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Franco AC, Campanello PI, Villalobos-Vega R, Bustamante M, Miralles-Wilhelm F. 2006. Nutrient availability constrains the hydraulic architecture and water relations of savannah trees. *Plant, Cell and Environment* 29: 2153–2167.
- Buhtz A, Kolasa A, Arlt K, Walz C, Kehr J. 2004. Xylem sap protein composition is conserved among different plant species. *Planta* 219.
- Cernusak LA, Winter K, Turner BL. 2011. Transpiration modulates phosphorus acquisition in tropical tree seedlings. *Tree Physiology* 31: 878–885.
- Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12: 351–66.
- Cleveland CC, Townsend AR, Taylor P, Alvarez-Clare S, Bustamante MMC, Chuyong G, Dobrowski SZ, Grierson P, Harms KE, Houlton BZ, *et al.* 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis: Nutrients, climate and tropical NPP. *Ecology Letters* 14: 939–947.
- Condit R, Engelbrecht BMJ, Pino D, Perez R, Turner BL. 2013. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences* 110: 5064–5068.
- Coomes DA, Jenkins KL, Cole LE. 2007. Scaling of tree vascular transport systems along gradients of nutrient supply and altitude. *Biology Letters* 3: 87–90.
- Cruziat P, Cochard H, Améglio T. 2002. Hydraulic architecture of trees: main concepts and results. *Annals of forest science* 59: 723–752.

- Eller C, de V. Barros F, R.L. Bittencourt P, Rowland L, Mencuccini M, S. Oliveira R. 2018. Xylem hydraulic safety and construction costs determine tropical tree growth: Tree growth vs hydraulic safety trade-off. *Plant, Cell & Environment*.
- Espino S, Schenk HJ. 2011. Mind the bubbles: achieving stable measurements of maximum hydraulic conductivity through woody plant samples. *Journal of experimental botany* 62: 1119–32.
- Gessler A, Schaub M, McDowell NG. 2017. The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* 214: 513–520.
- Gleason SM, Westoby M, Jansen S, Choat B, Hacke UG, Pratt RB, Bhaskar R, Brodribb TJ, Bucci SJ, Cao K-F, *et al.* 2015. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytologist*: n/a-n/a.
- Goldstein G, Bucci SJ, Scholz FG. 2013. Why do trees adjust water relations and hydraulic architecture in response to nutrient availability? *Tree Physiology* 33: 238–240.
- Hagen-Thorn A, Armolaitis K, Callesen I, Stjernquist I. 2004. Macronutrients in tree stems and foliage: a comparative study of six temperate forest species planted at the same sites. *Annals of Forest Science* 61: 489–498.
- Harvey HP, Van Den Driessche R. 1997. Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiology* 17: 647–654.
- Heineman KD, Turner BL, Dalling JW. 2016. Variation in wood nutrients along a tropical soil fertility gradient. *New Phytologist* 211: 440–454.
- Iwai H, Usui M, Hoshino H, Kamada H, Matsunaga T, Kakegawa K, Ishii T, Satoh S. 2003. Analysis of sugars in squash xylem sap. *Plant and cell physiology* 44: 582–587.
- Jacobsen AL. 2005. Do Xylem Fibers Affect Vessel Cavitation Resistance? *PLANT PHYSIOLOGY* 139: 546–556.
- Jin J, Tang C, Sale P. 2015. The impact of elevated carbon dioxide on the phosphorus nutrition of plants: a review. *Annals of Botany* 116: 987–999.
- John R, Dalling JW, Harms KE, Yavitt JB, Stallard RF, Mirabello M, Hubbell SP, Valencia R, Navarrete H, Vallejo M. 2007. Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences* 104: 864–869.
- Johnson DM, Wortemann R, McCulloh KA, Jordan-Meille L, Ward E, Warren JM, Palmroth S, Domec J-C. 2016. A test of the hydraulic vulnerability segmentation hypothesis in angiosperm and conifer tree species (N Phillips, Ed.). *Tree Physiology* 36: 983–993.
- Johnsna CM, Vieirab IC, Zarinc DJ, Frizanoa J, Johnsna AH. 2001. Carbon and nutrient storage in primary and secondary forests in eastern Amazonia. *Forest Ecology and Management* 147: 252.

- Kamil B. 2016. MuMIn: Multi-Model Inference. R package version 1.15.6. <http://CRAN.R-project.org/package=MuMIn>
- Kerkhoff AJ, Fagan WF, Elser JJ, Enquist BJ. 2006. Phylogenetic and Growth Form Variation in the Scaling of Nitrogen and Phosphorus in the Seed Plants. *The American Naturalist* 168: E103–E122.
- Ketterings, QM, Coe, R, Noordwijk, M, Ambagau, Yakub, Palm, CA. 2001. Reducing uncertainty in the use of allometric biomass equations for predicting above-ground tree biomass in mixed secondary forests. *Forest Ecology and Management* 146:199-209.
- Larjavaara M, Muller-Landau HC. 2010. PERSPECTIVE: Rethinking the value of high wood density: Rethinking the value of high wood density. *Functional Ecology* 24: 701–705.
- Lynch JP, Ho MD, phosphorus L. 2005. Rhizoeconomics: Carbon costs of phosphorus acquisition. *Plant and Soil* 269: 45–56.
- Malavolta, E, Vitti, GC, Oliveira SA. 1997. Avaliação do estado nutricional de plantas: princípios e aplicações. Ed. Potafos 2nd Edition. Piracicaba 319p.
- Martin AR, Erickson DL, Kress WJ, Thomas SC. 2014. Wood nitrogen concentrations in tropical trees: phylogenetic patterns and ecological correlates. *New Phytologist* 204: 484–495.
- Martin-StPaul NK, Longepierre D, Huc R, Delzon S, Burlett R, Joffre R, Rambal S, Cochard H. 2014. How reliable are methods to assess xylem vulnerability to cavitation? The issue of ‘open vessel’ artifact in oaks. *Tree Physiology* 34: 894–905.
- Meerts P. 2002. Mineral nutrient concentrations in sapwood and heartwood: a literature review. *Annals of Forest Science* 59: 713–722.
- Morris H, Gillingham MAF, Plavcová L, Gleason SM, Olson ME, Coomes DA, Fichtler E, Klepsch MM, Martínez-Cabrera HI, McGlenn DJ, *et al.* 2017. Vessel diameter is related to amount and spatial arrangement of axial parenchyma in woody angiosperms. *Plant, Cell & Environment*.
- Morris H, Plavcová L, Cvecko P, Fichtler E, Gillingham MAF, Martínez-Cabrera HI, McGlenn DJ, Wheeler E, Zheng J, Ziemińska K, *et al.* 2015. A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. *New Phytologist*: n/a-n/a.
- Nardini A, Salleo S, Jansen S. 2011. More than just a vulnerable pipeline: xylem physiology in the light of ion-mediated regulation of plant water transport. *Journal of Experimental Botany* 62: 4701–4718.
- Pammenter NW, Vander Willigen C. 1998. A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree physiology* 18: 589–593.
- Pereira L, Bittencourt PRL, Oliveira RS, Junior MBM, Barros FV, Ribeiro RV, Mazzafera P. 2016. Plant pneumatics: stem air flow is related to embolism - new perspectives on methods in plant hydraulics. *New Phytologist*: n/a-n/a.

Pereira L, Domingues-Junior AP, Jansen S, Choat B, Mazzafera P. 2017. Is embolism resistance in plant xylem associated with quantity and characteristics of lignin? *Trees*.

Pereira L, Mazzafera P. 2012. A low cost apparatus for measuring the xylem hydraulic conductance in plants. *Bragantia* 71: 583–587.

Pereira L, Flores-Borges DNA, Bittencourt PRLB, Mayer JLS, Kiyota E, Araújo P, Jansen S, Freitas RO, Oliveira RS, Mazzafera P. The chemical nature of xylem pit membranes based on infrared nanospectroscopy. Submitted to *Plant Physiology*.

Pfautsch S, Renard J, Tjoelker MG, Salih A. 2015. Phloem as Capacitor: Radial Transfer of Water into Xylem of Tree Stems Occurs via Symplastic Transport in Ray Parenchyma. *Plant Physiology* 167: 963–971.

Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team. 2014. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-118. <http://CRAN.R-project.org/package=nlme>

Poorter H, Jagodzinski AM, Ruiz-Peinado R, Kuyah S, Luo Y, Oleksyn J, Usoltsev VA, Buckley TN, Reich PB, Sack L. 2015. How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. *New Phytologist* 208: 736–749.

Poorter L, McDonald I, Alarcón A, Fichtler E, Licona J-C, Peña-Claros M, Sterck F, Villegas Z, Sass-Klaassen U. 2010. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytologist* 185: 481–492.

Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmerler J, *et al.* 2010. Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* 7: 1515–1541.

Quesada CA, Phillips OL, Schwarz M, Czimczik CI, Baker TR, Patiño S, Fyllas NM, Hodnett MG, Herrera R, Almeida S, *et al.* 2012. Basin-wide variations in Amazon forest structure and function are mediated by both soils and climate. *Biogeosciences* 9: 2203–2246.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>

Rowland L, da Costa ACL, Galbraith DR, Oliveira RS, Binks OJ, Oliveira AAR, Pullen AM, Doughty CE, Metcalfe DB, Vasconcelos SS, *et al.* 2015. Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature*.

Rowland, L *et al.* 2017. Drought and tree size alter total woody tissue respiration and its growth and maintenance components in tropical forests. *New Phytologist*, accepted.

Ruivo MLP, Cunha ES. 2003. Mineral And Organic Components In Archaeological Black Earth And Yellow Latosol In Caxiuang, Amazon, Brazil. *WIT Transactions on Ecology and the Environment* 64.



- Samuelson LJ, Farris MG, Stokes TA, Coleman MD. 2008. Fertilization but not irrigation influences hydraulic traits in plantation-grown loblolly pine. *Forest Ecology and Management* 255: 3331–3339.
- Sardans J, Peñuelas J. 2013. Tree growth changes with climate and forest type are associated with relative allocation of nutrients, especially phosphorus, to leaves and wood: Tree stoichiometry and growth. *Global Ecology and Biogeography* 22: 494–507.
- Schenk HJ, Espino S, Romo DM, Nima N, Do AYT, Michaud JM, Papahadjopoulos-Sternberg B, Yang J, Zuo YY, Steppe K, *et al.* 2017. Xylem Surfactants Introduce a New Element to the Cohesion-Tension Theory. *Plant Physiology* 173: 1177–1196.
- Schuldt B, Leuschner C, Brock N, Horna V. 2013. Changes in wood density, wood anatomy and hydraulic properties of the xylem along the root-to-shoot flow path in tropical rainforest trees. *Tree physiology*: 161–174.
- Secchi F, Pagliarani C, Zwieniecki MA. 2017. The functional role of xylem parenchyma cells and aquaporins during recovery from severe water stress: Response of xylem parenchyma cells to embolism. *Plant, Cell & Environment* 40: 858–871.
- Sperry JS, Donnelly JR, Tyree MT. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment* 11: 35–40.
- Teskey RO, Saveyn A, Steppe K, McGuire MA. 2007. Origin, fate and significance of CO<sub>2</sub> in tree stems. *New Phytologist* 0: 071120093824001–???
- Tuomi J, Kytöviitta MM & Hardling R. 2001. Cost efficiency of nutrient acquisition and the advantage of mycorrhizal symbiosis for the host plant. *Oikos* 92:62-70.
- van Raij, BV, Andrade JC, Oliveira, Cantarella H., Quaaggio, JA. 2001. Análise química para avaliação de fertilidade de solos tropicais. Instituto Agronômico de Campinas 285p.
- Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W. 2010. Lignin Biosynthesis and Structure. *PLANT PHYSIOLOGY* 153: 895–905.
- Venturas MD, Mackinnon ED, Jacobsen AL, Pratt RB. 2015. Excising stem samples underwater at native tension does not induce xylem cavitation: No evidence for a tension-cutting artefact. *Plant, Cell & Environment* 38: 1060–1068.
- Whitehead DL, Quicke GV. 1960. The nitrogen content of grass lignin. *Journal of the Science of Food and Agriculture* 11: 151–152.
- Wieder WR, Cleveland CC, Smith WK, Todd-Brown K. 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience* 8: 441–444.
- Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EV, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* 92: 1616–1625.

Yamada Y, Awano T, Fujita M, Takabe K. 2011. Living wood fibers act as large-capacity “single-use” starch storage in black locust (*Robinia pseudoacacia*). *Trees* 25: 607–616.

Zanne AE, Oberle B, Dunham KM, Milo AM, Walton ML, Young DF. 2015. A deteriorating state of affairs: How endogenous and exogenous factors determine plant decay rates (R Bardgett, Ed.). *Journal of Ecology* 103: 1421–1431.

Zanne AE, Westoby M, Falster DS, Ackerly DD, Loarie SR, Arnold SEJ, Coomes DA. 2010. Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. *American Journal of Botany* 97: 207–215.

Zieminska K, Butler DW, Gleason SM, Wright IJ, Westoby M. 2013. Fibre wall and lumen fractions drive wood density variation across 24 Australian angiosperms. *AoB PLANTS* 5: plt046-plt046.

### Discussão

**Berry ZC, White JC, Smith WK. 2014.** Foliar uptake, carbon fluxes and water status are affected by the timing of daily fog in saplings from a threatened cloud forest. *Tree Physiology* 34: 459–470.

**Beysens D, Muselli M, Milimouk I, Ohayon C, Berkowicz S, Soyeux E, Mileta M, Ortega P. 2006.** Application of passive radiative cooling for dew condensation. *Energy* 31: 2303–2315.

**Binks O., Meir P., Rowland L, Costa ACL, Bitencourt PRL, Eller C, Salles G, Soza A, Ferreira L, Vasconcelos SS, Oliveira R, Mencuccini M.** Foliar uptake in Amazon rainforest trees and its impact on hydraulic stress. Submitted to *New Phytologist*.

**Bond WJ. 2010.** Do nutrient-poor soils inhibit development of forests? A nutrient stock analysis. *Plant and Soil* 334: 47–60.

**Eller CB, Lima AL, Oliveira RS. 2013.** Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *The New phytologist* 199: 151–161.

**Eller CB, Lima AL, Oliveira RS. 2016.** Cloud forest trees with higher foliar water uptake capacity and anisohydric behavior are more vulnerable to drought and climate change. *New Phytologist* 211: 489–501.

**Gessler A, Schaub M, McDowell NG. 2017.** The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* 214: 513–520.

**Johnsna CM, Vieirab IC, Zarinc DJ, Frizanoa J, Johnsna AH. 2001.** Carbon and nutrient storage in primary and secondary forests in eastern Amazoãnia. *Forest Ecology and Management* **147**: 252.

**Lopes AP, Nelson BW, Wu J, Graça PML de A, Tavares JV, Prohaska N, Martins GA, Saleska SR. 2016.** Leaf flush drives dry season green-up of the Central Amazon. *Remote Sensing of Environment* **182**: 90–98.

**Martin CE, von Willert D. 2000.** Leaf Epidermal Hydathodes and the Ecophysiological Consequences of Foliar Water Uptake in Species of *Crassula* from the Namib Desert in Southern Africa. *Plant Biology* **2**: 229–242.

**Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, et al. 2013.** New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**: 167.

## Declaração – Bioética e Biossegurança



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### DECLARAÇÃO

Em observância ao §5º do Artigo 1º da Informação CCPG-UNICAMP/001/15, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "*Ecophysiology and Ecohydrology of a Tropical Montane Forest and a Tropical Raniforest and a Tropical Cloud Forest*", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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Data: 16 de Fevereiro de 2018

# Declaração – Direitos Autorais

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As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **Ecophysiology and Ecohydrology of a Tropical Rainforest and a Tropical Cloud Forest**, não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 16 de fevereiro de 2018

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