DEBORAH MATTOS GUIMARÃES APGAUA

FUNCTIONAL ANATOMY AND WATER TRANSPORT STRATEGIES OF RAINFOREST PLANTS

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Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Engenharia Florestal, área de concentração em ecologia, para a obtenção do título de Doutora.

Orientador

Dr. Rubens Manoel dos Santos

Coorientadora

Dr. Susan G. W. Laurance

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GENERAL ABSTRACT

Tropical rainforests are key terrestrial ecosystems involved in the maintenance of earth's biodiversity and carbon budget but are sensitive to climate change. Because rainforests typically occur in environments of high rainfall, climate change induced drought events is likely to result in extensive plant mortality, resulting in shifts of community composition and stand biomass. Lowland tropical rainforests are of particular concern, as their vulnerability to drought is still not well understood. How species in these rainforests will cope with such droughts will dependent, among others factors, on the strategies that these plants use to transport water. Based on that, I adopted both a species- and ecosystem-level approaches in this thesis to study hydraulic-related functional traits of plants. The structure of my thesis is as follows - the first part consists of a general introduction and literature review, and the second part consists of two articles (one published and the other in revision) pertaining to my research. These studies were developed during two years of an internship resulted from a collaboration between Federal University of Lavras, Brazil, and James Cook University, Australia, and were conducted in tropical lowland rainforest in Daintree National Park, northeast Australia. In the first manuscript, I used a multidisciplinary approach involving vegetation ecology, plant physiology and anatomy to conduct a study on water use strategies of eight species of rainforest trees. In the second manuscript, I investigated how wood traits relates to leaf water use efficiency across 90 plant species in six functional groups (Mature-phase trees, Understorey trees and –shrubs, Pioneer trees and –shrubs, and vines). Overall, my results suggest that different plant functional groups exhibit various contrasting water use strategies, and also that leaf-level physiological processes and wood anatomy are coordinated. Models for predicting changes in vegetation during climate change scenarios can benefit from a biophysical approach involving the use of wood and leaf anatomical trait data combined with physiological measurements (i.e. sap flow rates, intrinsic water-use-efficiency). Such integrated approaches are becoming increasingly useful for contextualizing plants responses to drought, particularly for species-rich ecosystems such as tropical lowland rainforests.

Keywords: Lowland tropical rainforests. Climate change. Functional traits. Hydraulic traits. Plant functional groups.

RESUMO GERAL

Florestas tropicais úmidas são importantes ecossistemas terrestres envolvidos na manutenção da biodiversidade e balanço de carbono na Terra, mas são sensíveis às mudanças climáticas. Por ocorrem tipicamente em ambientes de alta pluviosidade, secas causadas pelas mudanças climáticas podem levar a um aumento da mortalidade, resultando em alterações na composição da comunidade e na biomassa da floresta. As florestas tropicais úmidas de terra baixa são de especial preocupação, uma vez que sua vulnerabilidade à seca ainda não é bem compreendida. Porém, sabe-se que a resposta das plantas à esta condição irá depender, entre outros fatores, das estratégias utilizadas para o transporte de água. Diante disso, utilizou-se nesta tese tanto abordagens a nível de espécies quanto a nível de comunidade para estudar traços funcionais relacionados com as estratégias hidráulicas das plantas. A presente tese está estruturada na seguinte forma - a primeira parte consiste na introdução geral e na revisão de literatura, e a segunda parte consiste em dois artigos (um publicado e outro em revisão) relacionados à esta pesquisa. Estes estudos foram desenvolvidos durante dois anos de intercâmbio resultado de colaboração entre a Universidade Federal de Lavras, Brasil, e a James Cook University, Austrália e foram conduzidos em floresta tropical úmida de terra baixa localizada em Daintree National Park, nordeste da Austrália. No primeiro manuscrito, utilizou-se abordagem multidisciplinar envolvendo ecologia da vegetação, fisiologia e anatomia de plantas para conduzir um estudo estratégias do uso da água em oito espécies de árvores de floresta úmida. No segundo manuscrito, foi investigado como traços funcionais da madeira se relacionam as estratégias do uso da água em 90 espécies pertencentes à seis grupos funcionais (árvore de fase madura, árvore de sub-bosque, árvore pioneira, arbusto de sub-bosque, arbusto pioneiro e lianas). Em geral, os resultados sugerem que diferentes grupos funcionais de plantas exibem muitas estratégias contrastantes do uso da água, e também que processos fisiológicos a nível de folha e anatomia da madeira são coordenados. Modelos para predizer mudanças na vegetação durante mudanças climáticas podem se beneficiar de uma abordagem biofísica envolvendo o uso de traços anatômicos da madeira e das folhas combinados com medidas fisiológicas (ex. taxas do fluxo de seiva, eficiência intrínseca do uso da água). Estas abordagens integradas estão se tornando cada vez mais úteis para se contextualizar as respostas das plantas à seca, particularmente em ecossistemas ricos em espécies como as florestas úmidas de terra baixa.

Palavras-chave: Floresta úmida de terra baixa. Mudanças climáticas. Traços funcionais. Traços hidráulicos. Grupos funcionais de plantas.

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SUMMARY

FIRST PART

1. GENERAL INTRODUCTION

Tropical rainforests rank among the most important terrestrial ecosystems on Earth. In addition to the well-established fact that they support the richest biological diversity of any terrestrial community (HEYWOOD, 1995), tropical rainforest also play crucial roles in the global carbon budget and cycling (MALHI; GRACE, 2000). Being distributed in the highly productive tropical zone has also placed these forest systems under huge pressures for clearing for agriculture and other human land use (RUDEL, 2013). However, the most pressing concern for the future of tropical rainforest is the potential negative impacts of anthropogenic climate change. Despite having existed for over 60 millions years (JHONSON; ELLIS, 2002; MORLEY, 2000), tropical rainforest appear to be highly vulnerable to climatic instability, and particularly to drought (ENGELBRECH et al., 2007; PHILLIPS et al., 2009).

Water is a common resource for plants adapted to the rainforest ecosystem, and how efficient a given species is at conducting water will determine the species/individual-level influence on forest productivity and carbon gain (FAN et al., 2012; FARQUHAR et al., 1989). Recently, various studies have demonstrated negative impacts of drought on tropical rainforests. Drought events have been linked to change in vegetation dynamics by increasing tree and seedling mortality (ALLEN et al., 2010; CONDIT; HUBBELL; FOSTER, 1995; EDWARDS; KROCKENBERGER, 2006; PHILLIPS et al., 2009) and also through modifying ecological processes like plant growth and recruitment (LAURANCE et al., 2009; ZHAO; RUNNING, 2010).

Given these alarming concerns and the fact that plant hydraulics play a key role in the adaptive biology of rainforest plants, there is a growing need for understanding plant water use strategies in rainforest ecosystems. For the purpose of this thesis, I define water use strategies as the combination of hydraulic-related traits that a plant possesses. Specifically, these relate

specifically to wood xylem traits involved in the transport of water through a plant (SCHOLZ et al., 2013; TYREE; ZIMMERMANN, 2002).

Many studies on plant hydraulics to date are physiological in their focus, and are often limited to a few species at a time due to the time investment necessary for making physiological measurements. While these studies are of great importance for understanding plant-level responses, there is an increasing need to understand plant hydraulics from an ecosystem-level perspective. Consequently, there is a necessity for increasing species coverage, particularly in species-rich tropical ecosystems, and this may be achievable by adopting a trait-based approach to delineating water use strategies.

Moreover, the rainforest biome is comprised of complex strata with different life forms and successional stages (plant functional groups) having contrasting light demands and structural requirements. To the best of my knowledge, the water use strategies of these plant functional groups have not been comprehensively examined in a single study. Understanding the differences in water use strategies between functional groups in a rainforest ecosystem can therefore provide a more holistic framework for predict changes in vegetation during climate change scenarios.

The aims of my thesis are therefore to examine:

1. How do traits related with plant water transport vary in co-occurring lowland tropical rainforest trees?
2. How do wood hydraulic traits vary across different plant functional groups in a lowland tropical rainforest?

2. LITERATURE REVIEW

2.1 **Climate Change**

Since biophysical processes on earth are interconnected, changes in temperature and rainfall affecting carbon and water cycles will have direct influences on vegetation (QIN et al., 2014). For instance, a rise in temperature has been documented to result in more frequent wildfires and drought in some regions (CHRISTENSEN et al., 2007). Several droughts such as those related to the El Niño Southern Oscillation (ENSO) effect caused by an increase of sea surface temperature (TRENBERTH, 1997), are predicted to have severe negative impacts on forests across the globe, and particularly those located in tropical regions (INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE IPCC, 2007). These effects constitute a big concern for earth's biodiversity.

Two general drought patterns have been already documented in the world's tropical rainforests: prolonged droughts caused by the annual lack of wet season, or an increase in dry season severity and duration (ARAGÃO et al., 2008; LAURANCE; ANDRADE; LAURANCE, 2010; MARENGO et al., 2005; VAN NIEUWSTADT; SHEIL, 2005). Typically, tropical rainforests have a dry season not exceeding 2-3 months, and little if any tree damage occurs during these naturally short periods of water deficit. However, when droughts are more prolonged or intense than the expected dry season for tropical regions, increase tree and seedling death can occur, as was observed in the 1997/98 El Niño drought across large parts of Amazonia and Borneo (ARAGÃO et al., 2008; VAN NIEUWSTADT; SHEIL, 2005). In the past two decades, the volume of research on the effects of climate change on tree mortality has increased significantly (ADAMS et al., 2009; ALLEN et al., 2010; CHOAT et al., 2012; DALE et al., 2001; HILKER et al., 2014). However, still little is

known about the functional trait behavior that characterize plant species susceptibility to drought.

2.2 Tropical Rainforest vulnerability to drought

Tropical rainforests are among the most ecologically complex and biodiverse ecosystems on the planet, and harbor the bulk of the world's woody plant diversity (GENTRY, 1992; RICHARDS, 1996; SLIK et al., 2015). Tropical landscapes are also notable for their extremely high productivity. For this reason, they contribute significantly to the global carbon budget and cycling in the planet, and many studies have been dedicated to estimate forest annual net carbon and biomass (CHAVE et al., 2005; CRAMER et al., 2004; MALHI; GRACE, 2000; PHILLIPS et al., 1998). Extensive plant mortality in the tropics resulting from climate change can therefore have impacts on atmosphere carbon dioxide, building up future greenhouse gas concentrations (CRAMER et al., 2004).

Many studies have shown how tropical rainforest ecosystems are vulnerable to extensive dry periods. For instance, Van Nieuwstadt and Sheil (2005) described an 20 to 26% increase in tree mortality in eighteen 1.8ha forest monitoring plots in Borneo after an entensive drought. These negative effects also continued two years post-drought. In another study conducted in twenty 1-ha plots located on Amazon rainforest, Laurance et al. (2009) detected an the increase in tree mortality associated with dry periods resulting from climate change. In a seven-year drought experiment in Amazon, Costa et al. (2010) found that annual tree mortality in a plot with drought treatment was double that of a nearby control plot. This study also documented a much lower wood productivity and loss in carbon compared to the control plot.

Until now, there has been no targeted and comprehensive investigation of water use strategies of different functional groups (i.e. understorey and

pioneer shrubs, and vines) within a tropical ecosystem. Plant functional traits can help predict the most adaptable and the most sensitive plant species to drought (APGAUA et al., 2015; BARBOSA, 2008; HOEBER et al., 2014; SKELTON; WEST; DAWSON, 2015) and serve as a sensitive good indicator of climate change effects on vegetation.

2.3 Functional traits as a tool for understanding plant water use strategies

For the purpose of this thesis, functional traits are therefore defined any anatomical, morphological and physiological trait that represents ecological strategies and determine how plants respond to environmental factor (PÉREZ-HARGUINDENGUY et al., 2013). Functional traits can be used to test hypothesis at various levels of organization (species to ecosystems), bypassing the limitations that high-level taxonomic classifications can issue on those studies (CORNELISSEN et al., 2003). The utility of functional traits have resulted in standardized protocols (CORNELISSEN et al., 2003; PÉREZ-HARGUINDEGUY et al., 2013; SCHOLZ et al., 2013) that have been compiled to guide the type of data collected for comparison and compilation in global data bases (i.e. TRY Plant Trait Database) (KATTGE et al., 2011). For the past two decades therefore, plant ecologists have routinely used functional traits to test ecological theory, and to understand plant ecological strategies and adaptation to environmental stress. (PÉREZ-HARGUINDEGUY et al., 2013; WESTOBY, 1998).

Investigating traits specifically related to how plants conduct water is a logical approach for understanding how drought events affect tropical rainforest plants requires. Water is involved in many vital plant metabolic processes (i.e. photosynthesis, transpiration and growth). During photosynthesis for instance, plants need to open the stomata to allow the entry of CO_2 and this process results

in water loss. This loss necessitates drawing water from the soil, establishing a plant hydraulic system that involves a soil-plant-atmosphere continuum (COWAN, 1965). While plant physiologists have had a long tradition studying plant hydraulics (BRODRIBB; FIELD; JORDAN, 2007; GLEASON et al., 2016; TYREE; ZIMMERMANN, 2002), ecologists have only recently started to incorporate plant hydraulics into ecological studies. Wood and leaves are plant organs directly involved in plant hydraulics. Their biophysical and functional anatomy traits (i.e. wood density, xylem vessel size, leaf area and leaf dry matter content) can be used to characterize the strategies used by different species of plants to optimize water conductivity. Other physiological process (i.e. sap flow rates, intrinsic water use efficiency, growth rates) can be used as response variables for modelling how biophysical and functional anatomy traits affect water transport.

2.4 Hydraulic traits: wood and leaf economic spectrum

The evolution of vessels provided angiosperms an efficient water conductivity that could be key reason for their success in terrestrial biomes (PAMMENTER; MIDGLEY; BOND, 2004; TYREE; ZIMMERMANN, 2002).

Wood traits, such as wood density and xylem anatomical traits, serve various ecological functions like the mechanism for support, water conductance and storage, and growth ability (BAAS et al., 2004; FAN et al., 2012), and have come to known as part of the worldwide wood economic spectrum (Fig. 1) (CHAVE et al., 2009; POORTER et al., 2010). For example, wood density is correlated with plant performance and successional behavior of plants (CHAVE et al., 2009). Fast growing and colonizing species are therefore often observed to have low wood densities (CHAVE et al., 2006; WRIGHT et al., 2003). Importantly, wood density is has also been positively correlated with drought resistance (HACKE et al., 2001; JACOBSEN et al., 2005; PRATT et al. 2007)

through increasing the strength of fibres associated with vessels and possibly increasing the resistance of sapwood to xylem embolism (HACKE et al., 2001; JACOBSEN et al., 2005). However, Fan et al. (2012) have shown that xylem traits (i.e. vessels diameter) can predict plant performance even better than wood density in 40 species of Asian tropical trees. Hoeber et al. (2014) also found a correlation between xylem traits and growth rates in a tropical semi- dry forest, but found wood density as a reliable indicator for this environment response.

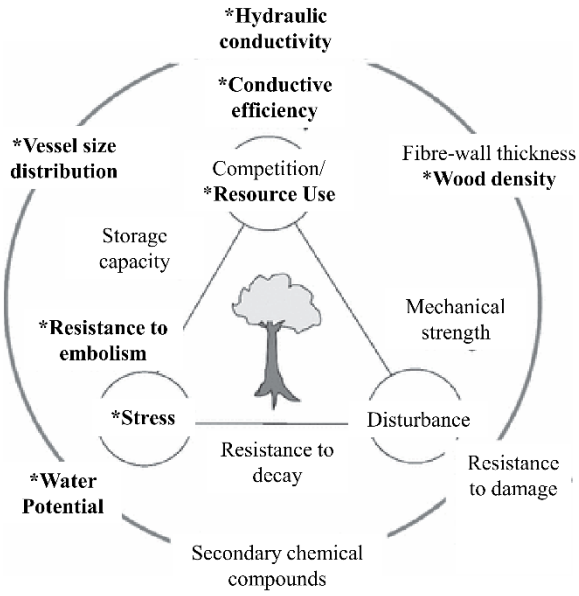

Fig.1 The role of wood in major plant ecological functions (competitive ability, resistance to stress and disturbance). The outer circle relates the main wood traits associated with these wood properties (see also Table 1) Traits examined in this thesis are in bold with an asterisk (*). Figure modified from Chave et al. (2009).

Vessel diameters in the xylem are important for water conductance efficiency because according to the Hagen-Poiseulle law, the flow rate of water

through a vessel is proportional to the fourth power of the radius of the vessel (TYREE; EWERS, 1991; TYREE; ZIMMERMANN, 2002). The cohesion-tension theory (DIXON, 1914) postulates that water flows through plant vessels under negative pressure gradient generated by a transpirational stream (TYREE; ZIMMERMANN, 2002). Because vessels sizes can differ greatly within and across species, plants theoretically exhibit a trade-off between safety and efficiency, coordinating differently in contrasting environments (GLEASON et al., 2016). Big vessel, whilst efficient for water transport, are at a higher risk of cavitation, or the breaking of the water column formed by different pressure between the external environment and inside the vessel (TYREE; ZIMMERMANN, 2002). The results of this breakage may be irreversible, due to the formation of bubbles, or embolism. On the other hand, a small vessel is theoretically much safer, but will have a much lower capacity for water conductance. Consequently, plants exhibit various trait strategies to avoid this condition.

Leaves are the sites of transpiration and the main organ of photosynthesis in plants, and leaf traits that play important functional roles have therefore come to be known as part of the leaf economical spectrum (DONOVAN et al., 2011; WRIGHT et al., 2004). A number of leaf traits are also of relevance to plant hydraulics (PÉREZ-HARGUINDEGUY et al., 2013). For example, leaf area, leaf dry matter content and leaf vein density play important roles in the control of transpiration rates (PÉREZ-HARGUINDEGUY et al., 2013; SACK; FROLE, 2006). In addition, plant photosynthesis and hydraulic processes are intrinsically linked (BRODRIBB; FIELD, 2000; BRODRIBB; FIELD; JORDAN, 2007). Leaf carbon isotope discrimination for instance, reflects plant intrinsic water–use efficiency, which is a measure how much water is lost during the process of transpiration per unit of CO_2 fixed during photosynthesis (DAWSON et al., 2002; FARQUHAR et al., 1989).

3 CONCLUSION

Drought is a worrisome effect of climate change that can have deleterious effects on terrestrial ecosystems, such as increased tree mortality and changes in the rainforest dynamics. While studying the effects of drought on plants have traditionally been within the domain of plant physiologists, the number of ecological studies examining the topic are on the rise. These studies often involve multidisciplinary approaches such as ecological modelling, and the use of functional traits as proxies for plant function. The targeted use of hydraulic wood and leaf functional traits is a useful way of contextualizing plants responses to drought, and can be particularly useful in species rich ecosystems such as in a tropical rainforest. Such trait data will also be of great utility for modelling species distributional shifts in tropical regions in climate change scenarios.

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SECOND PART

MANUSCRIPT 1

FUNCTIONAL TRAITS AND WATER TRANSPORT STRATEGIES IN LOWLAND TROPICAL RAINFOREST TREES

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ABSTRACT

Understanding how tropical rainforest trees may respond to the precipitation extremes predicted in future climate change scenarios is paramount for their conservation and management. Tree species clearly differ in drought susceptibility, suggesting that variable water transport strategies exist. Using a multi-disciplinary approach, we examined the hydraulic variability in trees in a lowland tropical rainforest in north-eastern Australia. We studied eight tree species representing broad plant functional groups (one palm and seven eudicot mature-phase, and early-successional trees). We characterised the species' hydraulic system through maximum rates of volumetric sap flow and velocities using the heat ratio method, and measured rates of tree growth and several stem, vessel, and leaf traits. Sap flow measures exhibited limited variability across species, although early-successional species and palms had high mean sap velocities relative to most mature-phase species. Stem, vessel, and leaf traits were poor predictors of sap flow measures. However, these traits exhibited different associations in multivariate analysis, revealing gradients in some traits across species and alternative hydraulic strategies in others. Trait differences across and within tree functional groups reflect variation in water transport and drought resistance strategies. These varying strategies will help in our understanding of changing species distributions under predicted drought scenarios.

Keywords: drought, flow rate, leaves, plant anatomy, rainforest, trees, water resources

RESUMO

A compreensão de como árvores de florestas úmidas tropicais podem responder aos extremos pluviométricos previstos em futuros cenários de mudanças climáticas, é essencial para sua conservação e manejo. Espécies arbóreas diferem claramente em termos de susceptibilidade à seca, sugerindo que existe variação em transporte da água. Utilizando-se abordagem multidisciplinar, foi examinada a variabilidade hidráulica em árvores de Floresta úmida de terra baixa no nordeste da Austrália. Foram estudadas oito espécies arbóreas representando grupos funcionais (uma palmeira e sete eudicotiledônias de estágios sucessionais primário e tardio). Foi caracterizada o sistema hidráulico das espécies por meio de taxas máximas do volume e velocidade do fluxo de seiva utilizando-se o método de razão de calor. Também foram medidas taxas de crescimento e vários traços do caule, vasos e folhas. Medidas do fluxo de seiva apresentaram variabilidade limitada, apesar das espécies de estágio sucessional primário e a palmeira apresentarem alta média da velocidade de seiva relativa à maioria de espécies de estagio sucessional tardio. Traços do caule, vasos e folhas foram fracos indicadores das medidas do fluxo de seiva. Entretanto, esses traços exibiram diferentes associações em análises multivariadas, revelando gradientes entre espécies para alguns traços e estratégias hidráulicas alternativas em outros. Diferenças nos traços entre e dentro de grupos funcionais de árvores refletem variações em estratégias para o transporte de água e para resistência à seca. Essas variações em estratégias ajudarão no nosso entendimento da mudança da distribuição de espécies sob cenários de seca previstos.

Palavras-chave: seca, taxa de fluxo, folhas, anatomia de plantas, floresta úmida, árvores, recursos hídricos

1 INTRODUCTION

One of the major threats to earth's biodiversity are precipitation extremes predicted in future climate change scenarios [1]. In particular, tropical forests are likely to experience more frequent and severe droughts and increased aridity [2, 3], which can result in high levels of hydraulic stress in trees, loss of biomass and increased mortality [4, 5]. Drought is also likely to shape species' distributions in tropical forests [6, 7]. The resilience of tropical forests to such events will therefore depend collectively on the strategies trees use to transport water and respond to water deficits.

Tree water transport can be studied by measuring rates of volumetric sap flow and sap velocities [8, 9]. Over the past decade, researchers have been adopting an increasingly multidisciplinary approach to study plant water transport incorporating the measurement of several stem and leaf traits [10, 11]. Such functional traits encompass morphological and physiological features that regulate the ecological functioning of a species [12]. Leaf area and leaf length to breadth ratio (i.e. leaf slenderness) for instance, determines the available area for transpiration [13]. Other leaf traits such as leaf mass per area are strongly correlated with key biological processes, including growth rate [14] and photosynthetic capacity [15], which in turn may be directly or indirectly related to plant water use [16].

Wood density is a key functional trait that is positively correlated with drought resistance [17] by increasing the strength of fibres associated with vessels [18], and by increasing the resistance of sapwood to xylem embolism [19]. However, the universality of this relationship is in question as even within a single biome, for example, rainforests, trees exhibit a wide range of wood densities [20, 21]. Rather, differences in wood density may relate more to adjustments in vessel characteristics such as vessel cross-sectional size, density

and fractions (i.e. frequency and cross-sectional area occupied by open vessel spaces respectively per unit area) [21, 22].

More important than having a modulating effect on wood density, stem vessel size distribution, density and fractions affect the hydraulic conductivity of xylem [23, 24]. The different combinations of vessel sizes and densities can also reflect different strategies used to deal with water deficit [25]. Other anatomical traits such as the amount and arrangement of parenchyma in wood are also increasingly being recognized for their functional significance relating to drought adaptation [21, 25, 26]. Sapwood area and the ratio of sapwood to leaf areas (i.e. Huber values) also have important roles in modulating water transport and tree growth [27, 28]. Collectively, these stem traits are important structural aspects of the hydraulic architecture of a plant [29].

Since both leaf and stem traits are involved in plant water relations, understanding how trees may respond to water deficit requires an understanding how these traits vary across species [30, 31]. Given the high tree species richness in the tropics, we hypothesise a commensurate variety of trait strategies involved in the common function of water transport and drought resistance. In recent years, ecologists have used multivariate methods to examine various aspects of these relationships [22, 32] and to characterise water use trait strategies across species [11, 26, 31].

An ongoing rainfall exclusion experiment in a permanent plot of lowland tropical rainforest in northern Australia provided an opportunity to study the water transport trait strategies of tropical trees. The study species are being monitored *in situ* with sap flow sensors to determine their rates of water use prior to the implementation of rainfall exclusion. Understanding how water transport trait strategies vary between species and how this affects sap flow will provide an important context for the rainfall-exclusion study. We therefore

characterized and examined the trait strategies related to water transport in eight co-occurring species of lowland tropical rain forest trees.

2 MATERIALS AND METHODS

Ethics Statement

Permission to sample vegetation was not necessary as the study site was on land owned by the James Cook University. Internal approval from James Cook University was granted for performing the experiment on the property. No protected species were sampled.

2.1 Study site and species

Our study site is located in a one-hectare permanent plot in lowland tropical rainforest at the Daintree Rainforest Observatory, Cape Tribulation (16°06′20″S, 145°26′40″E) in north-eastern Australia. We selected seven common eudicot and one monocot palm (>8 tree stems/ha $\geq$10 cm DBH), representing different functional groups [33] (Table1). These species belong to different genera and families, thereby preventing bias due to close phylogenetic relatedness [34]. For brevity, we henceforth refer to our study species by their genus. We sampled four mature individuals (>60% potential height) of each species, with the exception of *Castanospermum* for which resources limited sampling to two individuals. Our sample selection was complemented with a canopy crane assessment to ensure that we selected individuals with well-illuminated crowns, that were not overtopped by other species, and which had limited liana load. In the case of *Myristica*, which is an understorey tree, all the individuals we selected were partially overtopped by canopy trees (60% to 70% crown exposed). However the variation in crown exposure of the four selected

individuals was minimal, and in all cases well-illuminated branches were accessible by the crane.

Table 1. Tree species used in the study, their functional groups and high-order trait characteristics.

Species	**Family**	**Functional group**	**DBH(cm) (mean, ±SD)**	**Height(m) (mean, ±SD)**	**Wood density (g cm^{-3}) (mean, ±SD)**
Alstonia scholaris (L.) R.Br.	Apocynaceae	Early successional, canopy tree	23.73, ±3.7	19.3, ±3.7	0.33, ±0.03
Elaeocarpus angustifolius Blume	Elaeocarpaceae	Early successional, canopy tree	32.13, ±11.84	20.9, ±5.1	0.49, ±0.07
Argyrodendron peralatum (F.M.Bailey) Edlin ex J.H.Boas	Malvaceae	Mature-phase, canopy tree	35.92, ±3.52	28.9, ±4.5	0.81, ±0.07
Castanospermum australe A.Cunn. ex Mudie	Fabaceae	Late successional to mature-phase, canopy tree	85.25, ±59.11	31.4, ±2.3	0.62, ±0.01
Endiandra microneura C.T.White	Lauraceae	Mature-phase, canopy tree	32.93, ±13.74	22.4, ±4	0.68, ±0.05
Myristica globosa (Warb.) W.J.de Wilde	Myristicaceae	Mature-phase, subcanopy tree	33.5, ±4.55	21.7, ±4.3	0.5, ±0,02
Syzygium graveolens (F.M.Bailey) Craven & Biffin	Myrtaceae	Mature-phase, canopy tree	54.22, ±8.3	27.9, ±2.2	0.56, ±0.06
Normanbya normanbyi (W.Hill) L.H.Bailey	Arecaceae	Mature-phase, subcanopy palm tree	14.68, ±0.59	16.6, ±1.4	1.46, ±0.02

Functional grouping and canopy occupancy of the species is based on Goosem and Tucker [33, p.35] and field observation

2.2 Traits

We measured physiological, morphological and anatomical traits related to plant water use and growth (Table 2).

Table 2. Traits evaluated in the current study and their hypothesized ecological relevance to tree water use.

Traits	**Unit**	**Ecological relevance**
Maximum sap flow rate	$cm^3\ hour^{-1}$	Sap conductance
Maximum sap flow velocity	$cm\ hour^{-1}$	Sap conductance
Annual basal area incremente	$cm^2\ year^{-1}$	Plant growth
Sapwood área	cm^2	Sap conductance
Sapwood vessel área	μm^2	Sap conductance
Sapwood vessel density	mm^2	Sap conductance
Sapwood vessel fraction	No unit	Sap conductance
Sapwood vessel diameter	µm	Sap conductance
Theoretical specific conductivity	$kg\ s^{-1}\ MPa^{-1}$	Sap conductance
Vulnerability index	No unit	Sap conductance and susceptibility to vessel cavitation
Leaf área	cm^2	Transpiration and photosynthesis
Leaf dry matter contente	$mg\ mg^{-1}$	Structural support and leaf water storage
Leaf mass per unit area	$mg\ mm^{-2}$	Structural support and growth
Leaf slenderness	$cm\ cm^{-1}$	Control of water status
Leaf thickness	Mm	Resistance to leaf drying
Huber value	$cm^2\ cm^{-2}$	Sap conductance and transpiration
Min Leaf water potential	Mpa	Physiological measure of the leaf water status
Max Leaf water potential	Mpa	Physiological measure of the leaf water status
Carbon isotope ratio	‰	Physiological measure of intrinsic water-use-efficiency in leaves
Intrinsic water-use-efficiency	$\mu mol\ mol^{-1}$	Physiological measure of intrinsicwater-use-efficiency in leaves

We obtained sap flow measurements by the heat ratio method [35] using commercially-available sap flow meters (Model: SFM1; ICT International) [36]. The sap flow meters were installed at 1.3 m height on the bole, with the exception of buttressed trees (*Argyrodendron*) on which the devices were installed 50 cm above the buttress. Sap flow data was used for a four to nine day time window at the end of the dry season in November 2013 as this coincided with a period where all equipment was functioning simultaneously. The total rainfall for the nine-day period was 27 mm, and there was no rainfall recorded for a week prior to this. Meteorological data was obtained from the Bureau of Meteorology [37] for the Cape Tribulation Store weather station (no. 31012), which is < 1 km northeast of the study site. For each individual, we computed the mean maximum rates of sap flow and sap velocities over four to nine complete days.

As a measure of tree growth, we calculated the mean annual basal area increment (BA_i) of our study individuals using a 12-year (2001-2015) dataset from the permanent one-hectare plot. BA_i was obtained by subtracting the most recent from the earliest basal area measurement available, and then dividing the difference with the number of years that had elapsed between those two measurements. We restricted BA_i comparisons to the seven eudicot species.

From the individuals sampled for sap flow, we measured stem and leaf functional traits, which are variously correlated with plant growth rate, light-use-efficiency, water-use-efficiency and relative drought resistance [12] (Table 2).

Two cores, each *ca*. 5 cm long, were obtained with an increment corer from each individual tree at breast height. We measured using callipers the depth of sapwood, which was determined visually by a colour change in the sapwood/heartwood interface. We used this to calculate sapwood area by subtracting the cross-sectional area of the non-sapwood area from that of the trunk (excluding bark). “Sapwood area” for *Normanbya* was calculated from the

entire cross-sectional area of the palm stem at 1.3 m DBH, excluding the bark. Due to the hard wood, coring for sapwood samples was also not feasible for *Normanbya*. We therefore collected wood wedge samples at 1.3 m DBH from *Normanbya* individuals of a similar diameter to those used in sap flow measurements. The sapwood samples were sanded and polished with increasing grits of sanding paper until anatomical structures were visible. Sapwood vessels from the first two centimeters of sapwood towards the bark were examined with a stereo microscope (30x magnification; Nikon SMZ 745T) and photographed (Nikon DS-Fi2). On the digital photographs, vessels were measured for area (vessel area: VA) and density (vessel density: VD) using imaging software GIMP 2.8.10 [38].

From the vessel areas and cross sectional areas of digital images, we computed the vessel fraction (VF = VA × VD: total cross-sectional area used for sap transport). The idealized vessel diameters (V_{dia}) was calculated for each species and we used the Hagen–Poiseuille equation to calculate the theoretical specific xylem hydraulic conductivity (henceforth theoretical specific conductivity) as: $K_s = \pi/128\eta A_{\text{cross sectional area}} \text{ x } \Sigma D^4$, where η is the viscosity (1.002×10^{-9} MPa s^{-1}) and ρ is the density of water (998.23 kg m^{-3}) respectively at 20°C [23]. We also derived a vulnerability index (VI = V_{dia}/VD: susceptibility to cavitation) [39] for each individual. Wood density was measured on adjacent 5 to 10 mm length of sapwood core segments from the same cores sampled for the vessel measurements using the water displacement method [20]. For the palm *Normanbya*, we used wood wedge samples (see above).

We used the canopy crane to access leaf and branch material in the canopy. Leaf water potential (Ψ_L was measured at regular intervals from predawn (0500-0600 h) to 1950 h within a week in May 2014, using a pressure chamber [12]. The total rainfall for the week prior to collection dates was 52

mm, and there was no rainfall for a week prior to this period [37]. For our purposes, we only use the averages of the minimum and maximum Ψ_L as indicators of the leaf water status at the driest time of the day and resting state water relations respectively [12, 40]. Milky exudate in *Alstonia scholaris* obscured pressure chamber readings so we used literature Ψ_L values from a related species, *Alstonia macrophylla* Wall. [41]. Like *A. scholaris*, *A. macrophylla* is a fast growing rainforest successional species [42]. For leaf area, leaf slenderness (defined as the ratio of the leaf length to leaf breadth), leaf mass per area (LMA) and leaf dry matter content (LDMC), five to 20 replicates per individual of sun-exposed leaves were obtained from the tree canopy. For the compound-leaved *Castanospermum*, leaflets were taken to be the functional unit equivalent to leaves. Leaf areas were obtained from scans processed in imaging software GIMP. Leaf thickness was measured using a calliper, avoiding major veins. These leaves were weighed fresh and then weighed after drying 60°C for a week. LMA was the ratio of leaf dry weight to oven dried mass and LDMC was leaf dry mass divided by fresh mass. For leaf carbon isotope ratio ($\delta^{13}C$) determination, leaves were ground finely using a bead mill grinder and analysed at the Terrestrial Ecohydrology laboratory, School of the Environment, University of Technology, Sydney. We calculated the intrinsic water-use-efficiencies (WUE_i) of each species from $\delta^{13}C$ values after Werner et al. [43]. For Huber values we collected terminal branches with leaves and measured the total leaf areas of all the leaves on the branch, and also the cross sectional area of the cut branch. Huber values were then computed by dividing the branch sapwood area (excluding pith area) by leaf areas.

2.3 Data Analyses

We analysed the data using both univariate and multivariate statistics. Variables were checked for normality and transformed where necessary before

analysis. Univariate one-way ANOVAs were performed for each trait and significant differences between species were determined by Tukey HSD tests (confidence level of 0.05). From 30 trees, we identified major gradients in functional traits using Non-metric multidimensional scaling (NMDS) ordination [44]. To improve ordination performance, traits were standardized. Monte Carlo randomization tests (100 runs) were used to determine whether ordination axes explained significantly more variation than expected by chance. When testing for correlations between individual functional traits and ordination axes, Bonferroni-corrected alpha values were used to reduce the experiment-wise error rate ($p = 0.1/28 = 0.00357$).

We used linear mixed effects models (with Restricted Maximum Likelihood) to examine relationships between sap flow measures and annual basal area increment (response variables) as a function of leaf and stem traits. We used the two NMDS axes as fixed factors in the models to resolve the high levels of inter-correlations between the leaf and stem traits. Other fixed factors included in the saturated models were individual tree characteristics: height and DBH. Species were included as a random factor, accounting for seven tree species due to the exclusion of the palm *Normanbya*. All modelling was performed in R [45], using the package nlme [46] following a standard protocol for data exploration [47].

For each response variable, the most supported set of predictors was inferred using an Akaike's information-theoretic approach [48] corrected for sample size, and the top five models which included >95% of Akaike weight are presented. To obtain a measure of relative support of each model combination, we also computed their Akaike weight *w*, which refers to the probability of a model combination being the best supported one among a given model set. Inferences were drawn based on all plausible models.

3 RESULTS

3.1 Sapwood anatomical observations

With the exception of the palm, all the study species are eudicot trees with diffuse porous wood, and had light-coloured parenchyma tissue appearing either as bands of varying widths or in patches. Early-successional trees (*Alstonia*, *Elaeocarpus*; Fig 1a, b) have scattered vessels with relatively thin radial parenchyma bands. Mature-phase trees differ drastically in wood anatomy, particularly with reference to patterns of parenchyma-vessel association and vessel density. *Endiandra* (Fig 1e) and *Myristica* (Fig 1f) have solitary vessels, and irregularly-spaced bands of radial parenchyma. *Argyrodendron*, *Castanospermum* and *Syzygium* (Fig 1c, d, g respectively) has vessels distinctively associated with parenchyma. In the case of *Castanospermum*, the vessels are consistently encapsulated in patches of abundant parenchyma tissue (aliform parenchyma) which are sometimes confluent (Fig 1c). Notably also, *Syzygium* has the most densely-packed vessels among all the study species (Fig 1g). The vascular system of the single palm *Normanbya* differs drastically from those of the other functional groups, consisting of dark scattered vascular bundles within a matrix of light-coloured ground tissue. Each vascular bundle is comprised largely of dense fibres with a single large vessel is situated at the distal end (into trunk) (Fig 1h).

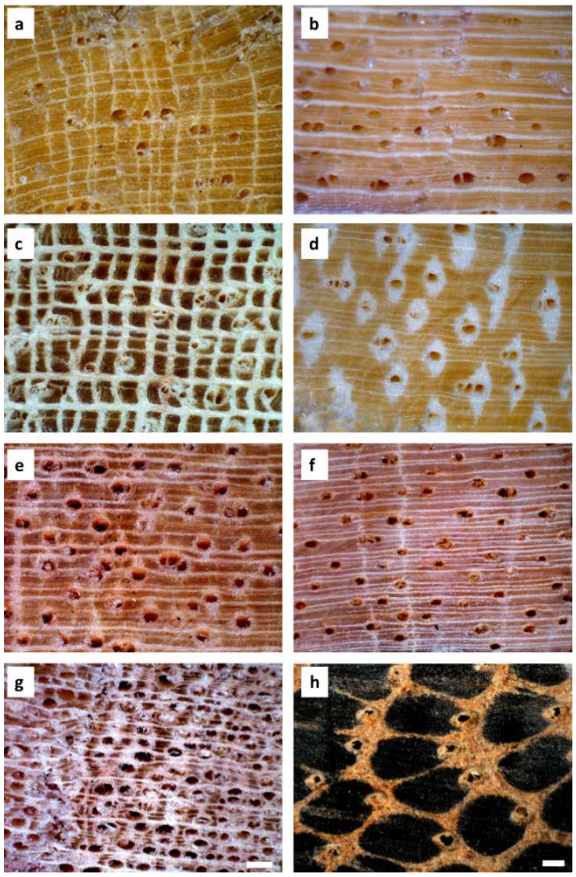

Fig 1. Contrasting wood anatomical features (a-h) of tropical lowland rainforest trees from Daintree, Australia. Early successional trees (a) *Alstonia scholaris* and (b) *Elaeocarpus angustifolius* with light coloured wood and scattered vessels; mature-phase trees (c) *Argyrodendron peralatum*, (d) *Castanospermum australe*, (e) *Endiandra microneura*, (f) *Myristica globosa*, and (g) *Syzygium graveolens* with varying vessel and parenchyma arrangements, and; palm (h) *Normanbya normanbyi* with dark fiber bundles. The white scale bar in *Syzygium* equal 0.2 mm and serves for all images except for *Normanbya* where it equals 0.25mm.

3.2 Sapflow and Relative Growth

We observed differences among functional groups in maximum sap velocity but not within sap flow rate or annual basal area increment (Fig 2; Table in S1 Table). Maximum sap velocity showed two distinct responses with low averages for the mature-phase species (*Argyrodendron*, *Castanopermum*, *Endiandra* and *Myristica*) and high sap velocities for the early-successional (*Alstonia*, *Eleaocarpus*), the palm and mature-phase *Syzygium* (One-way ANOVA: $F_{7,22} = 5.54$, $p < 0.001$). High and low averages for max sap flow rates were recorded for both early successional and mature-phase species ($F_{7,22} = 4.436$, $p = 0.0033$) respectively. Annual basal area increments were similar across all functional groups, except for remarkably high values for one early-successional tree ($F_{6,19} = 6.834$, $p < 0.001$).

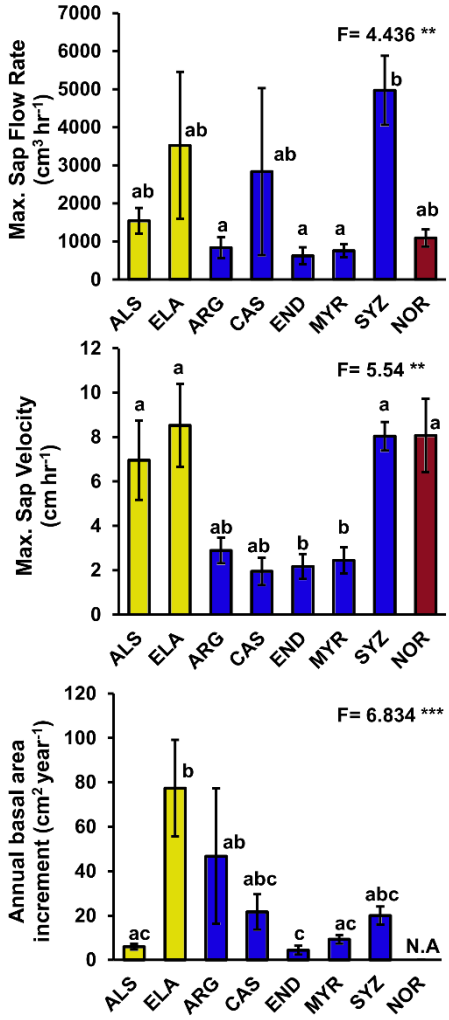

Fig 2. Means (±S.E) of sap flow measures and annual basal area increment of tropical lowland rainforest tree species from Daintree, Australia. Species codes are as follows for early successional (yellow bars), mature-phase (blue bars) and palm species (maroon bars): *Alstonia scholaris* (ALS); *Elaeocarpus angustifolius* (ELA); *Argyrodendron peralatum* (ARG); *Castanospermum australe* (CAS); *Endiandra microneura* (END); *Myristica globosa* (MYR); *Syzygium graveolens* (SYZ); *Normanbya normanbyi* (NOR). The palm species NOR was excluded from the annual basal area increment graph as it does not exhibit secondary growth comparable with the

other seven eudicot trees. One-way ANOVA F-values are given and significance levels are indicated by asterisks as follows: P<0.05*, <0.01**, <0.001***. Numerator degrees of freedom and denominator error degrees of freedom are 7 and 22 respectively for sap flow measures and 6 and 19 respectively for annual basal area increment. Significant differences between species are indicated by different letters (Tukeys HSD, $p < 0.05$).

3.3 Stem traits

We observed differences among functional groups with the single palm species responding significantly differently from the early-successional and mature-phase trees in the following traits (Fig 3; Table in S1 Table): vessel area ($F_{7,22} = 47.56$, $p < 0.001$), vessel density ($F_{7,22} = 94.58$, $p < 0.001$), vulnerability index ($F_{7,22} = 101.7$, $p < 0.001$), and theoretical specific conductivity ($F_{7,22} = 18.86$, $p < 0.001$). Not surprisingly, wood density differed significantly among early-successional, mature-phase and the palm species ($F_{7,22} = 87.26$, $p < 0.001$). Sapwood area reflected tree size but no functional groups pattern ($F_{7,22} = 4.36$, $p = 0.004$) and Huber value showed no difference across species ($F_{7,22} = 1.256$, $p = 0.316$) and is not included in Fig 3.

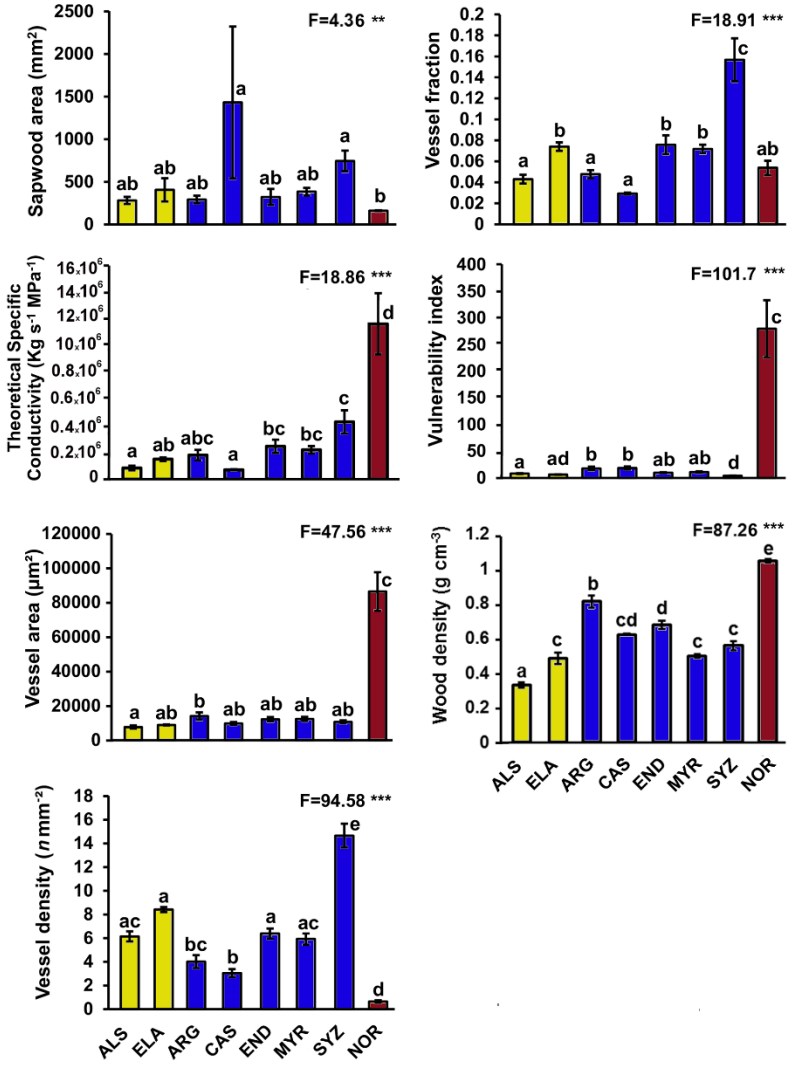

Fig 3. Means (±S.E) of stem traits of tropical lowland rainforest tree species from Daintree, Australia. Species and colour codes follows Fig 2. One-way ANOVA F-values are given and significance levels are indicated by asterisks as follows: P<0.05*, <0.01**, <0.001***. Numerator degrees of freedom and denominator error degrees of freedom are 7 and 22 respectively for all variables. Significant differences between species are indicated by different letters (Tukeys HSD, $p < 0.05$).

3.4 Leaf traits

We observed few differences among functional groups in leaf traits (Fig 4; Table in S1 Table). One mature-phase species had a significantly higher LMA than all other species ($F_{7,22}$ = 13.43, p < 0.001) and the single palm species distinctively different from other functional groups with respect to leaf slenderness ($F_{7,22}$ = 128.1, p < 0.001). Leaf thickness showed no difference across species ($F_{7,22}$ = 0.553, p = 0.785) and is not included in Fig 4.

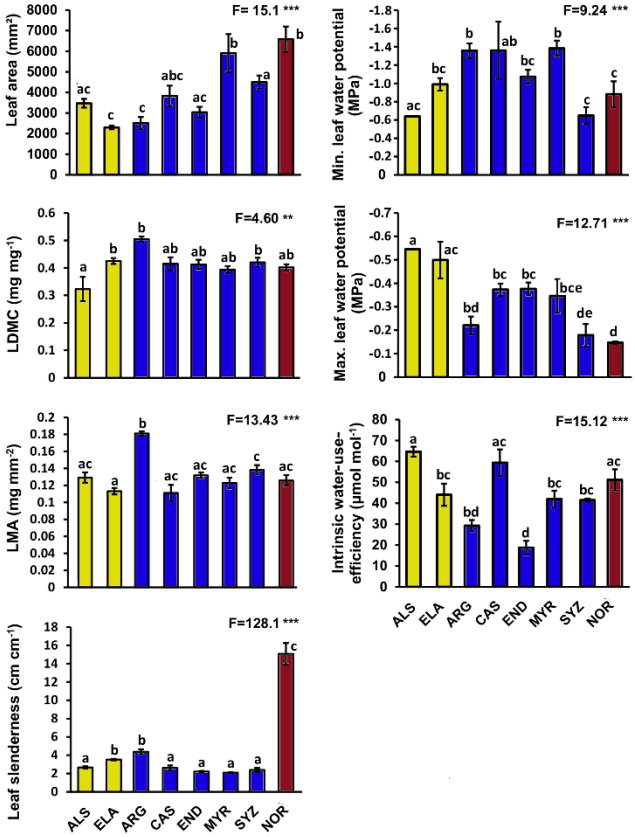

Fig 4. Means (±S.E) of leaf traits of tropical lowland rainforest tree species from Daintree, Australia. Species and colour codes follows Fig 2. One-way ANOVA F-values are given and significance levels are indicated by asterisks as follows: P<0.05*, <0.01**, <0.001***. Numerator degrees of freedom and denominator error degrees of

freedom are 7 and 22 respectively for all variables. Significant differences between species are indicated by different letters (Tukeys HSD, $p < 0.05$).

3.5 Coordination among functional traits and sap flow response modelling

We explored the coordination of functional traits across 30 rainforest trees (seven eudicot tree species and one palm species) using non-metric multidimensional scaling ordination analysis (NMDS). Two ordination axes collectively explained 86.5% of the total variation in the functional trait data set (Fig 5a). Axis 2, which captured 73.5% of the variation, clearly distinguished a gradient of early-successional, mature-phase and palm trees. This gradient showed the functional coordination of seven of the 14 water-use traits examined in this study (Fig 5a; Table 3), with low values for an early-successional species (*Alstonia*), moderate values for a group of mature-phase species (and one early-successional species) and high values for a palm species. The lack of interspecific variation among the five mature-phase species and one early-successional species along this axis is notable. Axis 1 explained 13% of the total variation and described a gradient among species that does not reflect obvious functional grouping but demonstrates an orthogonality between leaf volume (measured as LDMC) and maximum leaf water potential. Along the Axis 1 gradient, we observe interspecific clustering of trees within four mature-phase species, with the remaining four overlapping.

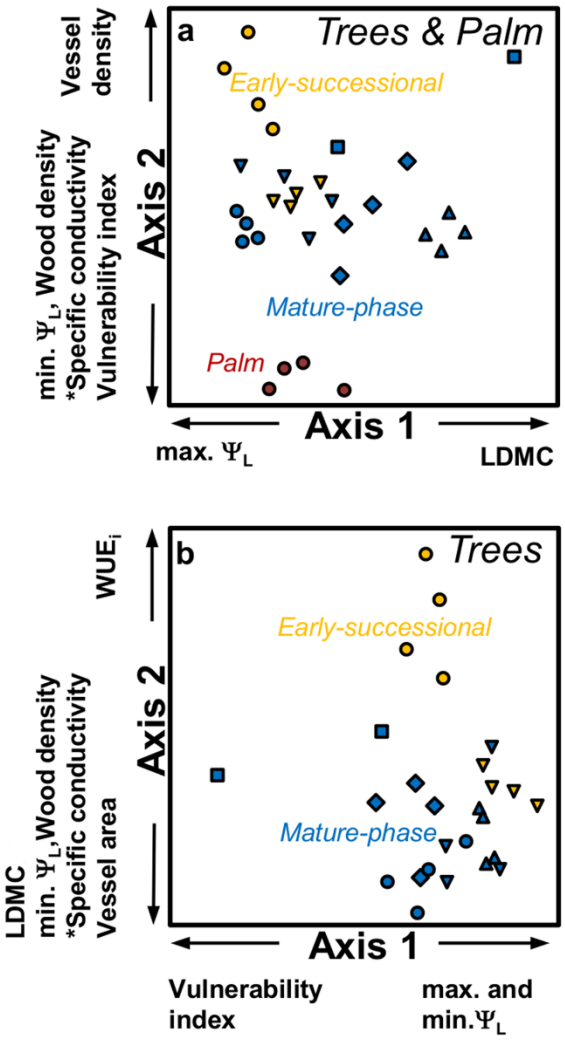

Fig 5. Non-metric multidimensional scaling ordinations of traits of tropical lowland rainforest species including (a) and excluding (b) the palm species. Traits and their abbreviations (in parentheses) include: vessel area, vessel density, *theoretical specific conductivity, wood density, leaf dry matter content (LDMC), leaf slenderness, minimum and maximum leaf water potentials (min ΨL, max ΨL respectively), intrinsic water-use-efficiency (WUEi) and vulnerability index. Species symbols are as follows: Secondary-successional species – *Alstonia scholaris* (yellow circles); *Elaeocarpus angustifolius*

(yellow inverted triangles); Mature-phase species – *Argyrodendron peralatum* (blue circles); *Castanospermum australe* (blue squares); Endiandra microneura (blue inverted triangles); Myristica globosa (blue diamonds); *Syzygium graveolens* (blue triangles), and; Palm – *Normanbya normanbyi* (maroon circles). The arrows by the axes indicate significant positive or negative Pearson correlations between individual traits and axes.

Table 3. Pearson correlations for functional traits with two ordination axes produced by Non-metric multidimensional scaling (NMDS).

Traits	**NMD with *Normanbya***		**NMDS without *Normanbya***	
	Axis 1	**Axis 2**	**Axis 1**	**Axis 2**
Leaf dry matter contente	**0.548**	-0.39	-0.004	**-0.773**
Leaf slenderness	0.061	**-0.753**	n.s	n.s
Leaf water potential (Max.)	**-0.669**	-0.105	**0.715**	0.41
Leaf water potential (Min.)	-0.297	**-0.715**	**0.646**	**-0.564**
Vessel área	0.011	**-0.862**	-0.14	**-0.779**
Vessel density	-0.23	**0.587**	**0.694**	-0.057
Vessel fraction	n.s	n.s	**0.608**	-0.478
Theoretical specific conductivity	-0.224	**-0.89**	0.37	**-0.721**
Wood density	0.485	**-0.798**	0.088	**-0.772**
Water-use-efficiency	n.s	n.s	-0.277	**0.691**
Vulnerability index	0.171	**-0.687**	**-0.665**	-0.153

Only traits exhibiting significant associations with at least one axis are listed. Values in bold were significant using a Bonferroni-corrected alpha value (p = 0.00357) (See also Fig 5a and 5b).

Palms lack sapwood and growth rings and are likely to be distinctly different from eudicot trees with respect to hydraulic architecture. Hence, we further explored the robustness of our functional coordination among traits, by considering only eudicot trees (26 individuals) in a second ordination analysis

(Fig 5b). The second ordination explained 83% of the total variation in the data and showed remarkable similarities with the first (Fig 5b). Axis 2, distinguished a similar gradient from early successional to mature-phase species, capturing 53.5% of the total variation. We observed four of the seven functional traits that defined this gradient in the earlier ordination also coordinating and influencing the position of trees in this analysis (Table 3). Along this axis only an early successional species (*Alstonia*) showed interspecific variation, but most overlapped, demonstrating the natural variation within species and the importance of within species replication. Similarly, there was no interspecific variation detected among the species in Axis 1 which explained 29.5% of the variation and described a gradient of leaf water potential (maximum and minimum) and vessel fraction and density at one end, and vulnerability index at the other end. Interestingly, the individual tree identified as vulnerable is the largest individual on the one-hectare plot measuring 127 cm DBH.

Sap flow velocity and annual basal area increment were poorly predicted by stem and leaf traits as ordinated variables from NMDS and individual tree characteristics (DBH and height) when nested within species (Table 4). Sap flow velocity values were low and similar across four species and high and variable across three species (Fig 2), but these patterns were not reflected in the ordination gradients. With respect to annual basal area increment these were overall low and similar across six species and high in a single species (*Elaeocarpus*), also a pattern not reflected in the ordination axes or other functional traits examined.

Table 4. Top candidate models that predict mean maximum sap velocity and mean annual basal area increment across seven tropical lowland rainforest tree species in Daintree, northeast Australia.

Response variable	**Intercept**	**NMDS axis 1**	**NMDS axis 2**	**DBH**	**AICc**	**ΔAICc**	***w***	***w*$_1$/*w*$_i$**
Sap velocity	0.690				-0.8	0.00	0.450	
	0.552		0.520		0.3	1.06	0.265	1.698
	0.657	0.073			1.9	2.67	0.118	2.246
	0.759			-0.044	3.1	3.86	0.065	1.815
	0.530	0.052	0.518		3.3	4.06	0.059	1.102
(Σw – top 5 models)	*0.957*	*0.177*	*0.324*	*0.065*				
Basal area incremente	1.187				32.7	0.00	0.315	
	1.365		-0.673		33.5	0.75	0.217	1.452
	0.341			0.536	33.9	1.17	0.176	1.233
	1.143	0.098			34.1	1.35	0.160	1.100
	-0.437	0.842		0.786	34.5	1.74	0.132	1.212
(Σw – top 5 models)	*1.000*	*0.292*	*0.217*	*0.308*				

AICc refers to Akaike Information criterion corrected for small sample size, ΔAICc to the difference between each model's AICc and the minimum AICc found, *w* to Akaike weights, and w_1/w_i to evidence ratios where w_1 is the Akaike weight of the best fitting model. Akaike weights may be interpreted as relative model probabilities. Models with a higher evidence ratio are less likely to be the best model. The summed weights (Σw) for predictors is the relative likelihood that the predictor should form part of the model [48]. The top candidate models include ≥95% of Akaike weights**.**

4 DISCUSSION

In the wet lowland rainforests of northern Australia, we found a coordination of stem and leaf water-use characteristics that followed an ecological gradient in our species. We found seven traits (minimum leaf water potential, leaf slenderness, wood density, theoretical specific conductivity, vessel area and density, and vulnerability index) that were positively correlated with the gradient from early successional to mature-phase species to palm trees. Importantly among the five mature-phase species we studied, there was considerable trait variation within species and hence a marked lack of interspecific variation among species, which suggests the importance of replication within species for eco-physiological studies. Despite detectable differences in relative growth rates and sap flow values among some species, our data on the stem and leaf functional traits important for water transport were poor predictors in explaining this variation.

4.1 Various stem and leaf trait associations effect water transport in trees

Our species and individual trees were selected to span a range of wood density and tree size values in order to detect trait variations. We found large overlaps across species in maximum rates of sap flow and velocities across species, which may be expected for plants growing in the same environment [49, 50, 51]. Our linear mixed effects models showed that tree features and the functional coordination of stem and leaf traits were not good predictors of sap flow velocity and basal area incremental growth . One explanation for this lack of predictability is that the variation exhibited within species was greater or equal to the variation between species.

Our NMDS however, revealed various gradients and associations of traits that may reflect contrasting strategies in water transport traits. For instance, leaf carbon isotope ratios reflect a plant’s intrinsic water-use-efficiency

(WUE_i) [43], and can differentiate early-successional from mature-phase species [52]. Indeed, WUE_i appears to segregate early-successional species *Alstonia* from the clustering of mature-phase species (*Argyrodendron*, *Endiandra* and *Syzygium*) (Fig 5).

In their multivariate analyses, Worbes et al. [11] showed leaf $\delta^{13}C$ as having an analogous influence as vessel area, whereas we found an opposite relationship between vessel area and WUE_i (based on leaf $\delta^{13}C$). This disparity could reflect intrinsic differences in the adaptations to water deficit between the drier tropical forest type examined by Worbes et al. [11] and the humid tropical forest in the current study. In addition, Worbes et al. [11] sampled deciduous species, which were not represented in our sampled species.

A further potential coordination of leaf and stem traits was the relationships among leaf volume (dry matter content), minimum leaf water potential and theoretical specific conductivity from our ordinations. Similar reports of coordination between the stem and leaf economic spectra, was reported in Méndez-Alonzo et al. [32] and Kröber et al. [31] in tropical dry forest and in a common garden experiment respectively. We hypothesised that a high vessel area or vessel density may be associated with large stem water storage capacity, which may in turn lead to a less negative maximum and minimum water potential (Ψ_L). For tropical deciduous species, Worbes et al [11] reported that vessel size and density are linked to the most negative Ψ_L values as the driving force for water transport. Other traits such as leaf vein [53] and stomatal densities [54] may contain more relevant information for establishing the link between the leaf and stem economic spectra and therefore deserve measuring in future studies.

4.2 Trait strategies for water transport and drought resistance

Water transport and drought resistance involves a complex interplay of physiological processes [40, 55, 56], intrinsic tree architectural factors (e.g. xylem tapering) [57] and environmental factors (e.g. soil moisture, seasonality, vapour pressure deficits) [58] that we were unable to fully account for or measure. Nevertheless, we can summarize from the available data the water transport strategies and discuss empirically the potential drought resistance of our study species with relevance to their functional groups.

The early-successional species (*Alstonia*, *Elaeocarpus*) have relatively high WUE_i and low VIs, which suggests a relatively high drought tolerance relative to the other study species. These trait combinations may also allow these species to be in exposed habitats experiencing larger vapour pressure deficits (VPDs) whilst minimizing embolism risk. These species also tended to have higher maximum sap velocities, possibly reflecting their exposure to larger VPDs and light levels in more exposed environments characteristic of early-successional species.

The studied mature-phase species exhibited some observable variations in trait strategies. The mature-phase tree *Castanospermum* had intermediate wood density, high WUE_i values, the largest sapwood area, and was associated with the highest VIs of the eudicot trees. Conspicuously, the wood anatomy of *Castanospermum* was unique in having significant portions of parenchyma surrounding the vessels and this appears to be a common trait in trees from the Fabaceae [59, 60]. Borchett and Pockman [61] suggest that such anatomical features buffer the stem from abrupt water pressure changes during water loss or gain. Therefore, the high VI in *Castanospermum* may be compensated by having large quantities of sapwood water storage that can be utilized to repair embolisms [56, 62, 63], or to mitigate against the development of low xylem

water potentials. Such a feature suggests a drought-avoiding rather than drought-resisting strategy for this species [21].

The mature-phase canopy species *Endiandra* and *Argyrodendron* had among the lowest annual basal area increments, relatively low sap velocities and maximum rates of sap flow, low WUE_i values, and relatively high VIs. This reflects a potentially low drought resistance. However, the conspicuous vessel-parenchyma association in *Argyrodendron* suggests that this species has higher water storage and possibly better ability to cope with drought than *Endiandra*. The sub-canopy tree *Myristica* had relatively low sap velocities and annual basal area increment, probably reflecting the low-light environment and low vapour pressure deficit that occurs in the sub-canopy of closed forests. However, it has intermediate WUE_i values and VIs, which might reflect the reduced evaporative demands [64] of being in the forest sub-canopy.

Surprisingly, we found a relatively high sap velocity and maximum rate of sap flow, moderate WUE_i, and also extremely low VI (due to the high vessel packing per unit area) and the largest vessel density and vessel fraction in the mature-phase tree *Syzygium*. Such low VIs would be expected to appear in taxa of xerophytic environments [24] but ironically in *Syzygium*, this high vessel packing per unit area may confer greater physiological safety to drought than one with wider and fewer vessels. Such a strategy could work by restricting air embolisms to smaller and more localized sections of the sap flow column in the event of in high VPDs during a drought event [24, 65]. However, this may also compromise the species hydraulic efficiency via increased resistance to water transport [17, 66].

Our only sample of a palm, *Normanbya*, had stem, vessel, and leaf traits that differed drastically from species of other functional groups. From these observations and on the basis of well documented differences between the hydraulic architecture of palm and eudicot trees [67], we can infer a drastically

different water transport and drought resistance strategy for *Normanbya* from our other eudicot study species. Unlike eudicot trees, palms lack of sapwood and growth rings, and the arrangement of the vascular system is predetermined [68]. The relatively high WUE_i, high sap velocity and large vessel areas relative to our eudicot study species, suggests that *Normanbya* has a very efficient hydraulic system to maintain a large conducting capacity with a minimal vascular investment [67], especially where water is not limiting. However, these characteristics also suggest that this species will be vulnerable to droughts.

5 CONCLUSION

Increased incidence and durations of drought are predicted scenarios for the future of lowland tropical rainforest, and underpins the importance of understanding the strategies that rainforest trees may use to cope with such conditions. Studying trees representing different functional groups, we found, with some differences, little variation in maximum sap velocities and rates of sap flow across species. We have demonstrated however within and across functional groups, that tropical lowland rainforest trees exhibit variable trait strategies for water transport. This is achieved through various associative extents in stem and leaf traits. In particular, quantitative wood anatomical features (vessel areas and densities) have a bearing on plant water transport and should therefore be examined as an informative trait in field-based ecophysiological studies involving the measurement of sap flow. Also, quantitative studies on vessel traits in conjunction with physiological measures can provide useful metrics for estimating a species drought resistance. This information will serve as an important context for an ongoing rainfall exclusion experiment in place, and will complement our understanding of changing species distributions of rainforest trees under various climate change scenarios.

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MANUSCRIPT 2

PLANT FUNCTIONAL GROUPS WITHIN A TROPICAL FOREST EXHIBIT DIFFERENT HYDRAULIC STRATEGIES

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ABSTRACT

Understanding plant water transport strategies in forest ecosystems is crucial for contextualizing community-level adaptations to drought, especially in lifeform-rich tropical forests. To provide this context, we explored how wood traits influence hydraulic function across different plant functional groups in a lowland tropical rainforest. Wood traits in 90 species from six functional groups (mature-phase, understorey and pioneer trees; understorey and pioneer shrubs; vines) were measured and related to intrinsic water-use efficiency as a measure physiological performance. We also examined vessel size distribution patterns across species to determine tradeoffs in hydraulic safety versus efficiency. Wood trait variation defined plant functional groups. Vines and to a lesser extent pioneer-trees and -shrubs exhibited a wide variance of vessel sizes, with large vessels ensuring hydraulic efficiency, and a complement of small vessels ensuring hydraulic safety. Contrastingly, understorey-trees and -shrubs exhibited greater safety mechanisms with high wood densities and numerous narrow vessels. Underpinning these trends, vessel dimensions were important predictors of intrinsic water-use efficiency. We conclude that tropical rainforest plant functional groups possess distinct hydraulic strategies. Using plant functional groups as a framework for hydraulic studies can therefore enhance cross-study comparability, and provide a sound basis for modeling species responses to drought.

Keywords: plant hydraulic strategies, plant functional anatomy, tropical plant lifeforms, tropical rainforest, vines, wood anatomical traits, wood functions, xylem conductivity

RESUMO

A compreensão das estratégias das plantas para transporte de água em ecossistemas florestais é crucial para contextualizar adaptações à nível de comunidade à seca, especialmente em ambientes ricos em formas de vida como as florestas tropicais. Para promover este contexto, foram investigados como os traços da madeira influenciam a função hidráulica entre diferentes grupos funcionais em uma floresta úmida tropical de terra baixa. Traços da madeira em 90 espécies de seis grupos funcionais (árvore de fase madura, árvore de sub-bosque, árvore pioneira, arbusto de sub-bosque, arbusto pioneiro e lianas) foram medidos e relacionados à eficiência-intrínseca-do-uso-da-água como medida de performance fisiológica. Foram examinados também o padrão de distribuição de classes diaméricas dos vasos do xilema para determinar compensações entre segurança versus eficiência hidráulica. Variação nos traços da madeira definiram os grupos funcionais de plantas. Lianas e em escala menor árvores e arbustos pioneiros exibiram ampla variância em tamanhos de vasos, com grandes vasos assegurando eficiência hidráulica, e pequenos vasos complementares garantindo a segurança hidráulica. Árvores e arbustos de sub-bosque, por sua vez, exibem maior mecanismo de segurança com alta densidade da madeira e muitos vasos estreitos. Apoiando este comportamento, as dimensões dos vasos foram importantes preditores da eficiência-intrínseca-do-uso-da-água. Conclui-se que grupos funcionais de plantas em floresta úmida possuem estratégias hidráulicas distintas. Utilizando grupos funcionais de plantas como estrutura para estudos hidráulicos pode, portanto, reforçar a comparação entre estudos e promover uma base sólida para modelar a resposta de espécies perante à seca.

Palavras-chaves: estratégias hidráulicas de plantas, anatomia functional de plantas, forma de vida de plantas tropicais, floresta úmida tropical, lianas, traços anatômicos da madeira, funções da madeira, condutividade do xilema

1 INTRODUCTION

Prolonged drought events due to climate change present a significant threat to tropical forests adapted to high precipitation regimes (Choat *et al.*, 2012; Duffy *et al.*, 2015). Given that differential drought sensitivity shapes tree distributions in tropical forests at both regional and local scales (Engelbrecht *et al.*, 2007; Condit *et al.*, 2013), the dominant forest trees at a local area can be expected to be adapted only to a relatively narrow environmental range (Sperry *et al.*, 2002). Although trees represent the dominant lifeforms, rainforest ecosystems are also species-rich in other plant functional groups, such as vines, light-demanding pioneer and shade tolerant trees and shrubs, reflective of niche differentiation across these groups (Grime, 1974; Richards, 1996; Leigh, 1999). Clearly, an understanding of the diverse ecological strategies that different plant functional groups use to conduct water is needed to set a foundation for predicting plant drought adaptation at an ecosystem level.

For the past two decades, plant ecologists have routinely used functional traits as proxies for understanding plant ecological strategies (Westoby,1998; Westoby *et al.*, 2002; Pérez-Harguindeguy *et al.*, 2013). Traits, such as wood density and its anatomical structure, reflect the multiple tradeoffs inherent in tissues critical to diverse ecological functions (i.e. structural support, water conductance and storage, and growth) (Baas *et al*., 2004; Fan *et al*., 2012), and have come to be known as part of the worldwide wood economic spectrum (Chave *et al*., 2009; Poorter *et al*., 2010). Wood density has often been linked with xylem vulnerability (Hacke *et al*., 2001; Van Nieuwstadt & Sheil, 2005; Phillips *et al*., 2010), where species with high density wood may have ascribed better resistance to cell wall collapse and the ability to withstand lower water potentials in conditions of water deficit. More detailed work has suggested that wood density and vessel traits describe two distinct ecological axes (Preston *et*

al., 2006; Ziemińska *et al*., 2013), and that wood density is a property almost entirely disconnected from anatomical traits related to water conductance (Zanne *et al*., 2010). It is anatomical features of wood xylem (i.e. water conducting tissue) such as vessel size, density, fraction, grouping that have direct bearing on the efficiency of water conductance through plant stems (Tyree & Zimmermann, 2002; Loepfe et al. 2007; Zanne *et al*., 2010; Scholz et al. 2013) (Fig. 1).

Because the water conductivity of vessels increases to the fourth power of diameter, wider vessels are much more conductive than narrower ones (Tyree & Zimmermann, 2002), but at the same time, are likely more prone to drought-induced cavitation (e.g. Zhu & Cao, 2009; Blackman *et al*., 2010; Cai &Tyree, 2010; Carlquist, 2012). This phenomenon has often been referred to as the hydraulic safety vs. efficiency tradeoff (Tyree & Zimmermann, 2002). Some authors have therefore also proposed that the heterogeneity of vessel sizes within in a plant stem represents a safety mechanism enabling the plant to continue transporting water even if some vessels have embolized (Baas *et al*., 2004; Mauseth & Stevenson, 2004; Sperry *et al*., 2008).

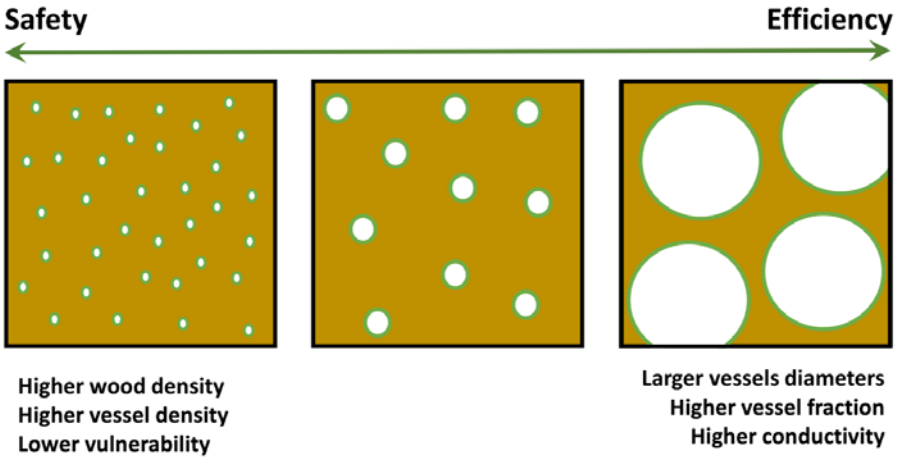

Fig. 1. Schematic representing different water transport strategies that may be present in plant stem xylem. The white holes represent water-conducting vessel lumen and the relative number of vessels, conduit lumen diameters and fractions underpin different hydraulic safety-efficiency strategies.

The diverse range of microclimates within structurally complex rainforest environments (Pearcy, 1987) is likely to drive differences in the coordination of functional traits across different plant functional groups. Understoreys of tropical rainforests are generally heavily shaded with ephemeral sunflecks (Canham *et al.*, 1990) resulting in lower vapor pressure deficits and moderated diel temperature range as compared to the forest canopy. Plants in the understory can also be exposed to relatively destructive falling debris (van Gelder *et al.*, 2006), making an investment in structural support important. Among the sun-exposed plant functional groups, mature-phase and pioneer species occupy different regeneration niches (Bazzaz, 1979), which may necessitate trait tradeoffs between support and growth in these groups.

Despite these ecological differences in plant functional groups, the majority of studies examining plant hydraulics have focused on trees (Christensen-Dalsgaard *et al.*, 2007; Poorter *et al.*, 2010; Zach et al., 2010;

Gleason *et al.*, 2012), and usually only on a limited sample of the flora in an ecosystem. The few comparative studies to date have mostly compared trees and vines (Zhu & Cao, 2009), trees and shrubs (Martínez-Cabrera *et al*., 2011; McCulloh *et al.*, 2015), or lifeforms within the same genus or family (Fisher & Ewers, 1995; Baas *et al*., 2004). Some comparisons have been made between mature-phase species and pioneers (McCulloh *et al*., 2011) but these have been limited in species coverage. To our knowledge, there has been no attempt to characterize and compare how plant functional groups within a discrete ecological setting vary in terms of their hydraulic strategies.

One way to link functional wood anatomy to plant physiological performance is to model plant water transport (e.g. sap flux measures) as a response to vessel traits (e.g. James *et al*., 2003; Schuldt *et al*., 2010; Apgaua *et al*., 2015). However, due to the time and labour intensive nature of these measurements, such an approach is only applicable to a limited range of species at a given time.

In lieu of sap flux measures, a useful indicator of plant physiological performance in ecological studies is long-term integrated or intrinsic water-use efficiency (WUE_i) (Poorter, 2005) – a plant's photosynthetic production (i.e. carbon gain) rate relative to the rate at which it transpires water to the atmosphere (Farquhar et al. 1989; Cernusak *et al.*, 2007). WUE_i can be determined for a large number of samples with relative ease from leaf carbon isotope ($\delta^{13}C$) discrimination, where a higher (less negative) $\delta^{13}C$ indicates a high WUE_i (Dawson *et al*., 2002; Seibt et al., 2008; Fichot *et al*., 2009; Cernusak *et al*., 2013).

Pertinently, various authors have demonstrated that the successional status of tropical rainforest trees are associated with differences in leaf carbon isotope signature (and hence WUE_i) (Huc *et al.*, 1994; Bonal et al., 2007). Leaf WUE_i is also correlated with other hydraulic physiological measures such as mid-day

leaf water potentials (Bonal *et al*., 2000, 2007), and is therefore suitable as a physiological response to our hydraulic strategies defined by functional anatomical traits.

We hypothesize that plant functional groups within a tropical rainforest biome will vary in their hydraulic strategies and demonstrate contrasting hydraulic safety mechanisms. To test this hypothesis, we compared the stem functional anatomy of 90 species representing six woody plant functional groups in a tropical rainforest with different growth environments and lifeform. We also modelled the relationship between wood traits and plant intrinsic water-use efficiency.

2 MATERIALS AND METHODS

2.1 Study site and species

Our study site is located at the Daintree Rainforest Observatory (16°06′20″S 145°26′40″E, 50 m a.s.l.; Laidlaw *et al*., 2007; Tng *et al.*, 2016) in a lowland rainforest adjacent to the Daintree National Park in north-eastern Australia. The site experiences a tropical climate, with mean temperatures of 24.4^{o}C and a relatively high annual average rainfall of 4900 mm annum^{-1} (Bureau of Meteorology, 2015). The forest type at the site has a complex vertical profile, with canopy heights ranging from 24 to 33m (Liddell *et al*., 2007), and a wide variety of plant lifeforms (Tracey, 1992). Soils are of relatively high fertility, developed over metamorphic and granitic colluvium (Bass *et al.*, 2011).

Within this lowland rainforest, we collected 90 co-occuring woody plant species belonging to 77 genera and 40 families (Fig. 2). These species were selected to maximize phylogenetic spread across angiosperm lineages, and represent commonly occurring mature-phase and pioneer species within the Daintree Rainforest Observatory. We selected these 15 species to be replicates

for each of our six woody plant functional groups, according to their habit and successional preferences. These groups include: 1) pioneer shrubs, 2) pioneer trees, 3) understorey shrubs, 4) understorey trees, 5) mature-phase trees and 6) vines. We defined shrubs as woody plants that attain reproductive maturity below six meters height, and only rarely exceed this height (Hyland *et al.*, 2010; Tng *et al.*, 2016). Vines were restricted to liana species with woody growth (Gentry, 1991). Pioneer shrubs and trees were defined as species requiring a high-light environment at maturity (rainforest margins, gaps or emergent positions above a canopy) (Bazzaz, 1979; Richards, 1996). Our delineation of species into these discrete groups is based on available height and long-term tree demographic data from published studies conducted both at the site (Laidlaw *et al.*, 2007; Tng *et al.*, 2016), the broader wet tropics region of north-eastern Australia (Bradford et al., 2014), and on compiled records of ecological strategy (Hyland *et al.*, 2010; Goosem & Tucker, 2013). We restricted sampling to mature and healthy individuals.

As we aimed to cross-examine woody tissue from species of different statures and habits, sampling methods were adjusted to suit different plant functional groups. From trees, we collected wood wedges at breast height (1.3m). For shrubs, we sampled destructively, collecting stem sections at the base of the plant from 5 to 10 cm above the soil level and avoiding basal branches where present. For vines, we traced the shoots back to where stems were approximately 1 - 1.5 cm in diameter, and made stem collections of approximately 5 cm length.

Leaf carbon isotope samples were obtained from the same individuals sampled for wood, and we limited our sampling to well-illuminated mature leaves. For understorey trees and shrubs, collecting leaves shaded under a canopy was unavoidable, but we took care to sample leaves from individuals

from their outer canopy that were not self-shaded. Fieldwork was conducted during the dry seasons (May to August) in 2014 and 2015.

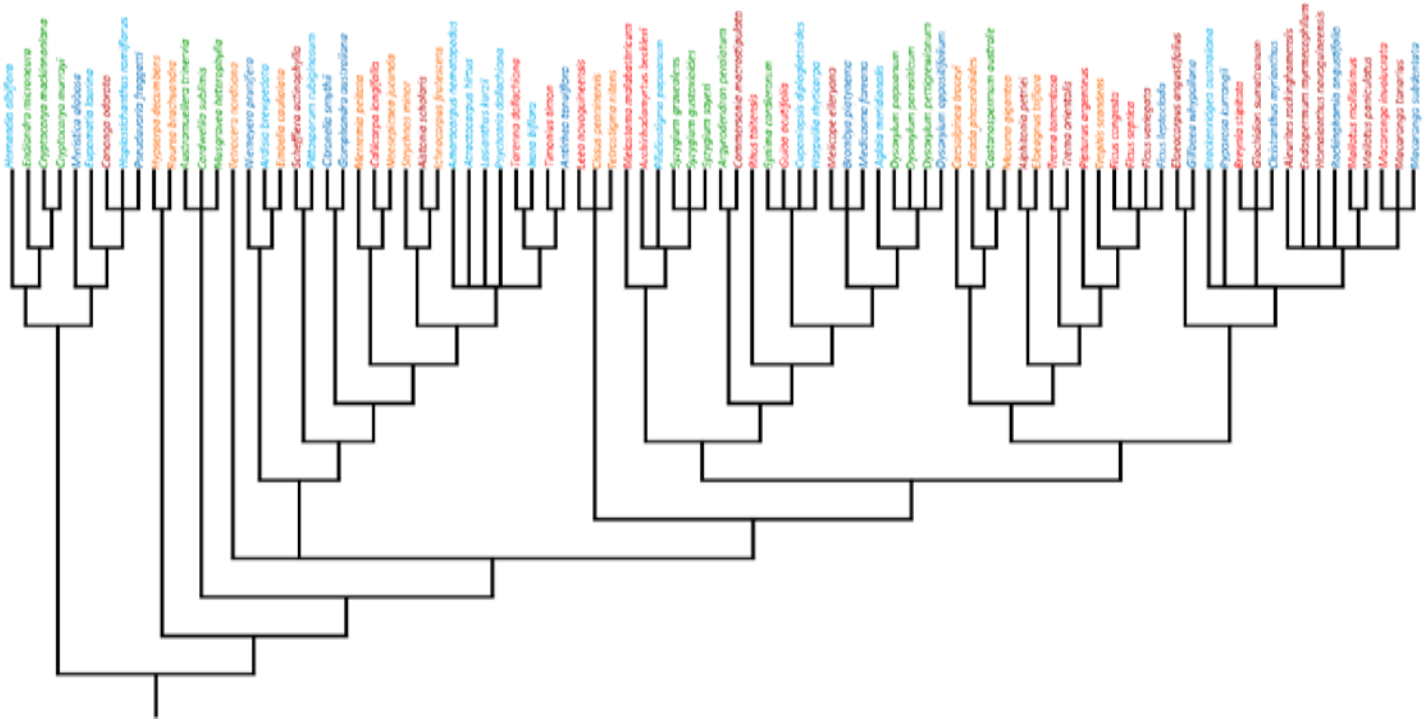

Fig. 2. Evolutionary relationships for the 90 tropical plant species studied in north Queensland, Australia. The cladogram is based on the Angiosperm Phylogeny Group (APG) III classification. Colors indicate our six woody plant functional groups (Mature-phase Trees - GREEN; Understorey Trees - DARK-BLUE;

Understorey Shrubs - LIGHT-BLUE; Pioneer Trees - DARK-RED; Pioneer Shrubs - BRIGHT-RED; Vines - ORANGE).

2.2 Trait sampling

We measured wood traits related to plant water transport (Table 1) in one individual of each of our 90 study species. After removing bark, we used part of each sapwood sample to obtain two replicate wood density measurements for each species. In some samples where pith was present, we scraped this pith tissue off after splitting wood sections longitudinally through the middle, and used the two wood sections for wood density determination. Wood fresh volume was measured using the Archimedes principle after which the samples were oven-dried at 70°C for at least 48 hours. We then measured the dry weight of the wood samples and calculated the wood density as wood dry mass per unit of fresh volume.

Table 1. Traits measured in the current study across 90 lowland rainforest plant species.

Traits	**Symbol**	**Unit**	**Range**	**Relevance to plant hydraulics**
Wood density	WD	g cm^{-3}	0.29-0.92	Provides resistance to cavitation.
Sapwood Vessel diameter	*D*	µm	21.21-504.1	The diameter of a vessel lumen that determines the area available for water conductance
Sapwood max diameter	d_{max}	µm	29.22-706.57	The largest vessel lumen diameter
Hydraulically weighted diameter	d_h	µm	21.88-448.56	Denotes the mean diameter that vessels need to be if it is to have the same total conductivity for the same number of conduits as the sampled stem
Sapwood Vessel density	VD	mm^{-2}	1.97-241.87	The number of vessels within a given area of sapwood
Sapwood Vessel Fraction	VF	No unit	0.02-0.39	The area occupied by vessel lumen within a given area of sapwood
Theoretical Specific Conductivity	K_s	kg s^{-1} MPa^{-1}	$7.03x10^{5}$-$3.16x10^{9}$	A theoretical calculation of water conductivity based on the Hagen–Poiseuille equation

Traits	Symbol	Unit	Range	Relevance to plant hydraulics
Carlquist vulnerability Index	VI	No unit	0.09-260.35	Index calculated by dividing vessel diameter by vessel density. Species with narrow and many vessels thus show low values in agreement with the often observed trade-off between vessel size and cavitation resistance
Vessel multiple fraction	V_{mf}	No unit	0.174-1.000	Calculated by dividing number of groups of vessels by number of vessels. Low indices denote high interconnectivity of vessels and potentially higher hydraulic safety and conductivity
Carbon isotope ratio	δ13C	‰ VPDB	-37.71 - -27.00	Measured from leaves, this reflects average intercellular CO_2 [ ]
Intrinsic Water-use-efficiency	WUEi	μmol mol^{-1}	0.83-84.59	Measure of carbon gain per unit of water use, calculated from leaf carbon and CO_2 isotope signatures, and atmospheric CO_2 [ ] Hypothesized to be linked with vessel-based hydraulics.

To soften wood to make anatomical sections, we soaked our stem wedges or sections in a one-part alcohol:three-part glycerin: one-part water solution for 24 hours in a 60˚C oven. We sectioned wood samples with a GSL1 portable microtome (Gärtner *et al.*, 2014), stained the sections with Toluidine blue, and mounted them onto microscope slides with glycerin jelly for examination. Sapwood vessels from the outermost two centimeters of sapwood of each species, and whole stem section in vine species with only c.1 cm stem diameter were examined with a light microscope (40x and 100x magnification; Nikon ECLIPSE Ci-L) and photographed with a mounted digital camera (Nikon DS-Fi2). We processed the digital images using imaging software GIMP (v2.8.10) and colored 60-100 vessel lumens from at least three images per species. To obtain vessel density (VD), we subsampled these colored vessels from three digital square frames of a standardized area, counting and measuring the area of all vessel lumens that fell within the frame. We used the imaging software Image J to count and measure vessel lumen areas (VA) from the colored vessel lumens.

We calculated vessel fraction (VF) as the product of vessel area and vessel density (VA x VD). Idealized vessel diameter was calculated from the vessel area, and from these we obtained mean (d) and maximum diameters (d_{max}) for each species. Additionally, we calculated the hydraulically weighted diameter (d_h) of each species, using the equation: $(\Sigma d^4/N)^{0.25}$, where N is the number of vessels (Tyree and Zimmermann, 2002). This measure denotes the mean diameter that vessels need to be if it is to have the same total conductivity for the same number of conduits as the sampled stem. As d_{max} and d_h were strongly correlated with d and showed effectively similar patterns (see Fig. S1; Table S3), we excluded d_{max} and d_h from further analyses.

We used the Hagen–Poiseuille equation (Tyree and Zimmermann, 2002) to calculate the theoretical specific xylem hydraulic conductivity (K_s) per unit of

cross sectional area (henceforth theoretical conductivity or conductivity per unit of cross sectional area) as: $K_s = \pi \Sigma d^4/128\eta A_{\text{cross sectional area}}$, where η is the viscosity ($1.002 \times 10{-}9$MPa s^{-1}) (Tyree & Ewers, 1991). We also derived a vulnerability index (VI = *d*/VD: susceptibility to cavitation) (Carlquist, 1977) for each species.

Finally, as vessel clustering may enhance hydraulic efficiency, we calculated an index related to vessel grouping, the vessel multiple fraction (V_{mf}), which is the number of groups of vessels (i.e. vessels in direct contact with one another) divided by the total number of vessels (Scholz *et al.*, 2013). A V_{mf} value approximating 1 denotes a tendency towards solitary vessels.

To visualize hydraulic safety-efficiency mechanisms, we constructed vessel diameter class distributions of each species, sorted into proportions (%) of vessels contributing to each diameter class, and also the averages of these proportions across all 15 species in each functional group. Firstly, we sorted vessels of each species into 28 diameter classes of 25 μm intervals, with the exception of the last class (> 650 μm). We averaged the vessel diameters of each class across all 15 species in each functional group, and calculated the percentage frequency of vessels within each class. We also calculated the skew for the averaged diameter class distribution.

To obtain intrinsic water-use efficiencies (WUE_i) of each species, we used a standard algorithm provided by Werner *et al.* (2012), which calculates WUE_i from user-supplied source air CO_2 concentrations and the $\delta^{13}C$ values of leaf samples and source air CO_2. Previous studies have shown that WUE_i estimates from leaf carbon isotope content should be corrected for the contribution of the carbon isotope composition of respired CO_2 in closed-canopy forests (Da Silveira *et al.*, 1989). We measured carbon isotope ratios ($\delta^{13}C$) from leaf samples, which were ground finely using a bead mill grinder and analysed at the Terrestrial Ecohydrology laboratory, School of the Environment, University of

Technology, Sydney or at the School of Plant Biology, University of Western Australia.

We used a custom-built sampling manifold to collect data on CO_2 concentrations and isotopic composition through the forest canopy. Continuous monitoring with a Picarro G2131-i Analyzer (Picarro Inc., Santa Clara, CA, USA) allowed us to obtain the average daily (0600 to 1800 hrs) CO_2 concentrations as well their $\delta^{13}C$ values at 0.5, 1.5, 3, 22, and 44 m for a month during the 2014 dry season (Table S2). Depending on the canopy height positions or light exposure of individual species, we used corresponding CO_2 concentrations and $\delta^{13}C$ values in the algorithm. We used the CO_2 concentrations and their $\delta^{13}C$ values at the 44m collection point for vines, mature-phase trees, and pioneer trees and shrubs; at 22m for understorey trees, and at 1.5m for understorey shrubs. As $\delta^{13}C$ values were strongly correlated with WUE_i (Pearson correlation: $r = 0.847$, $P < 0.0001$), we use only the latter in our analyses.

2.3 Data Analyses

To compare the differences among plant functional groups, we analyzed the data using both univariate and multivariate statistics. We averaged the trait values within species and then for each functional group. Variables were checked for normality and transformed where necessary before analysis. A one-way ANOVA was performed for each trait and significant differences between functional groups were determined by Tukey HSD tests ($\alpha = 0.05$). We used Pearson correlations to examine inter-trait correlations. To examine the generality of the correlation patterns between traits and to visualize the relationships between our functional groups multivariately, we performed a principal components analysis (PCA) with the measured traits.

We examined the influence of species evolutionary histories on the observed trait differences between plant functional groups by comparing the results of anovas and PCAs with and without phylogenetic correction. A phylogenetic tree for our 90 species was constructed using the PHYLOMATIC v.3 utility (Webb & Donoghue, 2005), based on the R20120829 phylogenetic tree for plants, derived from the Angiosperm Phylogeny Group III reconstruction (APG, 2009), with polytomies applied within most families and genera (Fig. 2). For this analysis, branch lengths were scaled to 1. We conducted phylogenetic anovas and post-hoc tests for each trait, using the phylanova function, and phylogenetic PCA on the trait dataset using the phylANOVA function in the *phytools* package in R. The phylogenetic ANOVA and PCA results were essentially similar to the normal set of analyses (Table S4 and S5 respectively); therefore, we report only the latter.

To determine the relationship between plant intrinsic water-use efficiency (WUE_i) and vessel traits across our 90 study species, we used a generalized linear model to fit WUE_i as a response to our wood and vessel traits. The model used a Gaussian distribution and an identity link, and we followed a standard protocol of data exploration (Zuur *et al*., 2010). After excluding collinear variables, the final set of explanatory variables consisted of vessel diameter ($\log_{10}(x)$), vessel multiple fraction and plant functional group as a categorical variable. All linear models were performed in R (R Development Core Team, 2011) using the nlme package.

3 RESULTS

Plant functional groups exhibited significant differences between all wood traits (Fig. 3a-g; One-way ANOVA, all $p<0.0001$; see Table S4 for complete results). Most of these results were due to high or low mean trait

values in two functional groups: understorey shrubs and vines. Trait differences across plant functional groups were not consistent, however, which suggests varying hydraulic strategies across these groups.

3.1 Trait variation across plant functional groups

In general, mature-phase trees were similar to pioneer trees in all wood traits with the exception of wood density (Fig. 3a). In contrast, understorey trees differed from mature-phase and pioneer trees in most wood traits except vessel fraction (Fig. 3d). Conspicuously, understorey trees had lower theoretical conductivities and low vulnerability indices (Fig. 3e,f). Together understorey and pioneer tree groups had significantly lower vessel multiple fractions, indicating high vessel connectivity (Fig. 3g).

Understorey shrubs presented contrasting trait strategies from pioneer shrubs, and from all other functional groups in general, having significantly higher wood and vessel densities (Fig. 3a,c), but much lower vessel diameters and fractions, theoretical conductivities and vulnerability indices (Fig. 3b,d-f). Pioneer shrubs, alternatively, exhibited many trait similarities with trees and vines such as wood density, vessel fraction, vulnerability index and vessel multiple fraction, with the exception of having lower vessel diameters and theoretical conductivities than pioneer trees and vines (Fig. 3b, e-f) and lower vessel densities than understorey trees (Fig. 3c).

Vines were noteworthy in a number of respects to their wood traits. Firstly, they were similar to pioneer trees and shrubs in wood traits, particularly wood density (Fig. 3a). Although the mean vessel diameter of vines was not significantly different from mature-phase and pioneer trees, vines exhibited by a large margin the widest vessels across all functional groups. For example, vessels in stem sections of *Mucuna gigantea* (max. diameter: 706.6μm) and two other outliers species were plainly visible to the naked eye. The same three

species had correspondingly high vulnerability indices (Fig. 3f), although again vines as a group did not differ from pioneer tree and shrubs.

Most conspicuously, vines had significantly the highest vessel fractions (Fig. 3d) and consequently also the highest theoretical conductivities across all functional groups (Fig. 3e). Vessel multiple fractions was highest in vines, denoting low vessel connectivities, although the trait means did not differ significantly from mature-phase trees and shrub groups.

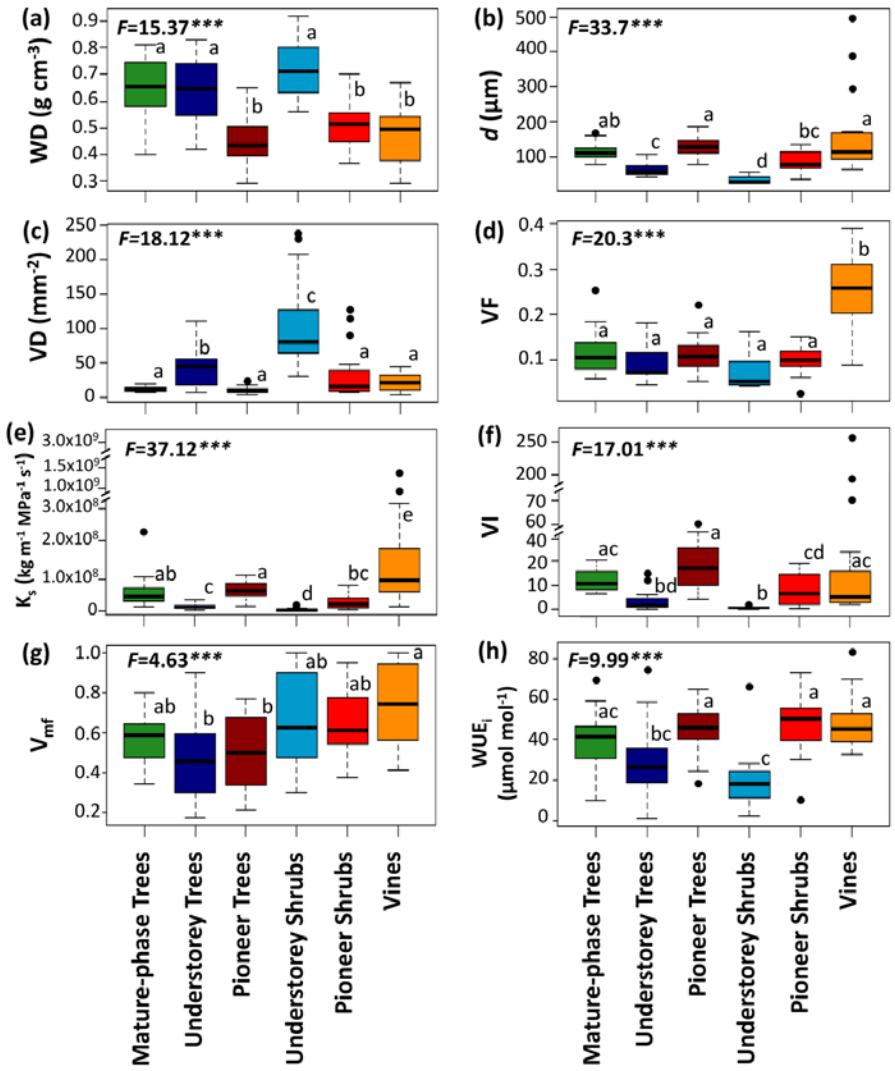

Fig. 3. Boxplots showing (a) wood density, (b) vessel lumen diameter, (c) vessel density, (d) vessel lumen fraction, (e) theoretical conductivity, (f) vulnerability index, (g) vessel multiple fraction, and (h) intrinsic water use efficiency of six woody plant functional groups in a lowland tropical rainforest. Each box encompasses the 25th to 75th percentiles; the median is indicated by the boldest vertical line and the other vertical lines outside the box indicate the 10th and 90th percentiles. Dots indicate outliers. One-way ANOVAs were performed on the data and significant differences between groups are indicated by different letters based on Tukey HSD tests at a 0.05

confidence level. Numerator degrees of freedom and denominator error degrees of freedom are 5 and 84 respectively for all measures.

A multivariate analysis of 90 rainforest plant species ordinated trait space identified three major ordination axes that explained 92.3% of the data variation (Fig. 4). The first PCA axis (62.0%) identified a gradient of functional groups with vines and pioneer species on the positive side of the axis 1 and coordinating with traits of vessel diameter, theoretical conductivity and vulnerability index and on the negative side of the axis shrubs and understorey trees coordinating with vessel and wood density. Of the second axis (16.0%) vines are positively associated with the axis and correlated with vessel fraction and vessel density and negatively associated with mature and pioneer trees and the traits wood density, vulnerability index and vessel multiple fraction. The third axis (14.4%) (not illustrated in Fig. 4) was defined primarily by vessel multiple fraction.

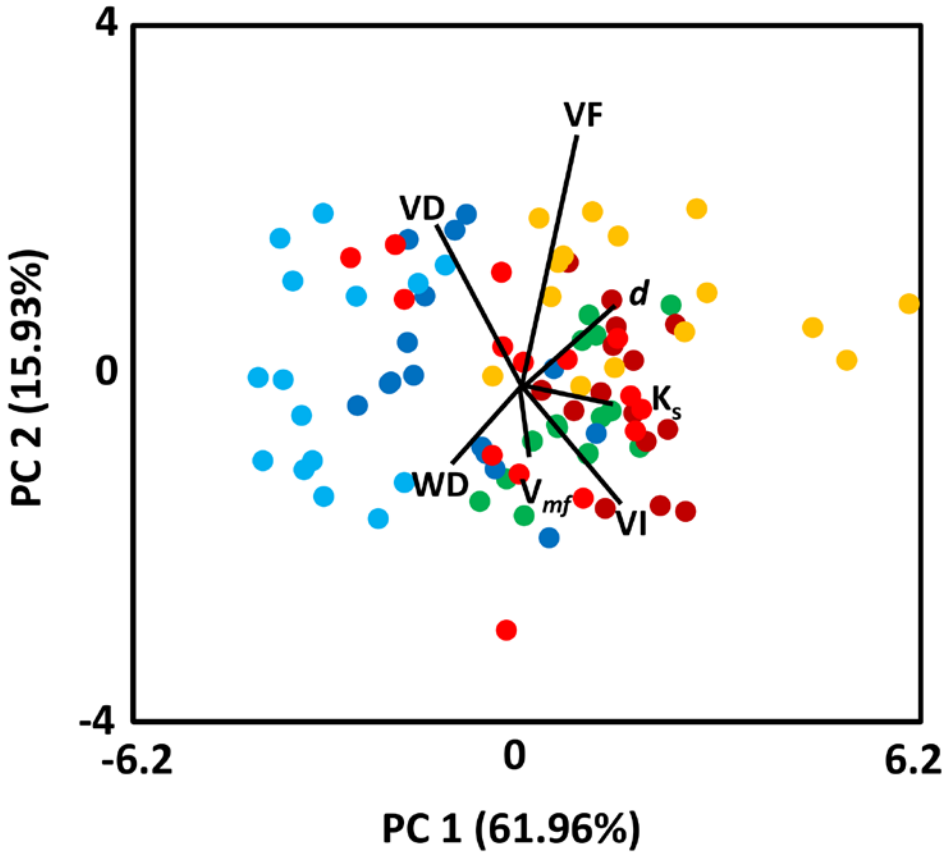

Fig. 4. Principal components analysis of wood trait means of 90 tropical lowland rainforest plant species. Trait weightings are plotted as vectors whose length and direction represent the contribution of the variable in explaining the clustering pattern. Colors indicate our six woody plant functional groups (Mature-phase Trees - GREEN; Understorey Trees - DARK-BLUE; Understorey Shrubs - LIGHT-BLUE; Pioneer Trees - DARK-RED; Pioneer Shrubs - BRIGHT-RED; Vines - ORANGE).

3.2 Vessel diameter class distributions across plant functional groups

We observed important differences in the distribution of vessel diameter classes across the plant functional groups. Mature-phase and pioneer trees

exhibited similar patterns in vessel size class distributions (Fig. 5a,c), but understorey species, and to a smaller degree pioneer shrubs had a high percentage of vessels in the smaller diameter classes (Fig. 5b,e). Understorey shrubs in particularly, exhibited the narrowest range of vessel diameter classes, spanning only four classes, with ~30% of the vessels within the 25-50µm diameter class (Fig. 5d). Conspicuously, vines had the widest range of diameter size classes across all functional groups spanning the very small (<25µm) to very large (>650 µm) size classes (Fig. 5f; see Table S6 for complete results), which was six times wider than the range for understorey shrubs, and almost three times that of pioneer trees. Vines also exhibited the highest median vessel diameters (Fig. 5f), which was five times that of understorey shrubs (Table S6). All the functional group diameter class distributions had positive skews.

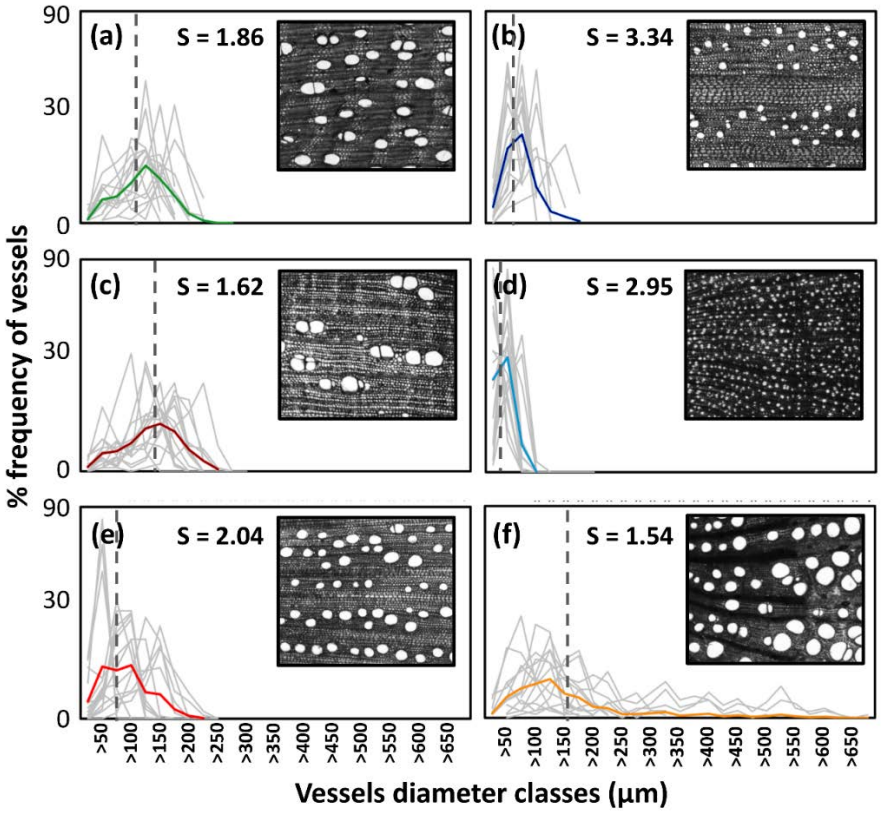

Fig. 5. Vessel diameter class distribution of plant functional groups (n=15 species per group) including (a) Mature-phase trees; (b) Understorey trees; (c) Pioneer trees; (d) Understorey shrubs; (e) Pioneer shrubs, and (f) Vines. Diameter class distributions for each species are depicted in grey lines and group mean vessel diameter classes are shown as colored lines. The dashed line refers to the median diameter of the group vessel distribution, and the skew (S) of this distribution is indicated. Insets show the cross-sections of stems sections (all 40-fold magnification) of species within each plant functional group representative of the median of group size class distributions: Mature-phase tree (*Syzygium gustavioides*); Understorey tree (*Citronella smythii*); Understorey shrub (*Hernandia albiflora*); Pioneer tree (*Macaranga tanarius*); Pioneer shrub (*Leea novoguineensis*); Vine (*Embelia cauliaculata*).

3.3 Intrinsic water-use efficiency and wood traits

Intrinsic water-use efficiency (WUE_i) was highest in vines and lowest in understorey shrubs of the 90 rainforest plant species in this study (Fig. 3h). Variation in WUE_i was best explained by a model that included only vessel diameter and plant functional group (Table 2; Fig. 6). Of the six plant functional groups included in the model, only understorey shrubs was a significant predictor of WUE_i (Table 2).

Table 2 Results of a generalized linear model (GLM) fitted with vessel trait variables to intrinsic water-use efficiency across 90 lowland woody rainforest species

Variable	**Estimate**	**S.E**	**t-value**	***P***
(Intercept)	-10.638	20.735	-0.513	0.609
[a]Vessel diameter	22.110	9.923	2.228	**<0.029***
[b]Vessel multiple fraction	2.600	6.684	0.389	0.698
Understorey trees	-8.860	6.056	-1.463	0.147
Pioneer trees	7.435	5.422	1.371	0.174
Understorey shrubs	-15.711	7.856	-2.000	0.049*
Pioneer shrubs	11.051	5.709	1.936	0.056
Vines	3.009	5.683	0.529	0.598

[a]Log_{10} transformed, [b]Arcsine transformed. Significance levels: *$P < 0.05$; ** $P < 0.001$; ***

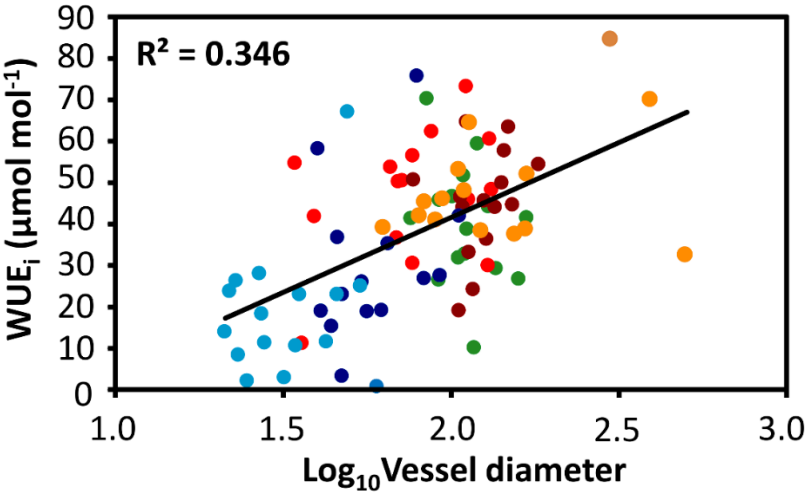

Fig. 6. Relationships between intrinsic water use efficiency (WUEi) and mean vessel diameter in 90 tropical plant species. An ordinary least-squares regression line is drawn and the R2 value of a linear regression is indicated. Colors indicate our six woody plant functional groups (Mature-phase Trees - GREEN; Understorey Trees - DARK-BLUE; Understorey Shrubs - LIGHT-BLUE; Pioneer Trees - DARK-RED; Pioneer Shrubs - BRIGHT-RED; Vines - ORANGE).

4 DISCUSSION

In the wet lowland rainforests of north-eastern Australia, we found significant variations in the wood traits of six plant functional groups that reflect a suite of hydraulic strategies. Our study of 90 plant species presents the first cohesive study of wood functional anatomy of different plant functional groups within a rainforest ecosystem, and how this relates to long-term integrated water-use efficiency. Wood trait values of our study species generally fall within previously published ranges for their respective lifeforms of trees (Tyree

& Zimmermann, 2002), shrubs (Martínez-Cabrera *et al*., 2009; Marques *et al*., 2015), and vines (Olson *et al*., 2014).

Some of the largest trait differences we observed were between the vine and understorey shrub functional groups, particularly in terms of vessel dimensions and theoretical conductivities. The generally low wood densities, wide vessels and large theoretical conductivities of vines relative to other functional groups may be related to the climbing habit and the need for rapid growth. Tyree & Ewers (1991) suggested that because vines use other plants for support, they can save on resource allocation to constructing tissue for mechanical support, thus allowing water conduction functions to be accentuated.

Contrastingly, understory shrubs had high wood densities, and the smallest vessels and conductivities, which could be related to small plant stature and slow-growing habit in the forest understory (Falster & Westoby, 2005; Rosell *et al.*, 2013; McCulloh *et al.*, 2015). Mature-phase, pioneer trees and pioneer shrubs were similar in most wood traits except for lower wood densities in the latter two groups, reflecting a well-documented strategy in pioneer species for maximizing growth at the expense of structural support (Meinzer *et al.*, 2003; McCulloh et al., 2011). And finally, understory trees were intermediate in many wood traits with mature-phase trees and understorey shrubs, probably reflecting plant height:vessel size scaling effects (e.g. Zach *et al.*, 2010; Olson *et al.*, 2014).

4.1 Tradeoffs between hydraulic efficiency and safety

A number of tradeoffs between vessel traits were apparent in the study, which helped to define water transport and also safety-efficiency strategies across plant functional groups. Understory shrubs had numerous small vessels and the highest wood densities – traits frequently associated with resistance to cavitation (Hacke *et al.*, 2001). On the other extreme, vines and to a lesser

extent pioneer trees and shrubs had large vessels and low vessel densities, leading to higher vessel diameter to number ratio (i.e. high vulnerability index). This relationship is well-characterized in the literature (Tyree & Zimmermann 2002; Poorter *et al.*, 2010; Zanne *et al.*, 2010) as a tradeoff between hydraulic efficiency and safety: species with few large vessels may have higher hydraulic conductivity, but also an increased potential cavitation risk.

The tendency for vessels within a stem to have non-random distributions can have important implications for hydraulic conductivity and safety. Previous studies have demonstrated that a higher conductivity could be hypothesized from higher vessel interconnectivity (Martínez-Cabrera *et al*., 2011; Lens *et al.* 2011; Fortunel *et al*., 2014). Contrary to our expectations, we found no evidence of high vessel connectivity (i.e. denoted by low vessel multiple fractions) being associated with high theoretical conductivities, and found instead that vessel multiple fraction was generally decoupled from other wood traits. This result was likely due to the influence of vines, which had the highest conductivities but which also had many solitary vessels with low vessel interconnectivity (high vessel multiple fractions). On the other hand, the tendency of vines to have solitary vessels, might also be interpreted as a safety mechanism against embolisms, since vulnerability to embolism increases with the connectivity of the xylem network (Loepfe *et al.* 2007; Martínez-Vilalta *et al.* 2012).

Few ecological studies have taken into consideration how the distribution of vessel sizes within stems may moderate this efficiency-safety tradeoff. In vines we found wide vessels intermixed with narrow vessels, as others have documented (e.g. Carlquist, 1991, Olson et al., 2014; Rosell & Olson, 2014), which may serve as a means of balancing high conductivity whilst maintaining hydraulic safety (Holbrook & Putz, 1996; Baas *et al*., 2004; Santiago *et al*., 2014). Narrow vessels in vines may provide a measure of conductive safety by permitting water conduction to continue if larger vessels are embolized

(Carlquist, 1991; Olson *et al.*, 2014). We also observed this behavior to a lower extent in pioneer trees and shrubs, which may explain their wider ecological tolerance relative to mature-phase trees. On this note, understory trees and shrubs with a redundancy of vessels in the very narrow diameter classes can be expected have high safety, but very low conductive efficiency (Tyree & Zimmermann, 2002).

4.2 Plant performance and vessel traits

To contextualize the trends we found in wood traits with an index of plant performance, we modelled the relationship between intrinsic water-use efficiency (WUE_i) and vessel features and functional groups. Across plant functional groups, we observed a positive relationship between WUE_i and vessel diameters. Since photosynthesis, gas exchange and water transport are intrinsically linked (Cruiziat *et al.*, 2002; Brodribb *et al.*, 2007), hydraulic limitation due to small vessels and lower conductivity also limits carbon gain (Bazzaz, 1979; Tyree & Zimmermann, 2002). This is corroborated by the observation that highly-illuminated functional groups (mature-phase and pioneer trees and vines) had large vessels and correspondingly high WUE_i, while understorey trees and shrubs under shaded rainforest canopies had numerous narrow vessels associated with a very low WUE_i.

The positive correlation that we observed between WUE_i and stem hydraulic conductivity differs from patterns observed previously for trees grown in exclusively in sunny environments (Panek, 1996; Cernusak & Marshall, 2001; Kerr *et al.*, 2015). In those cases, lower stem hydraulic conductivity was associated with less negative leaf $\delta^{13}C$, and therefore a higher WUE_i; thus WUE_i and hydraulic conductivity were negatively correlated.

The calculation of WUE_i from $\delta^{13}C$ is based upon the correlation between carbon isotope discrimination and the ratio of intercellular to ambient CO_2

concentrations. This ratio, in turn, reflects the balance between the supply of CO_2 to the leaf interior by stomatal conductance and its demand by photosynthesis. Our data show that for plant functional types that live in the heavily shaded rainforest understory, even though they have low stem hydraulic conductivity, the balance between supply and demand of CO_2 inside the leaf is still tipped in favor of a higher ratio of intercellular to ambient CO_2 concentrations, and therefore a lower WUE_i. This reflects the low photosynthetic demand for CO_2 caused by low irradiance. We conclude that the light environment in which different plant functional types grow in tropical rainforests plays a predominant role in structuring patterns of WUE_i among them.

4.3 Additional considerations

We explored the influence of phylogenetic relatedness upon wood traits and found repeated coordinated trait evolution across lineages. This is in agreement with other studies that have found wood anatomical traits to be phylogenetically labile at the species level (Zheng & Martínez-Cabrera 2013, Fortunel *et al.,* 2014), although it should be noted our study had very few same-genera samples, which may have helped to minimize any differences caused by phylogenetic relatedness (i.e. Silvertown & Dodd, 1997).

The study of wood functional anatomy is a rapidly growing field (Hartmann *et al.*, 2015), and we acknowledge that there are functional anatomical features affecting plant hydraulics that we were unable to take into account. These include vessel length distributions, vessel ultrastructural features such as pit membrane characteristics and perforation plate type and parenchyma fractions (e.g. Comstock & Sperry 2000; Christman & Sperry 2010; Jacobsen et al. 2012; Scholz *et al.*, 2013; Morris *et al.*, 2015). Integrating these additional

traits could be potential avenues of future investigation, and may be expected to further characterize hydraulic distinctiveness across plant functional groups.

5 CONCLUSIONS

We compared the functional anatomy of different plant functional groups within a rainforest ecosystem to characterize their water transport strategies and to understand the trade-offs between hydraulic traits that underpin their ecological differentiation. We found that lifeform (trees, shrubs or vines), and potentially light exposure strongly influence the hydraulic architecture of our plant functional groups. Vines in general also exhibited various differences in wood anatomy from the other plant functional groups, suggesting a highly different hydraulic strategy. Wood anatomy was a significant predictor of leaf intrinsic water use efficiency, and reflects the close co-ordination between wood and leaf hydraulics.

Our results exemplify the value of using plant functional group approaches as a robust comparative platform for understanding plant ecological strategies. There is scope for applying the functional anatomy trait data from this work to underpin physiological measures of stem and leaf drought vulnerabilities (e.g. Skelton *et al.*, 2015). This is particularly important for vines, and understorey-shrubs and -trees, which have traditionally received much less attention relative to mature-phase and pioneer trees. There is also potential for extending this work to tropical forest along rainfall gradients to provide a sound basis for modelling shifting species distributions.

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SUPPORTING INFORMATION

Table S1. Means and standard deviations of leaf and wood traits of the study species. Traits abbreviations: maximum sap flow rate (cm3 hour-1): SF; maximum sap velocity (cm hour-1): SV; Huber value: HV (unitless); Vessel area: VA (μm-2); Vessel density; VD (unitless); Vessel fraction: VF (unitless); leaf mass per area (g cm-2): LMA; leaf area (cm2): LA; leaf slenderness: LS (unitless); minimum and maximum leaf water potentials (MPa): LWPmin and LWPmax; Leaf carbon isotopes ratio (‰): δ13C; Water-use-efficiency (μmol mol-1): WUEi.

Trait	**Early successional**				**Mature-phase**										**Palm**	
	Alstonia scholaris		*Elaeocarpus angustifolius*		*Argyrodendron peralatum*		*Castanospermum australe*		*Endiandra microneura*		*Myristica globose*		*Syzygium graveolens*		*Normanbya normanbyi*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SF	787.30	1002.02	3527.19	3857.34	838.51	553.23	2834.07	3100.56	623.46	444.91	757.21	341.81	4969.94	1815.41	1092.93	449.88
SV	5.34	1.60	8.52	3.73	2.89	1.16	1.95	0.87	2.17	1.13	2.44	1.18	8.03	1.29	8.06	3.31
RGR	1.05	1.10	16.75	11.82	0.57	0.47	0.63	0.54	0.81	0.58	0.68	0.40	0.49	0.61	0.38	0.62
TSC	8.36x10^{5}	34263.17	150220.3	25629.55	180821.8	78142.70	70221.55308		246692.9	100208.0	220459.3	55309.56	430197.9	173464.4	1166024.3	459726.0
Vf	0.04	0.008	0.07	0.008	0.05	0.008	0.03	0.001	0.08	0.018	0.07	0.008	0.16	0.041	0.05	0.013
Va	7646.72	1861.62	8830.37	609.06	13987.65	4283.75	9701.76	1145.19	12159.32	2561.55	12325.55	2391.68	10618.66	1691.70	86346.92	22295.88
VD	6.14	0.86	8.42	0.41	4.02	1.10	3.05	0.48	6.39	0.86	5.93	0.96	14.66	2.01	0.64	0.20
SWA	196.30	139.86	406.36	272.33	292.82	90.40	1431.52	1258.82	322.87	187.77	385.35	87.92	744.53	242.51	161.44	10.68
LA	3473.10	422.03	2295.50	173.72	2506.98	601.75	3831.58	700.85	3034.30	530.68	5907.66	1846.64	4504.71	640.20	6580.51	1244.54
LMA	0.13	0.01	0.11	0.01	0.18	0.01	0.11	0.01	0.13	0.01	0.12	0.01	0.14	0.01	0.13	0.01
LDM C	0.32	0.09	0.43	0.02	0.50	0.02	0.42	0.03	0.41	0.03	0.39	0.03	0.42	0.03	0.40	0.02
LS	3	0.24	3.52	0.16	4.37	0.55	2.62	0.36	2.22	0.14	2.09	0.08	2.39	0.42	15.08	2.33
LT	0.36	0.06	0.33	0.14	0.36	0.14	0.31	0.01	0.29	0.05	0.28	0.08	0.37	0.10	0.30	0.03
HV	40.85	23.44	39.08	22.22	24.30	16.58	38.17	3.86	24.74	12.62	21.63	5.95	27.71	15.19	68.90	48.35
Min Ψ_L	-6.40	0.00	-9.89	1.37	-13.57	1.61	-13.59	4.46	-10.72	1.52	-13.82	1.69	-6.49	1.81	-8.82	2.82
Max Ψ_L	-5.45	0.00	-2.205	0.75	-3.73	0.51	-4.99	1.10	-3.76	0.55	-3.4575	1.44	-1.46875	0.10	-1.7875	0.95
$\delta^{13}C$	-28.84	0.43	-30.73	0.98	-32.09	0.49	-29.32	0.83	-33.06	0.60	-30.92	0.74	-30.96	0.13	-30.08	0.92
WUE_i	64.68	4.73	44.05	10.68	29.28	5.36	59.37	9.01	18.76	6.55	41.98	8.01	41.53	1.45	51.18	10.04

Table S2 Daytime (0600 to 1800hrs) gas characteristics sampled through the forest canopy at various heights at the Daintree Rainforest Observatory, Cape Tribulation, Australia. Data represent mean and range of daily averages sampled between 24th May and 22nd June 2015

Sampling height	CO_2 concentration (ppm)	δ13C (‰)
0.5m	451.6 ± 10.3	-9.0 ± 0.6
1.5m	439.9 ± 9.0	-8.4 ± 0.6
3.0m	431.2 ± 7.5	-7.9 ± 0.6
22m	416.5 ± 1.5	-7.0 ± 0.5
44m	418.4 ± 2.7	-7.1 ± 0.5

Table S3 Traits measured in the current study across 90 lowland rainforest plant species from six aplant functional groups (mature-phase trees, understorey trees, pioneer trees, understorey shrubs, pioneer shrubs, and vines)

Traits	Unit	Mature - phase Trees		Understorey Trees		Pioneer Trees		Understorey Shrubs		Pioneer Shrubs		Vines	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
WD	g cm^{-3}	0.652	0.111	0.633	0.130	0.452	0.096	0.722	0.105	0.509	0.099	0.465	0.019
D	µm	114.820	26.045	61.941	20.220	127.294	25.584	32.650	10.675	83.288	33.927	170.746	128.696
d_{max}	µm	176.615	37.045	93.783	31.827	193.099	36.134	49.261	16.081	124.118	53.737	285.837	176.420
d_H	µm	121.877	23.573	66.835	22.438	138.384	28.376	34.947	11.111	87.810	35.831	199.393	125.559
VD	mm^{-2}	11.391	3.544	41.285	28.33	10.469	5.951	107.460	68.829	35.845	42.148	21.207	14.252
VF	No unit	0.115	0.053	0.094	0.044	0.114	0.044	0.075	0.039	0.099	0.033	0.249	0.088
K_s	kg s^{-1} MPa^{-1}	$5.69x10^7$	$5.40x10^7$	1.12 $x10^7$	$7.6x10^6$	$5.83x10^7$	$2.40x10^7$	3.22 $x10^6$	$3.8x10^6$	2.54 x10	$2.20x10^7$	$4.52x10^8$	$8.3x10^8$
VI	No unit	12.222	6.155	3.575	4.485	19.730	15.406	0.531	0.444	7.836	7.181	40.631	78.819
V_{mf}	No unit	0.566	0.139	0.453	0.191	0.517	0.187	0.654	0.247	0.653	0.181	0.741	0.219
$\delta^{13}C$	‰ VPDB	-30.741	1.085	-32.832	2.531	-30.623	1.191	-35.139	1.520	-30.865	1.902	-30.286	1.306
WUE_i	µmol mol^{-1}	39.805	14.654	28.541	19.437	45.237	12.950	19.747	15.546	47.089	15.343	48.901	14.197

[a]Each plant functional group was represented by 15 replicate species. See Table 1 for trait abbreviations

Table S4 Pearson correlation coefficients for linear relationships between wood anatomical and hydraulic traits of the 90 studied rainforest species

	WD	*d*	d_{max}	d_h	VD	VF	K_s	VI	V_{mf}
D	**-0.589**								
d_{max}	**-0.576**	**0.982**							
d_h	**-0.577**	**0.969**	**0.976**						
VD	**0.466**	**-0.898**	**-0.877**	**-0.855**					
VF	**-0.434**	**0.532**	**0.530**	**0.546**	ns				
K_s	**-0.606**	**0.955**	**0.941**	**0.937**	**-0.729**	0.758			
VI	**-0.523**	**0.956**	**0.939**	**0.917**	**-0.985**	***0.263***	**0.828**		
V_{mf}	ns	ns	ns	ns	ns	ns	ns	ns	
WUE_i	***-0.337***	**0.541**	**0.529**	**0.542**	**-0.513**	***0.238***	**0.500**	**0.530**	ns

Significance levels: ns = non-significant; bold italic letters = $P < 0.05$; bold letters = $P < 0.001$; bold underlined letters = $P < 0.0001$. See Table 1 for trait abbreviations.

Table S5 Normal and phylogenetic One-way ANOVA results for wood trait comparisons between six woody plant functional groups from a lowland tropical rainforest (Significance test: **, P < 0.005; ***, P < 0.001; ns, non-significant)

Trait	**Normal ANOVA *F***	**Phylogenetic ANOVA *F***
WD	15.37***	15.37**
D	33.70***	33.98**
d_{max}	41.39***	41.39**
d_h	38.52***	38.52**
VD	18.12**	17.28**
VF	20.30***	14.43**
K_s	37.12***	37.12**
VI	17.01***	23.13**
V_{mf}	4.27**	4.27*
$\delta^{13}C$	19.88***	19.88**
WUE_i	9.99***	9.99**

[a]Numerator degrees of freedom and denominator error degrees of freedom are 5 and 84 respectively for all measures. See Table 1 for trait abbreviations

Table S6 Normal and phylogenetic principal components analysis (PCA) axis loadings for each wood trait of 90 species from six woody plant functional groups from a lowland tropical rainforest. The percentage of variance explained for each PCA axis is given in parentheses

	Normal PCA			Phylogenetic PCA		
Trait	**PC1(61.96)**	**PC2(15.93)**	**PC3(14.41)**	**PC1(59.94)**	**PC2(18.87)**	**PC3(13.47)**
WD	-0.331	-0.251	0.149	-0.690	0.262	0.131
D	0.476	-0.066	-0.001	0.991	0.048	0.008
VD	-0.417	0.456	0.111	-0.836	-0.501	0.213
VF	0.272	0.728	0.216	0.539	-0.742	0.368
Ks	0.461	0.203	0.071	0.944	-0.254	0.144
VI	0.448	-0.304	-0.077	0.923	0.347	-0.140
V_{mf}	0.041	-0.220	0.952	0.067	0.512	0.839

See Table 1 (Manuscript 2) for trait abbreviations

Table S7 Descriptive parameters of stem vessel size class (μm) distributions of different plant functional groups (group means) in a lowland tropical rainforest

Plant functional group	**Median**	**90th percentile**	**Skew**
Mature-phase Trees	110.06	148.04	1.86
Understorey Trees	63.19	79.85	3.34
Pioneer Trees	129.94	166.84	1.62
Understorey Shrubs	32.51	41.82	2.95
Pioneer Shrubs	82.83	106.85	2.04
Vines	150.96	215.28	1.54

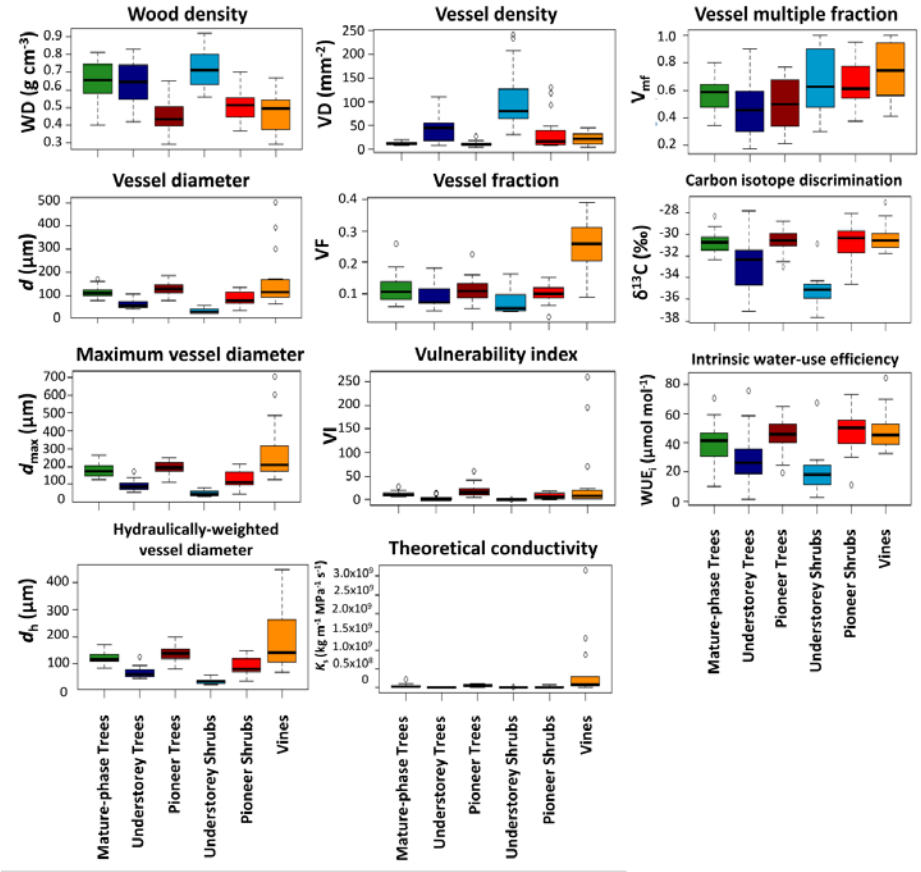

Fig. S1. Boxplots of traits measured in the study of 90 species from six woody plant functional groups in a lowland tropical rainforest. Each box encompasses the 25th to 75th percentiles; the median is indicated by the boldest vertical line and the other vertical lines outside the box indicate the 10th and 90th percentiles. Dots indicate outliers. Refer to Table 1 for trait abbreviations.