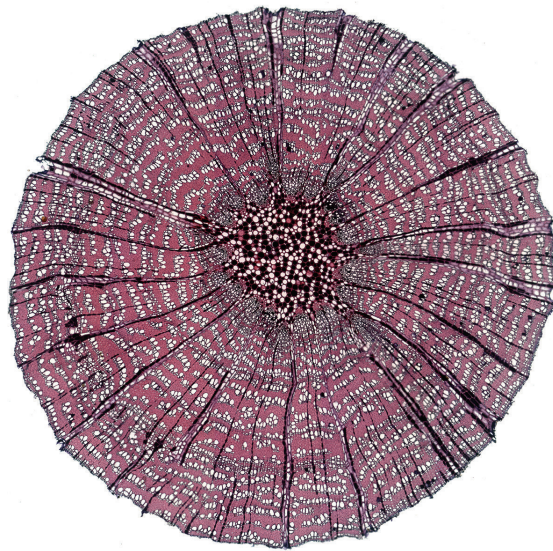


# **ANATOMICAL VARIATION IN TWIG WOOD ACROSS AUSTRALIAN ANGIOSPERMS**



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May 2014



# Contents

Contents .....	3
Figure list .....	6
Table list .....	8
Thesis abstract .....	9
Statement of candidate .....	11
Acknowledgements .....	13
Chapter 1 INTRODUCTION .....	15
1.1 Background .....	17
1.2 General approach .....	20
1.3 Thesis plan .....	21
1.4 Methodological note .....	22
1.5 References .....	26
Chapter 2 DISSECTING STEMS: WHAT CAN WOOD ANATOMY DISCLOSE ABOUT PLANT STRATEGIES? .....	31
2.1 Abstract .....	33
2.2 Introduction .....	33
2.2 Overview of functional processes and traits .....	38
2.3 Fibres .....	42
2.3.1 Fibre structure .....	42
2.3.2 Fibres and climate .....	44
2.3.3 Fibres and functional traits .....	45
2.3.4 Fibres in experimental studies .....	46
2.4 Parenchyma .....	47
2.4.1 Parenchyma structure .....	47
2.4.2 Parenchyma and climate .....	49
2.4.3 Parenchyma and functional traits .....	50
2.4.4 Parenchyma in experimental studies .....	51
2.5 Vessels .....	52
2.5.1 Vessel structure .....	52

2.5.2	Vessels and climate .....	54
2.5.3	Vessels and functional traits .....	56
2.5.4	Vessels in experimental studies .....	57
2.6	Synthesis: wood functional systems .....	58
2.6.1	Mechanical system .....	58
2.6.2	Metabolite transport and storage system .....	59
2.6.3	Hydraulic system.....	60
2.7	Conclusions.....	62
2.8	Acknowledgements .....	63
2.9	Figures .....	65
2.10	Tables.....	73
2.11	References .....	84
Chapter 3	FIBRE WALL AND LUMEN FRACTIONS DRIVE WOOD DENSITY VARIATION IN TWIGS ACROSS 24 AUSTRALIAN ANGIOSPERMS.....	97
3.1	Abstract .....	99
3.2	Introduction .....	99
3.3	Materials and Methods .....	102
3.4	Results .....	106
3.5	Discussion .....	109
3.6	Acknowledgements .....	113
3.7	Figures .....	115
3.8	Tables.....	129
3.9	References .....	130
3.10	Appendix 1 .....	137
3.11	Appendix 2 .....	142
Chapter 4	WOOD ANATOMICAL VARIATION LARGELY INDEPENDENT OF WOOD DENSITY IN TWIGS OF 69 AUSTRALIAN ANGIOSPERMS .....	151
4.1	Abstract .....	153
4.2	Introduction .....	154
4.3	Materials and Methods .....	158
4.4	Results .....	166



4.5	Discussion .....	175
4.6	Acknowledgements.....	183
4.7	Figures.....	185
4.8	Tables.....	207
4.9	References.....	209
4.10	Appendix.....	217
Chapter 5	DISCUSSION .....	229
5.1	Axes of variation.....	231
5.1.1	Wood density dimension .....	231
5.1.2	Anatomical variation independent of density.....	233
5.2	Limitations of this study and possible paths forward .....	236
5.3	Thesis context and significance .....	238
5.4	References.....	240

## Figure list

Figure 1-1	A map of Australia and study sites.....	23
Figure 1-2	Photographs of six study sites. ....	25
Figure 2-1	Fibres. ....	65
Figure 2-2	Parenchyma.....	67
Figure 2-3	Vessels.....	69
Figure 2-4	Vessels and tracheids.....	71
Figure 3-1	A twig cross-section of <i>Grevillea parallela</i> , Proteaceae.....	115
Figure 3-2	Tissue fractions for 24 species arranged in order of decreasing wood density. ....	117
Figure 3-3	Relationship between fraction of wood outside vessel lumens (fraction <sub>NV</sub> ) and the density of that non-vessel fraction (wood density <sub>NV</sub> ).....	119
Figure 3-4	Relationship between non-vessel density (wood density <sub>NV</sub> ) and fibre fraction in non-vessel area (fibre fraction <sub>NV</sub> ). ....	121
Figure 3-5	Relationships between non-vessel density (wood density <sub>NV</sub> ) and (a) fibre wall fraction in non-vessel area (fibre wall fraction <sub>NV</sub> ), and (b) fibre lumen fraction in non-vessel area (fibre lumen fraction <sub>NV</sub> ). ....	123
Figure 3-6	Relationship between the fraction of the non-vessel area that is fibre wall or is fibre lumen (a) and its schematic illustration (b). ....	125
Figure 3-7	Cross-sections through twigs of four species.....	127
Figure A3-1	Box plots showing comparisons in non-vessel wood density (wood density <sub>NV</sub> ), non-vessel fibre wall fraction (fibre wall fraction <sub>NV</sub> ) and non-vessel fibre lumen fraction (fibre lumen fraction <sub>NV</sub> ) between the four sites. ....	147
Figure A3-2	Overall tissue fractions for <i>Leucopogon ericoides</i> from cool-wet site and <i>L. ericoides</i> from cool-dry site.....	149
Figure 4-1	Illustration of wood tissues (a) and image analysis method used (b).....	185
Figure 4-2	Stack bar graph of tissue fractions across 69 species.....	187
Figure 4-3	Relationship between total fibre fraction and total parenchyma fraction. ....	189

Figure 4-4	Relationship between fibre wall fraction and fibre lumen fraction across 93 species (a) and a diagram illustrating this relationship (b). ....	191
Figure 4-5	Cross-sections through twigs of three low-density species.....	193
Figure 4-6	Box plots showing differences in anatomical traits and wood density between sites (and vegetation types).....	195
Figure 4-7	Relationships between parenchyma traits and vessel area. ....	197
Figure 4-8	Relationships between parenchyma traits, fibre wall fraction plus vessel wall fraction and modulus of elasticity. ....	199
Figure 4-9	Relationship between ray parenchyma fraction and pith area.....	201
Figure 4-10	Site and family comparisons for the 21 species from Myrtaceae and Proteaceae. ....	203
Figure 4-11	Bar graph representing estimated density of swollen cell wall material across 69 studied species. ....	205

## Table list

Table 2-1	Summary of variation in quantitative anatomical traits reported in the literature.....	73
Table 2-2	Relationships between fibre traits and climate.....	74
Table 2-3	Correlations between fibre and functional traits.....	75
Table 2-4	Relationships between parenchyma traits and climate.....	76
Table 2-5	Correlations between parenchyma and functional traits.....	78
Table 2-6	Relationships between vessel traits and climate. ....	79
Table 2-7	Correlations between vessel and functional traits. ....	82
Table 3-1	Species list and sites of collection. ....	129
Table A3-1	Details of the four sites sampled in this study. ....	137
Table A3-2	Wood density and tissue fractions of 24 species averaged across three replicates.....	138
Table A3-3	Wood density and tissue fractions of non-vessel proportion of 24 species averaged across three replicates. ....	140
Table A3-4	Site mean and standard deviation of wood density and tissue fractions. Values calculated on six species per site.....	145
Table A3-5	Site mean and standard deviation of wood density and tissue fractions of non-vessel proportion. Values calculated on six species per site. ....	145
Table 4-1	Site details.....	207
Table 4-2	Traits overview. ....	208
Table A4-1	Species and families. ....	217
Table A4-2	Tissue fractions: total parenchyma, axial parenchyma, ray parenchyma, total fibre, fibre wall, fibre lumen.....	218
Table A4-3	Tissue fractions: total vessel, vessel lumen, vessel wall, conduits <sub>15µm</sub> , mucilage canals.....	220
Table A4-4	Anatomical traits: axial parenchyma relative to total parenchyma, fibre wall relative to total fibre, pith area.....	222
Table A4-5	Vessel traits.....	224
Table A4-6	Non-anatomical traits. ....	226

## Thesis abstract

This thesis explores variation in wood anatomical structure and its potential ecological implications. Woody stems are major plant organs performing vital functions such as mechanical support, water transport, and metabolites storage. The various ways plants perform those functions are determined by wood anatomical structure. Despite this fundamental role of anatomy, surprisingly few studies have rigorously quantified anatomical traits, especially across a broad number of species. Moreover, twig wood has been especially overlooked in favour of main trunks. This thesis quantified anatomical variation across a relatively broad number of species in twig wood and explored the correlations of anatomy with other plant functional traits. The overarching aim was to contribute a quantitative anatomical understanding to our knowledge of plant functions, as they relate to plant ecological strategies.

Wood density has been considered a key plant functional trait, yet its anatomical nature is not entirely understood. First, anatomical underpinnings of density variation were quantified across 24 species with densities ranging from 0.37-0.83 g cm<sup>-3</sup>. Density proved mainly to be driven by the fraction of wood occupied by fibre walls. There was also substantial anatomical variation that was independent from density, representing mainly a trade-off between fibre and parenchyma fractions. The ecological significance of this dimension of variation is not understood. Since fibre-parenchyma variation was wider among lower-density species, anatomical variation in 69 low-medium density species (0.38-0.62 g cm<sup>-3</sup>) was quantified in order to capture this fibre-parenchyma trade-off more thoroughly. Potential correlates such as plant height, leaf area to sapwood area ratio, and modulus of elasticity were also measured. These other traits proved not to be well correlated with wood anatomy. For example, parenchyma fraction (an index of storage capacity) was expected to correlate with plant height (indexing canopy access to light), but it did not. The fraction of parenchyma (being soft, thin-walled tissue) was expected to covary with mechanical properties, but this expectation was only weakly supported. In addition to these two bodies of new empirical work, literature on anatomical knowledge from across diverse disciplines was synthesized. Other gaps in our current comprehension of wood anatomy and function in angiosperms were

highlighted. For example, the function of parenchyma has commonly been described as metabolite transport and storage. However, the available evidence suggests that, next to metabolite storage, parenchyma may play a major role in water storage or vessel refilling.

## Statement of candidate

I certify that the work in this thesis entitled: "Anatomical variation in twig wood across Australian angiosperms" has not previously been submitted for a degree to any other university or institution.

I also certify that the work described here is original and I performed the majority of data collection, data analysis, and writing involved in each chapter. I solely performed all anatomical work.

This thesis consists of three chapters, which are presented as draft manuscripts for submission to peer-reviewed journals. Their current status and contributions by people other than myself is outlined in the table below. Where my contribution is less than 100%, the remainder was contributed by the co-authors named. Chapter 1 (Thesis introduction) and Chapter 5 (Thesis discussion) have been written by myself only and are not intended for publication except as part of this thesis. In addition, numerous people participated in fieldwork, plant material processing, and research discussions. These are acknowledged in relevant chapters.

Chapter	Co-authors	Ziemińska's contribution (estimated %)			
		Concept	Data collection	Analysis	Writing
Chapter 2 Dissecting stems: what can wood anatomy disclose about plant strategies?	Current version written by Ziemińska only. Intended for submission, following contributions from prospective co-authors, including Steven Jansen, and further drafts.	80	100	100	100
Chapter 3 Fibre wall and lumen fractions drive wood density variation in twigs across 24 Australian angiosperms. (published in AoB PLANTS, 10.10.2013)	Don W Butler, Sean M Gleason, Ian J Wright, Mark Westoby	60	95	90	90
Chapter 4 Wood anatomical variation largely independent of wood density in twigs of 69 Australian angiosperms. (to be submitted to PLOS ONE)	Ian J Wright, Mark Westoby	80	100	90	90

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May 2014







## Acknowledgements

Comparative Ecology Lab led by Mark Westoby is an extremely nutritious environment to grow in, and I believe I am now a better researcher than I was a few years ago at the outset of my PhD. Many people contributed to my growth.

Firstly, I would like to thank my supervisor, Mark Westoby, for sharing with me his extraordinary knowledge and insightful perspectives, for his amazing generosity of time and energy in coaching me, and for patience in explaining the intricacies of plant strategies. Before my PhD, I studied plants in a relatively narrow context of comparative plant anatomy, but Mark has unveiled a broader picture of plant sciences. He sparked my excitement for plant ecology and showed how I can apply my anatomical skills to better understand how plants live. Thank you Mark!

I also would like to thank my co-supervisor, Ian Wright, who always has been amazingly supportive and generous with his help, constructive and inspiring feedback, and spot-on, crisp comments. Thanks also for encouragement to explore our curious world via logarithmic relationships!

I started my research at Macquarie University together with Sean Gleason and Don Butler. They have been fantastic colleagues and superb fieldwork companions, who introduced to me concepts in plant hydraulics and mechanics and were always happy to chat more about them. Many thanks!

The frequent lab meetings in the Comparative Ecology Lab created a great opportunity to discuss research carried out by others as well as my own, broadening my horizons, and introducing new to me, valuable perspectives. I believe the feedback I received from past and present members of the lab helped me to improve my research, as well as the way in which I communicate it. I am very grateful! Science aside, lab members have been a really friendly group of people, with whom it simply has been a pleasure to hang around, eat lunch and play croquet. Many thanks to: Chris Blackman, Don Butler, Yvonne Chang, Alicia Cook, Julia Cooke, Daniel Falster, Rich FitzJohn, Sean Gleason, Georges Kunstler, Claire Laws, Tanja Lenz, Marisa Nordenstahl, Yusuke Onoda, Barb Rice, Julieta Rosell, Andrea Stephens, Steph Stuart, Tiina Tosens, Wade Tozer, Lizzy Wenk, and Mark Westoby.

Big thank you to the people of Biological Sciences Department: the scientists, both staff and students, who shared their knowledge during multiple seminars and workshops allowing me to learn about the diverse world outside my own research and to learn new tools; academic staff, who have been very supportive in my PhD path; all the friendly administration team, especially Sam Newton, who made the things work just right. Thank you to Alison Downing, for excellent help with plant identification. Also, thanks to the Microscopy Unit for the hospitality and assistance during my anatomical work.

Many individuals helped in the field and the lab during my empirical work. These are acknowledged at the end of relevant chapters.

Research presented here would not be possible without financial support. Macquarie University scholarship allowed to carry out a significant majority of my work (Chapters 1-5), and the student grant from James Cook University allowed to use the canopy crane and research facilities at the Daintree Rainforest Observatory (Chapter 4). Australian Research Council grant awarded to Mark Westoby contributed to expenses of work presented in Chapter 3.

Finally, I would like to thank my partner, Chris, for his love, friendship, and support throughout my whole candidature. Thank you for inspiring discussions about science and life, and for playfulness, which has brightened up our sometimes too serious lives. Thank you for believing, together with me, that science is important.

## Chapter 1

# **INTRODUCTION**



Just as human anatomy has been fundamental in understanding human functions, plant anatomy can be a powerful tool for deciphering plant functions. This thesis explores the anatomical structure of wood that makes up plant stems in woody angiosperms. The overarching aim was to add quantitative anatomical knowledge to our exploration of plant function and plant ecological strategies.

In the Introduction, I describe the background of research presented in this thesis, explain general approach to the research questions, outline the thesis structure, and comment on several methodological issues.

## **1.1 Background**

All plants perform the same essential tasks: growth, water transport, photosynthesis, and reproduction. Yet they perform them differently. For example, some species are tall and others are short, some transport water more rapidly and others more slowly, some produce few large seeds and others many small ones, some produce expensive long-lived leaves and others cheap, throw-away leaves. In other words, plants have diverse ecological strategies. These strategies are underpinned by measurable functional traits such as for instance potential height, water conductivity, seed size, and leaf mass per area (Grubb 1998; Westoby et al. 2002; Grime 2006). The diversity of strategies is driven by climate, nutrient resources, and competition, and results in the stunning variability of plant organ structures we observe across different climates and also within a single location. There have been considerable advances in understanding leaf and seed strategies (e.g. Wright et al. 2004; Moles & Westoby 2006), but less so for stem strategies (Chave et al. 2009). Yet stems perform vital functions of water transport, mechanical support, and storage. Three major wood tissues perform these functions in angiosperms: vessels transport water, fibres give mechanical support, and parenchyma transports and stores carbohydrates and nutrients. The structure of those tissues varies broadly across species (IAWA Committee 1989; Carlquist 2001), suggesting a spread of ecological strategies being applied at the wood anatomical level. Potentially, by studying wood structure we can learn more about plant function and ecological strategies. In this thesis, I will concentrate on the angiosperms because they

make up an overwhelming majority of seed plants on Earth and their wood has significantly different anatomy than gymnosperm wood.

Wood anatomy is by no means a new discipline. On the contrary, it has been studied for many years starting in the 17<sup>th</sup> century when the first systematic wood descriptions were made by pioneers such as Malpighi, Grew and van Leeuwenhoek. There were no major and significant advances made in the 18<sup>th</sup>, and it was not until the 19<sup>th</sup> century when the discipline of plant anatomy gained renewed interest; especially with the discussions on the value of anatomical features for systematic purposes (mainly second half of 19<sup>th</sup> century). In that time, Rodlkofer initiated large-scale comparative studies and his student, Solareder, analysed anatomies of leaves and twig wood of over 1000 species from 140 families. However, these descriptions were of a qualitative nature. Plant anatomy was also widely studied by other researchers; their work together with Rodlkofer's and Solareder's was gathered in a comprehensive volume "Systematic anatomy of the Dicotyledons" ("Systematische Anatomie der Dikotyledonen", 1908) by Solareder. This work was taken a step further by Metcalfe and Chalk in the "Anatomy of the Dicotyledons" (1950). These authors included anatomical studies from the first half of the 20<sup>th</sup> century and updated wood descriptions with information about mature stems (Baas 1982). Gregory (1994) and Endress, Baas and Gregory (2000) have compiled comprehensive inventories of anatomical work carried out in the 20<sup>th</sup> century. Simultaneously, forestry studies have been making advances in wood descriptions conveniently gathered in forestry handbooks (e.g. Panshin & de Zeeuw 1980; Koch 1985; Ross 2010). However, the descriptions have encompassed commercial woods only, leaving a huge gap of unexplored species.

Much of this work has focused on comparative wood anatomy, where a set of qualitative and quantitative diagnostic features is applied to describe or identify given taxa. Features found helpful for identification are compiled in the very helpful handbook "IAWA list of microscopic features for hardwood identification" (IAWA Committee 1989). In addition to the abundant studies directed towards identification, ecological trends have been analysed (e.g. Baas 1986; Carlquist 2001), meaning especially the correlation of different traits with climate zones. These synthetic overviews have helped to establish some important functional trends, particularly related to vessels. At the same time,

many questions remain unresolved. Patterns and functions related to parenchyma are particularly elusive. The work of anatomists, both comparative and ecological, has been heavily concentrated on main stem wood, rather than on wood in twigs. Yet twig wood is under selection for its functions just as much as main stem wood. As the gateways to leaves, twigs are important to whole-plant functioning. This thesis concentrates on wood anatomical variation in twigs; twigs being defined as terminal branches with wood cylinder diameter of approximately 0.5 cm.

Although wood anatomy has been studied for many years, recent technological advances in microscopy and image analysis allow for increasingly better quantification and deeper understanding of plant structure (Martínez-Cabrera et al. 2009; Brodersen et al. 2013). Recently, anatomy has also become a more popular tool in studies investigating plant ecological strategies via plant functional traits (Jacobsen et al. 2007a; Mitchell et al. 2008; Gleason et al. 2012). Plant functional trait is a plant property that can be measured across species and used to compare them. Many functional traits, for example, leaf mass per area, seed mass or wood density, on the most basic level are in fact anatomical traits. Therefore, studying the detailed anatomy underpinning functional traits can be valuable in explaining plant ecological strategies.

The work in this thesis began from wood density ( $\text{g cm}^{-3}$ ). Density has routinely been measured in a vast number of studies and it has been suggested to be the key functional trait of stems (Chave et al. 2009). It is a good predictor of mechanical strength and stiffness (Panshin & de Zeeuw 1980; van Gelder, Poorter & Sterck 2006; Chave et al. 2009; Onoda, Richards & Westoby 2010). It has been implied to partly determine the trade-off between growth and survival (high-density species with slow growth rate but high survival rate; van Gelder, Poorter & Sterck 2006; Chave et al. 2009; Poorter et al. 2010). Wood density has also been shown to correlate with hydraulic traits (Meinzer et al. 2003, 2008; Santiago et al. 2004; Ackerly 2004; Bucci et al. 2004; Scholz et al. 2007; Jacobsen et al. 2007b, 2008; Pratt et al. 2007; Gotsch et al. 2010) and with life history traits (Putz et al. 1983; Enquist et al. 1999; Poorter et al. 2008, 2010; Kraft et al. 2010; Wright et al. 2010; Fan et al. 2012). It also has been suggested that many of those relationships are of correlative nature and wood maintenance costs are more likely to be causally linked with wood density (Larjavaara & Muller-Landau 2010). Additionally,

some reports have indicated density was associated with environment variables (Barajas-Morales 1985; Wiemann & Williamson 2002; Swenson & Enquist 2007; Martínez-Cabrera et al. 2009; Zhang et al. 2011), while others have found no relationship (ter Steege & Hammond 2001; Wiemann & Williamson 2002; Muller-Landau 2004). Substantial variation within the same climate has also been recorded (Wiemann & Williamson 2002; Muller-Landau 2004; Martínez-Cabrera et al. 2009). These diverse and sometimes conflicting results draw a picture of wood density being a black box rather than an informative indicator of plant functions. Logically, wood anatomical structure must directly affect wood density. Therefore, it seems appropriate to ask what are the basic anatomical components influencing density variation across angiosperm species. This question is not new, foresters and wood anatomists have discussed it previously, but only within small numbers of species (especially commercial woods) or only in qualitative terms. This issue has only begun to be studied by formal quantification across a substantial number of diverse species.

## **1.2 General approach**

This thesis aims to answer two basic and essential questions: what is the scope of trait variation and how are the traits interrelated? A third general question is also tackled, but to a lesser degree: how are the observed interrelations climate-specific?

Each scientific endeavour needs to start with observation. Here, anatomical variation in twig wood was quantified across 93 species (Chapters 3 and 4), and also other plant traits were measured (wood density, height, leaf area to sapwood area, modulus of elasticity). Quantifying trait variation and establishing the cost-benefit interdependencies between traits is a fundamental step towards understanding large-scale ecosystem and vegetation processes, and their distributions in the physical environment (Westoby & Wright 2006). The main task of this thesis is to contribute knowledge about twig wood to this fundamental step.

In the two empirical chapters (3 and 4), species sampling was spread across different environments, the main reason being to gather a wide spread of measured traits (both anatomical and non-anatomical) rather than to compare habitats. In Chapter



3, six species from four sites were collected, but the species selection was not strictly based on abundance or randomization. Given the selection procedure and the small number of species per site, sampling cannot be considered as leading to representative or reliable descriptions of average wood properties across all the species at a site. In Chapter 4, species were chosen within a narrow wood density range ( $0.4\text{--}0.6\text{ g cm}^{-3}$ ). Species collection from different environments was aimed mainly to achieve coverage of a large number of species and a high diversity of traits (both anatomical and non-anatomical). Again, sampling was not rigorously random nor based on abundance. Because of these sampling procedures and a lack of site replication, it would be misleading to draw strong conclusions about the sampled vegetation types and habitats. Nevertheless, site comparisons were carried out in both chapters and were carefully interpreted, keeping in mind the limitations of sampling methods.

This thesis applied a quantitative approach to exploring the anatomical basis of wood density variation in twigs of angiosperms. Substantial quantitative datasets were produced about anatomical variation in twigs. The overarching goal was to contribute an anatomical perspective in our quest to understand plant functions and ecological strategies. I aimed to achieve that via:

- Synthesizing and learning from the rich but scattered anatomical knowledge found in the literature (Chapter 2).
- Linking anatomical structure with plant functional traits, with special emphasis on wood density (Chapters 3 and 4).
- Describing plant anatomical variation in twigs in a quantitative way across a range of angiosperm species (Chapters 3 and 4).

### **1.3 Thesis plan**

This thesis contains a literature review (Chapter 2), two data chapters, for which I measured anatomical and ecological traits across a total of 93 woody species (Chapters 3 and 4), and a general discussion (Chapter 5).

In Chapter 2, I compiled quantitative evidence of anatomical variation that has been published on stems and twigs from a far more broad range of species, from

locations worldwide. Results from Chapters 3 and 4 of this thesis were also included. A particular emphasis was placed on quantitative features (e.g. tissue fractions, vessel size), but some qualitative features were also considered (e.g. presence of vessel helical sculpturing or vestured pits). This chapter's aim was to synthesise the scattered wealth of anatomical knowledge, to examine relationships between anatomy, climate, and plant functional traits, and to highlight key gaps in our current comprehension of anatomy and plant ecological strategies.

In Chapters 3 and 4, I showed that there were two orthogonal dimensions of variation. Certain anatomical traits drove density variation (Chapter 3), but other anatomical traits varied independently of density (Chapter 4). In Chapter 3, tissue proportions in 24 species ranging in density from 0.37 to 0.83 g cm<sup>-3</sup> were quantified. Sites were contrasted for latitude and for rainfall. I also observed substantial anatomical variation largely independent of wood density, particularly at mid-range density values (roughly 0.5-0.7 g cm<sup>-3</sup>). This finding inspired Chapter 4, where I quantified anatomical variation on a larger number of species (69), but in a more constrained range of density (0.4–0.6 g cm<sup>-3</sup>; i.e., the lower 2/3 of the range seen in Chapter 3). These two data chapters concentrated on tissue proportions because they are the most relevant to wood density, and can be functionally indicative.

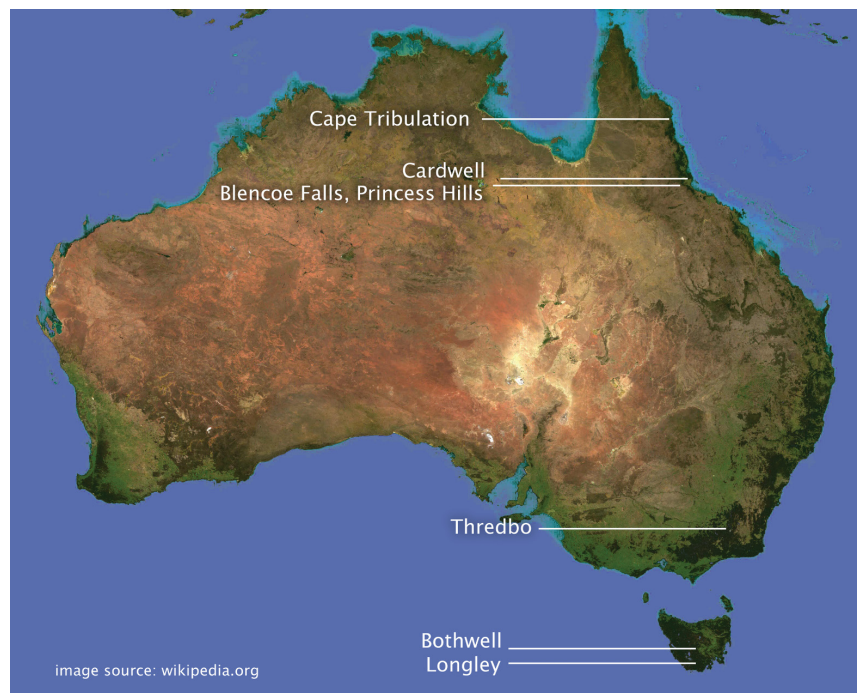
Finally, in Chapter 5, the overall meaning of my findings, their significance and implications are discussed. Limitations of this study and future research directions that should be priorities are also outlined.

## **1.4 Methodological note**

Significant variation in plant form and function is observed across diverse climates, but also across species within any single location. Accordingly, both types of variation were sampled in this work. Plant material was collected from seven sites containing several vegetation types: tropical rainforest and woodlands in North Queensland, temperate forests and woodlands in New South Wales and in Tasmania, Australia. Figure 1-1 is a map of Australia with the sites sampled and figure 1-2 shows photographs of six out of seven of the sampled locations. The omitted site (Princess

Hills, Queensland), was located within about 15 km from Blencoe Falls (Fig. 1-2d) and carried similar vegetation. Mean annual temperature and rainfall were also similar at these two sites. The other locations spanned broad gradients of temperature and rainfall. Both tree and shrub species were sampled on the basis that both growth forms produce secondary xylem and that there is a continuum of sizes from dwarf shrubs to tall trees rather than these being distinct categories.

Figure 1-1 A map of Australia and study sites.



There was one major methodological change between Chapters 3 and 4, which was related to image analysis. Anatomical studies are time-consuming, and often time needs to be traded-off with precision. In the two chapters where I quantified anatomical traits (Chapter 3 and 4), two different methods were applied. Manually colouring each tissue (Chapter 3) is precise but time consuming, whereas a grid method (Chapter 4) is less accurate but faster. The grid method allowed me to investigate a larger number of species, which increased the generality of findings. Figures 3-1 and 4-1 in the relevant chapters illustrate the two methods.

Figure 1-2 Photographs of six study sites: (a) Cape Tribulation, QLD (tropical rainforest), (b) Cardwell, QLD (tropical forest), (c) Thredbo, NSW (temperate forest), (d) Blencoe Falls, QLD (tropical woodland), (e) Longley, TAS (temperate forest) (f) Bothwell, TAS (temperate woodland). Photo (e) courtesy of Don Butler.



Figure 1-2      Photographs of six study sites.





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## Chapter 2

# **DISSECTING STEMS: WHAT CAN WOOD ANATOMY DISCLOSE ABOUT PLANT STRATEGIES?**

Kasia Ziemińska



## **2.1 Abstract**

Plants have myriad ways of living in different environments, as well as within the same location. So what can we learn about those ways of living from the anatomical structure of their wood? A wide variation in wood tissue properties across hundreds of species and diverse environments has been documented in the literature. Here, I compile quantitative evidence of wood anatomical variation across species and climates, and assess what is known or can be inferred about functional and ecological meaning of this variation. Our understanding of biological meaning of this variation varies among the different tissues. Vessels have been explored in the greatest detail. Although there are still unsolved questions, it is firmly known that the main vessel character, vessel lumen cross-sectional area (or diameter), is related to efficiency and, less firmly, to risks associated with transporting water. Perhaps most strikingly, we only poorly understand the biological meaning of variation in parenchyma, which occupies from 6% to over 60% of wood's volume. It is thought that parenchyma's primary functions are food transport and storage, yet it is not clear how these functions correspond to the wide spread of parenchyma proportions across species. It is possible that parenchyma may also be an important reservoir of water. Apart from gross tissue characteristics, there are also fine scale details, which potentially have important functional implications, such as intervessel or parenchyma pits. Quantification of anatomical tissues exposes interesting gaps in our understanding of wood functions, a whole plant functioning, and the diversity of wood strategies across a variety of species. Investigation into less explored anatomical structures, like parenchyma or pits, may clarify aspects of plant functional strategies that are not yet understood.

## **2.2 Introduction**

### **Background and aims**

Just as human anatomy has been essential in understanding human body functions, plant anatomy can give insight into plant functions. Here I gather the

quantitative evidence for anatomical variation across species and its relationships with the environment and functional traits.

Wood of angiosperms is composed of three major tissues: fibres, parenchyma, and vessels. Their general functions are respectively mechanical support, metabolite transport and storage, and water transport. Although those functions are believed to be universal across species, presumably the way they are carried out varies in different species and environments. In other words, plants use different anatomical strategies to perform those functions. There is an extraordinary variety of wood anatomical properties (e.g. Carlquist 2001, InsideWood 2004), which presumably underlies the diverse anatomical strategies.

Anatomical studies have been carried out with a variety of different objectives. Firstly, anatomy has long been used by foresters investigating wood properties important for commercial purposes (e.g. Panshin & de Zeeuw 1980; Koch 1985; Barnett & Jeronimidis 2009). Secondly, the wide diversity of wood structure has been described in a vast taxonomic literature (bibliography comprehensively compiled by Gregory 1994), which has given rise to careful wood anatomical character definitions (IAWA Committee 1989). Wood anatomists also have investigated links between anatomy and climate across hundreds of species (e.g. Carlquist & Hoekman 1985; Baas & Schweingruber 1987; Alves & Angyalossy-Alfonso 2000; Wheeler, Baas & Rodgers 2007). Thirdly, especially in recent years, wood anatomy has been rediscovered by ecologists aiming to better understand plant functions via functional traits (e.g. Jacobsen et al. 2005; Pratt et al. 2007; Poorter et al. 2010; Gleason et al. 2012). Finally, there have been experimental studies that incorporate anatomical observations with a view to explaining the mechanisms driving wood functions (e.g. Salleo et al. 2004; Lee, Holbrook & Zwieniecki 2012; Brodersen et al. 2013).

This review concentrates on relationships between anatomical traits, two major climate variables (temperature and rainfall), six hydraulic variables (water potential, conductivity, resistivity, embolism resistance, capacitance, and implosion resistance), and two mechanical variables (modulus of elasticity and modulus of rupture). However, it is important to note that other climatic and plant traits affect plant functions and strategies. Broad environmental or microclimatic factors, such as the length of growing

versus non-growing season, wind exposure, or light availability, will certainly have influence on shaping plant strategies. So also will plant traits, for example, plant height, leaf area to sapwood area ratio or rooting depth. The variables analysed here have been chosen for several reasons: plant variables needed to be measured on wood only, the variables needed to be potentially influential for plant function, and the variables needed to be widely reported in the cross-species literature. The aim of this review is not to explain the mechanisms of whole plant functioning, but rather to report and assess patterns across a diverse suite of species and their consistency across different studies. Among previous syntheses of the literature, some focused on functions (e.g. Gartner 1995a; Carlquist 2001, 2012; Tyree & Zimmermann 2002; Baas et al. 2004) and some on cross-species relationships (e.g. Baas 1986a, Baas & Wheeler 2011). However, these did not include functional traits or were carried out sufficiently long ago to justify a fresh review.

I seek to combine evidence generated from different research styles with special emphasis on cross-species quantitative reports. The overarching goal is to elucidate what is known so far about broad anatomical variation and its potential role in functions, and to consider what anatomical patterns may suggest about functions and plant strategies not yet or poorly established.

The review starts from a brief introduction to functional processes, as they are understood currently, and descriptions of traits that may be related to each process. Next, follow three sections on three major tissues: fibres, parenchyma, and vessels. Within each section, I 1) characterize the tissue in question, 2) describe how tissue properties vary with climate, 3) describe how tissue properties vary with other plant functional traits, and 4) discuss experimental studies elucidating the detailed processes happening in the given tissue. Finally, I synthesise and assess the evidence gathered, and draw conclusions.

## **Notes on methods**

This section comments on some recurring issues that arise when interpreting results in the literature.

Wood anatomy varies from main trunks to twigs within a plant (Panshin & De Zeeuw 1980; Koch 1985; Gartner 1995b). Although there is no clear cut-off on this continuum, it can be useful to recognize that main stems are different from twigs, and to treat them separately. Here, stems smaller than two cm in diameter are called twigs on the basis that this diameter class was most often used in the reviewed literature (e.g. Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4; Preston, Cornwell & DeNoyer 2006; Pratt et al. 2007; Gleason et al. 2012; Jacobsen et al. 2012). Other reports referred to main stems and branches that were substantially bigger than two cm (e.g. Panshin & de Zeeuw 1980; Koch 1985). Where no diameter was given, but the descriptions were referred to as being about mature or juvenile wood, mature wood was counted as stems and juvenile as twigs, even though the juvenile wood might possibly have been bigger than two cm diameter. Where no remark was made about wood diameter or maturity, it was assumed main stems were described, that being the most common practice.

In many publications, even those covering many species (e.g. Carlquist 1966; Wheeler, Baas & Rodgers 2007), each specimen was treated as a separate entity and analysis was run on all specimens rather than on species means. Sometimes the number of specimens was only a little greater than the number of species (e.g. Lens et al. 2004), indicating that few species were represented by more than one replicate. At other times the number of specimens was, for example, twice as large as species (van der Oever, Baas & Zandee 1981), but it was not clear whether there were two replicates per species, or the number of replicates was different for different species. Sometimes the number of specimens was not mentioned at all. Within one large dataset, the number of species was not reported, and the analysis was run on specimens (Wheeler, Baas & Rodgers 2007). Wheeler, Baas & Rodgers (2007) also occasionally included anatomical characteristics of genera rather than species. A further complication is that some studies reported the incidence of species (or specimens) within bands of a trait value, for example, classes of axial parenchyma abundance in relation to climate zones (Baas 1973; van der Graaff & Baas 1974; Baas & Schweingruber 1987; Baas et al. 1988; Alves & Angyalossy-Alfonso 2002; Jansen et al. 2004; Wheeler, Baas & Rodgers 2007), as opposed to actual measurements averaged across species within a given climate



category. For simplicity (and often following the authors of such papers), I abbreviate the description to saying for example ‘parenchyma was more abundant in the tropics’ meaning ‘the incidence of species or specimens with abundant parenchyma was higher in the tropics’.

In tables 2-1 to 2-7, I summarize trait variation and ecological and functional trends, and report the number of species and families a given relationship was described for. When several studies are cited with reference to a given relationship, I indicate the sum of species and families examined in all those studies. In rare cases, however, when families or species names were not mentioned in the literature, but species number was, I underestimated the number of families reported in the tables.

Two main climate factors influencing plant form and function are temperature and water availability. However, not all environmental surveys present those climatic data or locations, where the specimens were collected, often because this information was not available for studied samples, or because the specimens were collected from a botanic garden or arboretum. In those cases, other climatic proxies were used. Some studies used latitude, which is a reasonable surrogate for temperature except in mountain regions. Throughout the text, I apply the term ‘temperature’ or qualitative terms such as ‘warm’ or ‘cold’ when talking about both latitude and temperature. Similar to temperature, water conditions also have been reported variously either as annual rainfall (mm) or as a category (e.g. mesic, dry in Carlquist 1966; Baas & Schweingruber 1987; Alves & Angyalossy-Alfonso 2000). Similar to the case with temperature, I use the common language qualitative terms such as ‘wet’ or ‘dry’.

Vessels are pipe-like structures composed of lumen and wall. In certain works, it was not clear whether ‘vessel’ (as in ‘vessel size’ or ‘vessel proportion’) refers to that of the vessel lumen or of the total vessel (including lumen and wall). Because vessel wall proportion of xylem cross-section is generally low and varies little in comparison with vessel lumen proportion (Ziemińska et al. Chapter 3; Martínez-Cabrera et al. 2009), I include these cases along with cases where it was clear only vessel lumen was measured. For brevity, I will use ‘vessel size’ to refer to either vessel area or to vessel diameter. Fibre area or diameter (including both wall and lumen) is called ‘fibre size’ and fibre lumen area or diameter is referred to as ‘fibre lumen size’.

## 2.2 Overview of functional processes and traits

### Mechanical functions

A plant has to face a wide suite of internal and external stresses. It endures mechanical dangers of collapse under its own weight, of breakage from wind, falling debris, or snow, and of damage from animals. Moreover, the process of growth itself induces significant stresses called 'growth stresses'. They are a combination of stresses evoked during changing weight and geometry of plant organs and maturation stresses, which result from maturation of newly produced cells (Archer 1987; Thibaut & Gril 2003). There are various ways of achieving strength and stability: having large stem diameter (Larjavaara & Muller-Landau 2010; Anten & Schieving 2010; Butler et al. 2011), and/or adjusting and streamlining the geometry of the entire plant (Wainwright et al. 1982; Vogel 1989; Gartner 1991; Niklas & Speck 2001; Read & Stokes 2006; Butler et al. 2011), or strengthening root anchorage (Read & Stokes 2006). The other way to alter mechanical performance of a plant is to modify the quality of the building material – wood – by altering its anatomical structure or density (e.g. Beery, Ifju & McLain 1983; Koch 1985; Hepworth et al. 2002; Barnett & Jeronimidis 2009; Chave et al. 2009). Plant mechanical strategies have been reviewed elsewhere (Rowe & Speck 2005; Read & Stokes 2006), and here I focus on which anatomical traits affect the mechanical properties of wood and how those relationships vary across species. The specific mechanical stress in question is bending stress caused by, for example: wind, weight of organs in non-vertical branches, snow, falling debris, or animals.

The heterogenous cellular structure of wood and different cell orientation results in variable mechanical behaviour in different dimensions, perpendicular to each other. This pronounced property of wood is called anisotropy (as opposed to isotropy where material behaves in the same way in all dimensions). A subcategory of anisotropic materials is orthotropic materials. They have different properties in all three dimensions: longitudinal, radial, and tangential, as oppose to two dimensions only (axisymmetry, Niklas 1992). The orthotropy of wood requires specifying the direction of applied force. The focus of this review is bending stress, when the force is exerted perpendicular to the long axis of a stem.

The way in which bending stress acts on wood can be described in terms of its elasticity and strength. Elasticity defines plant response to temporary deformation and is expressed as modulus of elasticity (MOE, also referred to as Young's modulus). MOE measures the amount of force applied per given deformation of the material, and thus describes the slope of the force-deformation curve. The stiffer the material the higher is the MOE. The overall stiffness (resistance to bending) of an object is influenced both by the MOE of the material and by the shape of the object. Separate to stiffness is wood strength, which can be expressed as modulus of rupture (MOR). This measures the amount of force at the moment of wood rupture, a permanent deformation. Both MOE and MOR are usually measured in megapascals (MPa) and are usually strongly positively correlated with each other (e.g. Yang & Evans 2003; Jacobsen et al. 2005). In the sections organized by tissue type, I investigate how anatomical structure affects MOE and MOR.

Wood density is a very commonly measured functional trait, which represents the mass of dry wood per given volume ( $\text{g cm}^{-3}$ ). Although it is not a direct measure of mechanical performance, it is a good predictor of MOR and MOE (Chave et al. 2009). Wood density is not an unambiguous indicator of anatomical structure (e.g. Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4) yet, to some extent, it can be informative in the absence of detailed anatomical data.

## **Metabolite transport and storage functions**

Plants store metabolites, usually in the form of carbohydrates and nutrients (e.g. Lambers, Chapin & Pons 2008; Pallardy 2010). The benefits of storing carbon versus committing it promptly to growth have been widely debated (e.g. Chapin, Schulze & Mooney 1990; Körner 2003; Sala, Woodruff & Meinzer 2012). Storage might be valuable against future circumstances when carbon demand exceeds supply. Examples of such situations would include: spring before leafing (for deciduous species), after disturbance such as fires or storms that cause tissue loss (Pate et al. 1990; Bell, Pate & Dixon 1996), under short term limited light (Myers & Kitajima 2007), or during intensive reproduction (e.g. during masting periods, Chapin, Schulze & Mooney 1990). Sala, Woodruff & Meinzer (2012) also suggested that carbon storage may play an important role in maintaining water transport via refilling of embolised conduits. The amount of material stored has

been shown to vary across species, seasons, and climates (Barbaroux, Bréda & Dufrêne 2003; Hoch, Richter & Körner 2003; Körner 2003). For instance, Körner's (2003) synthesis indicated that on average carbon storage increased towards colder climate across 31 species sampled from four vegetation types: temperate deciduous, Mediterranean sclerophyll, tropical lower montane, and tropical lowland. As well as the costs of deferring growth, storage also incurs costs of translocation, chemical transformation, construction, and maintenance (Lambers, Chapin & Pons 2008; Pallardy 2010). There are certainly unsolved questions about storage and its importance. Studying storage tissue can potentially shed some light on storage strategies.

Carbon can be stored in the form of carbohydrates (soluble sucrose, non-soluble starch, and rarely fructans). Nutrients can be stored in various forms (mainly nitrogen and phosphorus in form of  $\text{NO}_3^-$ , amino acids, amides, or protein; Evert 2006; Lambers, Chapin & Pons 2008) but the largest quantities of storage material are carbohydrates (Pallardy 2010). Although metabolites can be stored in various organs such as roots, bark, stems, twigs, and leaves (Würth et al. 2005; Pallardy 2010), I focus on the subject of this review, the wood of stems and twigs.

Quantitatively, storage has mainly been measured as a concentration of non-structural carbohydrates (NSC) per dry weight or per volume. NSC are carbohydrates including starch, sucrose, glucose and others that are not involved in the structure of cell wall (cellulose and hemicellulose). On the basis that NSC can be stored in parenchyma (Essiamah & Eschrich 1985; Sauter & van Cleve 1989; van Bel 1990; Salleo, Trifilò & Lo Gullo 2006; Yamada et al. 2011), I aim to investigate the relationships between NSC content and this storage tissue.

## **Hydraulic functions and traits**

Water is a basic requirement for plant life. The main driving force for the ascent of water is a pressure difference between the roots, where water enters a plant, and the leaves, where water exits a plant. Water potentials are usually negative and expressed in megapascals (MPa, Tyree & Zimmermann 2002). Conductivity ( $\text{kg m s}^{-1} \text{MPa}^{-1}$  or  $\text{m}^4 \text{s}^{-1} \text{MPa}^{-1}$ ) describes how well water flows through the xylem and is measured as water flow rate ( $\text{kg s}^{-1}$  or  $\text{m}^3 \text{s}^{-1}$ ) per pressure difference ( $\text{MPa m}^{-1}$ , Tyree & Zimmermann 2002). The

reciprocal of conductivity is resistivity to the water flow ( $\text{MPa s m}^4$ , Sperry et al. 2007), and the higher the resistivity the more difficult it is for the water to flow. There are other obstacles a plant faces. Under very negative water potentials, cavities filled with water vapour can be created within a water column in a vessel (water changes its phase into gas as water vapour). This process is called 'cavitation' and it takes place during drought, or during freeze-thaw events when thawing water releases air bubbles (e.g. Lo Gullo & Salleo 1993; Cochard et al. 2001). Under continued negative tension, the gas bubble expands resulting in an embolised conduit, in other words a blocked conduit. Although the cavitation and embolism processes are strongly interlinked, they are not the same (Lens et al. 2013 nicely explain the details); nevertheless, 'cavitation' and 'embolism' have been used synonymously in the literature (e.g. Sperry et al. 1994; Davis, Sperry & Hacke 1999; Jacobsen et al. 2005; Choat et al. 2012). Plants vary in their susceptibility to embolism, some avoid it and some tolerate it. Embolism resistance (sometimes incorrectly referred to as 'cavitation resistance', Lens et al. 2013) is measured as a vulnerability curve, the percentage loss of conductivity as water potential becomes more negative. Most commonly, embolism resistance is expressed as P50, the water potential at which 50% of conductivity is lost (but mean embolism pressure also has been measured, e.g. in Lens et al. 2011). P80 and P88 (80% and 88% conductivity loss respectively) also have been reported and might be more relevant in certain situations (e.g. Markesteijn et al. 2011; Choat et al. 2012 and literature cited therein). The mechanisms for tolerating embolism include refilling of conduits (Zwieniecki & Holbrook 2009; Brodersen et al. 2013) or growing fresh conductive tissue. A third hydraulic characteristic is capacitance, which is the water storage capacity of wood. Stored water can be withdrawn and incorporated into a transpiration stream. Capacitance is measured as change in wood water content per change in water potential ( $\text{kg m}^{-3} \text{MPa}^{-1}$ ; Meinzer et al. 2003; Scholz et al. 2007). 'Implosion resistance' is considered to indicate the resistance of the conduit's walls to collapsing under negative pressure (Hacke et al. 2001). It is estimated from a measure of thickness of two adjacent conduit walls 't' divided by a conduit lumen diameter 'b' squared:  $(t/b)^2$ . I refer to this metric as 'double wall to lumen ratio'. Although double wall to lumen ratio has been measured in the literature (e.g. Hacke et al. 2001; Jacobsen et al. 2005, 2007; Pratt et al. 2007), to my

knowledge, there are no reports showing that implosion actually happens in woody stems although it has been documented in conifer leaves (Cochard et al. 2004; Brodribb & Holbrook 2005). Finally, wood density, introduced earlier, has been shown to correlate with hydraulic traits, for example, lower wood density is correlated with higher capacitance (Meinzer et al. 2003, 2008; Scholze et al. 2007; Pratt et al. 2007). Anatomical correlates of wood density (Ziemińska et al. Chapter 3; Fujiwara et al. 1991; Jacobsen et al. 2007; Martínez-Cabrera et al. 2009) can potentially be used to infer hydraulic functions.

## **2.3 Fibres**

### **2.3.1 Fibre structure**

Fibres are elongated cells whose primary role is mechanical support. They occur in virtually all woody angiosperm species although they may be very scarce or perhaps even absent in stem-succulent cacti and Crassulaceae (Carlquist 2001). Fibres are spindle-shaped and in cross-section they are approximately round and relatively small (Fig. 2-1a, b, c, d). They often stand out among all other cells by having the smallest diameter and relatively the thickest cell wall (Fig. 2-1b, d). However, there are instances where the thickness of fibre wall seems not significantly different from other tissues (Fig. 2-1c).

On average, fibres are the most abundant tissue (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4; French 1923 as cited by Panshin & de Zeeuw, 1980; Manwiler 1973 as cited by Koch 1985; Fujiwara et al. 1991; Pratt et al. 2007; Martínez-Cabrera et al. 2009; Poorter et al. 2010; Fichtler & Worbes 2012). Their proportion within wood ranged from 27% to 86% in stems of over 800 species from North America and China (French 1923 as cited in Panshin & de Zeeuw 1980; Manwiler 1973 as cited in Koch 1985; Zheng & Martínez-Cabrera 2013) and from 20% to 74% in twigs of nearly 100 species from eastern Australia (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4). Quantitative fibre tissue properties are summarised in Table 2-1. The proportion of wood that was occupied by fibre lumen ranged from 1% to 57% in stems of 61 shrub species from North and South Americas

(Martínez-Cabrera et al. 2009) and from 0.5% to 32% in twigs of 93 species from eastern Australia (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4). On average, fibre wall was a higher proportion of the cross-section than fibre lumen proportion, and it ranged from 16% to 73% in shrub stems (Martínez-Cabrera et al. 2009) and from 15% to 61% in twigs (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4).

Fibre diameter ranged from 11 to 44  $\mu\text{m}$  and fibre lumen diameter from 1.4 to 17.4  $\mu\text{m}$  in the stems of 54 trees from North America, from French Guyanan rainforest, and from an Indian *Eucalyptus* plantation (Manwiller 1973 as cited in Koch 1985; Ruelle et al. 2006; Dutt & Tyagi 2011). The range of fibre wall thickness spanned 1.2 to 6.0  $\mu\text{m}$  across 137 species from North America, Mexico, Japan, and India (Manwiller 1973 as cited in Koch 1985; Barajas-Morales 1985; Fujiwara et al. 1991; Dutt & Tyagi 2011). Ruelle et al. (2006) reported double wall thickness, with the corresponding single wall thicknesses ranging from 2.2 to 17.7  $\mu\text{m}$ . These observations referred to tension and opposite wood solely. Fibres with contrasting wall thickness are illustrated in Figure 2-1c and d.

It is commonly assumed by non-anatomists that fibres are dead. While this is often true, there are also reports of living fibres (Fahn & Leshem 1963; Lens, Smets & Jansen 2004; Yamada et al. 2011; Carlquist 2012). Carlquist (2001) also proposed a hypothesis of 'fibre dimorphism' where fibres diverge to non-living fibres or to parenchyma-like living fibres. Some fibres possess septa, which are thin, un lignified divisions perpendicular to the long axis of the cell. Those fibres are usually assumed to be alive (Carlquist 2001; Wheeler, Baas & Rodgers 2007). The incidence of living fibres might be underestimated because their living content may be removed during preparation of wood material for microscopy (Carlquist 2012).

Fibre walls contain bordered or simple pits. Pit number per wall area is variable. A pit is a cavity in the cell wall containing no secondary wall material. Two pits of adjacent cells are usually positioned opposite to each other and create tunnel-like connections between the lumens of two neighbouring cells. These are called pit-pairs. Bordered pits are funnel shaped where the narrow end faces the lumen of a cell and the wide end faces the corresponding wide end of a pit of a neighbouring cell (Fig. 2-1d with

bordered pits). Simple pits are approximately uniform in width. There is no secondary cell wall material in pits and a thin pit membrane forms the only border between the two cells. The membrane consists of middle lamella and the two primary walls of neighbouring cells, and it contains pores through which symplastic transport takes place (when a cell is alive). Another important characteristic of fibres is cellulose microfibril angle. This refers to cell wall ultrastructure: the angle is measured between the long axis of a stem and a cellulose microfibril of cell wall (usually the thickest, middle layer of the cell wall is measured; Boyd 1985; Dinwoodie 2000, Barnett & Bonham 2004). Cells of all tissues have cellulose microfibrils in their walls, but microfibril angle has mainly been measured in fibres, and in tracheids in gymnosperms.

### **2.3.2 Fibres and climate**

Studies that link proportions of fibres (or of fibre wall or lumen) with climate variables are scarce in the literature. One study has quantified fibre proportions in shrub stems from subtropical regions of Argentina and USA (Martínez-Cabrera et al. 2009). In that study, fibre lumen proportion was weakly inversely associated with temperature and positively with rainfall; while fibre wall proportion decreased slightly in higher rainfall sites (Table 2-2). The one work on twigs that I am aware of (my own, Chapter 4) reported similar results. Within the two sites with similar temperature but contrasting rainfall, fibre lumen fraction was higher in the wet site and fibre wall fraction was lower in that site. However, sampling was not necessarily representative at each site since in this study species were deliberately selected from a narrow wood density range (Ziemińska, Wright & Westoby Chapter 4).

The most widely measured fibre characteristics in relation to the climate have been fibre length and wall thickness (Table 2-2, data were found for main stems only). Overall, stem fibres tended to be longer in warmer (Carlquist 1966; van der Graaff & Baas 1974; Baas et al. 1988) and wetter locations (Carlquist 1966; Barajas-Morales 1985). However, the studies reporting a relationship with precipitation sampled only from two sites with contrasting rainfall in Mexico (Barajas-Morales 1985) or within Asteraceae only (Carlquist 1966). Also, Carlquist (1966) did not apply formal statistical tests. Less commonly measured traits were fibre size (diameter on a cross-section) and fibre wall to



lumen ratio (ratio of areas on a cross-section). Both increased with temperature across 61 shrub species, and wall to lumen ratio decreased with precipitation (Martínez-Cabrera et al. 2009).

Presence of fibres with either significantly bordered pits or minutely bordered to simple pits is considered to be of taxonomic value in wood identification (IAWA Committee 1989), and potentially may also play some functional role. The incidence of species with fibres with distinctly bordered pits (also called tracheids sensu Carlquist 1988, or fibre-tracheids sensu Baas 1986b) was higher in cold environments (e.g. temperate and boreal zones, Baas & Schweingruber 1987; Baas et al. 1988). They also tended to increase towards wetter environments but this trend did not seem to be strong across 505 northern hemisphere species (Baas & Schweingruber 1987).

### **2.3.3 Fibres and functional traits**

Reports linking fibre properties with functional traits are not common and the number of species tested has been limited (Table 2-3). Moreover, many of these studies have been carried out on twigs, in contrast to studies correlating fibres with climate, which have mostly been done on stems (Table 2-2).

Traditionally mechanical functions have been ascribed to fibres (Evert 2006). However, relatively few studies have demonstrated a relationship between specific fibre characteristics and the strength (MOR) or stiffness (MOE) of stems or twigs. One would expect that higher fibre proportion would increase strength and stiffness. However, fibre proportion was negatively correlated with MOE and MOR across five *Acer* species (Woodrum, Ewers & Telewski 2003). On the other hand, fibre wall proportion was positively related to MOR across 17 species of South African evergreen shrubs (Jacobsen et al. 2007). Fibre lumen size was shown to be higher in weaker and more elastic species (Woodrum, Ewers & Telewski 2003; Jacobsen et al. 2005, 2007). Intuitively, we would also expect higher strength and stiffness in woods with thick-walled fibres. This was the case across six shrub species (Jacobsen et al. 2005), but not across 17 other species (Jacobsen et al. 2007). Discrepancies between simple expectations and these actual results can potentially be explained as follows. Wood can be composed of a small fraction of thick walled fibres, resulting in low fibre wall as a fraction of the whole cross-section, and

potentially therefore low strength. And vice versa, wood with high fibre fraction can be composed of thin-walled fibres, also resulting in low fibre wall proportions, and hence low strength (Ziemińska et al. Chapter 3). Another trait correlated with both mechanical properties was microfibril angle. The bigger the angle between cellulose microfibrils and the long axis of the stem, the weaker (lower MOR) and more elastic (lower MOE) wood becomes (Evans & Ilic 2001; Yang & Evans 2003; Barnett & Bonham 2004).

Fibre traits were not correlated with hydraulic properties of wood such as conductivity and minimum water potential (midday water potential measured during peak dry season, Table 2-3; Jacobsen et al. 2007; Pratt et al. 2007; Rana et al. 2009). But embolism resistance has been shown to increase with fibre wall proportion and to decrease with fibre lumen size across six species (Jacobsen et al. 2005). There is no direct mechanism known that links fibre properties with embolism resistance, so presumably these two traits may be coordinated via some common selective factor acting on both of them. The other interesting trait associated with fibres is capacitance. This increased in species with higher proportions of fibre and vessel lumens and higher fibre lumen size across nine species (Pratt et al. 2007). Capacitance also has been shown to correlate inversely with wood density across 22 species from tropical forests of Panama (dry and wet) and Brazilian savannah (Meinzer et al. 2003, 2008; Scholz et al. 2007; Pratt et al. 2007). Stratton, Goldstein & Meinzer (2000) measured saturated water content in six species from Hawaiian dry forest (calculated as saturated mass minus dry mass divided by dry mass). This is an indicator of water storage but slightly different from capacitance (which is measured as change in relative water content per change of water potential, see section 2.2). In that study, saturated water content was inversely correlated with wood density. Low wood density results from high fibre lumen proportion (or high parenchyma proportion discussed in the following sections; Ziemińska et al. Chapter 3; Martínez-Cabrera et al. 2009). Overall, those results, although supported by only a few dozen species, support the view that fibre cell lumens can be used for water storage and contribute to capacitance.

### **2.3.4 Fibres in experimental studies**

The most common natural experiment affecting fibre properties is growth at an angle to the vertical gravity force such as in non-vertical branches or in leaning stems. In

such situations, angiosperms produce 'tension wood'. This type of wood has higher fibre fraction than in normal wood, and the fibres are longer in their axial dimension, have smaller diameter and thicker walls. Tension wood has been widely discussed, especially by foresters (e.g. Panshin & de Zeeuw 1980; Koch 1985; Barnett & Jeronimidis 2009). Increase in fibre wall proportion and decrease in fibre lumen proportion has also been shown experimentally when young trees of *Fagus silvatica* and *Alnus glutinosa* (4-10 cm diameter at breast height) were subjected to constant bending (Heinrich, Gärtner & Monbaron 2007).

Although fibres are generally considered dead cells they also have been reported to be alive and to store starch (Fahn & Leshem 1963; Carlquist 2012). In an interesting experiment on *Robinia pseudoacacia* stems, Yamada et al. (2011) found that fibres of the newly produced growth ring stored up to 80% of the starch in the wood. This starch was then used up during spring growth, followed by death of the fibres. In a similar vein, Wheeler et al. (2007) found in their global dataset of stems that species with septate fibres (living fibres) had less axial parenchyma. This suggested the two cell types might have common roles in storing starch and be substitutable, but this hypothesis has not been experimentally tested across a broad range of species.

Fibre water content has been investigated in a total of 12 Japanese tree species using cryo-scanning techniques (Umebayashi et al. 2008, 2010). Wood disks were collected before sunrise and immediately frozen in liquid nitrogen. Three or more outer growth rings were analysed on cryo-scanning electron micrographs. Fibres were observed (although not formally quantified) to contain water to a various degree in different species, from most fibres being empty (e.g. in *Rhus javanica*) to most fibres being filled with water (e.g. in *Fagus japonica*).

## **2.4 Parenchyma**

### **2.4.1 Parenchyma structure**

Parenchyma is a living tissue composed of cells of various shapes, whose primary function is transport and storage of carbohydrates and nutrients. It occurs in all angiosperm species. It is of two types: axial parenchyma, whose cells are generally

elongated parallel to the long axis of a stem (but approximately round in cross-section), and rays, which stretch radially, perpendicular to the long axis of a stem (Figures 2-2a, b, c, d). Rays can be composed of square, procumbent, or upright cells. On average parenchyma has been estimated to be the second most abundant tissue after fibres. Parenchyma proportion varied from 6% to 64% in stems (Martínez-Cabrera et al. 2009; Poorter et al. 2010; Zheng & Martínez-Cabrera 2013) and from 11% to 66% in twigs (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4). The quantitative summary of parenchyma features is presented in Table 2-1. Axial parenchyma proportion ranged from 0% to 32% in stems (French 1923 as cited in Panshin & de Zeeuw 1980; Manwiller 1973 as cited in Koch 1985; Martínez-Cabrera et al. 2009; Zheng & Martínez-Cabrera 2013) and from 1% to 33% in twigs (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4). Ray parenchyma occupied from 2% to 46% in stems (Myer 1922; French 1923 as cited in Panshin & de Zeeuw 1980; Manwiller 1973 as cited in Koch 1985; Petrić & Šćukanec 1975; Fujiwara et al. 1991; Martínez-Cabrera et al. 2009; Zheng & Martínez-Cabrera 2013) and from 6% to 41% in twigs (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4). Scarce and abundant axial and ray parenchyma are illustrated in Figure 2-2 c and d. Most often, parenchyma has thin cell walls. While this is typically true for axial parenchyma, rays can have substantial lignified cell walls. Walls can occupy roughly 20 to 70% of the ray volume and the thickness of cell walls varies from 1.2 to 3.5  $\mu\text{m}$  (Table 2-1; Fujiwara 1992). Axial parenchyma can be roughly divided into paratracheal parenchyma, which is in contact with vessels, and apotracheal parenchyma, which is without contact with vessels. However, even parenchyma that is not observed to touch a vessel in a given cross-section can in fact be in contact with it at some point along the length of the stem. Axial and ray parenchyma cells in direct contact with vessels are often referred to as 'contact cells' or 'vessel associated cells' (VAC; Czaninski 1977; Alves et al. 2004; Améglio et al. 2004; Evert 2006). Cells that are not in direct contact with vessels are called 'isolation cells' (Evert 2006). Different distribution and locations of axial parenchyma with regard to vessels and other cell types can be taxon specific (IAWA Committee 1989). Ray width, density (number of rays per mm), and height can vary greatly across species (IAWA Committee 1989). Pits in parenchyma cells are usually simple and relatively large. They are located

at various densities on different walls (radial or tangential) often depending on the neighbouring cell (Carlquist 2007, 2012).

## **2.4.2 Parenchyma and climate**

There are relatively abundant reports of parenchyma-climate relationships, as summarised in Table 2-4. Total parenchyma proportion (including both axial parenchyma and rays) was not related to climate in stems of 61 shrub species from Argentina and USA (Martínez-Cabrera et al. 2009), but tended to be higher in warmer and wetter locations in twigs of 69 species from eastern Australia (Ziemińska, Wright & Westoby Chapter 4). This latter result needs to be interpreted with caution because the sampling was concentrated in a narrow wood density range rather than being representative for a site.

Axial and ray parenchyma separately have exhibited stronger patterns with climate. Axial parenchyma has been shown to increase in abundance towards warmer locations in twigs of 69 species (Ziemińska, Wright & Westoby Chapter 4), in stems across hundreds of species worldwide (Baas 1973; Alves & Angyalossy-Alfonso 2002), and weakly across 61 shrubs (Martínez-Cabrera et al. 2009). However, Baas (1973) and Alves & Angyalossy-Alfonso (2002) reported parenchyma abundance qualitatively only. Additionally, Alves & Angyalossy-Alfonso (2002) noted that paratracheal parenchyma (in contact with vessels) increased in abundance towards warmer climate while apotracheal (away from vessels) decreased. Reports on ray parenchyma proportion are less common with one on stems (Martínez-Cabrera et al. 2009) and one on twigs (Ziemińska, Wright & Westoby Chapter 4). Ray proportion was higher in warmer and wetter sites in twigs (Ziemińska, Wright & Westoby Chapter 4), was not related to temperature, and weakly positively associated with rainfall in shrub stems (Martínez-Cabrera et al. 2009). Rays per mm were not related to precipitation across two sites with contrasting rainfall conditions (Barajas-Morales 1985). Ray height was higher in warmer and wetter regions (Carlquist 1966; Baas 1973; van der Graaff & Baas 1974; Barajas-Morales 1985; Lens et al. 2004). For ray width, either a weak positive relationship to temperature and rainfall or a lack of trend has been observed (Carlquist 1966; Baas 1973; Alves & Angyalossy-Alfonso 2002; Lens et al. 2004). Ray cell composition, such as the abundance of procumbent, upright,

square cells, or their combination did not correlate with the climate on a global scale (Alves & Angyalossy-Alfonso 2002; Wheeler, Baas & Rodgers 2007).

In summary, axial and ray parenchyma proportions, as well as ray height, tended to increase with temperature and somewhat less consistently with rainfall. Ray width has been reported both as positively related to temperature and rainfall and as not related (Carlquist 1966, Baas 1973, Alves & Angyalossy-Alfonso 2002, Lens et al. 2004).

### **2.4.3 Parenchyma and functional traits**

Parenchyma's main function is carbohydrate storage and on that basis, it might be expected that parenchyma proportion indicates storage capacity. However, to my knowledge no studies have been published that quantify the relationship between parenchyma proportion and non-structural carbohydrate content (NSC) in woody stems. A likely reason is the difficulty of data collection. NSC content may vary even during a day (Bucci et al. 2003), and probably more so over seasons and years and in response to events that lead stores to be mobilized (Chapin et al. 1990; Körner 2003). Even if we assume that at the highest NSC content all parenchyma space is used for storage, we still do not understand what the benefits are of storage in rays versus in axial parenchyma. And how the variation in proportion of those two tissues (Table 2-1) affects carbohydrate storage, mobilization, and transport strategies.

Mechanical performance has been investigated more often in relation to parenchyma traits than has storage, but still on only a quite limited number of species in total (Table 2-5). In twigs, higher total parenchyma proportion decreased with strength (MOR) across 17 species (Jacobsen et al. 2007) and less consistently decreased with stiffness (MOE) across 69 species with similar wood density values (Ziemińska, Wright & Westoby Chapter 4). Ray proportion was positively correlated with MOR across five *Acer* species with narrow density span (0.47–0.72 g cm<sup>-3</sup>; Woodrum, Ewers & Telewski 2003), but not across 69 species also with narrow density span (0.38–0.62 g cm<sup>-3</sup>; Ziemińska, Wright & Westoby Chapter 4).

Total parenchyma proportion has been found not to correlate with hydraulic properties such as embolism resistance, midday water potential, or potential conductivity (Jacobsen et al. 2007; Pratt et al. 2007; Poorter et al. 2010). We did not find

reports of a relationship between xylem parenchyma proportion and capacitance. However, several studies have found a negative relationship between capacitance or saturated water content and wood density (Stratton, Goldstein & Meinzer 2000; Meinzer et al. 2003, 2008; Scholz et al. 2007; see also section 2.3.3). Low wood density can be achieved either by higher fibre lumen proportion or by higher parenchyma proportion (Chapters 3 and 4 in this thesis) suggesting that capacitance may depend on either of these tissue properties (fibre lumen proportion was discussed in the last paragraph of section 2.3.3). Holbrook (1995) suggested that parenchyma could be a useful water storage reservoir provided that wood as a whole can adjust for volume changes associated with water movement in or out of parenchyma. Such adjustments could possibly take place in species with abundant parenchyma proportions and well interconnected parenchyma cells (Table 2-1, Fig. 2-2d).

#### **2.4.4 Parenchyma in experimental studies**

Parenchyma has been the subject of many experimental studies. Its cells have been shown to increase in respiratory activity, especially in contact cells, during spring in *Robinia pseudoacacia*, *Juglans regia* and *J. microcarpa* ('contact cells' are cells touching a vessel; Fromard et al. 1995; Alves et al. 2001). The increased respiratory activity was coordinated with the appearance of sucrose in the sap of *Acer saccharum* (Sauter, Iten & Zimmermann 1973).

The role of parenchyma embolism refilling was reviewed by De Boer & Volkov (2003). Since then new evidence has emerged showing the activity of aquaporins, amylases, and sugar transporter enzymes in living cells of wood (most likely parenchyma as living fibres are relatively scarce) and the relation of these enzymes to refilling of embolised vessels (Secchi & Zwieniecki 2011; Secchi, Gilbert & Zwieniecki 2011). Experiments with stems of *Laurus nobilis* demonstrated that vessel embolism repair was inhibited by girdling to remove phloem, suggesting that phloem might be an important driver for embolism repair (Salleo et al. 2004; Salleo, Trifilò & Lo Gullo 2006). In a synthesis of literature on vessel refilling, it has been postulated that solutes and water required for refilling would be transported from phloem to vessel via ray parenchyma (Nardini, Lo Gullo & Salleo 2011). However, recent reports question how common

refilling under negative pressure may occur (Sperry 2013; Wheeler et al. 2013). Wood water storage capacity has also been investigated experimentally (Borchert & Pockman 2005). During dehydration of excised branches of 15 species (13 tropical and two temperate) stem water potential declined slower in drought-avoiding species (density < 0.5 g cm<sup>-3</sup>) and faster in drought-tolerant species (density > 0.75 g cm<sup>-3</sup>). Anatomical properties were not measured in that work. Nevertheless, the results were consistent with the hypothesis that low-density species with high parenchyma (or high fibre lumen) can store water potentially important for stem water transport.

An experiment was carried out investigating ray parenchyma behaviour under mechanical load (Burgert, Bernasconi & Eckstein 1999). Ray proportion increased in *Fagus sylvatica* under constant force applied radially in stems, and rays positioned themselves parallel to the force direction. These results indicate rays are sensitive to mechanical stress. However, it was not clear whether rays increased or decreased the risk of mechanical failure.

## **2.5 Vessels**

### **2.5.1 Vessel structure**

Vessels are pipe-like conduits whose primary function is water transport, and they occur in a substantial majority of angiosperm species (although there are families whose species possess no vessels, e.g. Amborellaceae, Tetracentraceae, Trochodendraceae, Winteraceae; IAWA Committee 1989). A vessel is elongated parallel to the stem longitudinal axis and is composed of a number of vessel elements each of which is a separate dead cell (Fig. 2-3a and b). The boundaries between elements within a single vessel are called perforation plates and can be open (simple perforation plates, Fig. 2-3a) or can possess various sculpturing from parallel bars (scalariform perforation plates) to reticulate pattern or to almost sieve-like plates. Vessel internal walls can have helical sculpturing. Two neighbouring vessels are connected via pairs of bordered pits, funnel-shaped channels (similar in shape to the bordered pits in fibres). The narrow ends open towards the vessel lumen and the wide end is in contact with the wide end of the bordered pit in the neighbouring cell. A porous pit 'membrane' separates the two



wide ends of neighbouring pits. Pores in the membrane are the gateways for water travelling between neighbouring vessels.

When viewed on a cross-section, vessels are the most conspicuous wood feature because they are large, compared to the smaller cells of tissues surrounding them (Fig. 2-3c, d, e and f). Yet on average, they are only the third most abundant tissue after fibres and parenchyma. Their proportion ranged from 1% to 56% in stems (French 1923 as cited by Panshin & de Zeeuw 1980; Manwiller 1973 as cited by Koch 1985; Fujiwara et al. 1991; Martínez-Cabrera et al. 2009; Zheng & Martínez-Cabrera 2013) and from 1% to 23% in twigs (Ziemińska et al. Chapter 3; Ziemińska, Wright & Westoby Chapter 4; De Micco, Aronne & Baas 2008). Vessel quantitative characteristics are summarised in Table 2-1. Vessel lumen diameter varied widely across species from 16 to 267  $\mu\text{m}$  in stems (Barajas-Morales 1985; Lindorf 1994; Olson & Rosell 2013) and from 13 to 70  $\mu\text{m}$  in twigs (Jacobsen et al. 2012), excluding vines and lianas which tend to have even larger vessels (Jacobsen et al. 2012; Olson & Rosell 2013). Examples of vessel size diversity are shown in Figures 2-3c, d, e, and f. Vessel wall thickness varied surprisingly widely in stems ranging from 1 to 13  $\mu\text{m}$  across nearly 200 species worldwide (Barajas-Morales 1985; Baas et al. 1988; Baas et al. included only species from Oleaceae) and from 1 to 5  $\mu\text{m}$  in twigs of eight species (De Micco, Aronne & Baas 2008). Vessels can occur solitarily (Fig. 2-3c and d) or in groups (Fig. 2-3d and f). The number of vessels per group was reported to be very variable. For example, in the Californian flora it spanned from 1 to 150 vessels per group. However, the species with 150 vessels per group was the only extreme case and most species had up to five vessels per group with a mean of four vessels per group (Carlquist & Hoekman 1985; as cited in Rosell et al. 2007). Vessel groupings can be positioned radially, tangentially, or diagonally in relation to the xylem circumference.

In more than 30% of species, bordered pits are equipped with vestures, cell wall protrusions, which grow from the pit wall inwards into the pit chamber and/or near pit apertures (Jansen et al. 2004; excellent images are shown in that paper). Pit pairs between vessels and parenchyma are usually half-bordered, where the pit on the vessel side is bordered, and the pit on the parenchyma side is simple. The pit pairs vary in sizes and distribution.

Besides vessels, tracheids also transport water, but they occur only in a subset of angiosperm species. Tracheids are elongated cells similar in diameter to small vessels or fibres. Their wall thickness, pit size, and density resemble that of vessels (IAWA Committee 1989; Fig. 2-4a, b and c). They do not have perforation plates but regular end walls, and, in that sense, they are similar to conifer tracheids, although their pits have different structure. Tracheids often occur near vessels but can also form bands at the end of growth rings (IAWA Committee 1989; Carlquist 2001). Their proportions in wood were reported to be small and ranged from 0% to 8% in twigs of 24 species (Ziemińska et al. Chapter 3). Different types of tracheids have been recognized, depending on their location and distribution within the wood, and the definitions of these tracheids vary in the literature (Baas 1986b; Carlquist 1986). However, here for simplicity I will use the term 'tracheid' for all types. In cross-section, the distinction between tracheids and vessels is usually difficult or impossible to observe (for example, Fig. 2-4 b), but there are exceptions (for example, Fig. 2-4c or in Sano et al. 2011).

## **2.5.2 Vessels and climate**

Vessels have received the most attention in relation to climate among the three major tissues (Table 2-6). Their fraction has been found not to be related to temperature or to precipitation in both stems (61 species from Argentina and USA, Martínez-Cabrera et al. 2009) and twigs (120 species from eastern Australia; Gleason et al. 2012). The incidence of species with vessel groupings in stems increased towards warmer climate in Europe (Baas & Schweingruber 1987), but tended to decrease in Brazil (Alves & Angyalossy-Alfonso 2000). These discrepancies might possibly be explained by the fact that the warmest climate in Europe was also relatively dry (Mediterranean), but in Brazil, the warmest climate was in the Amazon area, which is certainly wetter than southern Europe. Similarly, Carlquist (1966) observed decreased number of vessels per group in warmer and wetter climates, among over 300 species within Asteraceae. He also observed that in species with libriform fibres (non-conductive tracheary elements) and no conductive tracheids, the degree of grouping (number of vessels per group) was higher in species growing in dry areas compared to mesic (Carlquist 1984).

Vessel proportion and vessel groupings have not received as much attention as vessel size, vessels per area, vessel element length, and helical sculpturing on vessel walls. Across almost 600 species worldwide, from four families, vessel size increased towards higher temperatures and vessels per area decreased (Carlquist 1966; Baas 1973; van der Oever, Baas & Zandee 1981; Baas et al. 1988). In some other surveys, the relationship between vessel size and temperature was positive but weak (van der Graaff & Baas 1974; Lens et al. 2004; Wheeler, Baas & Rodgers 2007) or not existent (Martínez-Cabrera et al. 2009). It is interesting that in a broad scale global dataset analysed by Wheeler, Baas & Rogers (2007) the relationship was weak. The authors ascribed this to the significant proportion of ring-porous species, with large early wood vessels, from temperate areas of northern hemisphere. Although this explanation is plausible, the authors did not analyse a dataset with ring-porous species excluded. In twigs, vessels tended to be larger in warmer areas across 69 Australian species (Ziemińska, Wright & Westoby Chapter 4). Vessel size to number ratio was also observed to increase towards warmer climates in twigs of 120 Australian species (Gleason et al. 2012). The relationships with rainfall were less clear. Vessel size in stems was positively correlated (Carlquist 1966; Barajas-Morales 1985), weakly positively correlated (Lens et al. 2004), or not related at all to rainfall (Martínez-Cabrera et al. 2009). In twigs, the relationship between vessel size and rainfall was positive across 79 species (Jacobsen et al. 2012) or did not exist (Ziemińska, Wright & Westoby Chapter 4). Moreover, Olson & Rosell (2012) showed that vessel size was mainly driven by the size of a plant rather than by a climate. However, since higher rainfall sites tend to be occupied by taller plants, vessel size could be related both to rainfall and to plant stature in parallel. Potentially, both plant size and vessel size are under selective pressure from climate.

Other commonly measured traits are vessel element length, helical sculpturing, and scalariform perforation plates. Vessel element length uniformly has been shown to increase with temperature (Carlquist 1966; Baas 1973; van der Graaff & Baas 1974; van der Oever, Baas & Zandee 1981; Baas et al. 1988; Lens et al. 2004; Wheeler, Baas & Rodgers 2007) and rainfall (Carlquist 1966; Barajas-Morales 1985; Jansen et al. 2004). Information on entire vessel length in twigs is much scarcer. Meta-analysis of 130 species has shown no relationship between vessel length and precipitation, while no

data for temperature were available (Jacobsen et al. 2012). Generally, helical sculpturing was more common in species growing in colder areas (Carlquist 1966; Baas 1973; van der Oever, Baas & Zandee 1981; Baas et al. 1988; Alves & Angyalossy-Alfonso 2000; Lens et al. 2004; Wheeler, Baas & Rodgers 2007), although no pattern was found across more than 500 species within Europe (Baas & Schweingruber 1987). The relationship with rainfall is less documented. Species with helical sculpturing were more common in drier locations (Carlquist 1966; Baas & Schweingruber 1987) or slightly more common in wetter sites in Brazil (Alves & Angyalossy-Alfonso 2000). The incidence of species with scalariform perforation plates has consistently been shown higher in cold and wet areas (Baas & Schweingruber 1987; Alves & Angyalossy-Alfonso 2000; Jansen et al. 2004; Wheeler, Baas & Rodgers 2007). The structure of vessel pits has also been under examination. Jansen's et al. (2004) survey of stems of almost 12000 species showed that more than 30% of analysed species possessed vestured pits, and they tended to be more prevalent in warmer and drier areas.

Information on tracheid presence has less coverage than vessels. An analysis of European flora indicated that vascular tracheids (tracheids intergrading with vessels as in Fig. 2-4b) were more common in warmer and drier locations (Baas & Schweingruber 1987). Zieminska, Wright & Westoby (Chapter 4) also measured the proportion of conduits with lumen diameter smaller than 15  $\mu\text{m}$ , which potentially could include tracheids. This proportion increased towards colder and/or drier environments.

### **2.5.3 Vessels and functional traits**

Vessel anatomical characteristics have been associated with many hydraulic traits, as well as with mechanical traits (Table 2-7). Conductivity, embolism resistance, and water potential have been the functional traits most commonly measured. The studies reported here investigated twigs, except where stem potential conductivity was calculated from vessel sizes using the Hagen-Poiseuille equation (Rana et al. 2009; Poorter et al. 2010; Zanne et al. 2010). In principle potential conductivity increases both with vessel lumen proportion and with vessel size (Rana et al. 2009; Poorter et al. 2010; Zanne et al. 2010), but in a broad scale comparison of 2230 species, the main driving component of conductivity was the ratio of vessel size to vessel number per area (vessel

size to number ratio,  $S$  in Zanne et al., 2010). This ratio was also an important component in measured conductivity in twigs among 120 species, accounting for more variation in conductivity than did vessel lumen proportion (Gleason et al. 2012).

The most commonly measured hydraulic trait has been embolism resistance. Overall, species with smaller conduits (Cochard & Tyree 1990; Wheeler et al. 2005; Hacke et al. 2006; Lens et al. 2011), shorter conduits (Hacke et al. 2006; Jacobsen et al. 2007; Lens et al. 2011), and higher double wall to lumen ratio (Hacke et al. 2001; Jacobsen et al. 2005; Pratt et al. 2007) have been shown to be more resistant. Interestingly, Lens et al. (2011) found that among seven *Acer* species the ones with more vessels per group were more resistant to embolism. Pit properties have also been linked with embolism resistance and air-seeding threshold (Jansen, Choat & Pletsers 2009; Lens et al. 2011).

Vessel proportion influenced the mechanical performance of wood. Wood strength and stiffness (MOR and MOE) decreased with higher vessel proportion (Jacobsen et al. 2005, 2007; Gleason et al. 2012).

#### **2.5.4 Vessels in experimental studies**

There have been myriad experiments linking vessel properties with hydraulic traits perhaps most comprehensively described in Tyree & Zimmermann's book (2002), and this topic could deserve a review on its own. Here, I point to only a few studies that take advantage of technological developments or that are novel since 2002. Water flow resistivity was measured on serially shortened stems (Sperry, Hacke & Wheeler 2005). As the stems were shortened, progressively fewer of the vessels had end-walls, and the declining resistivity to water flow thus measured the role of end-walls in hydraulic resistivity. Helical sculpturing on vessel walls has been experimentally shown to increase wettability (how well fluid adheres and spreads on a surface) in twigs of four species (Kohonen & Helland 2009). Evidence from dye-injection and cryo-scanning experiments suggested that vessel arrangement and type of porosity influences water transport patterns within and between growth rings (Umebayashi et al. 2008, 2010). Vessel arrangements are also important for embolism spread, which was visualised *in vivo* using high-resolution x-ray tomography (Brodersen et al. 2013).

Although vessels are traditionally considered a hydraulic tissue, they also affect mechanical properties. Bending tests were run on wood blocks of eight species, and the breakage was analysed microscopically (Beery, Ifju & McLain 1983). It was found that mechanical failure was initiated to a large degree in vessels. In a similar study, Hepworth et al. (2002) found that mechanical failure may depend more on arrangement than the properties of individual vessels. Large vessels in close vicinity to each other posed a higher risk than vessels further apart. Although interesting, these tests were carried on wood blocks cut from mature trees, and it remains unclear how this knowledge applies to stems and twigs of living trees.

## **2.6 Synthesis: wood functional systems**

Traditionally, functions have been ascribed to the relevant tissue: fibres play a mechanical role, parenchyma transports and stores food, and vessels transport water. While broadly this is true, it is also evident that tissues can influence more than one function. For this reason, we can organize our knowledge into systems: mechanical, storage, and hydraulic, where different tissues play a role in each of the systems, to some extent. In the following section, I synthesize the information gathered in this review with an overarching question: what are the anatomical influences on each system?

### **2.6.1 Mechanical system**

Wood plays a mechanical role, and its performance in this respect is typically measured in terms of wood strength (MOR) and stiffness (MOE). The properties of all tissues (fibres, parenchyma, and vessels) influence the mechanical behaviour of a given wood sample, but to varying extents.

Fibre wall proportion was positively related to mechanical strength (MOR) across twigs of 17 species (Jacobsen et al. 2007). Fibre wall proportion is perhaps the best explanatory variable among fibre traits (e.g. fibre wall thickness or lumen diameter) because it combines total fibre proportion with fibre wall thickness or fibre wall to lumen ratio. Fibre lumen plus vessel, vessel, and parenchyma proportions were

inversely correlated with MOR or MOE in twigs (Ziemińska, Wright & Westoby Chapter 4, 69 species; Woodrum, Ewers & Telewski 2003, 5 species; Jacobsen et al. 2005, 6 species, Jacobsen et al. 2007, 17 species; Gleason et al. 2012, 120 species), and ray parenchyma was positively correlated with MOR across five species (Woodrum, Ewers & Telewski 2003). MOR and MOE have been shown to be strongly associated with wood density across a broad comparison of 520 species (Chave et al. 2009). Because wood density is mainly driven by fibre wall and lumen proportions (Ziemińska et al. Chapter 3; Fujiwara et al. 1991; Jacobsen et al. 2007; Rana et al. 2009; Martínez-Cabrera et al. 2009), one can infer that those are also most important drivers of MOR and MOE along with some contribution from vessel and parenchyma traits. In addition or alternatively to modifying wood (building material) properties, plants also influence their mechanical performance via altering stem diameters (Larjavaara & Muller-Landau 2010; Anten & Schieving 2010; Butler et al. 2011).

## **2.6.2 Metabolite transport and storage system**

Parenchyma is the main storage tissue for carbohydrates (Esau 1977; Evert 2006). However, living fibres may be significantly involved in storage as was shown in *Robinia pseudoacacia* (Yamada et al. 2011). Living fibres were also observed to be more common in woods with scarcer parenchyma (Wheeler, Baas & Rodgers 2007; Carlquist 2012). I am not aware of any quantitative data on living fibre proportion (except of Yamada et al. 2011); nevertheless, the available evidence implies that parenchyma and living fibres may play similar roles.

Parenchyma proportion varied widely across species from 6% to over 60%. The degree of variation was even larger when axial and ray parenchyma were considered individually (0-52% and 2-46%, respectively). Despite this striking diversity, we do not really understand the functional significance of variation in parenchyma amount. In this review, I searched for correlations between parenchyma and non-structural carbohydrates content (NSC, starch and sugars). One might expect that larger parenchyma proportion would correspond to higher NSC content. However, I did not find any reports on this topic. Because NSC content varies diurnally, across seasons and climates (Bucci et al. 2003; Körner 2003; Sala, Woodruff & Meinzer 2012), it would be

difficult to link it with parenchyma properties, but not impossible. Despite this gap in our knowledge, some tentative patterns can be characterized. Across 31 species from four different temperatures of growing seasons, overall wood NSC content decreased towards warmer climates (Körner 2003). On the other hand, across more than 600 species parenchyma proportion tended to increase towards warmer climates (Table 2-4). Because data on NSC content across climates come from a relatively small species group compared to parenchyma data, conclusions must be tentative. Nevertheless, this discrepancy poses a question: does parenchyma proportion really reflect carbohydrate storage content or does parenchyma play some additional or alternative function? Potentially water storage, discussed in the next section, may be an equally important parenchyma function.

### **2.6.3 Hydraulic system**

The hydraulic system encompasses several aspects of water transport: conductivity, water potential, embolism resistance, vessel refilling, and capacitance. Some of those functions and their relation to anatomy have been well understood. For example, larger vessels are associated with higher conductivity and possibly lower embolism resistance (efficient but risky). Conversely small vessels have low conductivity but potentially are more resistant to embolism (inefficient but safe; Tyree & Zimmermann 2002). Presumably, embolism risk increases as the temperature and access to water decreases. Freeze-thaw embolism is more prone to occur in colder environments, which may explain why species from those environments tended to have smaller vessels (Table 2-6) and more small vessels or tracheids (Ziemińska, Wright & Westoby Chapter 4). However, the trend with rainfall is a little less consistent, and three out of six studies reported weak or no relationship between vessel size and rainfall. Potentially, other anatomical properties may play a role in secure water transport in drier environments. For example, Lens et al. (2011) found that in seven *Acer* species embolism resistance was higher in species with more vessels per group. And Carlquist (1966) found that species with more vessels per group were more abundant in drier areas. Additionally, it has been suggested that tracheids (conductive cells similar to vessels but smaller in diameter and without perforation plates) may add a safe pathway



for water transport (Carlquist 2012). It was found that tracheids were more common in drier areas in a comparison of 505 species from Europe (Baas & Schweingruber 1987). Ziemińska et al. (Chapter 4) quantified the proportion of conduits with lumen diameter < 15 µm, which presumably encompassed both small vessels and tracheids, and found this proportion to be higher in the drier site. Vessel pit characteristics are likely to be important as well. Jansen et al. (2004) observed vestured pits (pits with vessel wall protrusions) were more common in dry climates. This finding is consistent with the hypothesis that vestures may prevent excessive pit membrane deflection, therefore minimize the formation of larger membrane pores and decrease embolism spread (Zweypfenning 1978; Jansen et al. 2003).

Vessels are central to water transport relations; however, parenchyma and fibres may also play an important role. Embolised vessels can refill (e.g. Cochard et al. 2001; Améglio et al. 2002; Brodersen et al. 2013; Hao et al. 2013) and increasing evidence suggests that parenchyma is involved in the refilling process (Salleo et al. 2004; Alves et al. 2004; Salleo, Trifilò & Lo Gullo 2006; Secchi & Zwieniecki 2011). Although these studies advance our understanding of the mechanisms of vessel refilling, they are largely confined to a few species. Also, the extent to which embolism refilling under negative pressure takes place is debated (Sperry 2013; Wheeler et al. 2013). The question how parenchyma proportions, which vary widely across species (Table 2-1, Figure 2-2 c, d), are linked with vessel refilling remains unexplored. It is also not known whether the amount of stored carbohydrates affects the ability of vessels to refill. Supposedly, relatively small amounts of sugars are needed to trigger vessel refilling, and that would mean that the majority of parenchyma storage space could be used to store water (or carbohydrates).

Capacitance, an indicator of stored water that can be incorporated into a transpiration stream, may be an important component of water strategies. It was positively correlated with vessel plus fibre lumen proportion in nine Rhamnaceae species (Pratt et al. 2007). Additionally, it was also negatively correlated with wood density (Meinzer et al. 2003, 2008; Scholz et al. 2007). Lower-density species can have either high parenchyma proportion or high fibre lumen proportion (Ziemińska et al. Chapter 3 and 4). Potentially both tissues confer capacitance but it is not clear how the

two tissues would vary in their water release mechanisms. Holbrook (1995) suggested that water stored in extracellular spaces (including in the lumen of dead fibres) would be depleted quickly (before stem water potential would fall below -0.6 MPa, Tyree & Yang 1990). Potentially, water stored in xylem parenchyma might be of more significance but to my knowledge, this has not been tested.

## 2.7 Conclusions

1. Mechanical strength and stiffness arise from the composition of all tissues. Overall, more cell wall (mostly fibre walls) increases wood mechanical strength and stiffness, whereas more cell lumen of dead cells (vessels and fibres) and soft tissue (parenchyma) decreases them. These conclusions are based on studies within a relatively small number of species. However, wood density is also driven largely by the total amount of cell wall, and has been shown to correlate strongly with strength and stiffness across a large number of species.

2. Axial and ray parenchyma proportions vary widely across species. Although it is commonly thought that the main function of parenchyma is metabolite storage, there is little evidence that differences in storage requirements explain variation in amounts of parenchyma. On the contrary, parenchyma tends to increase towards warmer environments, while carbohydrate storage tends to increase towards colder climates. This suggests parenchyma may play an important role in other functions such as water storage. Increasing evidence also implies that parenchyma participates in vessel refilling. Yet it is not clear how the broad diversity in parenchyma properties across species and climates would influence refilling processes. The functional meaning of parenchyma properties and the wide variation of parenchyma amount across species and environments remains an open question.

3. Vessel size decreases towards colder climates and this is consistent with the explanation that smaller vessels are safer in environments prone to freeze-thaw events because they are less likely to embolise. We might also expect to find small vesselled species in dry climates because small vessels may confer some degree of embolism resistance in such areas. However, the evidence for this pattern is less solid, suggesting

other mechanisms might be in play (more vessels per group, more abundant tracheids, presence of vestured pits).

This chapter has highlighted the usefulness of anatomical information and the perspective it adds to consideration of plant ecological strategies. The review also pointed to some gaps in anatomical data, especially for broad scale quantitative measurements. Twig anatomical measurements were especially rare, despite the fact that many plant functional traits are measured exactly on twigs. The two following chapters aim to fill these gaps by rigorously quantifying anatomical variation in twigs across a relatively broad number of species from different environments. The attempt is also made to find associations between anatomical and plant functional traits such as wood density (Chapter 3), height, leaf area to sapwood area ratio, and modulus of elasticity (Chapter 4).

## **2.8 Acknowledgements**

I am very grateful to Mark Westoby, Ian Wright, and Sean Gleason for helpful and constructive comments on earlier versions of this draft.



## 2.9 Figures

Figure 2-1     Fibres.

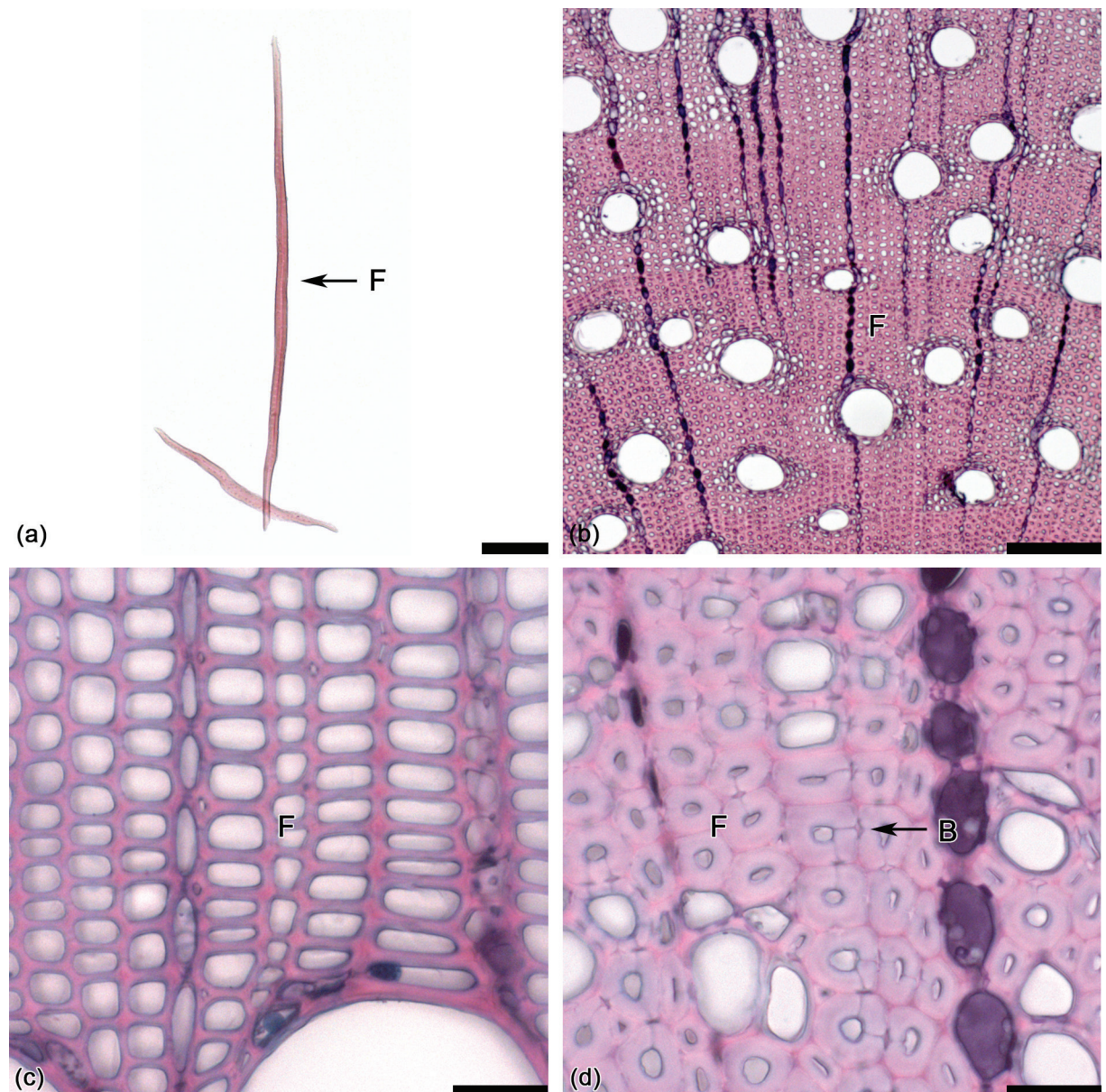


Figure 2-1     Fibres. (a) macerated fibre cell of twig of *Persoonia juniperina*, Proteaceae, scale bar 100  $\mu\text{m}$ ; (b) cross-section through a twig of *Eucalyptus amygdalina*, Myrtaceae, with most of the area occupied by fibres, scale bar 100  $\mu\text{m}$ ; (c) cross-section through a twig of *Alphitonia excelsa*, Rhamnaceae, with thin-walled fibres, scale bar 25  $\mu\text{m}$ ; (d) cross-section through a twig of *Persoonia juniperina*, Proteaceae, with thick-walled fibres, scale bar 25  $\mu\text{m}$ . F – fibre(s), B – bordered pit.





Figure 2-2 Parenchyma.

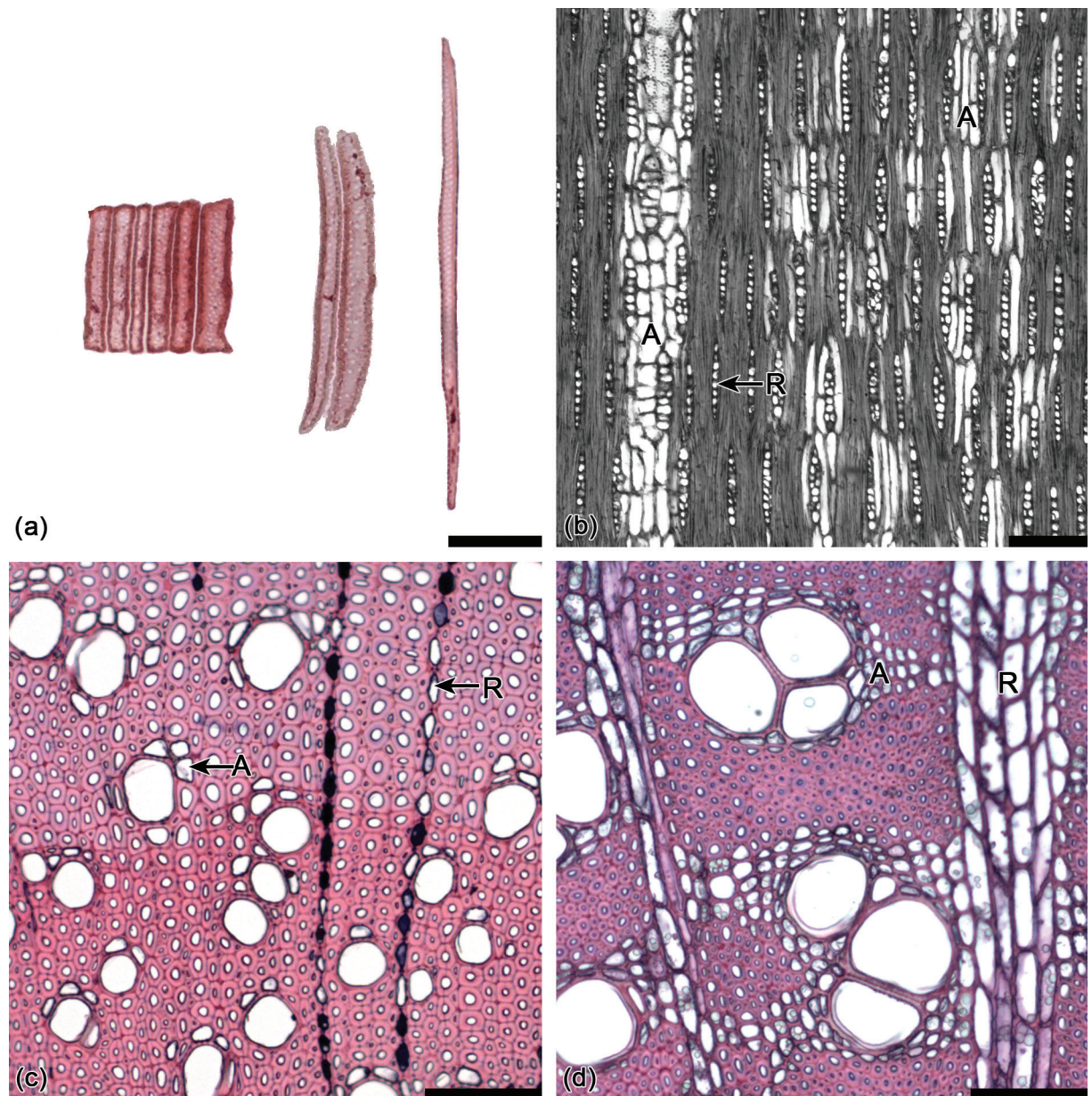


Figure 2-2 Parenchyma. (a) macerated parenchyma cells of twig of *Persoonia juniperina*, Proteaceae; (b) longitudinal tangential section through stem wood of *Dalbergia spruceana*, Fabaceae, with paratracheal axial parenchyma (square and rectangular cells to the left) and apotracheal axial parenchyma (elongated cells to the right); (c) cross-section through a twig of *Persoonia falcata*, Proteaceae, with scarce axial and ray parenchyma; (d) cross-section through a twig of *Grevillea parallela*, Proteaceae, with abundant axial and ray parenchyma; A – axial parenchyma, R – ray parenchyma. All scale bars 100 µm.

Figure 2-3 Vessels. (a) macerated vessel element cells of twig of *Persoonia juniperina*, Proteaceae; (b) longitudinal tangential section through stem wood of *Dalbergia cearensis*, Fabaceae, with a fragment of a vessel composed of vessel elements; (c) cross-section through a twig of *Epacris impressa*, Ericaceae, with very small, mostly solitary vessels; (d) cross-section through a twig of *Daviesia latifolia*, Fabaceae, with multiple vessels in group (small vessels intergrading with tracheids); (e) cross-section through a twig of *Eucalyptus amygdalina*, Myrtaceae, with solitary vessels; (f) cross-section through a twig of *Grevillea parallela*, Proteaceae, with large vessels and few vessels per group. PP – perforation plate (simple), VE – vessel element, V – vessel. All scale bars 100 µm.



Figure 2-3 Vessels. See figure caption on opposite page.

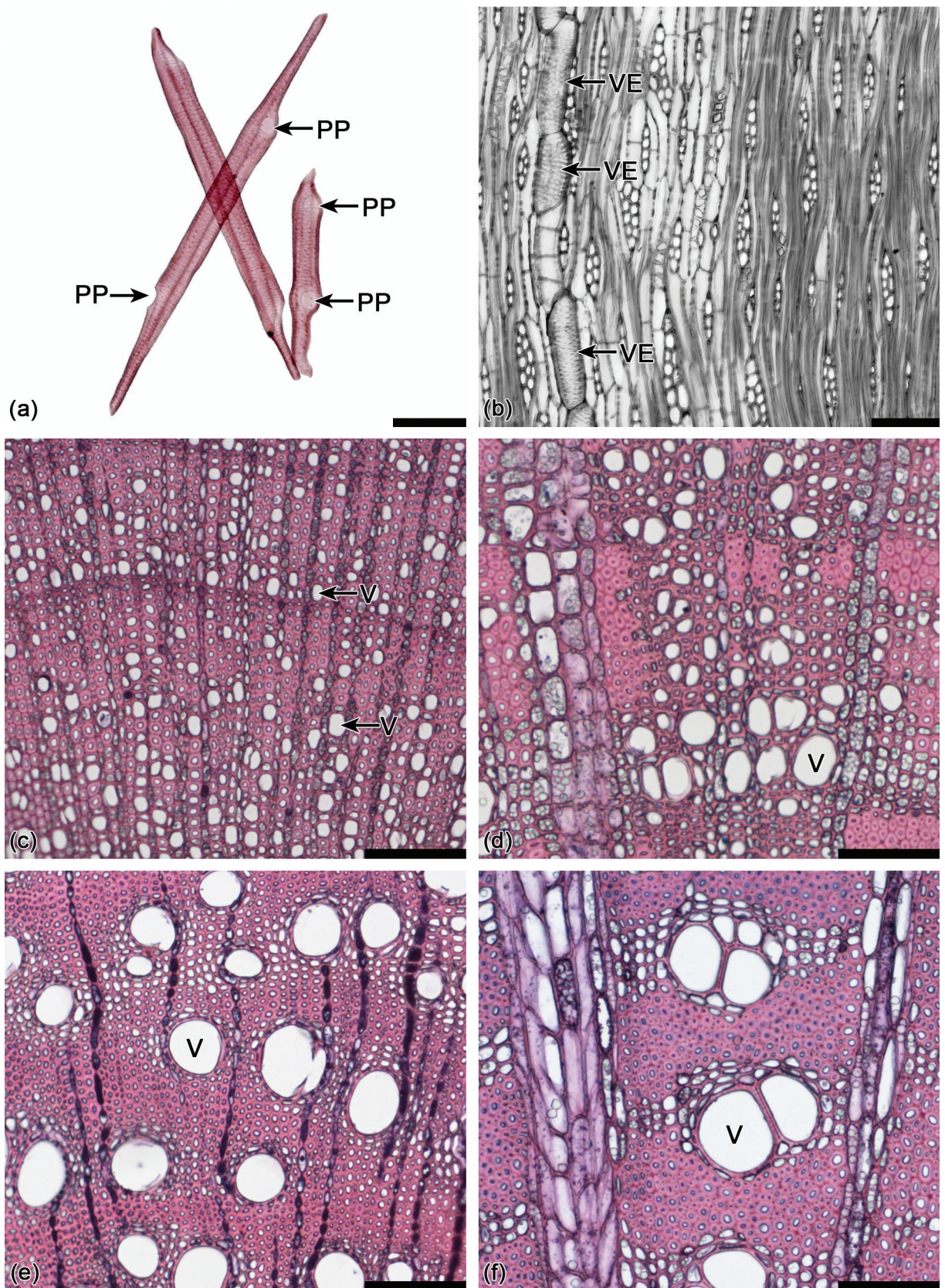






Figure 2-4 Vessels and tracheids.

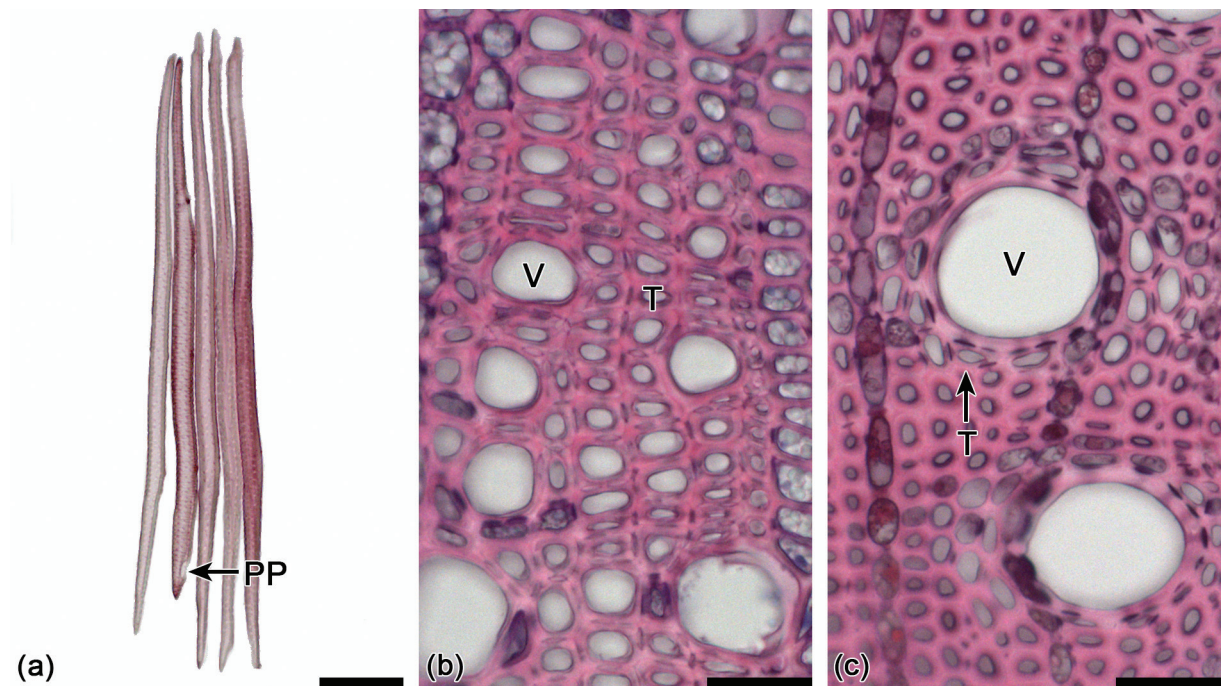


Figure 2-4 Vessels and tracheids. (a) macerated cells of twig of *Persoonia juniperina*, Proteaceae, with a small vessel (second cell from the left) among tracheids and fibres, scale bar – 100  $\mu\text{m}$ ; (b) cross-section through a twig of *Daviesia latifolia*, Fabaceae, with abundant tracheids intergrading with small vessels, scale bar – 25  $\mu\text{m}$ ; (c) cross-section through a twig of *Eucalyptus tenuiramis*, Myrtaceae, with tracheids surrounding a vessel, scale bar – 25 $\mu\text{m}$ ; PP – perforation plate, V – vessel, T – tracheid.



## 2.10 Tables

Table 2-1 Summary of variation in quantitative anatomical traits reported in the literature.

Trait	Units	Range in stems		Range in twigs		Number of species: stem; twig	References
		Low	High	Low	High		
Fibre proportion	%	26	86	20	74	836; 93	Stems: French 1923 as cited in Panshin & de Zeeuw 1980; Manwiller 1973 as cited in Koch 1985; Poorter et al. 2010; Zheng & Martínez-Cabrera 2013; Twigs: Ziemińska et al. Chapter 3 and 4
Fibre lumen proportion	%	1	57	0.5	32	61; 93	Stems: Martínez-Cabrera et al. 2009; Twigs: Ziemińska et al. Chapter 3 and 4
Fibre wall proportion	%	16	73	16	61	61; 93	Stems: Martínez-Cabrera et al. 2009; Twigs: Ziemińska et al. Chapter 3 and 4
Fibre diameter	µm	11	44	-	-	54; -	Stems: Manwiller 1973 as cited in Koch 1985; Ruelle et al. 2006; Dutt & Tyagi 2011
Fibre lumen diameter	µm	2	17	-	-	54; -	Stems: Manwiller 1973 as cited in Koch 1985; Ruelle et al. 2006; Dutt & Tyagi 2011
Fibre wall thickness	µm	1	6	-	-	137; -	Stems: Manwiller 1973 as cited in Koch 1985; Barajas-Morales 1985; Fujiwara et al. 1991; Dutt & Tyagi 2011
Parenchyma proportion, total	%	6	64	11	66	841; 93	Stems: Martínez-Cabrera et al. 2009; Poorter et al. 2010; Zheng & Martínez-Cabrera 2013; Twigs: Ziemińska et al. Chapter 3 and 4
Parenchyma proportion, axial	%	0	52	1	33	897; 93	Stems: French 1923 as cited in Panshin & de Zeeuw 1980; Manwiller 1973 as cited in Koch 1985; Martínez-Cabrera et al. 2009; Zheng & Martínez-Cabrera 2013; Twigs: Ziemińska et al. Chapter 3 and 4
Parenchyma proportion, ray	%	2	46	6	41	1022; 93	Stems: Myer 1922; French 1923 as cited in Panshin & de Zeeuw 1980; Manwiller, 1973 fide Koch, 1985; Petrić & Šćukanec 1975; Fujiwara 1992; Martínez-Cabrera et al. 2009; Zheng & Martínez-Cabrera 2013; Twigs: Ziemińska et al. Chapter 3 and 4
Vessel proportion	%	1	56	1	23	947; 101	Stems: French 1923 as cited in Panshin & de Zeeuw 1980; Manwiller 1973 as cited in Koch 1985; Fujiwara et al. 1991; Martínez-Cabrera et al. 2009; Poorter et al. 2010; Zanne et al. 2010; Zheng & Martínez-Cabrera 2013; Twigs: De Micco et al. 2008; Ziemińska et al. Chapter 3 and 4
Vessels per group	-	1	150	-	-	207; -	Carlquist & Hoekman 1985 as cited in Rosell et al. 2007
Vessel lumen diameter	µm	16	267	13	70	215; 80	Stems: Barajas-Morales 1985; Lindorf 1994; Olson & Rosell 2012; Twigs: Jacobsen et al. 2012 and literature cited therein
Vessel wall thickness	µm	1	13	1	5	191; 8	Stems: Baas et al. 1988; Barajas-Morales 1985 Twigs: De Micco et al. 2008
Tracheid lumen diameter	µm	3	9	-	-	15; -	Stems: Sano et al. 2011

Table 2-2 Relationships between fibre traits and climate.

Anatomical trait	Unit	Relationship with		Organ	Number of species (families)	Climate	Location	Statistical method	Reference
		Temp	Rain						
Fibre lumen proportion	%	↘	↗	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009
Fibre wall proportion	%	-	↘	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009
Fibres with bordered pits	presence	↘↘	↗	Stem	642 (72)	Tropical to boreal	Worldwide	Visual examination of bar graphs	Baas & Schweingruber 1987; Baas et al. 1988
Fibre length	µm	↗↗↗↗↗	↗↗↗↗	Stem	686 (38)	Tropical to temperate	Worldwide	Visual examination of scatterplots, bar graphs and trait values, t-test, correlation	Carlquist 1966; van der Graaff & Baas 1974; Barajas-Morales 1985; Baas et al. 1988; Lens et al. 2004
Fibre size	µm	↗	na	Stem	31 (1)	Tropical to temperate	Worldwide	Visual examination of scatterplots	van der Oever et al. 1981
Fibre wall thickness	class or µm	↗↗-	↘- -	Stem	637 (69)	Tropical to temperate	Worldwide	Pearson's Standardised Residues, t-test, correlation, visual examination of scatterplots	van der Oever et al. 1981; Barajas-Morales 1985; Alves & Angyalossy-Alfonso 2002; Martínez-Cabrera et al. 2009
Fibre wall to lumen ratio	-	↗	↘	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009

Notes: Temp = temperature. 'Temperature' indicates either reported mean annual temperature (°C) or latitude. Where a study reported only latitude | provisionally interpreted this as a correlation with temperature (low latitudes corresponding to high temperatures and vice versa). Rain = rainfall. 'Rainfall' indicates either actual rainfall (mm) or a category (e.g. dry, wet). The arrows refer to the direction of relationship between anatomical and climate variables: ↗ positive, ↘ weak positive, ↘ negative, ↘ weak negative, '-' no relationship and 'na' no data was available. Each symbol represents given relationship in one study i.e. two positive arrows indicate that the positive relationship was found in two independent studies. 'Bar graphs' refer to an incidence of species with given trait value plotted against climate category. Number of species and families is the sum of all species and families studied by the cited authors.

Table 2-3 Correlations between fibre and functional traits.

Anatomical trait	Unit	Functional trait	Unit	Relation-ship	Organ	Number of species (families)	Reference
Fibre proportion	%	MOE	MPa	↗	Twig	5 (1)	Woodrum et al. 2003
Vessel lumen + fibre lumen proportion	%	MOR	MPa	↗	Twig	5 (1)	Woodrum et al. 2003
		MOR	MPa	↗	Twig	6 (3)	Jacobsen et al. 2005
Fibre lumen proportion	%	Capacitance	$\Delta RWC \Delta P^{-1}$	↗	Twig	9 (1)	Pratt et al. 2007
		Conductance, potential	$mm^2 Pa^{-1} s^{-1}$	-	Stem	5 (1)	Rana et al. 2009
Fibre wall proportion	%	MOR	MPa	↗	Twig	17 (9)	Jacobsen et al. 2007
		Embolism resistance	MPa	↗	Twig	6 (3)	Jacobsen et al. 2005
Fibre lumen diameter	$\mu m$	Conductivity	$kg m^{-1} s^{-1} MPa^{-1}$	--	Twig	26 (10)	Jacobsen et al. 2007; Pratt et al. 2007
		MOR	MPa	↗↗	Twig	23 (12)	Jacobsen et al. 2005, 2007
		MOE	MPa	↗	Twig	5 (1)	Woodrum et al. 2003
Fibre wall thickness	$\mu m$	Embolism resistance	MPa	↗	Twig	6 (3)	Jacobsen et al. 2005
		Conductivity	$kg m^{-1} s^{-1} MPa^{-1}$	-	Twig	17 (9)	Jacobsen et al. 2007
		Water potential, midday	MPa	-	Twig	17 (9)	Jacobsen et al. 2007
		Capacitance	$\Delta RWC \Delta P^{-1}$	↗	Twig	9 (1)	Pratt et al. 2007
		MOR	MPa	↗-	Twig	23 (12)	Jacobsen et al. 2005, 2007
		Conductivity	$kg m^{-1} s^{-1} MPa^{-1}$	-	Twig	17 (9)	Jacobsen et al. 2007
		Water potential, midday	MPa	-	Twig	17 (9)	Jacobsen et al. 2007
Fibre wall to lumen ratio	-	Conductance, estimated	$mm^2 Pa^{-1} s^{-1}$	-	Stem	5 (1)	Rana et al. 2009
		MOR	MPa	↗	Twig	5 (1)	Woodrum et al. 2003
		MOR	MPa	↗	Stem	3 (1)	Yang & Evans 2003
		MOE	MPa	↗↗	Stem	4 (1)	Yang & Evans 2003, Evans & Illic 2001

Notes: MOE – modulus of elasticity, MOR – modulus of rupture. The arrows refer to the direction of relationship between anatomical and functional traits: ↗ positive, ↘ negative, '-' no relationship.

Table 2-4 Relationships between parenchyma traits and climate.

		Relationship with		Organ	Number of species (families)	Climate	Location	Statistical method	Reference
		Temp	Rain						
Parenchyma proportion, total	%	-	-	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009
Parenchyma proportion, axial	%	↗	↗	Twig	69 (26)	Tropical to temperate	Australia	Kruskal-Wallis test	Ziemińska et al. Chapter 4
Parenchyma proportion, axial apotracheal	class or %	↗↗↗	↘	Stem	633 (48)	Tropical to temperate	Worldwide	Pearson's Standardised Residues, correlation, in Baas (1973) method not clear	Baas 1973; Alves & Angyalossy-Alfonso 2002; Martínez-Cabrera et al. 2009
Parenchyma proportion, axial apotracheal	%	↗	-	Twig	69 (26)	Tropical to temperate	Australia	ANOVA	Ziemińska et al. Chapter 4
Parenchyma proportion, axial paratracheal	presence	↘	na	Stem	491 (22)	Tropical to temperate	Brazil	Pearson's Standardised Residues applied to the incidence of species with a given trait value within a climate type	Alves & Angyalossy-Alfonso 2002
Parenchyma proportion, axial paratracheal	presence	↗	na	Stem	491 (22)	Tropical to temperate	Brazil	Pearson's Standardised Residues applied to the incidence of species with a given trait value within a climate type	Alves & Angyalossy-Alfonso 2002
Parenchyma proportion, ray	%	-	↗	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009
Rays per mm	%	↗	↗	Twig	69 (26)	Tropical to temperate	Australia	Kruskal-Wallis test	Ziemińska et al. Chapter 4
Ray height, multiseriate	mm <sup>-1</sup>	na	-	Stem	54 (21)	Tropical	Mexico	t-test	Barajas-Morales 1985
Ray height	µm	↗↗	↗↗	Stem	443 (2)	Tropical to temperate	Worldwide	Visual examination of values in climate categories, correlation	Carlquist 1966; Lens et al. 2004
Ray height	µm	↗↗	↗	Stem	187 (36)	Tropical to temperate	Worldwide	t-test, visual examination of bar graphs, in Baas (1974) methods not clear	Baas 1973; van der Graaff & Baas 1974; Barajas-Morales 1985



		Relationship with		Organ	Number of species (families)	Climate	Location	Statistical method	Reference
		Temp	Rain						
Ray width, multiseriate	number of cells or $\mu\text{m}$	↗-	↗-	Stem	443 (2)	Tropical to temperate	Worldwide	Visual examination of values in climate categories, correlation	Carlquist 1966; Lens et al. 2004
Ray width	number of cells	-	na	Stem	572 (21)	Tropical to temperate	Worldwide	Pearson's Standardised Residues, in Baas (1974) methods not clear	Baas 1973; Alves & Angyalossy-Alfonso 2002

Notes: Temp – temperature. 'Temperature' indicates either reported temperature (°C) or latitude. In case a study reported only latitude I classified it as 'temperature' (low latitudes confer high temperatures and vice versa). Rain – rainfall. 'Rainfall' indicates either actual rainfall (mm) or a category (e.g. dry, wet). The arrows refer to the direction of relationship between anatomical and climate variables: ↗ positive, ↘ weak positive, ↗ negative, ↘ weak negative, 'na' no relationship and 'na' no data available. Each symbol represents a relationship in one study i.e. two positive arrows indicate that the positive relationship was found in two independent studies. 'Bar graphs' refer to an incidence of species with given trait value plotted against climate category. Number of species and families is the sum of all species and families studied by the cited authors. \* number corresponds to the specimen rather than species (i.e. some species may have been represented by a few specimens and each specimen is counted as a separate entity).

Table 2-5 Correlations between parenchyma and functional traits.

Anatomical trait	Unit	Functional trait	Unit	Relation- ship	Organ	Number of species (families)	Reference
Parenchyma proportion, total	%	MOR	MPa	↗	Twig	17 (9)	Jacobsen et al. 2007
		MOE	MPa	↗	Twig	69 (26)	Ziemińska et al. Chapter 4
		Embolism resistance	MPa	-	Twig	9 (1)	Pratt et al. 2007
		Water potential, midday	MPa	-	Twig	17 (9)	Jacobsen et al. 2007
		Conductivity, potential	kg m <sup>-1</sup> s <sup>-1</sup> MPa <sup>-1</sup>	-	Stem	42 (21)	Poorter et al. 2010
Parenchyma proportion, axial	%	MOE	MPa	-	Twig	69 (26)	Ziemińska et al. Chapter 4
Parenchyma proportion, ray	%	MOR	MPa	↗	Twig	5 (1)	Woodrum et al. 2003
		MOE	MPa	-	Twig	69 (26)	Ziemińska et al. Chapter 4

Notes: The arrows refer to the direction of relationship between anatomical and functional traits: ↗ positive, ↘ negative, ↗ weak negative, '–' no relationship.

Table 2-6 Relationships between vessel traits and climate.

Anatomical trait	Unit	Relationship with		Organ	Number of species (families)	Climate	Location	Statistical method	Reference
		Temp	Rain						
Vessel proportion	%	-	-	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009
Vessel groupings	presence	-	-	Twig	120 (25)	Tropical to temperate	Australia	Correlation	Gleason et al. 2012
		↘↗	-	Stem	996 (93)	Tropical to boreal	Brazil, Europe, Cyprus, Madeira	Pearson's Standardised Residues, visual examination of bar graphs	Baas & Schweingruber 1987; Alves & Angyalossy-Alfonso 2000
Vessels per group	-	↗	↗	Stem	328 (1)	Tropical to temperate	Worldwide	Visual examination of trait values within climate categories	Carlquist 1966
Vessel size	µm or µm <sup>2</sup>	↗↗↗ ↗↗↗ ↗↗ ↗ <sub>-</sub>	↗↗↗ - -	Stem	859 (165) + 5663* (258)	Tropical to arctic	Worldwide	Visual examination of a scatterplots, bar graphs, and trait values in a climate category, correlation, t-test	Carlquist 1966; Baas 1973; van der Graaff & Baas 1974; van der Oever et al. 1981; Barajas-Morales 1985; Baas et al. 1988; Lens et al. 2004; Wheeler et al. 2007; Martínez-Cabrera et al. 2009
Hydraulically weighted diameter	µm	↗	↗-	Twig	148 (min 26)	Tropical to temperate	Worldwide	Correlation, Kruskal-Wallis test	Jacobsen et al. 2012 and literature cited therein; Ziemińska et al. Chapter 4
Vessels per area	mm <sup>2</sup> or class	-	-	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009
		↗	-	Twig	69 (26)	Tropical to temperate	Australia	ANOVA	Ziemińska et al. Chapter 4
Vessel wall thickness	µm	↗↗↗ ↗↗↗ ↗ <sub>-</sub>	↗↗↗ - -	Stem	531 (167) + 5663* (258)	Tropical to arctic	Worldwide	Visual examination of a scatterplots, bar graphs, and trait values in a climate category, correlation, t-test	Baas 1973; van der Graaff & Baas 1974; van der Oever et al. 1981; Barajas-Morales 1985; Baas et al. 1988; Lens et al. 2004; Wheeler et al. 2007; Martínez-Cabrera et al. 2009
		↗	-	Twig	69 (26)	Tropical to temperate	Australia	Kruskal-Wallis test	Ziemińska et al. Chapter 4
Vessel wall thickness	µm	↗	↗	Stem	85 (22)	Tropical to temperate	Worldwide	Visual examination of scatterplots, t-test	van der Oever et al. 1981; Barajas-Morales 1985

Anatomical trait	Unit	Relationship with		Organ	Number of species (families)	Climate	Location	Statistical method	Reference
		Temp	Rain						
Double wall to lumen ratio	$\mu\text{m}^2 \mu\text{m}^{-2}$	-	↗	Stem	61 (25)	Subtropical	Argentina, USA	Correlation	Martínez-Cabrera et al. 2009
Vessel length	m	na	-	Twig	48 (na)	Tropical to temperate	Worldwide	Correlation	Jacobsen et al. 2012 and literature cited therein
Vessel element length	class or $\mu\text{m}$	↗↗↗↗↗↗ ↗↗↗↗ ↗	↗↗↗↗↗↗	Stem	798 (40) + 5663* (258)	Tropical to arctic	Worldwide	Visual examination of scatterplots, bar graphs and trait values within a climate category, correlation, t-test	Carlquist 1966; Baas 1973; van der Graaff & Baas 1974; van der Oever et al. 1981; Barajas-Morales 1985; Baas et al. 1988; Lens et al. 2004; Wheeler et al. 2007
Vessel length	m	na	-	Twig	130 (31)	Tropical to temperate* *		Correlation	Jacobsen et al. 2012
Helical sculpturing on vessels	presence	↗↗↗↗ ↗↗↗↗ ↗↗ ↗-	↗↗↗↗ ↗↗↗↗ ↗↗ ↗-	Stem	1688 (98) + 5663* (258)	Tropical to arctic	Worldwide	Visual examination of scatterplots, bar graphs, trait values within a climate category, Pearson's Standardised Residues, correlation	Carlquist 1966; Baas 1973; van der Oever et al. 1981; Baas & Schweingruber 1987; Baas et al. 1988; Alves & Angyalossy-Alfonso 2000; Lens et al. 2004; Wheeler et al. 2007
Scalariform perforation plates	presence	↗↗↗↗ ↗	↗↗↗↗	Stem	96 (93) + 5663* (258) + 4370*** (na)	Tropical to arctic	Worldwide	Visual examination of scatterplots, bar graphs, trait values within a climate category, Pearson's Standardised Residues	Baas & Schweingruber 1987; Alves & Angyalossy-Alfonso 2000; Jansen et al. 2004; Wheeler et al. 2007
Vestured pits	presence	↗	↗	Stem	11843 (na)	Tropical to arctic	Worldwide	Visual examination of bar graph	Jansen et al. 2004
Vascular tracheid	presence	↗	↗	Stem	505 (71)	Subtropical to boreal	Europe, Cyprus, Madeira	Visual examination of bar graphs	Baas & Schweingruber 1987
Vasicentric tracheid	presence	-	-	Stem	505 (71)	Subtropical to boreal	Europe, Cyprus, Madeira	Visual examination of bar graphs	Baas & Schweingruber 1987
Tracheid length	$\mu\text{m}$	↗	↗	Stem	115 (1)	Tropical to temperate	Worldwide	Correlation (temperature), unclear test between three classes of rainfall	Lens et al. 2004

Notes: Temp – temperature. 'Temperature' indicates either reported temperature (°C) or latitude. In case a study reported only latitude I classified it as 'temperature' (low latitudes confer high temperatures and vice versa). Rain – rainfall. 'Rainfall' indicates either actual rainfall (mm) or a category (e.g. dry, wet). The arrows refer to the direction of relationship between anatomical and climate variables: ➤ positive, ➤ weak positive, ➤ negative, ➤ weak negative, 'na' no data available. Each symbol represents a relationship in one study i.e. two positive arrows indicate that the positive relationship was found in two independent studies. 'Bar graphs' refer to an incidence of species with given trait value plotted against climate category. Number of species and families is the sum of all species and families studied by the cited authors. \* indicates that this number corresponds to the specimen, not species, number (i.e. some species may have been represented by a few specimens and each specimen is counted as a separate entity). \*\* includes around 70 potted and garden plant species, both non-self supporting species and lianas (no relationship found when garden and potted species excluded), \*\*\* number of genera studied.

Table 2-7 Correlations between vessel and functional traits.

Anatomical trait	Unit	Functional trait	Unit	Relation- ship	Organ	Number of species (families)	Reference
Vessel + fibre lumens proportion	%	MOR	MPa	↗	Twig	6 (3)	Jacobsen et al. 2005
		Embolism resistance	MPa	↗	Twig	6 (3)	Jacobsen et al. 2005
		Conductivity	kg m <sup>-1</sup> s <sup>-1</sup> MPa <sup>-1</sup>	-	Twig	6 (3)	Jacobsen et al. 2005
		MOR	MPa	↗	Twig	17 (9)	Jacobsen et al. 2007
		MOE	MPa	↗	Twig	120 (25)	Gleason et al. 2012
Vessel proportion	%	Embolism resistance	MPa	-	Twig	9 (1)	Pratt et al. 2007
		Conductivity	kg m <sup>-1</sup> s <sup>-1</sup> MPa <sup>-1</sup>	↗	Twig	137 (34)	Jacobsen et al. 2007; Gleason et al. 2012
		Conductivity, potential	kg m <sup>-1</sup> s <sup>-1</sup> MPa <sup>-1</sup>	↗↗	Stem	2277 (447)	Rana et al. 2009; Poorter et al. 2010; Zanne et al. 2010
		Water potential, midday	MPa	↗ - -	Twig	148 (34)	Preston et al. 2006; Jacobsen et al. 2007; Gleason et al. 2012
		Embolism resistance	MPa	↗↗↗↗	Twig	53 (20)	Cochard & Tyree 1990; Wheeler et al. 2005; Hacke et al. 2006; Lens et al. 2011
Vessel size	μm	Conductivity, potential	kg m <sup>-1</sup> s <sup>-1</sup> MPa <sup>-1</sup>	↗↗	Stem	47 (22)	Rana et al. 2009; Poorter et al. 2010
		Embolism	%	↗	Twig	20 (6)	Mitchell et al. 2008
		Conductivity loss	%	↗	Twig	10 (8)	Davis et al. 1999
		Resistivity to flow	MPa s m <sup>-4</sup>	↗	Twig	19 (10)	Sperry et al. 2007
		Scalariform plate resistance	MPa s m <sup>-3</sup>	↗	Twig	13 (7 orders)	Christman & Sperry 2010
Hydraulically weighted diameter	μm	Vessel resistivity	MPa s m <sup>-4</sup>	↗	Twig	18 (12 orders)	Christman & Sperry 2010
		Water potential, midday	MPa	↗	Twig	11 (na)	Preston et al. 2006
		MOR	MPa	↗	Twig	17 (9)	Jacobsen et al. 2007
		Embolism resistance	MPa	-↗	Twig	32 (10)	Jacobsen et al. 2005; Jacobsen et al. 2007
		Conductivity	kg m <sup>-1</sup> s <sup>-1</sup> MPa <sup>-1</sup>	↗	Twig	6 (3)	Jacobsen et al. 2005
Vessel length, maximum	m	Water potential, midday	MPa	-	Twig	17 (9)	Jacobsen et al. 2007
		Embolism resistance	MPa	-↗	Twig	39 (16)	Jacobsen et al. 2007; Markesteijn et al. 2001

Anatomical trait	Unit	Functional trait	Unit	Relation-ship	Organ	Number of species (families)	Reference
Vessel length, mean	m	Embolism resistance	MPa	↗↘	Twig	36 (13)	Hacke et al. 2006, Lens et al. 2011
Vessel size to vessel number per area ratio	$\mu\text{m}^2 \mu\text{m}^2$	Conductivity	$\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$	↗	Twig	120 (25)	Gleason et al. 2012
Vessel surface area	$\mu\text{m}^2$	Conductivity, potential	$\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$	↗	Stem	2230 (425)	Zanne et al. 2010
Vessel number per area	$\text{mm}^{-2}$	Embolism resistance	MPa	↘	Twig	29 (13)	Hacke et al. 2006
		Conductivity, potential	$\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$	↗↘	Stem	47 (22)	Rana et al. 2009; Poorter et al. 2010
Number of all vessels / number of groups (one vessel being one group)	–	Water potential, midday	MPa	↗	Twig	11 (na)	Preston et al. 2006
Vessel wall thickness	$\mu\text{m}$	Embolism resistance	MPa	↗	Twig	7 (1)	Lens et al. 2011
		MOR	MPa	–	Twig	6 (3)	Jacobsen et al. 2005
		MOE	MPa	–	Twig	6 (3)	Jacobsen et al. 2005
		Embolism resistance	MPa	–	Twig	6 (3)	Jacobsen et al. 2005
		Water potential, midday	MPa	–	Twig	6 (3)	Jacobsen et al. 2005
Double wall to lumen ratio	$\mu\text{m}^2 \mu\text{m}^{-2}$	MOR	MPa	↗↗	Twig	22 (10)	Jacobsen et al. 2007; Woodrum et al. 2003
		Capacitance	$\Delta\text{RWC } \Delta\text{P}^{-1}$	↗	Twig	9 (1)	Pratt et al. 2008
		Embolism resistance	MPa	↗↗↗	Twig	51 (17)	Hacke et al. 2001 and literature cited therein; Jacobsen et al. 2005; Pratt et al. 2007
Intervessel wall thickness	$\mu\text{m}$	Conductivity	$\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$	– –	Twig	26 (10)	Jacobsen et al. 2007; Pratt et al. 2007
Scalariform perforation plate	presence	Water potential, midday	MPa	↘	Twig	17 (9)	Jacobsen et al. 2007
Intervessel pit chamber depth	nm	Embolism resistance	MPa	–	Twig	7 (1)	Lens et al. 2011
Intervessel pit membrane pore diameter	nm	Resistivity to flow	$\text{MPa s m}^{-4}$	↗	Twig	19 (10)	Sperry et al. 2007
Intervessel pit membrane thickness	nm	Embolism resistance	MPa	↘	Twig	7 (1)	Lens et al. 2011
		Air-seeding threshold	MPa	↘	Twig	26 (16)	Jansen et al. 2009
		Embolism resistance	MPa	↘	Twig	7 (1)	Lens et al. 2011
		Embolism resistance	MPa	↗	Twig	7 (1)	Lens et al. 2011

Notes: The arrows refer to the direction of relationship between anatomical and functional traits: ↗ positive, ↘ weak positive, ↗↘ negative, ↗↗↘ weak negative, '–' no relationship.

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## Chapter 3

# **FIBRE WALL AND LUMEN FRACTIONS DRIVE WOOD DENSITY VARIATION IN TWIGS ACROSS 24 AUSTRALIAN ANGIOSPERMS**

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Slightly modified version of this chapter has been published as a research article in AoB PLANTS on 10.10.2013



### 3.1 Abstract

Wood density is commonly considered a key plant trait affecting mechanical and physiological performance. Yet density is only one quantity describing multifunctional wood, and the association between density, wood functions and ecological strategies is still rather unclear. With the aim to better understand relationships between wood density, wood functions and ecological strategies, we investigated the anatomical underpinnings of wood density in trees and shrubs.

We measured wood density and anatomical traits in distal stems 4-10 mm diameter under bark in 24 Australian species of angiosperms. Eleven anatomical traits were quantified in this study including the proportions of wood components that are functionally different: fibres (wall and lumen), vessels (wall and lumen) and parenchyma (axial and ray). Density of wood that is outside vessel lumen and its tissue proportions were also calculated.

Wood density was mainly driven by the density of wood outside vessel lumens ( $\text{density}_{\text{NV}}$ ) rather than by vessel lumen fraction. In turn,  $\text{density}_{\text{NV}}$  variation was chiefly affected by fibre wall and lumen fractions. Considerable anatomical variation was observed at a given  $\text{density}_{\text{NV}}$ , especially among medium- $\text{density}_{\text{NV}}$  species (0.60-0.85 g cm<sup>-3</sup>); this range of medium  $\text{density}_{\text{NV}}$  roughly translates to 0.50-0.75 g cm<sup>-3</sup> of overall density. The anatomy of these species formed a continuum from low fibre lumen and medium parenchyma fraction to medium fibre lumen and low parenchyma fractions.

Our data suggest wood density is an emergent property influenced by a complex anatomy rather than an unambiguous functional trait, particularly in medium-density species. With much anatomical variation per given density, they likely represent a wide range of ecological strategies.

### 3.2 Introduction

Plants vary significantly in their ecological, physiological, and mechanical properties or traits both across climate zones and within sites (Westoby et al. 2002; Wright et al. 2004; Chave et al. 2009). This indicates there are multiple ways plant

species make a living. Different ways can be called plant ecological strategies (Westoby et al. 2002).

Wood density has been suggested as a key player in plant ecological strategies (Chave et al. 2009). Firstly, wood density has been linked with hydraulic strategies. Denser woods tend to tolerate more negative minimum water potentials (Santiago et al. 2004; Ackerly 2004; Bucci et al. 2004; Jacobsen et al. 2007b, 2008; Gotsch et al. 2010) and to have lower capacitance (Meinzer et al. 2003, 2008; Scholz et al. 2007; Pratt et al. 2007). Secondly, wood density has been associated with plant mechanical strategies where denser woods tend to be stiffer and more resistant to breakage per given wood diameter (Panshin & de Zeeuw 1980; Easterling et al. 1982; Zhang 1997; Dinwoodie 2000; Chave et al. 2009; Onoda, Richards & Westoby 2010). However, it also has been suggested that plants can build thicker stems to compensate for their lower density (Larjavaara & Muller-Landau 2010; Anten & Schieving 2010; Butler et al. 2011). Thirdly, denser woods might be more resistant to pathogen attacks (Augspurger & Kelly 1984; Romero & Bolker 2008). Additionally, wood density has been widely discussed in relation to life history strategies. For example, species with denser wood are more inclined to have higher survival rates (Putz et al. 1983; Kraft et al. 2010; Poorter et al. 2010). Growth rate is another important component of life history. Growth rate can be expected to negatively relate to wood density on the basis that higher investment in mass per volume should slow down growth (Enquist et al. 1999), and, while generally true, the correlation is not always strong (Poorter et al. 2008, 2010; Chave et al. 2009; Wright et al. 2010; Fan et al. 2012). Across species, wood density can vary with environmental factors such as temperature (Wiemann & Williamson 2002; Swenson & Enquist 2007; Martínez-Cabrera et al. 2009), and precipitation (Barajas-Morales 1985; Wiemann & Williamson 2002; Swenson & Enquist 2007; Martínez-Cabrera et al. 2009; Zhang et al. 2011), although not in all studies (ter Steege & Hammond 2001; Wiemann & Williamson 2002; Muller-Landau 2004). Despite this broad climate-related patterning, wood density also tends to vary quite widely among co-occurring species (Wiemann & Williamson 2002; Muller-Landau 2004).

There is an extraordinary variety of potential functional roles of wood density but also a number of unresolved questions. Considering this variety and these unknowns,



we face the question: does wood density really explain plant functions or is it rather an indirect element of ecological strategies (see also Larjavaara & Muller-Landau, 2010)? An informative way to tackle this problem would be to ask the basic question: what are the structural underpinnings of wood density variation? The premise of the work reported here was that if structural underpinnings of wood density variation were rigorously quantified and better understood, this might help to explain complexities in the functional implications of wood density.

Most studies of anatomical components of angiosperm wood density span only one or a few species (Schulz 1957; Taylor 1971; Taylor & Wooten 1973; Ezell 1979; Vurdu & Bensend 1980; Fukuzawa 1984; Bosman et al. 1994; Stokke & Manwiller 1994; McDonald, Williamson & Wiemann 1995; Lei, Milota & Gartner 1996; Denne & Hale 1999; Rana et al. 2009). However, comparisons across a broad number of species have also been made (Fujiwara et al. 1991; Fujiwara 1992; Jacobsen et al. 2007a; Martínez-Cabrera et al. 2009; Poorter et al. 2010), especially recently.

Angiosperm wood is a complex tissue composed of three main cell types: vessels that transport water, fibres responsible for mechanical strength, and parenchyma that stores and transports nutrients. These tissues have different structural characteristics and their relative proportions within wood influence wood density. Vessel lumens have essentially zero density; fibre walls, vessel walls, and parenchyma have positive density. Vessel fraction has variously shown either negative or no correlation with wood density (Preston, Cornwell & DeNoyer 2006; Jacobsen et al. 2007a; Mitchell et al. 2008; Martínez-Cabrera et al. 2009; Poorter et al. 2010; Zanne et al. 2010; Gleason et al. 2012). Parenchyma is another commonly occurring tissue, which has been reported to have positive, negative, or no relationship with density (Taylor 1969; Fujiwara 1992; Jacobsen et al. 2007a; Rana et al. 2009; Martínez-Cabrera et al. 2009; Poorter et al. 2010). Wood density is generally well correlated with fibre properties, especially fibre wall fraction (Fujiwara et al. 1991; Jacobsen et al. 2007a; Martínez-Cabrera et al. 2009). However, it is unclear how these different fibre traits are interrelated with each other and consequently how these interrelations influence wood density.

Most previous work linking anatomy with density has concentrated on vessels with relatively little attention given to the other tissues. Furthermore, among the studies

investigating all the major tissues (vessels, parenchyma, and fibres) only one focused on the wood of twigs in 17 angiosperm species studied by Jacobsen et al. (2007a). Twigs are important, being in direct spatial and functional contact with leaves and having been commonly subjected to physiological and ecological measurements. Moreover, twigs have been implied to differ in their density and tissue proportions from main stems (Fukuzawa 1984; McDonald, Williamson & Wiemann 1995; Lei, Milota & Gartner 1996; Bao et al. 2001). In this paper, we investigate wood from twigs from a wide range of angiosperm tree and shrub species growing in various environments (24 species from 4 sites in eastern Australia). We address two main unresolved issues: 1) Which fibre properties have the most decisive effect on wood density, and how are those properties interrelated with each other? 2) How do vessel and parenchyma proportions influence wood density?

### **3.3 Materials and Methods**

#### **Plant material and sites**

Four sites were chosen that spanned a wide range of temperature and aridity in eastern Australia (Table A3-1 in Appendix 1, page 137). The objective of site selection was to generate a broad range of trait values rather than to enable site comparisons. All carried natural, undisturbed vegetation growing on oligotrophic soils on flat to slightly sloping terrain. Two locations in Tasmania, at approximately 43°S, represented low mean annual temperature (MAT, 10.6°C), and two locations in Queensland near 18°S represented higher MAT (c. 22.5°C). Within each latitude, two locations were chosen so as to differ markedly in aridity index (AI; (Willmott & Feddema 1992), the ratio of mean annual precipitation (MAP) to potential evapotranspiration (PET). In both Queensland and Tasmania, the wetter site had an aridity index c. 1.0 and the drier site c. 0.6. MAP, MAT, and PET were obtained from GIS layers from the Australian Bureau of Meteorology.

At each of the four sites, six abundant and phylogenetically distinct woody eudicot species were chosen for sampling (species list in Table 3-1). The species sampled were chosen as follows. The list of observed species of trees and shrubs was composed,

and species were ranked from the most to the least abundant. Next, the six most abundant species were chosen. However, if these six species included two congeners then one of those was discarded and replaced with the next most abundant species that was not congeneric with any other included in the group. This procedure led to a group of species that are here called 'phylogenetically distinct'. One species was sampled at two sites, yielding a total of twenty-three species from eight families. Distal, sun-exposed twigs of trees and shrubs were collected from three replicate individuals per species. The diameter under bark of twig varied from 4 mm in plants with little pith to 10 mm in plants with higher pith content. Consequently, the diameter of wood, excluding bark and pith, was approximately 4-5 mm. This plant material is referred to here as twigs, although in several small shrub species 'twigs' were the main stems. Plant material was cut into segments 10-15 cm long and kept wet in sealed plastic bags. Wood density was measured within a week from collection; other parts of the same twigs were placed in fixative for later measurement of anatomical properties (details below).

## **Wood density**

Wood density was measured on segments 3–5 cm long for each twig sample. Bark and pith were removed and measurements were carried out on xylem only. Bark was scraped from twigs, and next, twigs were cut longitudinally dividing a twig into two to four pieces from which pith was scooped out. In this paper, we refer to xylem as 'wood'. After removing bark and pith, wood pieces were soaked in water for at least 48 hours prior to volume measurement. Then a beaker filled with water was placed on a balance (0.0001 g, Mettler AE 160). A thin wire platform was suspended in water so that it did not touch the side or bottom of the beaker. The balance was tared before each measurement, and a sample was gently placed on the platform. The mass of displaced water was read from the balance. From standard water density of  $1 \text{ g cm}^{-3}$  and knowing the mass of displaced water, we calculated sample volumes applying Archimedes buoyancy principle (e.g. one gram of displaced water equals one  $\text{cm}^3$  volume). Samples were then placed in paper envelopes and dried at  $70^\circ\text{C}$  for at least 72 hours. Wood density was calculated as the dry mass divided by water-saturated volume ( $\text{g cm}^{-3}$ ).

## Anatomy

To obtain anatomical cross-sections the material was first fixed in Formalin–Acetic Acid–Alcohol (FAA) for four weeks. FAA was prepared in proportions 5:5:90 (formalin : glacial acetic acid : 70% ethanol; Gerlach 1972). After four weeks, the fixative was replaced with 70% ethanol. This 70% ethanol was then replaced two more times within 10 days to further wash the fixative out. Final replacement of alcohol was used as long-term storage medium. Segments for image analysis were rehydrated to 50% ethanol and after 2-5 days to 30% ethanol. Cross-sections were cut with a sledge microtome (Reichert, Vienna, Austria) at 10-20 µm thickness using disposable blades (model A35, Feather Safety Razor Co. Ltd, Japan). For better contrast and tissue identification, sections were stained in safranin O (Gurr Microscopy Materials, BDH Chemicals Ltd, England) for lignified cell walls (10 mins) and Janus green B (Gurr's, London, England) for cytoplasm (10 mins). Safranin O solution used 2 g of stain in 100 ml distilled water (Ruzin 1999) and Janus green B used 0.1 g of stain and 1 ml of glacial acetic acid in 100 ml of distilled water (Conn et al. 1960). Sections were rinsed in distilled water after each staining session. Afterwards, they were mounted in glycerol on a slide, covered with a cover slip, and sealed with nail polish. Measurements were made only on cross-sections, but to assist in interpreting and identifying cell types, longitudinal tangential sections, longitudinal radial sections, and macerations were also made. For macerations, small shavings were placed in vials filled with Franklin's solution: glacial acetic acid and 6% hydrogen peroxide in proportions of 1:1 (Franklin 1945). These vials were loosely covered with Parafilm tape and heated in the oven at 60°C for 1-2 days. Tissues were then rinsed with distilled water, stained in safranin O (10 mins), and gently crushed onto a microscopic slide.

Microphotographs of cross-sections were taken at 100x and 400x magnifications using a digital camera (Scion Corporation, CFW-1310C, USA) attached to a light microscope (Olympus BX 50F, Olympus Co. Ltd., Japan) and image capturing software Scion Visicapture, version 1.4 (Scion Corporation). Two to three images of the same area at different focal planes were taken and stacked in Photoshop CS4 (Adobe Systems Incorporated, USA). Cross-sections were bigger than the field of view, therefore dozens (for 100x) or around ten (for 400x) images per cross-section were taken and then

merged in Photoshop, giving rise to images of whole cross-sections at 100x and of one narrow transect at 400x. Tissue cross-sectional areas and vessel traits were measured on one wedge shaped transect per replicate (100x; Fig. 3-1) and fibre characteristics (400x) on one rectangular transect, both stretching from pith to cambium. The radial transects were chosen to be the most representative for a section, and the tension wood was avoided where possible. The transect borders were approximately parallel to the rays and followed middle lamella so that no open cells were positioned on the borders. Tissue areas, vessels, and fibre walls were manually coloured in Photoshop using a Cintiq 21UX graphic tablet (Wacom Co., Ltd, Japan). Protoxylem and newly produced secondary xylem were excluded from analysis. Larger regions were inspected in species with larger vessels or with more variable structure in the tangential direction. The measured area ranged from 0.21-0.69 mm<sup>2</sup> for species from cool sites and from 0.31-1.9 for species from hot sites. On average, for each image there were 170 vessels measured ( $\pm 145$ ) of all sizes including vessel tails. Fibre, fibre wall, and lumen areas were measured for an average of 170 fibres per sample ( $\pm 57$ ) lying in two parallel rows from pith to cambium. Colour coded images were analysed with Image-Pro Plus, version 2.0.0.260 (Media Cybernetics Inc., USA).

Proportions of all major wood cell types (vessel wall and lumen, fibre wall and lumen, axial parenchyma, rays, and tracheids) and their properties (average vessel lumen area and average fibre lumen and wall area) were quantified. Traditionally, wood is considered a complex tissue composed of several cell types (Evert 2006). However, we refer to those cell types as 'tissues' for brevity and also because they perform distinctly different functions. Anatomical terminology follows 'IAWA list of microscopic features for hardwood identification' (IAWA Committee 1989). Vascular and/or vasicentric tracheids occurred in 13 species and are referred to hereafter as tracheids. Tracheids were first determined in macerated wood and then identified on a cross-section on the basis of number of pits, pit border diameter, and cell diameter (IAWA Committee, 1989; Sano et al., 2011). Cells with diameter similar to fibres, with bordered pits as common as on vessels, and with pit diameter similar to that of intervessel pits were counted as tracheids. Axial apotracheal and paratracheal parenchyma were collectively measured as axial parenchyma. None of the species studied here had storied rays. Tissue fractions

were expressed as the fraction of a tissue per cross-sectional area (Fig. 3-1). Tissue fractions of the area outside vessel lumen (non-vessel area) were calculated as tissue fraction multiplied by non-vessel area fraction. The term 'non-vessel' area is used for brevity and it includes: vessel walls, fibre walls and lumens, axial and ray parenchyma, tracheids. Non-vessel-lumen quantities are hereafter denoted by subscript 'NV', e.g. fibre wall fraction<sub>NV</sub>, wood density<sub>NV</sub>, etc. Fibre wall proportion in a given fibre was expressed as a proportion of the total fibre area. Fibre wall fraction was obtained by multiplying mean fibre wall proportion in a fibre by the fibre fraction per cross-section. Fibre lumen fraction was similarly calculated from mean fibre lumen proportion in a fibre multiplied by fibre fraction.

## Statistical analysis

We collected measurements for 23 species, one of which occurred at two sites and was considered as two entities, giving a total of 24 data points analysed. Measurements were carried out on three replicate individuals per species and the trait values were averaged for comparisons across species. Wood density, vessel lumen, sum of ray and axial parenchyma, ray parenchyma and fibre wall fractions of total wood area as well as non-vessel wood area were all approximately normally distributed (Shapiro-Wilk test,  $p < 0.05$ ). Vessel wall, axial parenchyma, tracheids, and fibre lumen fractions of total wood area as well as of non-vessel wood area were right-skewed and were transformed to generate approximately normally distributed variables. Log<sub>10</sub> transformations normalized all distributions with the exception of tracheid fraction and tracheid non-vessel fraction. We used ordinary least squares regression to assess bivariate relationships (SigmaPlot, Systat, San Jose, CA).

## 3.4 Results

Wood density and tissue proportions varied significantly across species as illustrated in Figure 3-2 (trait values in Table A3-2 in Appendix1). Wood density varied more than two-fold, from 0.37 to 0.83 g cm<sup>-3</sup>. Figure 3-2 shows tissue fractions averaged across all species (bar at the top) and for each individual species separately (the

remaining bars). The mean fibre fraction was  $0.52 \pm 0.09$  (hereafter numbers represent average fraction  $\pm$  one standard deviation). Fibre varied approximately two-fold across species and was the most abundant tissue type. Fibre fraction could be partitioned into fibre walls ( $0.45 \pm 0.08$ , two-fold variation; brown bars in Fig. 3-2) and fibre lumens ( $0.08 \pm 0.07$ , c. 60-fold variation; yellow bars). On average, parenchyma occupied 0.25 of wood cross-sectional area and varied almost three-fold across species. It consisted of axial parenchyma ( $0.10 \pm 0.05$ , 6-fold variation) and ray parenchyma ( $0.15 \pm 0.05$ , 4.5-fold variation). Vessels occupied 0.20, where  $0.15 \pm 0.03$  consisted of lumens (varying c. two-fold) and  $0.05 \pm 0.03$  of vessel walls, varying 4.5-fold. Tracheids occurred in just 13 of 24 species and occupied only small fractions ( $0.02 \pm 0.03$  averaged across all 24 species, 8.5-fold variation across the 13 species that had tracheids).

Alternatively, wood can simply be divided into two components: vessel lumen fraction and non-vessel fraction, which encompasses all tissues and their lumens other than vessel lumens. The density of the non-vessel fraction and tissue fractions within the non-vessel fraction are indicated hereafter by the subscript 'NV', e.g.  $\text{density}_{\text{NV}}$ , fibre fraction<sub>NV</sub>. Since vessel lumen has zero density, overall wood density is (by definition) the product of the non-lumen fraction density ( $\text{density}_{\text{NV}}$ ) and the non-vessel fraction itself (fraction<sub>NV</sub>):  $\text{density} = \text{density}_{\text{NV}} \times \text{fraction}_{\text{NV}}$  (Preston et al. 2006; Zanne et al. 2010). These three quantities can be log-transformed and the equation then becomes a sum:  $\log(\text{density}) = \log(\text{density}_{\text{NV}}) + \log(\text{fraction}_{\text{NV}})$ . Hence, when  $\text{density}_{\text{NV}}$  is plotted against fraction<sub>NV</sub> on log-log axes (Fig. 3-3), isolines of resulting overall wood density can be constructed and used to aid interpretation. Variation across species in  $\text{density}_{\text{NV}}$  (Y axis in Fig. 3-3) was four times greater than variation in fraction<sub>NV</sub> (X axis), and thus in this species set was a far stronger determinant of variation in overall wood density (direction across the isolines). Not surprisingly then,  $\text{density}_{\text{NV}}$  and overall density were tightly correlated with each other ( $r^2 = 0.95$ ,  $P < 0.001$ ). Vessel lumen fraction was only loosely (negatively) correlated with overall wood density ( $r^2 = 0.20$ ,  $P = 0.027$ ). Therefore, the following analyses concentrate entirely on  $\text{density}_{\text{NV}}$  and its anatomical components (trait values are given in Table A3-3 in Appendix 1).

Total fibre fraction<sub>NV</sub> (fibre lumen fraction<sub>NV</sub> plus fibre wall fractions<sub>NV</sub>) was unrelated to  $\text{density}_{\text{NV}}$  (Fig. 3-4). Species with the same fibre fraction<sub>NV</sub> varied widely in

wall proportion relative to lumen within a fibre (as indicated by the width of the ‘donut’ rings in Fig. 3-4). Figure 3-4 shows that lowest-density ( $<0.5 \text{ g cm}^{-3}$ ) species had high fibre fraction<sub>NV</sub>, but with fibres having low wall proportion (lower right of the graph). High-density<sub>NV</sub> species ( $>0.85 \text{ g cm}^{-3}$ ) also had high fibre fraction<sub>NV</sub>, but their fibres had large wall proportion (upper right of the graph). A substantial number of species located in the middle of the graph with medium density<sub>NV</sub> ( $0.60\text{--}0.85 \text{ g cm}^{-3}$ ) had variable fibre fraction<sub>NV</sub> and fibre wall proportion within a fibre. In addition, fibre wall proportion within a fibre was positively correlated with wood density<sub>NV</sub> ( $r^2 = 0.62$ ,  $P < 0.001$ ).

Density<sub>NV</sub> was positively correlated with fibre wall fraction<sub>NV</sub> ( $r^2 = 0.40$ ; Fig. 3-5a), and negatively with fibre lumen fraction<sub>NV</sub> ( $r^2 = 0.56$ ; Fig. 3-5b). Since the majority of species had low fibre lumen fraction<sub>NV</sub> (i.e., the data were right-skewed), the two species with highest fibre lumen fraction<sub>NV</sub> had strong influence on these relationships (lower left of Fig. 3-5a, lower right of Fig. 3-5b). That said, the correlations were still present across the other species considered on their own ( $r^2 = 0.26$  and  $P = 0.014$ ,  $r^2 = 0.18$  and  $P = 0.049$ , respectively). The species with the lowest fibre wall fraction<sub>NV</sub> (upper left in Fig. 3-5a) was *Daviesia latifolia*, which had a high amount (fraction of 0.07) of thick-wall tracheids. Presumably, this high fraction of tracheid wall contributed to the quite high density<sub>NV</sub> of this species (high, given its very low fibre fraction).

Next, we asked how fibre wall and lumen fractions<sub>NV</sub> were related to one another. Figure 3-6a shows an approximately triangular relationship between fibre wall fraction<sub>NV</sub> and fibre lumen fraction<sub>NV</sub>. The highest-density<sub>NV</sub> species ( $>0.85 \text{ g cm}^{-3}$ ; four large symbols, upper left of Fig. 3-6a) had high fibre wall fraction<sub>NV</sub> and low fibre lumen fraction<sub>NV</sub>. Medium-density<sub>NV</sub> species ( $0.60\text{--}0.85 \text{ g cm}^{-3}$ ; 18 medium-sized symbols in Fig. 3-6a) had variable fibre wall fraction<sub>NV</sub> and variable fibre lumen fraction<sub>NV</sub>. The two lowest-density<sub>NV</sub> ( $<0.50 \text{ g cm}^{-3}$ ) species had the highest fibre lumen fraction<sub>NV</sub> and low fibre wall fraction<sub>NV</sub> (two smallest symbols, lower right of Fig. 3-6a). The categories of high, medium- and low-density species are used here for easy reference, but in fact, the trait values are continuous and no clear boundaries can be indicated. The species with lowest fibre wall fraction<sub>NV</sub> (lower left in Fig. 3-6a) was *Daviesia latifolia* (see comment about tracheids above).



The second most abundant tissue, parenchyma, was not correlated with density<sub>NV</sub> and nor were its components, rays and axial parenchyma (all  $P > 0.7$ ). Similarly, vessel wall fraction<sub>NV</sub> was unrelated to density<sub>NV</sub>. Nevertheless, both parenchyma and vessel wall fractions<sub>NV</sub> indirectly affected density<sub>NV</sub>. Figure 3-6a illustrates that there was a considerable variation in density<sub>NV</sub> (indicated by symbol size) at a given fibre wall fraction<sub>NV</sub>, especially at lower wall fraction<sub>NV</sub> (lower half of the graph), and considerable variation in density<sub>NV</sub> at a given fibre lumen fraction<sub>NV</sub>, especially at lower lumen fraction<sub>NV</sub> (left half of the graph). These variations in density<sub>NV</sub> could be partially explained by parenchyma and vessel wall fractions<sub>NV</sub>. At a given fibre wall fraction<sub>NV</sub> density<sub>NV</sub> was positively correlated with parenchyma and vessel wall fractions<sub>NV</sub> ( $r^2 = 0.15$ ,  $P = 0.064$  and  $r^2 = 0.29$ ,  $P = 0.007$  respectively) and negatively with fibre lumen fraction<sub>NV</sub> ( $r^2 = 0.45$ ,  $P < 0.001$ ; all relationships tested against residuals from a regression of wood density<sub>NV</sub> on fibre wall fraction<sub>NV</sub>). Conversely, at a given fibre lumen fraction<sub>NV</sub> (i.e. tested against residuals from a regression of wood density<sub>NV</sub> on fibre lumen fraction<sub>NV</sub>), wood density<sub>NV</sub> was negatively correlated with parenchyma fraction<sub>NV</sub> ( $r^2 = 0.28$ ,  $P = 0.008$ ), not correlated with vessel wall fraction<sub>NV</sub> ( $P = 0.29$ ), and positively correlated with fibre wall fraction<sub>NV</sub> ( $r^2 = 0.40$ ,  $P < 0.001$ ). Additionally, parenchyma fraction<sub>NV</sub> was tightly negatively correlated with total fibre fraction<sub>NV</sub> ( $r^2 = 0.74$ ,  $P < 0.001$ ). The only remaining tissue, tracheids, occupied on average a very small fraction<sub>NV</sub> and was not subjected to detailed analysis.

### 3.5 Discussion

This study aimed to describe the anatomical components of wood density in twigs of 24 Australian trees and shrubs. Properties of fibres, the most abundant tissue, had the strongest effect on wood density variation as has been shown in some previous studies (Fujiwara et al. 1991; Jacobsen et al. 2007a; Martínez-Cabrera et al. 2009). However, our results contribute to a more comprehensive understanding of wood structure and its influence on density in twigs. In particular, for the first time, we quantitatively explain why fibre fraction does not correlate with density. We indicate which fibre properties are the most important drivers of density variation, and how

parenchyma indirectly influences density. We also are first, to our knowledge, to discuss in detail anatomical variation that can be independent of density.

## Wood density and its anatomical components

Density of tissue outside vessel lumens ( $\text{density}_{\text{NV}}$ ), rather than vessel lumen fraction, was the main driver of overall wood density variation. This result agrees with a comparison made across 584 species that considered main stem wood (Zanne et al. 2010), implying that  $\text{density}_{\text{NV}}$  determines overall density in both twigs (this study) and main stems similarly. Accordingly, discussion here is directed towards  $\text{density}_{\text{NV}}$  and individual tissue fractions within the non-vessel part of the wood (indicated by subscript 'NV', e.g. fibre wall fraction<sub>NV</sub>). We compare these results with the results for overall wood density reported by other studies, on the basis that overall wood density and  $\text{density}_{\text{NV}}$  are closely correlated ( $r^2 = 0.95$ ,  $P < 0.001$ , this study).

All tissue fractions<sub>NV</sub> influence  $\text{density}_{\text{NV}}$ , but fibre wall and lumen fractions<sub>NV</sub> are the most important. The strong influence of fibre wall fraction<sub>NV</sub> was due to both its high proportion and to its variability, while the influence of fibre lumen fraction<sub>NV</sub> was associated chiefly with its high variability (c. 60 fold). Other studies have consistently found a positive relationship between fibre wall fraction and density: in trunk wood among 50 Japanese trees (Fujiwara et al. 1991) and 61 North and South American shrubs (Martínez-Cabrera et al. 2009), and in twig wood of 17 South African shrubs (Jacobsen et al. 2007a). Fibre lumen fraction has received less attention but has also been shown (in concordance with our study) to have a negative relationship with wood density (Rana et al. 2009; Martínez-Cabrera et al. 2009). The second most abundant tissue, parenchyma, did not correlate with  $\text{density}_{\text{NV}}$  in our study nor in tree and shrub trunks (Martínez-Cabrera et al. 2009; Poorter et al. 2010), but correlated negatively with twig tissue density across 17 species (Jacobsen et al. 2007a). These discrepancies might be caused by variable densities of parenchyma tissue itself (Taylor 1969; Fujiwara 1992; Guilley & Nepveu 2003) or by various relationships of parenchyma relative to fibre wall and lumen fractions (see below). Plausibly, these discrepancies also stem from different relations between ray and axial parenchyma. We did not find cross-correlation between those two components of parenchyma nor were they individually related to density. In

contrast, Martínez-Cabrera et al. (2009) reported that ray and axial parenchyma were negatively correlated with each other and individually correlated with density (axial parenchyma positively related, rays, negatively). In that study, these links were strongly associated with environmental variables (MAP, MAT, and AI). These findings imply different functional trade-offs can be related to ray and axial parenchyma individually, and also that their link is affected by climate. Possibly these trade-offs may be more pronounced in trunk wood, as opposed to the twig wood studied here.

We did not find a direct relationship between parenchyma and density<sub>NV</sub>. Nevertheless, our results imply that parenchyma together with fibre lumens can influence density<sub>NV</sub> variation in a less direct way. At a given fibre wall fraction<sub>NV</sub>, density<sub>NV</sub> depended on the parenchyma fraction<sub>NV</sub> relative to fibre lumen fraction<sub>NV</sub>. Density<sub>NV</sub> was marginally positively correlated with parenchyma fraction<sub>NV</sub> and negatively with fibre lumen fraction<sub>NV</sub>. Parenchyma has higher density than fibre lumen, which has zero density. Therefore, higher parenchyma fraction<sub>NV</sub> relative to fibre lumen fraction<sub>NV</sub> increases density<sub>NV</sub>, and conversely, higher fibre lumen fraction<sub>NV</sub> decreases density<sub>NV</sub>. We note that parenchyma fraction<sub>NV</sub> was only weakly correlated with density<sub>NV</sub>, per given fibre wall fraction<sub>NV</sub>. This weak correlation possibly stems from variable parenchyma tissue densities (Taylor 1969; Fujiwara 1992; Guilley & Nepveu 2003).

Fibre fraction<sub>NV</sub> (sum of fibre wall and lumen fractions<sub>NV</sub>) was not associated with density<sub>NV</sub> because of wide variation in wall proportion within a fibre (top right to low right in Fig. 3-4). Poorter et al. (2010) suggested a similar explanation, but as far as we are aware, this issue has not been quantitatively clarified until now.

Anatomical traits that are not expressed as fractions of wood volume have less direct effects on density. We found density<sub>NV</sub> was correlated with fibre wall proportion within a fibre. Previous studies have also shown density can be related to fibre lumen diameter (Jacobsen et al. 2007a; Martínez-Cabrera et al. 2009), fibre wall thickness (Fujiwara et al. 1991; Martínez-Cabrera et al. 2009) and fibre wall to lumen ratio (Martínez-Cabrera et al. 2009). We believe those traits would deliver a more insightful understanding of wood density when combined with information about fractions. An example of such reasoning can be the above description of relationship between density<sub>NV</sub>, fibre fraction<sub>NV</sub>, and fibre wall proportion in a fibre.

## Variability of anatomical structures

Discussion so far has focused on wood density variation. However, we also found considerable anatomical variation within a given range of density. Hereafter, we use the arbitrary categories of 'medium', 'high' and 'low' density and tissue fractions. However, we observed a continuum of trait values and the categories are used only for convenience. Species with medium density<sub>NV</sub> (0.60–0.85 g cm<sup>-3</sup>) showed broader structural variability than high and low-density<sub>NV</sub> species (>0.85 and <0.5 g cm<sup>-3</sup>, respectively). The concept is illustrated in Figure 3-6b and the examples of cross-sections approximately corresponding to Figure 3-6b are shown in Figure 3-7. Species with high density<sub>NV</sub> (large symbols in Fig. 3-6a, top corner in Fig. 3-6b and 3-7a) had the highest fraction<sub>NV</sub> of fibre wall and small fibre lumen fraction<sub>NV</sub>. Their total fibre fraction<sub>NV</sub> was high and parenchyma fraction<sub>NV</sub> was low. In contrast, medium-density<sub>NV</sub> species had more variable fibre wall, fibre lumen and parenchyma fractions<sub>NV</sub> (medium symbols in Fig. 3-6a, middle in Fig. 3-6b and 3-7b, c). Consequently, a spectrum of possible architectures may be outlined where the two ends of the spectrum are: 1) low fibre, fibre lumen, and high parenchyma fractions<sub>NV</sub> (middle left in Fig. 3-6b, Fig. 3-7b) and 2) high fibre, medium fibre lumen, and low parenchyma fractions<sub>NV</sub> (middle right in Fig. 3-6b, Fig. 3-7c). The lowest-density species (<0.5 g cm<sup>-3</sup>, small symbols in Fig. 3-6a, bottom corner in Fig. 3-6b, Fig. 3-7d) in this study had high fibre lumen fraction<sub>NV</sub>. However, it is possible that low-density wood could also be composed of large parenchyma fractions<sub>NV</sub> and small fibre lumen fractions<sub>NV</sub>. More studies are needed to clarify the anatomies of low-density species. In the highest density species, the range of possible anatomies is physically constrained. More space has to be occupied by cell walls and this competition for space limits the variability of anatomies. To our knowledge, we are the first to set out this triangular scheme relating wood anatomy to density. Variability of structures in medium-density species implies that those species differ functionally, and there may be a wider range of ecological strategies available to these species.

## Twigs and main trunks comparisons

Caution is needed when comparing results of twigs with the results from main trunks. It is not proven that the relationships of wood density and anatomy are the same

at the branch level as at the trunk level. Taylor and Wooten (1973) found that in five species vessel and fibre fractions shifted in the same direction with plant height, but this was not the case for ray fraction. Other studies examining association between vessel lumen fraction and wood density across a large number of species showed no relationship in the main trunks (Martínez-Cabrera et al. 2009; Poorter et al. 2010; Zanne et al. 2010), but negative relationship in twigs (Preston et al. 2006; Jacobsen et al. 2007a; Mitchell et al. 2008; Gleason et al. 2012). Such disparities indicate that the relationship between wood density and tissue fractions may conceivably be different in main trunks than in twigs, yet it is unclear how generally this is so.

## Conclusions

Wood density has been proposed as a key plant functional trait and is related to ecological strategies (Chave et al. 2009), but relatively little is known about the underpinnings of these relationships. This study provides anatomical bases for wood density variation. It shows that wood density depends on anatomical structure, but also a range of very different structures can result in very similar wood density, especially among species with medium density<sub>NV</sub> (here, 0.60–0.85 g cm<sup>-3</sup>). This conclusion suggests that there may be a wider range of ecological strategies among such species. Taken together, these findings imply that twig wood density should not be considered as an unambiguous indicator of plant ecological strategies. We hope this research will enhance interpretation and design of ecological studies related to wood density.

## 3.6 Acknowledgements

Many individuals have helped throughout this study to whom we are very grateful. We thank Judy and Eddie Howitt, John and Jill Bignell, National Parks and State Forests staff in Queensland and Tasmania for their hospitality and sites access. Parks and Wildlife Service of Tasmania and Queensland gave permission for plant material collection. Macquarie University Microscopy Unit kindly provided facilities to carry out anatomical work. Many thanks to Steven Jansen for valuable anatomical discussions and comments on earlier version of this manuscript. Wendy Noble and Luen-Hsien Chang

also provided useful feedback on earlier version of this manuscript. We are grateful to the Comparative Ecology Lab members for constructive conversations about the research presented here. This work has been supported by the Macquarie University Research Excellence Scholarship and Australian Research Council Discovery Project grant awarded respectively to KZ and MW.

### 3.7 Figures

Figure 3-1 A twig cross-section of *Grevillea parallela*, Proteaceae.

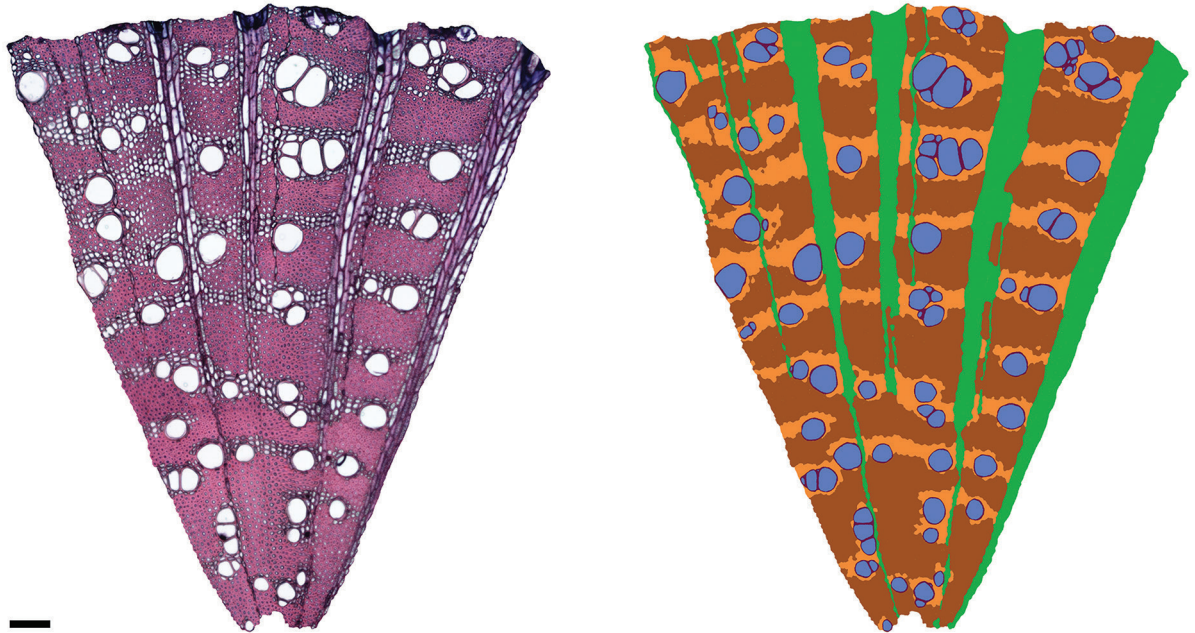


Figure 3-1 A twig cross-section of *Grevillea parallela*, Proteaceae. Radial sector of stained section is shown on the left side and the processed image on the right. Colours in the processed image denote different tissue types: blue - vessel lumen, purple - vessel wall, green - rays, orange - axial parenchyma, brown - fibres. Scale bar corresponds to 100  $\mu\text{m}$ .





Figure 3-2 Tissue fractions for 24 species arranged in order of decreasing wood density.

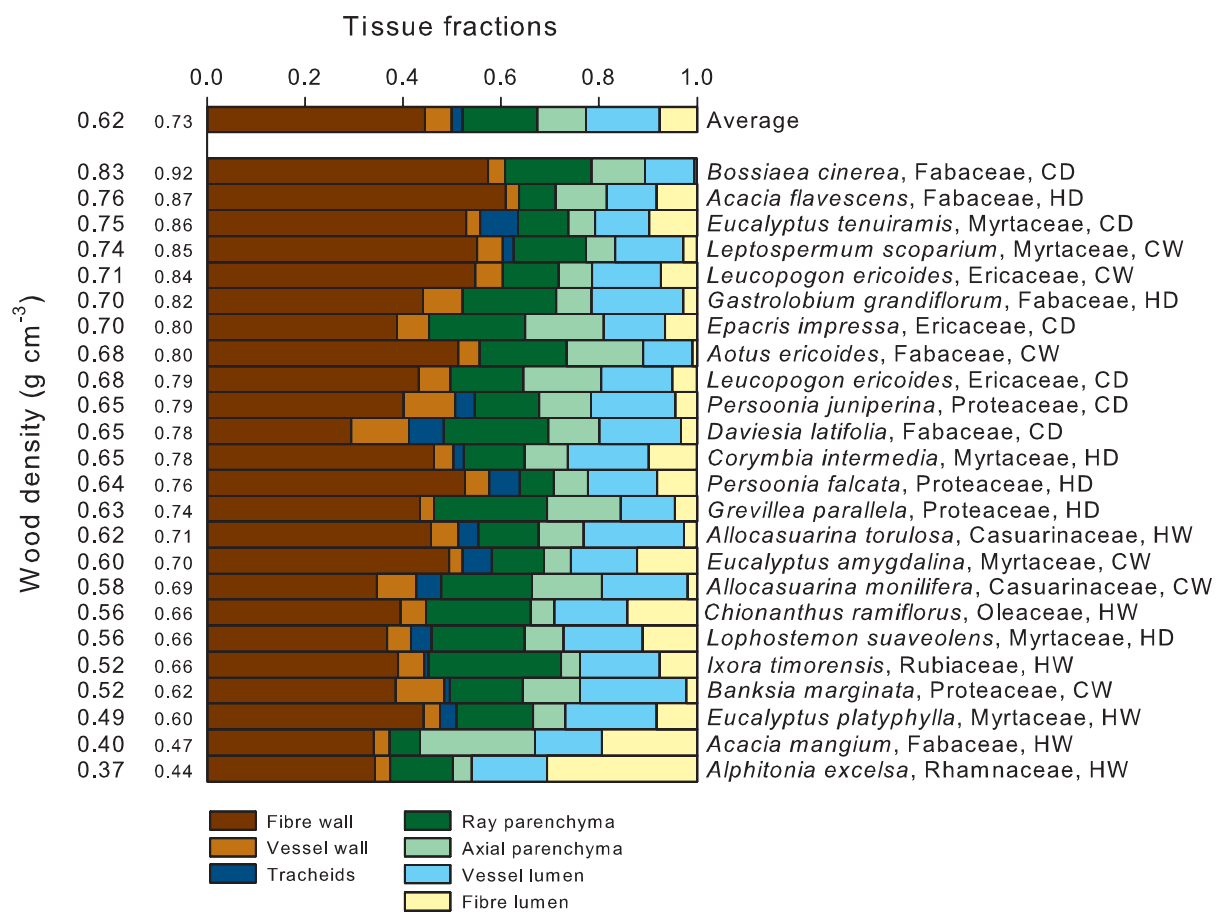


Figure 3-2 Tissue fractions for 24 species arranged in order of increasing wood density (from bottom to top). Large wood density numbers indicate total wood density whereas small numbers indicate non-vessel density. Mean tissue fractions across all species are shown in the bar at the top of the figure. Letters next to family name indicate sites of collection: CW – cool-wet, CD – cool-dry, HW – hot-wet, HD – hot-dry. *Leucopogon ericoides* occurred in two sites and is treated here as two separate entities.



Figure 3-3 Relationship between fraction of wood outside vessel lumens ( $\text{fraction}_{\text{NV}}$ ) and the density of that non-vessel fraction ( $\text{wood density}_{\text{NV}}$ ).

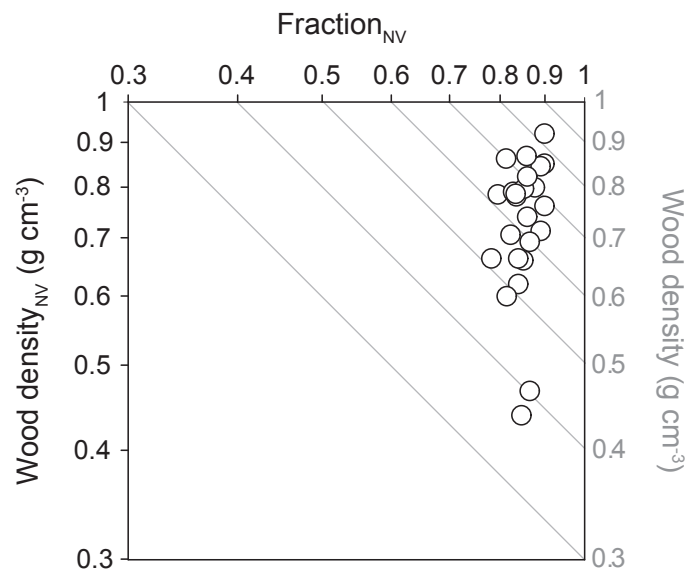


Figure 3-3 Relationship between fraction of wood outside vessel lumens ( $\text{fraction}_{\text{NV}}$ ) and the density of that non-vessel fraction ( $\text{wood density}_{\text{NV}}$ ) among 24 Australian species.  $\text{Fraction}_{\text{NV}} = 1 - \text{vessel lumen fraction}$ . Each circle represents a different species (mean value from three replicates). Diagonal isolines represent contours of overall wood density, which increases towards the upper right. All axes are log scaled.



Figure 3-4 Relationship between non-vessel density (wood density<sub>NV</sub>) and fibre fraction in non-vessel area (fibre fraction<sub>NV</sub>).

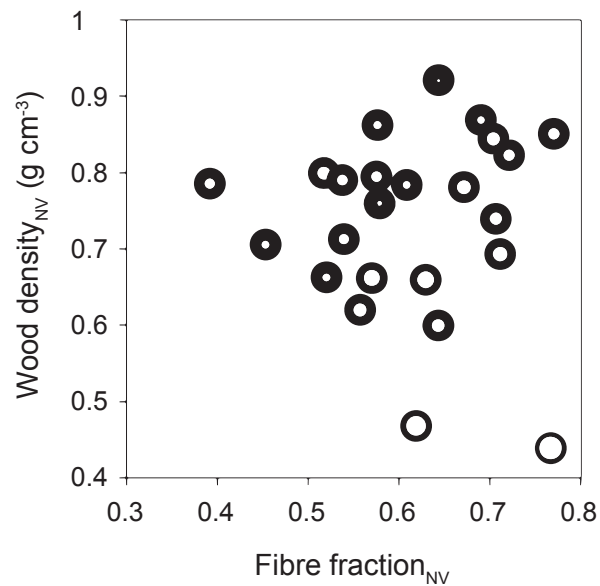


Figure 3-4 Relationship between non-vessel density (wood density<sub>NV</sub>) and fibre fraction in non-vessel area (fibre fraction<sub>NV</sub>). Each 'donut' circle symbolizes one species. The width of the donut border (black) represents fibre wall proportion within an individual fibre and the width of the hole (white) represents fibre lumen proportion. These proportions were estimated from individual fibres (as fibre wall area – or lumen area, divided by total fibre area), for 75–314 fibres per replicate (mean 170), then across three replicates per species.



Figure 3-5 Relationships between non-vessel density (wood density<sub>NV</sub>) and (a) fibre wall fraction in non-vessel area (fibre wall fraction<sub>NV</sub>), and (b) fibre lumen fraction in non-vessel area (fibre lumen fraction<sub>NV</sub>). Each circle represents a different species (mean value from three replicates). Grey lines denote standard deviation. \*\*\*  $P < 0.001$

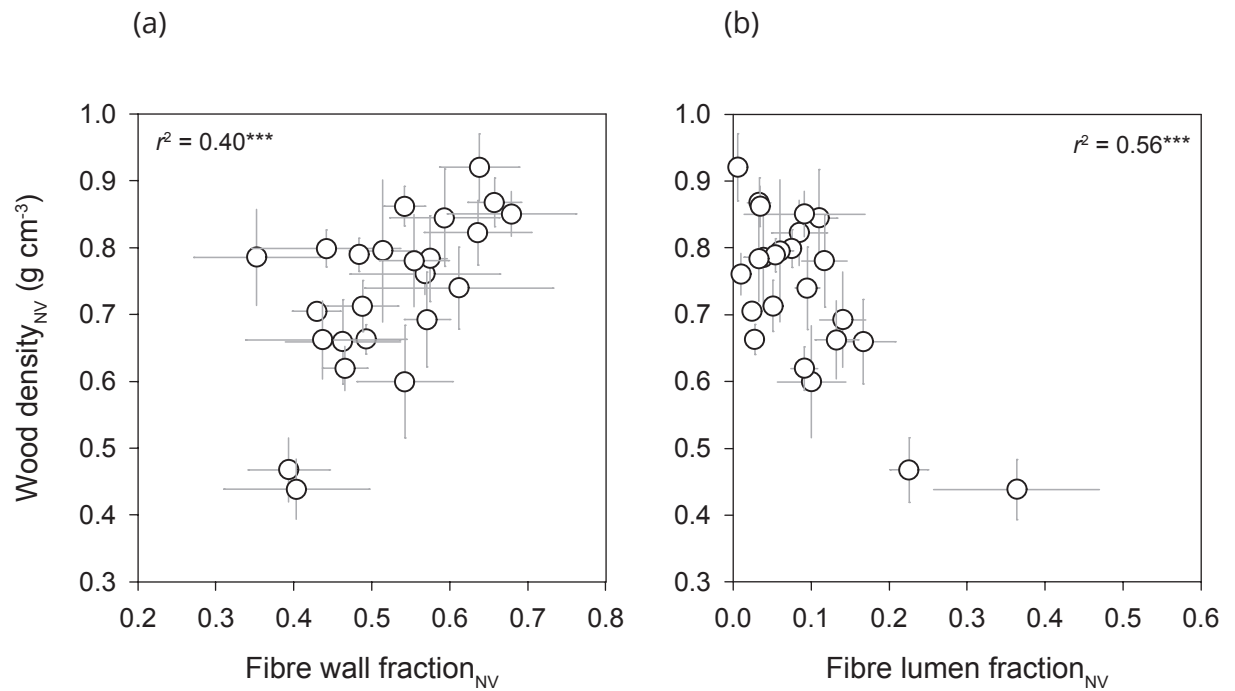


Figure 3-6 Relationship between the fraction of the non-vessel area that is fibre wall or is fibre lumen. (a) Each symbol represents a different species (mean value from three replicates). Symbol diameter is proportional to non-vessel wood density (wood density<sub>NV</sub>) with the biggest symbol indicating highest density<sub>NV</sub> (0.92 g cm<sup>-3</sup>) and the smallest symbol indicating lowest density<sub>NV</sub> (0.44 g cm<sup>-3</sup>). (b) A schematic representation of graph (a) flipped clockwise by 45°. The diagram represents four cross-sections of potential anatomical structures in low, medium- and high-density species. Each hexagon within the four squares indicates a fibre cell consisting of dark fibre wall and bright fibre lumen. The green area on the right of each square indicates parenchyma. Wood density<sub>NV</sub> increases towards the top of the diagram.



Figure 3-6 Relationship between the fraction of the non-vessel area that is fibre wall or is fibre lumen (a) and its schematic illustration (b). See figure caption on opposite page.

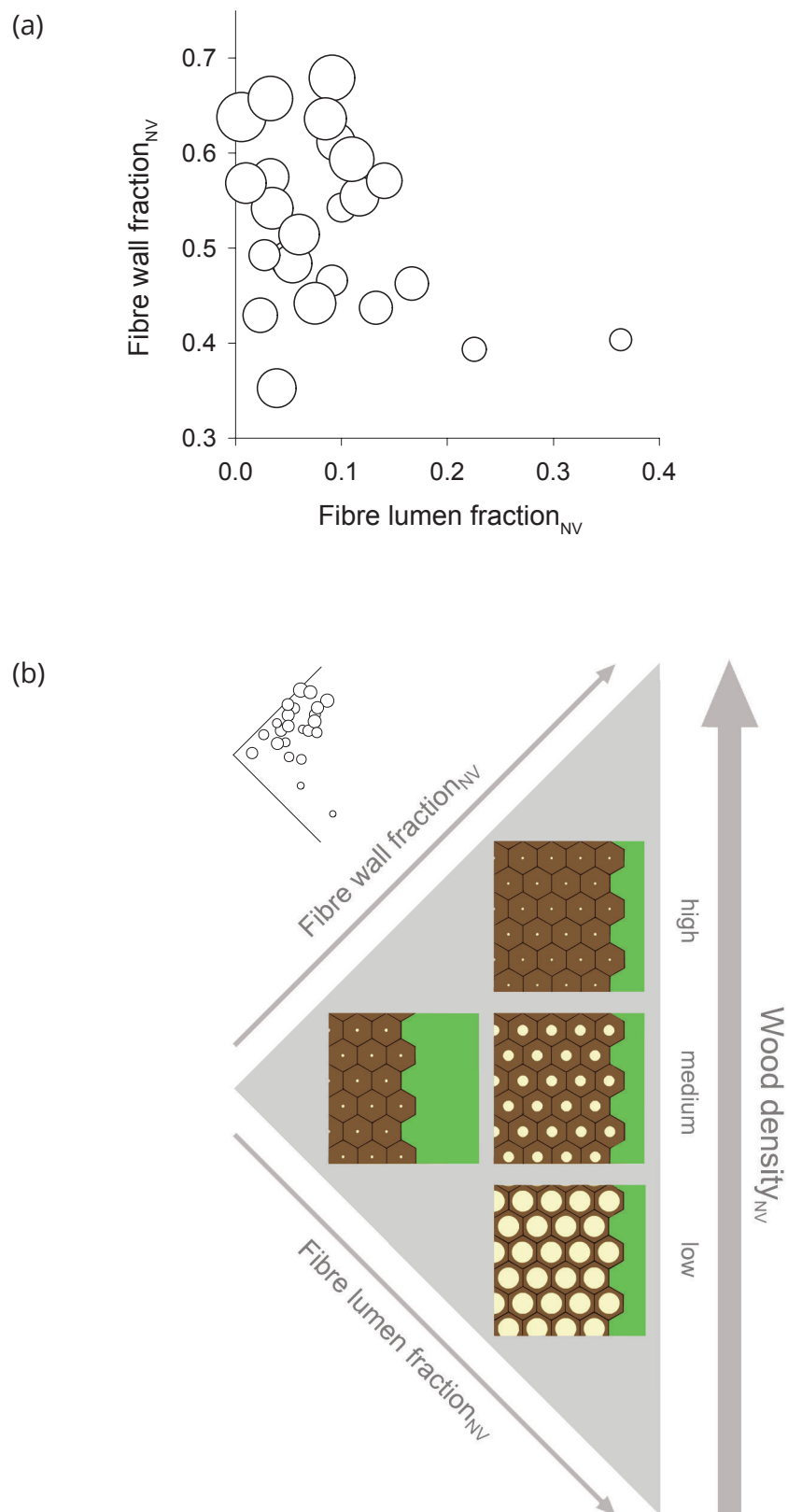
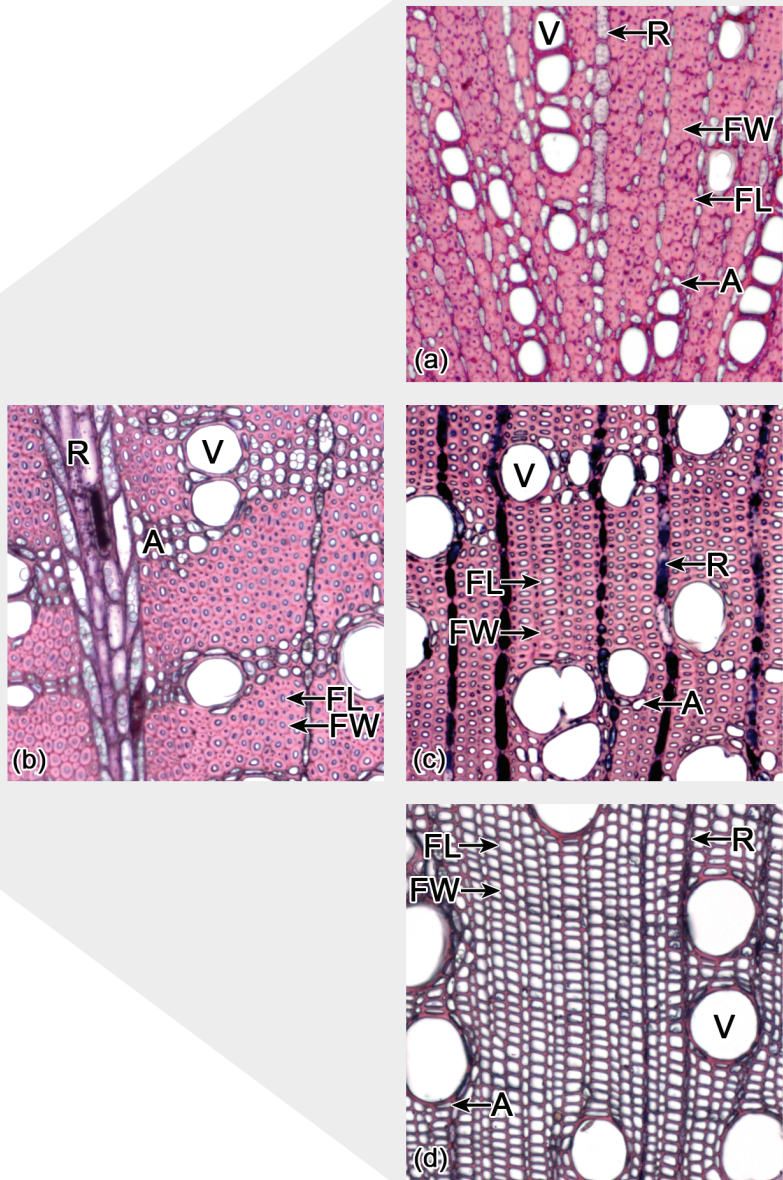


Figure 3-7 Cross-sections through twigs of four species. The triangle arrangement corresponds to the one in Figure 3-6b and so the cross-sections are examples of respective anatomies drawn in that figure. (a) *Bossiaea cinerea* (Fabaceae, wood density  $0.83 \text{ g cm}^{-3}$ ), (b) *Grevillea parallela* (Proteaceae, wood density  $0.63 \text{ g cm}^{-3}$ ), (c) *Corymbia intermedia* (Myrtaceae, wood density  $0.65 \text{ g cm}^{-3}$ ), (d) *Alphitonia excelsa* (Rhamnaceae, wood density  $0.37 \text{ g cm}^{-3}$ ). V – vessels, FW – fibre wall, FL – fibre lumen, A – axial parenchyma, R – ray parenchyma. Scale bar corresponds to  $100 \text{ }\mu\text{m}$ .

Figure 3-7 Cross-sections through twigs of four species.



Non-vessel tissue fractions (overall tissue fractions)			
	Fibre wall	Fibre lumen	Parenchyma
(a)	0.64 (0.57)	0.01 (0.01)	0.32 (0.29)
(b)	0.49 (0.43)	0.05 (0.05)	0.43 (0.38)
(c)	0.55 (0.46)	0.12 (0.10)	0.26 (0.21)
(d)	0.40 (0.34)	0.36 (0.31)	0.20 (0.18)



## 3.8 Tables

Table 3-1 Species list and sites of collection.

Site	Species name	Family
Cool-wet	<i>Allocasuarina monilifera</i>	Casuarinaceae
	<i>Aotus ericoides</i>	Fabaceae
	<i>Banksia marginata</i>	Proteaceae
	<i>Eucalyptus amygdalina</i>	Myrtaceae
	<i>Leptospermum scoparium</i>	Myrtaceae
	<i>Leucopogon ericoides</i>	Ericaceae
Cool-dry	<i>Bossiaea cinerea</i>	Fabaceae
	<i>Davesia latifolia</i>	Fabaceae
	<i>Epacris impressa</i>	Ericaceae
	<i>Eucalyptus tenuiramis</i>	Myrtaceae
	<i>Leucopogon ericoides</i>	Ericaceae
	<i>Persoonia juniperina</i>	Proteaceae
Hot-wet	<i>Acacia mangium</i>	Fabaceae
	<i>Allocasuarina torulosa</i>	Casuarinaceae
	<i>Alphitonia excelsa</i>	Rhamnaceae
	<i>Chionanthus ramiflorus</i>	Oleaceae
	<i>Eucalyptus platyphylla</i>	Myrtaceae
	<i>Ixora timorensis</i>	Rubiaceae
Hot-dry	<i>Acacia flavescens</i>	Fabaceae
	<i>Corymbia intermedia</i>	Myrtaceae
	<i>Gastrolobium grandiflorum</i>	Fabaceae
	<i>Grevillea parallela</i>	Proteaceae
	<i>Lophostemon suaveolens</i>	Myrtaceae
	<i>Persoonia falcata</i>	Proteaceae

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### 3.10 Appendix 1

Table A3-1 Details of the four sites sampled in this study.

	Hot-wet Cardwell QLD	Hot-dry Princess Hills QLD	Cool-wet Lower Longley TAS	Cool-dry Bothwell TAS
Sampling time	August 2009	November 2009	March 2009	March 2009
Biome	tropical dry forest	savannah	woodland	woodland
Longitude	146.16°S	145.52°S	147.18°S	147.04°S
Latitude	18.48°E	18.25°E	42.98°E	42.39°E
Altitude (m)	50	595	280	420
MAP(mm)	1925	1106	964	547
MAT(°C)	24.1	21.3	11.3	10
AI(MAP/PET)	1.026	0.601	1.153	0.646

MAP – mean annual precipitation, MAT – mean annual temperature, AI – aridity index, PET – potential evapostranspiration, QLD – Queensland, TAS – Tasmania

Table A3-2 Wood density and tissue fractions of 24 species averaged across three replicates.

Site	Species		Wood density (g cm <sup>-3</sup> )	Fibre	Fibre wall	Fibre lumen	Parenchyma	Axial parenchyma	Ray parenchyma	Vessel lumen	Vessel wall	Tracheids	Fibre wall proportion in a fibre
Cool-wet	<i>Allocasuarina monilifera</i>	AV	0.58	0.37	0.35	0.02	0.33	0.15	0.19	0.18	0.08	0.05	0.95
		SD	0.05	0.05	0.05	0.00	0.05	0.01	0.06	0.07	0.03	0.01	0.02
	<i>Aotus ericoides</i>	AV	0.68	0.52	0.51	0.01	0.33	0.16	0.18	0.10	0.04	-	0.98
		SD	0.03	0.09	0.09	0.00	0.06	0.05	0.04	0.02	0.02	na	0.01
	<i>Banksia marginata</i>	AV	0.52	0.41	0.39	0.02	0.27	0.12	0.15	0.22	0.10	0.01	0.95
		SD	0.02	0.05	0.05	0.01	0.01	0.02	0.02	0.02	0.02	0.00	0.01
	<i>Eucalyptus amygdalina</i>	AV	0.60	0.62	0.49	0.12	0.16	0.05	0.11	0.14	0.03	0.06	0.80
		SD	0.05	0.06	0.03	0.03	0.04	0.01	0.03	0.02	0.01	0.03	0.03
	<i>Leptospermum scoparium</i>	AV	0.74	0.59	0.56	0.03	0.20	0.06	0.15	0.14	0.05	0.02	0.95
		SD	0.04	0.03	0.03	0.01	0.03	0.01	0.04	0.01	0.01	0.01	0.02
	<i>Leucopogon ericoides</i>	AV	0.71	0.62	0.55	0.07	0.18	0.07	0.11	0.14	0.06	-	0.88
		SD	0.02	0.10	0.08	0.03	0.04	0.03	0.01	0.03	0.02	na	0.04
Cool-dry	<i>Bossiaea cinerea</i>	AV	0.83	0.58	0.57	0.01	0.29	0.11	0.18	0.10	0.03	-	0.99
		SD	0.04	0.05	0.05	0.00	0.04	0.02	0.03	0.01	0.00	na	0.00
	<i>Davesia latifolia</i>	AV	0.65	0.33	0.29	0.03	0.32	0.10	0.21	0.17	0.12	0.07	0.91
		SD	0.05	0.09	0.07	0.02	0.06	0.02	0.04	0.03	0.02	0.02	0.05
	<i>Epacris impressa</i>	AV	0.70	0.45	0.39	0.07	0.36	0.16	0.20	0.12	0.07	-	0.85
		SD	0.04	0.09	0.09	0.00	0.06	0.07	0.01	0.02	0.01	na	0.03
	<i>Eucalyptus tenuiramis</i>	AV	0.75	0.63	0.53	0.10	0.16	0.06	0.10	0.11	0.03	0.08	0.84
		SD	0.04	0.05	0.07	0.02	0.05	0.03	0.03	0.04	0.01	0.02	0.04
	<i>Leucopogon ericoides</i>	AV	0.68	0.49	0.44	0.05	0.31	0.16	0.15	0.15	0.07	-	0.90
		SD	0.10	0.05	0.05	0.01	0.05	0.02	0.03	0.03	0.01	na	0.03
	<i>Persoonia juniperina</i>	AV	0.65	0.45	0.40	0.04	0.24	0.11	0.13	0.17	0.11	0.04	0.90
		SD	0.02	0.01	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02
Hot-wet	<i>Acacia mangium</i>	AV	0.40	0.54	0.34	0.19	0.30	0.23	0.06	0.14	0.03	-	0.64
		SD	0.04	0.06	0.05	0.02	0.05	0.05	0.01	0.01	0.00	na	0.04
	<i>Allocasuarina torulosa</i>	AV	0.62	0.48	0.46	0.03	0.21	0.09	0.12	0.21	0.06	0.04	0.95
		SD	0.05	0.02	0.02	0.01	0.01	0.03	0.02	0.01	0.01	0.01	0.01
	<i>Alphitonia excelsa</i>	AV	0.37	0.65	0.34	0.31	0.17	0.04	0.13	0.15	0.03	-	0.53
		SD	0.03	0.04	0.08	0.08	0.05	0.01	0.04	0.02	0.00	na	0.12
	<i>Chionanthus ramiflorus</i>	AV	0.56	0.54	0.40	0.14	0.26	0.05	0.21	0.15	0.05	-	0.74
		SD	0.04	0.10	0.08	0.04	0.05	0.02	0.04	0.03	0.02	na	0.04
	<i>Eucalyptus platyphylla</i>	AV	0.49	0.52	0.44	0.08	0.22	0.07	0.16	0.19	0.03	0.03	0.85
		SD	0.05	0.09	0.06	0.04	0.04	0.01	0.03	0.03	0.01	0.01	0.05
	<i>Ixora timorensis</i>	AV	0.52	0.47	0.39	0.08	0.31	0.04	0.27	0.16	0.05	0.01	0.84
		SD	0.04	0.03	0.02	0.02	0.03	0.01	0.02	0.04	0.01	0.00	0.03

Site	Species		Wood density (g cm <sup>-3</sup> )	Fibre	Fibre wall	Fibre lumen	Parenchyma	Axial parenchyma	Ray parenchyma	Vessel lumen	Vessel wall	Tracheids	Fibre wall proportion in a fibre
Hot-dry	<i>Acacia</i>	AV	0.76	0.69	0.61	0.08	0.18	0.10	0.07	0.10	0.03	-	0.88
	<i>flavescens</i>	SD	0.03	0.01	0.07	0.07	0.01	0.00	0.01	0.01	0.01	na	0.10
	<i>Corymbia</i>	AV	0.65	0.56	0.46	0.10	0.21	0.09	0.12	0.17	0.04	0.02	0.83
	<i>intermedia</i>	SD	0.09	0.08	0.06	0.03	0.03	0.02	0.02	0.04	0.01	0.01	0.04
	<i>Gastrolobium</i>	AV	0.70	0.47	0.44	0.03	0.26	0.07	0.19	0.19	0.08	-	0.94
	<i>grandiflorum</i>	SD	0.02	0.03	0.03	0.01	0.02	0.02	0.03	0.01	0.01	na	0.02
	<i>Grevillea</i>	AV	0.63	0.48	0.43	0.05	0.38	0.15	0.23	0.11	0.03	-	0.90
	<i>parallela</i>	SD	0.04	0.03	0.04	0.01	0.03	0.02	0.03	0.01	0.00	na	0.03
	<i>Lophostemon</i>	AV	0.56	0.48	0.37	0.11	0.27	0.08	0.19	0.16	0.05	0.04	0.76
	<i>suaveolens</i>	SD	0.06	0.09	0.09	0.02	0.06	0.03	0.03	0.02	0.00	0.02	0.06
	<i>Persoonia</i>	AV	0.64	0.61	0.53	0.08	0.14	0.07	0.07	0.14	0.05	0.06	0.86
	<i>falcata</i>	SD	0.07	0.12	0.12	0.01	0.04	0.02	0.03	0.04	0.01	0.05	0.04

Notes: all values, except for wood density, refer to fractions and are unitless. Two traits in two furthest right columns are the properties of individual fibres. All other values are the fractions of tissues within a studied radial sector. AV – species average, SD – standard deviation, ‘-’ indicates that the tracheids were not observed.

Table A3-3 Wood density and tissue fractions of non-vessel proportion of 24 species averaged across three replicates.

Site	Species		Wood density (g cm <sup>-3</sup> )	Non-vessel	Fibre	Fibre wall	Fibre lumen	Vessel wall	Parenchyma	Axial parenchyma	Ray parenchyma	Tracheids
Cool-wet	<i>Allocasuarina monilifera</i>	AV	0.70	0.82	0.45	0.43	0.02	0.10	0.41	0.18	0.23	0.06
		SD	0.01	0.07	0.03	0.03	0.01	0.04	0.06	0.02	0.07	0.01
	<i>Aotus ericoides</i>	AV	0.76	0.90	0.58	0.57	0.01	0.05	0.37	0.18	0.20	-
		SD	0.03	0.02	0.09	0.10	0.00	0.02	0.07	0.06	0.04	na
	<i>Banksia marginata</i>	AV	0.66	0.78	0.52	0.49	0.03	0.13	0.34	0.15	0.19	0.01
		SD	0.02	0.02	0.05	0.05	0.01	0.03	0.02	0.03	0.03	0.00
	<i>Eucalyptus amygdalina</i>	AV	0.69	0.86	0.71	0.57	0.14	0.03	0.19	0.06	0.12	0.07
		SD	0.07	0.02	0.06	0.03	0.03	0.01	0.04	0.01	0.04	0.04
	<i>Leptospermum scoparium</i>	AV	0.87	0.86	0.69	0.66	0.03	0.06	0.23	0.07	0.16	0.03
		SD	0.04	0.01	0.03	0.03	0.02	0.01	0.04	0.02	0.03	0.01
	<i>Leucopogon ericoides</i>	AV	0.82	0.86	0.72	0.64	0.08	0.07	0.21	0.08	0.13	-
		SD	0.05	0.03	0.09	0.07	0.04	0.03	0.06	0.04	0.02	na
Cool-dry	<i>Bossiaea cinerea</i>	AV	0.92	0.90	0.64	0.64	0.01	0.04	0.32	0.12	0.20	-
		SD	0.05	0.01	0.05	0.05	0.00	0.00	0.05	0.02	0.04	na
	<i>Davesia latifolia</i>	AV	0.79	0.83	0.39	0.35	0.04	0.14	0.38	0.12	0.26	0.09
		SD	0.07	0.03	0.10	0.08	0.02	0.03	0.07	0.02	0.06	0.03
	<i>Epacris impressa</i>	AV	0.80	0.88	0.52	0.44	0.08	0.07	0.41	0.18	0.22	-
		SD	0.03	0.02	0.10	0.09	0.01	0.01	0.08	0.08	0.00	na
	<i>Eucalyptus tenuiramis</i>	AV	0.84	0.89	0.70	0.59	0.11	0.03	0.18	0.06	0.11	0.09
		SD	0.07	0.04	0.05	0.07	0.02	0.01	0.05	0.03	0.03	0.02
	<i>Leucopogon ericoides</i>	AV	0.80	0.85	0.57	0.51	0.06	0.08	0.37	0.19	0.18	-
		SD	0.11	0.03	0.04	0.04	0.02	0.02	0.07	0.03	0.04	na
	<i>Persoonia juniperina</i>	AV	0.79	0.83	0.54	0.48	0.05	0.13	0.29	0.13	0.16	0.05
		SD	0.02	0.02	0.01	0.01	0.01	0.02	0.03	0.02	0.02	0.02
Hot-wet	<i>Acacia mangium</i>	AV	0.47	0.86	0.62	0.39	0.23	0.04	0.34	0.27	0.07	-
		SD	0.05	0.01	0.06	0.05	0.02	0.00	0.06	0.06	0.01	na
	<i>Allocasuarina torulosa</i>	AV	0.78	0.79	0.61	0.57	0.03	0.07	0.27	0.11	0.16	0.05
		SD	0.06	0.01	0.02	0.02	0.01	0.01	0.01	0.04	0.03	0.02
	<i>Alphitonia excelsa</i>	AV	0.44	0.85	0.77	0.40	0.36	0.04	0.20	0.05	0.15	-
		SD	0.05	0.02	0.06	0.09	0.11	0.00	0.06	0.01	0.05	na
	<i>Chionanthus ramiflorus</i>	AV	0.66	0.85	0.63	0.46	0.17	0.06	0.31	0.06	0.25	-
		SD	0.06	0.03	0.10	0.07	0.04	0.03	0.07	0.03	0.06	na
	<i>Eucalyptus platyphylla</i>	AV	0.60	0.81	0.64	0.54	0.10	0.04	0.27	0.08	0.19	0.04
		SD	0.08	0.03	0.09	0.06	0.04	0.01	0.06	0.01	0.05	0.02
	<i>Ixora timorensis</i>	AV	0.62	0.84	0.56	0.47	0.09	0.06	0.37	0.05	0.32	0.01
		SD	0.03	0.04	0.03	0.03	0.02	0.02	0.03	0.02	0.02	0.00



Site	Species		Wood density (g cm <sup>-3</sup> )	Non-vessel	Fibre	Fibre wall	Fibre lumen	Vessel wall	Parenchyma	Axial parenchyma	Ray parenchyma	Tracheids
Hot-dry	<i>Acacia</i>	AV	0.85	0.90	0.77	0.68	0.09	0.03	0.20	0.12	0.08	-
	<i>flavescens</i>	SD	0.03	0.01	0.01	0.08	0.08	0.01	0.01	0.00	0.01	na
	<i>Corymbia</i>	AV	0.78	0.83	0.67	0.55	0.12	0.05	0.26	0.11	0.15	0.03
	<i>intermedia</i>	SD	0.07	0.04	0.06	0.04	0.03	0.01	0.04	0.02	0.03	0.02
	<i>Gastrolobium</i>	AV	0.86	0.81	0.58	0.54	0.03	0.10	0.32	0.09	0.24	-
	<i>grandiflorum</i>	SD	0.03	0.01	0.03	0.03	0.01	0.01	0.03	0.03	0.03	na
	<i>Grevillea</i>	AV	0.71	0.89	0.54	0.49	0.05	0.03	0.43	0.17	0.26	-
	<i>parallela</i>	SD	0.04	0.01	0.04	0.05	0.01	0.00	0.04	0.03	0.03	na
	<i>Lophostemon</i>	AV	0.66	0.84	0.57	0.44	0.13	0.06	0.32	0.10	0.23	0.05
	<i>suaveolens</i>	SD	0.06	0.02	0.10	0.10	0.03	0.01	0.07	0.03	0.04	0.03
	<i>Persoonia</i>	AV	0.74	0.86	0.71	0.61	0.09	0.06	0.16	0.08	0.08	0.07
	<i>falcata</i>	SD	0.06	0.04	0.11	0.12	0.02	0.01	0.05	0.02	0.04	0.06

Notes: the subscript 'NV' is omitted for brevity; all values, except for wood density, are unitless. AV – species average, SD – standard deviation, '-' indicates that the tracheids were not observed.

## 3.11 Appendix 2

### Site comparisons

Six most abundant species of trees and shrubs, three replicates each, from four sites were collected and the mean site values were compared using one-way ANOVA. Traits analysed included wood density, overall tissue fractions (vessel lumen, vessel wall, total parenchyma, axial parenchyma, ray parenchyma, fibre wall, fibre lumen, and tracheids), non-vessel wood density, and non-vessel tissue fractions (the same tissues as for overall fractions). The term 'non-vessel' refers to wood fraction outside vessel lumen, and non-vessel traits are denoted hereafter by a subscript 'NV'.

Overall wood density and non-vessel density (wood density<sub>NV</sub>) were significantly lower in the hot-wet site than in the three other sites (ANOVA,  $F_{3,23} = 7.94$ ,  $P < 0.001$  and  $F_{3,23} = 7.26$ ,  $P = 0.002$ , respectively, Fig. A3-1) and there was no significant difference between those three sites. All the other traits, overall fractions, and non-vessel fractions did not differ significantly between the sites in their mean or median (site means are in Tables A3-4 and A3-5). The exceptions were fibre lumen fraction and non-vessel fibre lumen fraction (fibre lumen fraction<sub>NV</sub>). Fibre lumen fraction was marginally significantly higher in hot-wet site than in cool-wet and cool-dry sites (ANOVA,  $F_{3,23} = 3.113$ ,  $P = 0.080$  and  $P = 0.085$ , respectively). There was no significant difference among all the other site comparisons. Similar to fibre lumen fraction, fibre lumen fraction<sub>NV</sub> was marginally significantly higher in hot-wet site than in cool-wet and cool-dry sites (ANOVA,  $F_{3,23} = 3.263$ ,  $P = 0.071$  and  $P = 0.073$ , respectively). Interestingly, fibre wall fraction<sub>NV</sub>, although being the main driver of wood density<sub>NV</sub> variation, did not differ significantly between the sites. However, the box plots (Fig. A3-1) indicate that the pattern of wood density<sub>NV</sub> variation across the sites was relatively consistent with the pattern of fibre lumen fraction<sub>NV</sub> variation. The only inconsistency occurred between cool-dry sites and probably resulted from the fact that the species with lowest fibre wall fraction<sub>NV</sub> (*Daviesia latifolia*) had high proportion of thick-walled tracheids. This might have caused its high density<sub>NV</sub> (high per given non-vessel fibre wall fraction).

The most prominent results from this comparison were that wood density and wood density<sub>NV</sub> were significantly lower in hot-wet site than in other sites (Fig. A3-1).

Previous studies across a larger number of species reported mixed results. A few works have found that wood density increased with mean annual temperature (MAT, Wiemann & Williamson 2002; Martínez-Cabrera et al. 2009, together almost 400 species) and decreased with mean annual precipitation (Barajas-Morales 1985; MAP, Martínez-Cabrera et al. 2009, together 281 species). Similar, but very weak trends, were also found by Swenson & Enquist (2007) across more than 4000 species. Several studies found no relationship (Wiemann & Williamson 2002; Muller - Landau 2004) or positive one with MAP (Zhang et al. 2011, 618 species). Zhang et al. (2011) also reported no significant correlation with MAT.

We found that the hot-wet site had significantly higher fibre lumen fraction and fibre lumen fraction<sub>NV</sub> than other sites (Fig. A3-1). Martínez-Cabrera et al. (2009) reported similar results across 61 shrub species from North and South Americas. In that study, fibre lumen fraction was positively related to MAP, similar to our study where within the two hot sites, the wetter one had higher fibre lumen fraction (yet this was not the case within the cool sites). Fibre lumen fraction was negatively associated with MAT (Martínez-Cabrera et al. 2009), whereas in our study, fibre lumen fraction and fibre lumen fraction<sub>NV</sub> were positively associated with MAT within the two wet sites, but not within the dry ones. Other traits correlated with climate included total fibre fraction (positively to MAP, Fichtler & Worbes 2012), fibre wall fraction (negatively to MAP and not related to MAT), ray fraction (positively to MAP and not related to MAT), axial parenchyma (negatively to MAP and positively to MAT, Martínez-Cabrera et al. 2009). Total parenchyma fraction decreased with increasing MAP in tropical trees (Fichtler & Worbes 2012), but was not associated across subtropical and tropical environments (Martínez-Cabrera et al. 2009; this study). Vessel lumen and vessel wall fractions varied independently of climate (Martínez-Cabrera et al. 2009; Fichtler & Worbes 2012; this study).

Lack of consistent patterns in wood density and tissue traits across temperature and precipitation gradients may be caused by several factors. First, sampling design varied across the studies, and collected samples were not always representative for the given site (e.g. Wiemann & Williamson 2002; this study). Second, the range of environmental gradients differed. For example, the two sites compared by Barajas-

Morales (1985), who found negative relationship between density and precipitation, were located in the tropics and had similar MAT but different MAP. In contrast, Zhang et al. (2011), who found a positive relationship between density and precipitation, included wider temperature range and vegetation types from boreal to tropical forests. Thirdly, it is likely that temperature and precipitation are not independent but rather interdependent factors influencing wood density and anatomy shifts (Chave et al. 2009). In addition, other environmental variables, for example seasonality, also may play a role.

Table A3-4 Site mean and standard deviation of wood density and tissue fractions. Values calculated on six species per site.

Site		Wood density (g cm <sup>-3</sup> )	Fibre	Fibre wall	Fibre lumen	Parenchyma	Axial parenchyma	Ray parenchyma	Vessel lumen	Vessel wall	Tracheids	Fibre wall proportion in a fibre
Cool-wet	AV	0.64	0.52	0.48	0.05	0.25	0.10	0.15	0.15	0.06	0.02	0.92
	SD	0.09	0.11	0.09	0.04	0.08	0.05	0.03	0.04	0.03	0.03	0.07
Cool-dry	AV	0.71	0.49	0.44	0.05	0.27	0.12	0.16	0.14	0.07	0.02	0.90
	SD	0.07	0.11	0.10	0.03	0.06	0.04	0.04	0.03	0.04	0.01	0.05
Hot-wet	AV	0.49	0.53	0.39	0.14	0.25	0.09	0.16	0.17	0.04	0.01	0.75
	SD	0.09	0.06	0.05	0.10	0.05	0.08	0.07	0.03	0.01	0.02	0.15
Hot-dry	AV	0.66	0.55	0.47	0.07	0.24	0.09	0.15	0.14	0.05	0.02	0.86
	SD	0.07	0.09	0.08	0.03	0.08	0.03	0.07	0.03	0.02	0.03	0.06

Notes: the subscript 'NV' is omitted for brevity; all values, except for wood density, are unitless. AV – site average, SD – standard deviation.

Table A3-5 Site mean and standard deviation of wood density and tissue fractions of non-vessel proportion. Values calculated on six species per site.

Site		Wood density (g cm <sup>-3</sup> )	Non-vessel	Fibre	Fibre wall	Fibre lumen	Parenchyma	Axial parenchyma	Ray parenchyma	Vessel wall	Tracheids
Cool-wet	AV	0.75	0.85	0.61	0.56	0.05	0.29	0.12	0.17	0.07	0.03
	SD	0.08	0.04	0.11	0.09	0.05	0.09	0.05	0.04	0.04	0.03
Cool-dry	AV	0.82	0.86	0.56	0.50	0.06	0.32	0.13	0.19	0.08	0.04
	SD	0.05	0.03	0.11	0.10	0.03	0.08	0.05	0.05	0.04	0.04
Hot-wet	AV	0.59	0.83	0.64	0.47	0.16	0.29	0.10	0.19	0.05	0.02
	SD	0.13	0.03	0.07	0.07	0.12	0.06	0.09	0.09	0.02	0.02
Hot-dry	AV	0.77	0.86	0.64	0.55	0.09	0.28	0.11	0.17	0.05	0.02
	SD	0.08	0.03	0.09	0.09	0.04	0.10	0.03	0.08	0.03	0.03

Notes: the subscript 'NV' is omitted for brevity; all values, except for wood density, are unitless. AV – site average, SD – standard deviation.



Figure A3-1 Box plots showing comparisons in non-vessel wood density (wood density<sub>NV</sub>), non-vessel fibre wall fraction (fibre wall fraction<sub>NV</sub>), and non-vessel fibre lumen fraction (fibre lumen fraction<sub>NV</sub>) between the four sites.

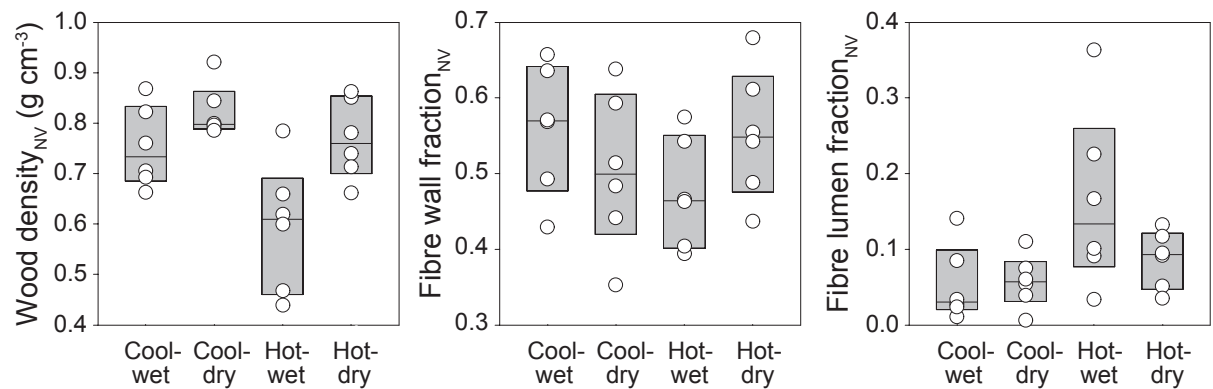


Figure A3-1 Box plots showing comparisons in non-vessel wood density (wood density<sub>NV</sub>), non-vessel fibre wall fraction (fibre wall fraction<sub>NV</sub>), and non-vessel fibre lumen fraction (fibre lumen fraction<sub>NV</sub>) between the four sites. The black line inside the grey box is a median. The box top and bottom boundaries indicate upper and lower quartile, and the circle symbols are individual species (species mean calculated from three replicates per species).





# **Comparison of *Leucopogon ericoides* (Ericaceae) from cool-wet and cool-dry sites**

Across the species set studied here, one species, *Leucopogon ericoides*, occurred in two sites: cool-wet and cool-dry. T-test was used to compare means of measured traits between *L. ericoides* from the two sites.

*L. ericoides* from cool-dry site had significantly more total parenchyma fraction ( $P = 0.023$ ), ray parenchyma fraction ( $P = 0.088$ ), and axial parenchyma fraction ( $P = 0.014$ ). Significant differences were also found in non-vessel total fibre fraction, (higher in cool-wet site,  $P = 0.061$ ), non-vessel total parenchyma fraction (higher in cool-dry site,  $P = 0.037$ ), and non-vessel axial parenchyma fraction (higher in cool-dry site,  $P = 0.021$ ).

Figure A3-2 Overall tissue fractions for *Leucopogon ericoides* from cool-wet site and *L. ericoides* from cool-dry site.

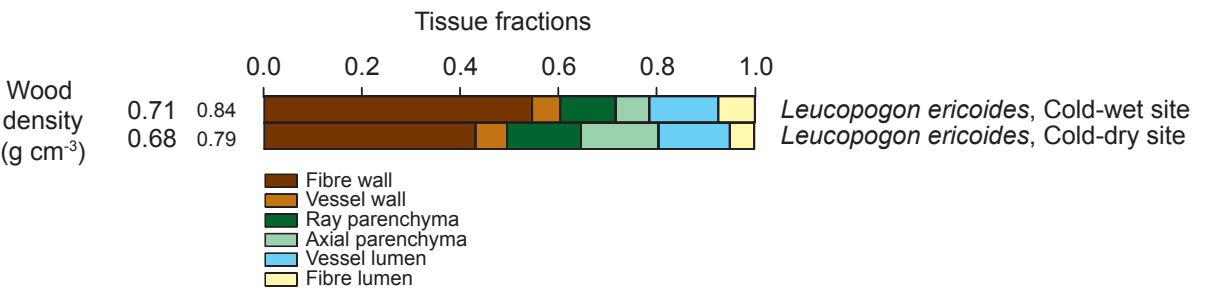


Figure A3-2 Overall tissue fractions for *Leucopogon ericoides* from cool-wet site and *L. ericoides* from cool-dry site. Overall wood density is indicted in large numbers on the left side of the graph, and non-vessel density is indicated in small numbers on the left side of the graph. Tissue fractions are at the top of the graph. Sampling sites are on the right side of the graph.

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## Chapter 4

# **WOOD ANATOMICAL VARIATION LARGELY INDEPENDENT OF WOOD DENSITY IN TWIGS OF 69 AUSTRALIAN ANGIOSPERMS**

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## 4.1 Abstract

Wood density has been suggested to be a key functional trait but does one value of wood density correspond to a single ecological strategy or to many? Species with the same density can have different anatomies and hence probably different ecological strategies. However, the extent of this anatomical variation has not been quantified on a large scale nor has it been linked with ecological strategies. Here, we aim to assess the magnitude of anatomical variation largely independent of wood density and explore its potential ecological implications.

Wood tissue fractions (fibre lumen and wall, axial and ray parenchyma, vessel lumen and wall, conduits with maximum lumen diameter below 15 $\mu$ m), pith area, and vessel properties in twigs were quantified across 69 species of angiosperm trees and shrubs. Wood density variation of analysed species was limited, in the range c. 0.4-0.6 g cm<sup>-3</sup> (1.6 fold). To search for potential ecological correlates of anatomical variation the species were sampled across rainfall and temperature contrasts, and some other plant traits were measured (height, leaf area to sapwood area ratio, and modulus of elasticity).

All anatomical traits varied more than wood density. The most variable traits were pith area (nearly 550-fold variation), ratio of vessel cross-sectional mean area (called here 'vessel area') to vessel number per area (almost 258-fold variation), fraction of conduits with maximum diameter smaller than 15  $\mu$ m (63-fold variation) and axial parenchyma fraction (26-fold variation). We also found considerable anatomical variation stretched along a strong trade-off between total parenchyma (axial parenchyma + ray parenchyma) and total fibre fractions (fibre wall + fibre lumen;  $r = -0.86$ ,  $P < 0.001$ ). Total parenchyma occupied a fraction of 0.12 to 0.66, and total fibre fraction ranged from 0.20 to 0.74 across studied species. Vessel properties (e.g. vessel area) correlated very weakly or not at all with parenchyma traits (total parenchyma, axial and ray parenchyma fractions) suggesting weak or no functional link between vessel area and parenchyma abundance. Axial parenchyma fraction and vessel area increased towards the equator. Surprisingly, however, vessel area did not differ significantly between the sites with similar temperature but contrasting rainfall. We did not find any clear relationships between parenchyma and measured functional traits.

Overall, there seems to be at least three axes of variation in xylem, substantially independent of each other: a wood density spectrum, a fibre-parenchyma spectrum, and a vessel area spectrum. Despite these efforts to understand it, the fibre-parenchyma spectrum does not yet have any clear or convincing ecological interpretation.

## 4.2 Introduction

There is a fascinating variety of wood anatomical structures (Baas 1982; Carlquist 2001; InsideWood 2004; Wheeler 2011) indicating plants have many ways of making a living or many 'strategies' (Grubb 1998; Westoby et al. 2002; Grime 2006). Plant hydraulic strategies, which link water transport with vessel traits, have received much of the attention (e.g. Zanne et al. 2010; Martínez-Cabrera et al. 2011; Gleason et al. 2012; Jacobsen et al. 2012). Strategies related to other wood tissues such as fibres, parenchyma and tracheids have received relatively less attention from functional ecologists. We have recently intensified our efforts to quantitatively describe the variation of proportions of those tissues across a wide number of species, especially non-forestry species (Fujiwara et al. 1991; Fujiwara 1992; Jacobsen et al. 2007; Martínez-Cabrera et al. 2009; Fichtler & Worbes 2012; Fortunel et al. 2013; Zheng & Martínez-Cabrera 2013). In this paper, we aim to describe anatomical variation of all main tissues across a broad number of species and to discuss their potential roles in ecological strategies. We specifically concentrate on anatomical diversity largely independent of wood density. This is because recent reports suggest a considerable variation in this dimension (Ziemińska et al. Chapter 3; Poorter et al. 2010), where functional implications remain unexplored.

Generally, wood is composed of several tissues, whose main functions are considered to be: 1) mechanical support performed by fibres, 2) carbohydrate and nutrient storage and distribution carried out by parenchyma, and 3) water transport via vessels (Evert 2006, wood tissues are shown on a cross-section in Figure 4-1a). We are still learning about the full spectrum of wood tissue functions, their specific mechanisms, and ecological implications.

Fibres (Fig. 4-1a), which mechanically support wood, are usually the most abundant tissue (Ziemińska et al. Chapter 3; Pratt et al. 2007; Martínez-Cabrera et al. 2009; Poorter et al. 2010; Fichtler & Worbes 2012, Fortunel et al. 2013). Parenchyma (Fig. 4-1a) is on average the second most abundant tissue (Ziemińska et al. Chapter 3; Martínez-Cabrera et al. 2009; Poorter et al. 2010; Fichtler & Worbes 2012, Fortunel et al. 2013) and it has multiple functions. Parenchyma has been shown to store metabolites (Sauter & van Cleve 1989; van Bel 1990; Evert 2006; Yamada et al. 2011) and has been suggested to store water (Holbrook 1995; Olson 2003; Chapotin, Razanameharizaka & Holbrook 2006; Lambers, Chapin & Pons 2008). It can participate in pathogen defence (Romero & Bolker 2008) and potentially in vessel refilling (Secchi & Zwieniecki 2011; Nardini, Lo Gullo & Salleo 2011). Finally, it may contribute to mechanical stiffness (Beery, Ifju & McLain 1983; Burgert & Eckstein 2001; Woodrum, Ewers & Telewski 2003) or aid in twisting and bending of lianas (Schenck as cited in Haberlandt 1914). Vessels (Fig. 4-1a) are usually the third most abundant tissue (Ziemińska et al. Chapter 3; Martínez-Cabrera et al. 2009; Poorter et al. 2010; Fichtler & Worbes 2012, Fortunel et al. 2013). Water transport is strongly affected by vessel cross-sectional area (that is the average area of vessels, not vessel fraction) and vessel number per area (Zanne et al. 2010), more so than by vessel fraction. This is because the water conductivity scales in response to the fourth power of vessel lumen diameter (from the Hagen-Poiseuille equation), and plants vary more widely with regard to vessel area to number ratio than with regard to vessel lumen fraction ( $S$  is vessel area to vessel number ratio and  $F$  is vessel lumen fraction in Fig. 2 in the broad survey compiled by Zanne et al. 2009). Finally, there are tracheids, the least abundant tissue (Ziemińska et al. Chapter 3) and in fact rarely quantified. Tracheids occur in a subset of species, which usually grow in cold and/or dry climates (Wheeler, Baas & Rodgers 2007). Their influence on wood density variation is not known. Tracheids in angiosperms are suggested to function as auxiliary water transport in case of significant dysfunction of vessels (embolised due to drought or freeze-thaw events, Carlquist 1984) and/or water storage (Sano et al. 2011; Carlquist 2012), but, to our knowledge, their contribution to those processes has not been quantified.

There are numerous reports on wood density variation and extensive discussion of how it might relate to ecological strategies ( $\text{g cm}^{-3}$ , review by Chave et al. 2009). Wood

density is obviously directly influenced by wood anatomy (e.g. Ziemińska et al. Chapter 3; Fujiwara et al. 1991; Jacobsen et al. 2007; Rana et al. 2009; Martínez-Cabrera et al. 2009; Fortunel et al. 2013), but cannot be seen as an indicator of any single anatomical trait exclusively (Ziemińska et al. Chapter 3). Anatomically, variation in wood density among species is mainly driven by fibre wall and lumen properties (Ziemińska et al. Chapter 3; Fujiwara et al. 1991; Jacobsen et al. 2007; Rana et al. 2009; Martínez-Cabrera et al. 2009; Fortunel et al. 2013) creating one dimension of variation. However, there is also a second dimension of variation that is largely independent of wood density or largely orthogonal to it, because species with similar density can have very diverse anatomies (Ziemińska et al. Chapter 3; Poorter et al. 2010). Yet little is known about this dimension of variation and how it might potentially relate to ecological strategies. In the present work, we concentrate on this dimension of variation by selecting species for study within a fairly narrow wood density range (0.4-0.6 g cm<sup>-3</sup>). This density range encompasses values studied previously (Ziemińska et al. Chapter 3), but in that study they were represented by a few species only. Here, we aim to look at a wider set of species to better quantify this dimension of variation.

Neither parenchyma fraction (axial + ray) nor fibre (wall + lumen) fraction is related to density variation but they are strongly negatively correlated with each other (Ziemińska et al. Chapter 3; Poorter et al. 2010; Fichtler & Worbes 2012). This suggests there is a dimension of variation running from high parenchyma fraction to high fibre fraction (but with a good share of the fibre being lumen in lower-density species), and that this dimension is substantially independent from wood density variation (Ziemińska et al. Chapter 3). Previous work on twigs showed that this dimension was widest in medium-density species (roughly 0.50–0.75 g cm<sup>-3</sup>, Ziemińska et al. Chapter 3). However, in that study only three sampled species had low density (roughly <0.5 g cm<sup>-3</sup>), and logically a wide variation in fibre and parenchyma fractions should be possible in low-density wood. It is also not known whether the fibre-parenchyma spectrum of variation has any links with hydraulic properties of wood. The present study focuses on the fibre-parenchyma spectrum of variation in twigs in low- and medium-density species (0.4-0.6 g cm<sup>-3</sup>) and on its links (if any) with other functional traits, including those related to hydraulic and mechanical properties.



We set out to address three broad groups of questions: 1) What is the nature of anatomical variation in low- to medium-density woods across a broad range of angiosperm species? 2) How much of the anatomical variation between species takes the form of variation across different climates, versus how much occurs as variation among coexisting species? To answer this group of questions we sampled species from three contrasting vegetation types: tropical rainforest, tropical woodland, and temperate forest (more detail in the 'Materials and Methods' section). 3) What can be inferred about the ecological or functional meaning of this variation? Within each broad group, we had more specific questions and hypotheses:

1) We aimed to describe the scale and axis of anatomical variation across low- to medium-density species (c.  $0.4\text{--}0.6\text{ g cm}^{-3}$ ), with special emphasis on understanding variation in the lower-density species (roughly  $< 0.5\text{ g cm}^{-3}$ ). The twig wood of low-density species studied so far in this thesis (two species) was composed of high fibre fraction together with relatively low parenchyma fraction (Ziemińska et al. Chapter 3). Yet a structure with high parenchyma fraction and low fibre fraction should also be a possible way to produce low overall wood density (as, for example, in stems of some rainforest trees studied by Poorter et al. 2010). Here we investigated a larger number of low-density species (23 species  $< 0.5\text{ g cm}^{-3}$ ) to assess the span of anatomical variation.

2) Three sites were chosen in order to explore links between the climate and anatomical variation: two tropical sites (rainforest and woodland) and one temperate site (temperate forest); they provided a contrast between temperatures (tropical sites versus temperate site), and within the tropics, a contrast of higher versus lower rainfall (rainforest versus woodland). Qualitative records report the incidence of species with abundant axial parenchyma increases towards the tropics in mature wood (Baas 1973; Wheeler et al. 2007), but this trend has not been assessed quantitatively, especially so in twigs. Additionally, relationships between axial and ray parenchyma and rainfall have only been quantified in one study on mature shrub stems of 61 species (axial parenchyma being weakly negatively associated with rainfall, ray parenchyma weakly positively associated; Martínez-Cabrera et al. 2009). We hypothesized higher fractions of parenchyma tissues would occur in the drier of the two tropical sites on the basis that high storage capacity for water or nutrients might be necessary to survive the stressful

dry season or to accelerate growth, flower, and fruit production during a shorter growing season. We also anticipated larger vessel area in the sites nearer the equator as found by many previous authors, (e.g. Baas 1973; van der Oever, Baas & Zandee 1981; Wheeler et al. 2007), and within the tropics we expected larger vessels in the wetter site (Barajas-Morales 1985; Jacobsen et al. 2012).

3) We searched for potential ecological implications of anatomical variation: a) Hypothetically, parenchyma fraction might be positively correlated with vessel size traits (average vessel area, hydraulically weighted diameter, vessel area to vessel number ratio) across species because large vessels are more prone to embolism (Davis, Sperry & Hacke 1999; Pittermann & Sperry 2003), and parenchyma has been suggested to participate in refilling after embolism (Secchi & Zwieniecki 2011; Nardini et al. 2011). This trend might be especially crucial within sites prone to drought or to freeze-thaw events. It is important to mention, however, that it has been questioned whether refilling under negative pressure is common (Sperry 2013; Wheeler et al. 2013). b) In addition, we asked whether anatomical traits are related to the ratio of leaf area to sapwood cross-sectional area, an indicator of potential transpiration demand (leaf area) relative to potential water supply capacity (sapwood area; Meinzer et al. 2008; Gleason et al. 2012). c) Higher carbohydrate storage has been shown to increase survivorship under shade stress in seedlings of seven species growing in moist forest in Panama (Myers & Kitajima 2007). On the basis, that parenchyma fraction might indicate storage capacity, we asked whether adult plants growing in the light-stressed environment of forest understory would have higher storage capacities in the form of more parenchyma. d) Finally, ray parenchyma fraction has been shown to increase with mechanical stiffness (Woodrum et al. 2003). We investigated whether this was also the case across species studied here. Additionally, we asked whether other parenchyma traits (total parenchyma, axial parenchyma) might be related to stiffness.

## **4.3 Materials and Methods**

## Plant material and sites

Three sites were chosen along the east coast of Australia, in New South Wales (NSW) and Queensland (QLD), in such a way as to give rise to contrasts both of temperature and of precipitation (site details in Table 4-1). A cool temperate forest site located in Kosciuszko National Park, NSW (36.48°S) was compared to two warm sites in Queensland: a tropical woodland in Girringun National Park (18.16°S, located approximately 15 km from Princess Hills site sampled in Chapter 3) and a tropical wet rainforest in Daintree National Park (16.10°S). The two tropical sites have similar mean annual temperature of c. 22°C but they vary markedly in mean annual precipitation (4230 mm and 995 mm respectively), with the lower-rainfall woodland also having a much longer dry season (Table 4-1). Mean annual temperature of the temperate forest site (c. 7°C, being at 1300m elevation) is approximately 15°C lower than at the tropical sites, and mean annual precipitation is 1835 mm of rainfall or equivalent snow. A canopy crane at the Daintree Rainforest Observatory (James Cook University) was used to collect twigs from tall trees. Shorter species were collected from the ground, and all species were collected within one kilometre from the crane. Species in the tropical woodland and the temperate forest were collected within a radius of c. 5 kilometres (with two species within c. 30 km) from the coordinates provided in Table 4-1. These sites were less rich in species, hence the wider search radius.

Twigs of more than 100 species of trees and shrubs were collected in total, from three replicate individuals per species. Three species overlapped with those studied in Chapter 3, but sampling and processing were separate. Over 40 species collected in the rainforest were targeted using a dataset of twig wood density as a guide (dataset compiled by S.A. Stuart, unpublished). From each of the two remaining sites, the tropical woodland and the temperate forest, approximately 30 of the most abundant species were collected. Wood density was measured on all sampled species (three replicates each with a few exceptions stated below), and only species with mean density between 0.38 and 0.62 g cm<sup>-3</sup> were selected for anatomical quantification. The lower density boundary was equal to the lowest-density measured across species sampled here. The higher cut-off was set so that each site was represented by at least ten species. This selection process resulted in the total of 69 species described here, spanning 48 genera

and 26 families (Table A4-1 in the Appendix). All species are evergreen except for four deciduous trees from the rainforest site (*Dysoxylum pettigrewianum*, *Ficus variegata*, *Palaquium galactoxylum*, and *Wrightia laevis*). Two rainforest species are pioneers (*Leea indica* and *Mallotus paniculatus*), and one species from the temperate woodland (*Exocarpos strictus*) is a hemi-parasitic shrub, parasitising roots of neighbouring trees, at least in the early phases of life.

Upper branches one metre long were collected and processed within 24 hours. A branch was divided into four main adjacent sections, using a wood diameter of c. 0.5 cm (under bark and excluding pith, i.e. the radius stretched from cambium to the outside of the pith) as a reference point. From the top of the branch the sections were as follows: 1) the segment above wood diameter of c. 0.5 cm for measurements of leaf area to sapwood area ratio, 2) a segment c. 10 cm long below 0.5 cm diameter for anatomical measurements, 3) a segment c. 5 cm long for wood density measurement, and 4) the remaining segment for mechanical tests (see details on specific measurement methods below). Occasionally, it was impossible to collect upper branches from tall canopy species (*Eucalyptus* and *Corymbia* species in the temperate forest and the tropical woodland). In those instances, shorter trees or lower branches were sampled.

## Height

The height of each individual tree and shrub sampled was measured using a measuring tape dropped from the crane gondola for tall species (at the rainforest site) or a measuring tape and a clinometer for shorter species at the rainforest site and for all species at the tropical woodland and temperate forest sites. Additionally, maximum heights of species were recorded from Flora of Australia (*Flora of Australia Online* 2013), from taxonomic literature, or in the absence of those, from online reports.

## Wood density

Within 24 hours from collection, bark and pith were removed, and wood was soaked in water for 48 hours. Wood volume was then measured using the buoyancy principle of Archimedes. A container filled with water was placed on a precision balance

and a thin wire platform was suspended in water so that it did not touch any walls or the bottom of the container. A twig was delicately placed on the wire platform and the mass of displaced water was recorded. The balance was tared before each measurement. The mass of displaced water was then used to calculate the volume of a twig assuming standard water density of  $1.0 \text{ g cm}^{-3}$ . Next, twigs were dried for at least 72 hours at  $105^{\circ}\text{C}$ , and mass was measured on a precision balance. The density was calculated as dry mass divided by the volume of soaked wood ( $\text{g cm}^{-3}$ ).

## Mechanical traits

Mechanical measurements were carried out within two weeks of collection on three replicates for each species (except for: *Dysoxylum parasiticum* and *D. pettigrewianum* from the tropical rainforest, four replicates, *Pouteria xerocarpa* from the tropical rainforest, one replicate, *Palaquium galactoxylum* from the tropical rainforest, two replicates, *Pimelea linifolia* from the tropical woodland, one replicate, and *P. linifolia* from the temperate forest, two replicates, *Grevillea glauca*, one replicate). Between collection and testing, twigs were stored in sealed plastic bags in a cool room where available ( $4^{\circ}\text{C}$ ) or in an air conditioned room (around  $20^{\circ}\text{C}$ ). A material testing machine (Model 5542, Instron Corporation, Canton, MA, USA) was used to carry out a three point bending test. Segments of twigs with length at least 20 times greater than diameter (including bark and pith) were used to measure modulus of elasticity (MOE).

## Anatomical traits

The anatomical traits measured were: average vessel area (called here 'vessel area'), pith area, and the fractions of cross-section contributed by each of vessel lumen, vessel wall, axial parenchyma, ray parenchyma, fibre wall, fibre lumen, conduits with maximum lumen diameter below  $15 \mu\text{m}$  (see below), and mucilage canals. Anatomical definitions followed 'IAWA list of microscopic features for hardwood identification' (IAWA Committee 1989). We acknowledge that, traditionally, angiosperm wood is referred to as a complex tissue composed of various cell types: vessels, parenchyma, and fibres (Evert 2006). Nevertheless, the various cell types are called here 'tissues' for brevity and

because they differ in morphology and functions. In addition to vessels, we established a category of conduits with maximum lumen diameter below 15  $\mu\text{m}$  (called hereafter conduits<sub>15 $\mu\text{m}$</sub> ). These were cells with overall diameter similar or in between that of small vessels and fibres. The walls resembled those of vessels in thickness, pit diameter, and a distance between pits on a circumference of a cell wall (IAWA Committee 1989; Sano et al. 2011). These cells would have been either tracheids or small vessels, however we were unable to confidently categorize them as one or the other from the cross-sections, hence the category conduits<sub>15 $\mu\text{m}$</sub> . In one species, *Pimelea linifolia*, cells that could have been either thin-walled fibres or parenchyma were observed. Those cells formed irregular patterns from a variable thickness band occurring off to one side off the pith to composing an entire growth ring and, as such, resembling ground tissue. The thickness of their walls varied from that of fibres to that of ray cells. Their diameters ranged from those similar to fibres to as large as vessel diameters. Nuclei and starch were not observed in those cells; pits and transverse walls were found only in a couple of cells. The transition between fibres and these cells within a growth ring was gradual, and no clear cut-off was observed in wall thickness or cell diameter. However, a clearer difference between axial parenchyma cells associated with vessels and the problematic cells was noted as the former ones had abundant simple pits and the latter ones did not. Based on those observations, the problematic cells were counted as fibres. These cells might be an example of fibre dimorphism where parenchyma-like fibres (or parenchyma) could have originated from libriform fibres (i.e. fibres with small, slit-like pits), and the two cell types are similar in appearance (Carlquist 2001).

All anatomical traits were measured on three replicates per species (except for: *Dysoxylum parasiticum* and *D. pettigrewianum* from the tropical rainforest, four replicates, *Palaquium galactoxylum*, two replicates, *Pouteria xerocarpa*, one replicate, and *Grevillea glauca*, one replicate). The twigs were placed in FAA fixative (formalin : acetic acid : 70% alcohol in proportions 5:5:90; Gerlach 1972) within 24 hours after collection. After four weeks, the fixative was replaced with 70% ethanol. The ethanol solution was replaced twice more through the following week with the third change of ethanol serving as a storing medium. Before sectioning, samples were placed in 50% ethanol and kept in an oven for up to 72 hours at 40-50°C. This treatment helped to soften the wood. Cross-

sections 10-50  $\mu\text{m}$  thick were cut using a sledge microtome (Reichert, Vienna, Austria) and disposable blades (model A35, Feather Safety Razor Co. Ltd, Japan). The sections were mounted in glycerol. High resolution photographs were taken at total 100x magnification using a Nikon digital camera (model DXM 1200F, Nikon Corporation, Japan) mounted on a light microscope (Olympus BX 50F, Olympus Co. Ltd., Japan) and Nikon ACT-1 imaging software (version 2.62, Nikon Corporation, Japan). Each image had dimensions 3840 x 3072 pixels and was saved in tif format. One to two of the most representative radial sectors were chosen and photographed in a sequence from pith to bark. The sectors avoided tension wood where possible. Where the focus was uneven within a field of view, several photographs were taken at different focal lengths. These photographs were then stacked together in Photoshop CS4 (Adobe Systems Incorporated, USA). Next, the photographs capturing the whole radial sector were merged as a sequence resulting in one image of the whole sector. The sector analysed was bounded by pith, rays, and bark.

Vessel lumens were coloured in Photoshop and then measured in Image-Pro Plus version 2.0.0.260 (Media Cybernetics Inc., USA). Approximately 30 to 500 vessels per replicate falling within a radial sector were measured. The protoxylem and newly produced vessels were excluded from measurements. To measure tissue fractions a grid of points 300 pixels (84.3  $\mu\text{m}$ ) apart in horizontal and vertical directions was overlaid over the image of the radial sector (Fig. 4-1b). A minimum 300 points for each sample were analysed. Each point was colour coded according to the tissue it fell into using Photoshop. Then image analysis software was used to count points and estimate tissue fractions (number of points of a given tissue divided by the total number of analysed points). Digital calipers were used to measure two perpendicular diameters of pith on freshly collected twigs, and then the area was calculated as an ellipse.

Additional anatomical traits were calculated as follows. Total parenchyma fraction was the sum of axial and ray parenchyma (called here 'total parenchyma'). Total fibre fraction was the sum of fibre lumen and wall fractions (called here 'total fibre'). Also calculated were axial parenchyma proportion relative to total parenchyma, fibre wall proportion relative to total fibre fraction, hydraulically weighted vessel diameter ( $D_H$ ), number of vessels per area ( $N$ ), and the average vessel area to number of vessels per

area ratio ( $S$ ; called 'vessel area to number ratio' for brevity). Axial parenchyma proportion relative to total parenchyma indicates how much space within total parenchyma is devoted to axial rather than to ray. Similarly, fibre wall proportion relative to total fibre fraction indicates how much total fibre space was occupied by fibre wall relative to lumen.  $D_H$  was calculated from the mean diameters for individual vessels as  $D_H = (\sum \text{diameter}^5) / (\sum \text{diameter}^4)$  (Sperry et al. 1994). Number of vessels per area ( $N$ ,  $\text{mm}^{-2}$ ) was calculated as vessel lumen fraction in the cross-section divided by arithmetic average of vessel lumen area ( $A$ ,  $\text{mm}^2$ ). The vessel area to vessel number ratio ( $S$ ,  $\text{mm}^4$ ; Zanne et al. 2010) was calculated as vessel lumen area ( $A$ ) divided by number of vessels per area ( $N$ ).

## Leaf traits

Leaf traits were measured on three replicates per species, on leaves from twigs with wood diameter of 0.5 cm (wood diameter measured under bark and excluding pith). In five species, one, two, or four replicates per species were measured (see 'Anatomical traits' section for the exceptions). For species with compound leaves, the rachis was included in measurements. For each species specific leaf area (SLA) was measured on at least seven simple leaves or one to two compound leaves.

The leaves were placed in a sealed plastic bag within 24 hours from collection, and stored in a refrigerator for up to a week and a half. Next, they were positioned under transparent plexiglass and photographed (Pentax K100 DSuper, Pentax Ricoh Imaging Company, Japan). The photographs were enhanced in Photoshop and the total area of leaves was measured in Image-Pro Plus. After the photographs were taken, the leaves were placed in paper bags and dried in a drying oven at 70°C for at least 72 hours. Leaf dry mass was then measured on a precision balance. SLA was calculated as mass of dried leaves divided by their leaf area.

Leaf area to sapwood area ratio (LA:SA) was measured at approximately 0.5 cm of wood diameter (under bark and excluding pith). One twig per replicate and three replicates per species were used (with few exceptions listed above). The two diameters of wood, perpendicular to each other, were noted using digital calipers, and sapwood area was calculated as an ellipse. The pith and bark were excluded from the



measurement. All leaves from this segment, except for the ones used for SLA, were placed in paper bags and dried at 70°C for at least 72 hours. Next, mass was measured on a precision balance, and the total leaf area on the shoot was calculated as SLA multiplied by the mass of all leaves. The total leaf area at wood diameter of 0.5cm was the sum of the area used for SLA and the remaining leaves. LA:SA was calculated by dividing the total area of leaves by the sapwood cross-sectional area calculated from the two diameters.

## Statistical analysis

Sixty-nine species were analysed, three replicates per species (except for particular cases mentioned above), and species arithmetic trait averages were used in analysis. Many traits showed approximately normal distributions across species (Shapiro-Wilk test,  $p < 0.05$ ): vessel lumen fraction, ray fraction, fibre lumen fraction, fibre wall fraction, total vessel fraction (vessel lumen + wall), total parenchyma fraction (axial + ray parenchyma), total fibre fraction (fibre lumen + wall), axial parenchyma relative to total parenchyma proportion, fibre wall relative to total fibre proportion, fibre wall + vessel wall fraction. The remaining traits did not exhibit normal distribution across species: height and maximum height, pith area, density, LA:SA, modulus of elasticity (MOE), vessel wall fraction, axial parenchyma fraction, conduits<sub>15µm</sub> fraction, mucilage canals fraction, vessel area (A), vessel area to number ratio (S), vessel number per area (N), and hydraulically weighted diameter ( $D_H$ ). Among those traits, log-transformations normalized the distributions of three traits only: vessel wall fraction, N and MOE. Consequently, for ease and clarity of analysis we did not use transformed values but rather ran non-parametric tests. To explore covariance among traits we used Pearson's correlation coefficients (for normally distributed variables) and Spearman's rank correlation coefficient (if one or both variables were non-normally distributed). Scatterplots were used for visual interpretation. To compare the sites one-way ANOVA was used (Holm-Šidák post-hoc test) for normally distributed traits with equal variances. For the traits not distributed normally or where there were unequal variances, Kruskal-Wallis test was used (Dunn's post-hoc test).

Additionally, two-way ANOVA was run to examine the effects of family and site on trait variation. This analysis was performed on species belonging to Myrtaceae and Proteaceae, as these were the only two families present in all three sites.

## 4.4 Results

### Overview of traits

Our first main question was about the scope of anatomical variation in species within a narrow density range (between 0.38 and 0.62 g cm<sup>-3</sup>, 1.6- fold variation). First, we report on this topic as well as on variation in other measured traits (height, modulus of elasticity, and leaf area to sapwood area ratio).

Among anatomical traits the most variable were pith area (almost 547-fold variation) and vessel area to number ratio  $S$  (>250-fold variation), followed by the fraction of conduits with maximum diameter smaller than 15  $\mu\text{m}$  (called here 'conduits<sub>15 $\mu\text{m}$</sub>  fraction', 63-fold variation) and the axial parenchyma fraction (26-fold variation, trait summaries and abbreviations are in Table 4-2). Among non-anatomical traits, the most variable were plant height, with 48-fold variation, and leaf area to sapwood area ratio (almost 30-fold variation). The least variable anatomical traits were fibre wall fraction, fibre wall proportion of total fibre fraction, total fibre fraction, total vessel fraction, and mucilage canals fraction, all with variation approximately 3.5-fold or less. The narrow variations in density (1.6-fold) and in fibre wall fraction were to be expected because for this study species were chosen within a relatively narrow band of densities (0.38-0.62 g cm<sup>-3</sup>).

Overall, there was a considerable variation in anatomical traits across species, which is illustrated in the stack bar graph (Fig. 4-2). On average, fibres including fibre lumen and wall were the most abundant tissue with mean fraction of 0.45 and 3.7-fold variation. Fibre wall fraction averaged at 0.32 (with 3.5-fold variation), and fibre lumen fraction averaged at 0.13 (roughly 16-fold variation). The second most abundant tissue was total parenchyma (axial and ray) with mean fraction of 0.35 and almost 6-fold variation (0.12-0.66). Since in this study we were mainly interested in parenchyma variation across species, Figure 4-2 is ordered accordingly from the highest total

parenchyma fraction to the lowest (green bars). Parenchyma components, axial and ray, occupied 0.14 (26.3-fold variation) and 0.21 (6.8-fold variation) respectively. Vessels (lumen + wall) occupied on average 0.18 of wood cross-section and varied 3.4-fold. Their lumen fraction was 0.13 with almost 4-fold variation and wall fraction was 0.04 with more than 5-fold variation. Conduits<sub>15µm</sub> occurred in 26 species (38% of all sampled species) and occupied 0.06 averaged across the 26 species with the conduits<sub>15µm</sub> present with more than 60-fold variation (or 0.02 averaged across all species). Mucilage canals occurred only in three species from Lauraceae (*Cryptocarya murrayi*, *C. mackinnoniana* and *Litsea leefeana*) and they occupied 0.01 averaged across the three species (or 0.0006 averaged across all species). This canal fraction was not included in subsequent analysis.

## Anatomical variation

Next, we inquired about the details of anatomical variation, especially fibre and parenchyma properties. Total fibre fraction was strongly negatively correlated with total parenchyma fraction ( $r = -0.86$ ,  $P < 0.001$ , Fig. 4-3). Fibre components, wall and lumen, were not correlated with each other ( $r = 0.11$ ,  $P = 0.38$ , but see the following paragraphs for more details). The components of parenchyma, axial and ray, were not associated with each other either ( $r = 0.19$ ,  $P = 0.11$ ).

Fibre wall fraction and fibre lumen fraction varied independently from each other (species represented by black circles in Fig. 4-4a), but considered together with species from a previous study (Ziemińska et al. Chapter 3; grey circles) the data points were distributed roughly in a triangle (Fig. 4-4a). Highest-density species (largest bubbles, Fig. 4-4a) had large fibre wall fraction and small fibre lumen fraction (top left of the graph). As the density decreased, the variability of fibre wall and lumen fractions increased and many anatomical combinations were observed: from medium fibre wall fraction and small fibre lumen fraction (e.g. 0.40 and 0.05 respectively) via medium fibre wall fraction and high fibre lumen fraction (e.g. 0.40 and 0.30 respectively) to low fibre wall and lumen fractions (e.g. 0.20 and 0.05 respectively). The terms 'high', 'medium' and 'low' are used for convenience only and correspond to the diagram in Fig. 4-4b. In fact, a continuum of values was observed. Trait values are listed in the Appendix (Tables A4-2, A4-3, A4-4, A4-5, and A4-6).

Total fibre fraction is the sum of fibre wall and fibre lumen fractions. Therefore, isolines can be drawn in Figure 4-4a (grey diagonal lines) to indicate total fibre fraction and to aid in interpretation. Total fibre fraction increased from the bottom left corner of the graph (isoline with fraction of 0.2) towards the diagonal of the graph (isoline with fraction of 0.7). The lowest-density species (smallest bubbles in Fig. 4-4a) varied widely in fibre fraction, and the width of this variation decreased towards highest-density species (largest bubbles, top left of Fig. 4-4a). Also, per given fibre fraction (along the isolines) lower density species (smaller bubbles) tended to have higher fibre lumen fraction relative to fibre wall.

Total fibre fraction was strongly negatively correlated with total parenchyma fraction (see the first paragraph in 'Anatomical variation' section). Correspondingly, species in lower left of Figure 4-4a (low fibre fraction) tended to have high parenchyma fraction. Species positioned along the centre diagonal isoline tended to have low parenchyma fraction. A schematic diagram in Figure 4-4b represents a summary of those relationships (see Discussion for more details), and Figure 4-5 shows cross-sections through low-density woods with various anatomies represented in the diagram Figure 4-4b.

Despite these general trends, inconsistencies were also observed. Species with similar total fibre, fibre wall, and lumen fractions sometimes differed in densities. This can be seen in Figure 4-4a (bubbles that are near to each other but differ in their size), but it is more informative to examine in the stack bar graph showing all the tissue components (Fig. 4-2). For example, two species at the top of Figure 4-2, *Cardwellia sublimis* and *Argyrodendron peralatum*, had similar structure but moderately different densities (0.47 and 0.58 g cm<sup>-3</sup>, respectively). This could possibly be explained by differences in starch content. Starch content was not formally quantified, but we rarely observed starch granules in *C. sublimis* versus frequently in *A. peralatum*. Starch density is high, c. 1.5 g cm<sup>-3</sup> (Gordon 1987; Rodriguez-Perez et al. 2011), so the amount of starch could potentially affect overall wood density. Alternatively or in addition to the starch effect, dissimilar densities might have resulted from parenchyma wall and/or conduits<sub>15µm</sub> wall. We did not quantify these fractions, but the thickness of the wall of axial parenchyma may be lower than that of rays (Carlquist 2007), and the proportion of

wall within ray parenchyma was found to be substantial across 50 woody angiosperm species (20 to 70%; Fujiwara 1992).

One species, *Pimelea linifolia*, was found at two sites (the tropical woodland and the temperate forest). *P. linifolia* growing in the temperate forest was significantly taller ( $t = -4.44$ ,  $P = 0.01$ ) than *P. linifolia* from the tropical woodland and had almost two-fold lower fibre lumen fraction ( $t = 8.02$ ,  $P = 0.003$ ) and a smaller total fibre fraction ( $t = 3.66$ ,  $P = 0.03$ ), but a larger proportion of fibre wall relative to total fibre fraction ( $t = -2.85$ ,  $P = 0.046$ ). *P. linifolia* from the tropical woodland measured larger vessel area, vessel area to number ratio and vessel hydraulically weighted diameter ( $t = 5.45$ ,  $P = 0.0045$ ,  $t = 4.02$ ,  $P = 0.016$  and  $t = 4.40$ ,  $P = 0.012$ , respectively), and correspondingly smaller vessel number per area ( $t = -3.39$ ,  $P = 0.023$ ). These differences in vessel properties could possibly be compensated by the fraction of conduits<sub>15µm</sub> ( $t = 4.09$ ,  $P = 0.015$ ), which was three times smaller in the tropical woodland (the site where vessel area was larger). Additionally, median total parenchyma fraction was marginally significantly lower in the tropical woodland (Mann-Whitney test,  $P = 0.10$ ; however, the species from the two sites had unequal variance,  $P < 0.05$ ). There were no significant differences in the other traits measured.

All trait values of species averages are listed in the Appendix (Tables A4-2, A4-3, A4-4, A4-5, and A4-6).

## Site comparisons

Our second main goal was to explore anatomical patterns across the three different climates and vegetation types: tropical wet rainforest (roughly: wet and warm climate), tropical woodland (dry and warm climate), and temperate forest (wet and cool climate; see Table 4-1 for site details). We were specifically interested in parenchyma and vessel trends.

Median of total parenchyma fraction was highest in the rainforest and did not differ between the tropical woodland and temperate forest sites (Kruskal-Wallis,  $H_{2,68} = 20.55$ ,  $P < 0.001$ , Fig. 4-6a). Mean axial parenchyma fraction was higher in the rainforest than in the temperate forest and was not different between the rainforest and the tropical woodland (ANOVA,  $F_{2,68} = 3.33$ ,  $P < 0.05$ ; Fig. 4-6b). It did not differ significantly

between the temperate forest and the tropical woodland either. Ray parenchyma fraction median was higher in the rainforest than in the two other sites, between which there was no difference (Kruskal-Wallis test,  $H_{2,69} = 17.22$ ,  $P < 0.001$ , Fig. 4-6c).

Vessel area median was smallest in the temperate forest but did not differ significantly between the rainforest and the tropical woodland sites (two warm sites of contrasting rainfall; Kruskal-Wallis test,  $H_{2,68} = 22.15$ ,  $P < 0.001$ , Fig. 4-6d). We also found that conduits<sub>15 $\mu$ m</sub> occurred in only two species from the rainforest (two of 41 sampled), and they were more common in the two other sites (ten of 11 species in the tropical woodland and 14 of 17 species in the temperate forest). ANOVA (Kruskal-Wallis analysis on ranks) of conduits<sub>15 $\mu$ m</sub> fraction was run on all species. Analysis revealed significant differences in median conduits<sub>15 $\mu$ m</sub> fraction ( $H_{2,25} = 46.15$ ,  $P < 0.001$ ). While post-hoc tests indicated significant differences between the tropical rainforest and woodland ( $Q = 4.215$ ,  $P < 0.05$ ), and between the tropical rainforest and the temperate forest ( $Q = 29.33$ ,  $P < 0.05$ ). There was no significant difference between the tropical woodland and the temperate forest.

Pith area median was largest in the rainforest and did not differ significantly between the tropical woodland and the temperate forest (Kruskal-Wallis,  $H_{2,68} = 35.56$ ,  $P < 0.001$ ; post-hoc tests: the rainforest vs. the tropical woodland,  $Q = 4.33$ ,  $P < 0.5$ , the rainforest vs. the temperate forest,  $Q = 5.05$ ,  $P < 0.5$ , the tropical woodland vs. the temperate forest,  $Q = 0.03$ ,  $P > 0.5$ ).

Species with density  $< 0.5 \text{ g cm}^{-3}$  were uncommon in the tropical woodland (one of 11 species) and the temperate forest site (one of 17 species; in fact it was the same species, *Pimelea linifolia*, that was collected in both sites and had density  $< 0.5 \text{ g cm}^{-3}$ ; Fig. 4-6f). In comparison, more than half of the species collected at the tropical rainforest site had density  $< 0.5 \text{ g cm}^{-3}$  (23 of 41 species). Wood density mean was significantly lower in the rainforest than in the two other sites between which there was no significant difference (ANOVA,  $F_{2,68} = 11.67$ ,  $P < 0.001$ ). In interpreting these results, it needs to be remembered that these species were not sampled at random from all species present, but selectively from the medium and low range of wood density. Median height was greater in the rainforest than in the two other sites, and there was no significant difference between those sites (Kruskal-Wallis,  $H_{2,68} = 38.10$ ,  $P < 0.001$ ). The

median of leaf area to sapwood area ratio was significantly higher in the rainforest than in the two other sites, which did not differ (Kruskal-Wallis,  $H_{2,68} = 42.29$ ,  $P < 0.001$ ). The median of modulus of elasticity was significantly higher in the temperate forest than in the rainforest and the tropical woodland, which did not differ from each other (Kruskal-Wallis,  $H_{2,68} = 21.95$ ,  $P < 0.001$ ).

## **Trait correlations across species**

Our third main objective was to assess potential ecological interpretations of anatomical variation across the studied species. We investigated relationships between parenchyma, vessels, and the three ecological traits: leaf area to sapwood area ratio, height, and modulus of elasticity.

Parenchyma properties measured (axial parenchyma, ray parenchyma, total parenchyma fractions, and the proportion of axial parenchyma relative to total parenchyma) were very weakly or not at all related to vessel traits (vessel area, hydraulically weighted diameter, vessel area to number ratio) or to leaf area to sapwood area ratio, either across all species or within sites. Axial parenchyma fraction tended to increase with vessel area ( $r = 0.31$ ,  $P = 0.009$ , Fig. 4-7a), vessel area to number ratio ( $r = 0.36$ ,  $P = 0.002$ ), and hydraulically weighted diameter ( $r = 0.29$ ,  $P = 0.013$ ) and to decrease with number per area ( $r = -0.35$ ,  $P = 0.003$ ). Ray fraction was not associated with vessel properties (Fig. 4-7b). The correlations between total parenchyma and vessel properties were similar to those between axial parenchyma and vessel properties, but with slightly smaller  $r$  values (total parenchyma fraction versus: vessel size,  $r = 0.30$  and  $P = 0.013$ ; vessel area to vessel number per area ratio,  $r = 0.30$  and  $P = 0.01$ ; vessel number per area,  $r = -0.29$  and  $P = 0.015$ ; no relationship with hydraulically weighted diameter).

Maximum height was weakly or not at all correlated with parenchyma properties either across sites or within sites. Across all sites, maximum height tended to increase with axial parenchyma ( $r = 0.36$ ,  $P < 0.01$ ) and, consequently, with total parenchyma ( $r = 0.25$ ,  $P < 0.05$ ), but there was no relationship with ray fraction ( $r = 0.05$ , ns). Within the tropical rainforest, axial parenchyma tended to increase with maximum height ( $r = 0.34$ ,  $P < 0.05$ ), while ray and total parenchyma fractions varied independently. There were no significant relationships within the two other sites. The results were similar but slightly

weaker when *Pimelea linifolia* was excluded from analysis (it was difficult to tell apart axial parenchyma from fibres in that species, which might have affected measurements; see 'Materials and Methods' for details). Leaf area to sapwood area had a weak tendency to increase with total parenchyma fraction and ray fraction ( $r = 0.33$ ,  $P = 0.006$  and  $r = 0.34$ ,  $P = 0.004$  respectively), but was not related to axial parenchyma fraction.

Vessel properties were well correlated with height, maximum height, and leaf area to sapwood area. Vessel area, vessel area to number ratio, and hydraulically weighted diameter increased with height ( $r = 0.64$ ,  $r = 0.55$ ,  $r = 0.52$ , respectively, all  $P < 0.001$ ), maximum height ( $r = 0.65$ ,  $r = 0.58$ ,  $r = 0.57$ , all  $P < 0.001$ ), and with leaf area to sapwood area ratio ( $r = 0.58$ ,  $r = 0.52$ ,  $r = 0.54$ , respectively, all  $P < 0.001$ ). Correspondingly, vessel number per area decreased with height, maximum height, and leaf area to sapwood area ratio ( $r = -0.47$ ,  $r = -0.50$ ,  $r = -0.48$ , respectively,  $P < 0.001$ ).

Modulus of elasticity (MOE) was weakly negatively associated with total parenchyma and axial parenchyma fractions ( $r = -0.41$ ,  $P < 0.001$  and  $r = -0.34$ ,  $P < 0.01$ , respectively, Fig. 4-8a, b) and not associated with ray fraction ( $r = 0.24$ ,  $P = 0.049$ , Fig. 4-8c) across all species. The strongest anatomical components of MOE variation were fibre wall fraction plus vessel wall fraction ( $r = 0.50$ ,  $P < 0.0001$ , Fig. 4-8d) and fibre wall fraction ( $r = 0.47$ ,  $P < 0.001$ ), where more elastic species tended to have lower wall fractions. MOE varied almost independently from wood density ( $r = 0.27$ ,  $P < 0.05$ ), remembering however that only part of the range of wood density was sampled in this study. Within sites, the effects of parenchyma tissues were somewhat different. Axial parenchyma fraction correlated negatively with MOE in the rainforest ( $r = -0.35$ ,  $P < 0.05$ ), but was not associated within the two other sites. While ray fraction was correlated negatively in those two sites (the woodland,  $r = -0.63$ ,  $P < 0.05$ , the temperate forest,  $r = -0.59$ ,  $P < 0.05$ ), but not correlated in the rainforest. Interpretation of these results requires additional caution because MOE variation within species was often larger than across species, especially so for the temperate forest site (see error lines in Fig. 4-8).

Pith area was positively correlated with ray parenchyma fraction (Pearson correlation, pith area log transformed,  $r = -0.44$ ,  $P < 0.001$ , Fig. 4-9) across the three



sites, but not within the sites. Considering direct contact between these two wood components, it is plausible they might be functionally interlinked.

## **Myrtaceae and Proteaceae comparisons**

Twenty-one species from two families, Myrtaceae and Proteaceae, were sampled considering all three sites. For this subset of species, effects of family and site on trait variation were examined using two-way ANOVA and Holm-Šidák post-hoc tests. The results should be interpreted with caution because the sample sizes within site and family combinations were not equal, and species were not sampled randomly, but within a narrow wood density range.

In Myrtaceae, total parenchyma fraction was on average lower than in Proteaceae (fraction of 0.31 and 0.44 respectively,  $F_{2,20} = 7.21$ ,  $P = 0.02$ , Fig. 4-10a). The fraction was higher in the tropical rainforest (0.52) than in the tropical woodland (0.34, post-hoc test:  $t = 3.47$ ,  $P = 0.007$ ) and the temperate forest (0.27, post-hoc test:  $t = 4.06$ ,  $P = 0.003$ ). There was no significant difference between the tropical woodland and temperate forest sites (post-hoc test:  $t = 1.22$ ,  $P = 0.24$ ). The effects of family and site on total parenchyma fraction were independent ( $P = 0.60$ ). The two components of total parenchyma, ray and axial parenchyma, followed, however, different patterns compared to total parenchyma fraction. Axial parenchyma, was interdependently influenced by family and site ( $F_{2,20} = 4.27$ ,  $P = 0.03$ ). The average axial parenchyma fraction in Myrtaceae was 0.16 and in Proteaceae was 0.17, and within those two families, the fraction followed different pattern across the three sites. Within Myrtaceae, axial parenchyma fraction was higher in the tropical rainforest than in the tropical woodland ( $t = 5.10$ ,  $P < 0.001$ ) and temperate forest ( $t = 5.10$ ,  $P < 0.001$ ), and there was no significant difference between the two latter sites ( $t = 1.24$ ,  $P = 0.23$ ). Whereas, in Proteaceae axial parenchyma fraction in the tropical rainforest and tropical woodland was higher than in the temperate forest ( $t = 3.27$ ,  $P = 0.01$  and  $t = 2.75$ ,  $P = 0.03$ , respectively, Fig. 4-10b). The fraction in the two tropical sites did not differ significantly ( $t = 0.74$ ,  $P = 0.47$ ). Ray fraction was on average smaller in Myrtaceae (0.15) than in Proteaceae (0.27,  $F_{2,20} = 14.72$ ,  $P = 0.002$ ), and there was no significant difference between the three sites across all species ( $F_{2,20} = 2.57$ ,  $P = 0.11$ ) nor within either family

(Fig. 4-10c). The effects of family and site were independent ( $F_{2,20} = 0.23$ ,  $P = 0.80$ ). Total fibre fraction varied conversely to total axial parenchyma fraction. Site and family had independent effects on the variation of this fraction ( $F_{2,20} = 1.07$ ,  $P = 0.37$ ). On average, total fibre fraction was higher in Myrtaceae (0.46) than in Proteaceae (0.36;  $F_{2,20} = 5.45$ ,  $P = 0.034$ ), opposite to total parenchyma fraction (Fig. 4-10d). Fraction in the temperate forest (0.52) was higher than the fraction in the tropical forest (0.27;  $t = 4.03$ ,  $P = 0.003$ ) and not significantly different from the fraction in the tropical woodland (0.44;  $t = 1.53$ ,  $P = 0.15$ ). The two latter sites differed significantly from each other ( $t = 3.10$ ,  $P = 0.015$ ).

Vessel average area was affected interdependently by both family and site ( $F_{2,20} = 9.43$ ,  $P = 0.002$ , Fig. 4-10e). The mean vessel area was on average  $0.00145 \text{ mm}^2$  in Myrtaceae and  $0.00115 \text{ mm}^2$  in Proteaceae. Within Myrtaceae, there was no significant difference in vessel area among the three studied sites. Whereas in Proteaceae vessel area was significantly bigger in the tropical forest and in the tropical woodland than in the temperate forest ( $t = 5.83$ ,  $t = 5.18$ , respectively, both  $P < 0.001$ ). There was no significant difference between the two tropical sites ( $t = 1.05$ ,  $P = 0.31$ ). Conduits<sub>15 $\mu$ m</sub> were not present in three Myrtaceae species from the tropical rainforest and two Proteaceae species, one from the tropical rainforest and one from the tropical woodland. The interaction between the site and family effects was marginally significant ( $F_{2,20} = 3.36$ ,  $P = 0.06$ , Fig. 4-10f). The average fraction of conduits<sub>15 $\mu$ m</sub> was almost the same in Myrtaceae and Proteaceae (0.034 and 0.033 respectively;  $F_{2,20} = 0.003$ ,  $P = 0.96$ ). However, the fraction followed different patterns within the families across the three sites. Within Myrtaceae, conduits<sub>15 $\mu$ m</sub> fraction was significantly lower in the tropical rainforest than in the tropical woodland and the temperate forest ( $t = 3.12$ ,  $P = 0.02$  and  $t = 2.31$ ,  $P = 0.07$  respectively). There was no significant difference between the tropical woodland and the temperate forest sites ( $t = 0.12$ ,  $P = 0.91$ ). While in Proteaceae, conduits<sub>15 $\mu$ m</sub> fraction was significantly larger in the temperate forest than in the two other sites (the tropical woodland,  $t = 3.48$ ,  $P = 0.007$ , the tropical rainforest,  $t = 3.87$ ,  $P = 0.005$ ). There was no significant difference between the two tropical sites ( $t = 0.66$ ,  $P = 0.52$ ).

## 4.5 Discussion

In this study, we aimed to describe anatomical variation approximately orthogonal to wood density and to explore possible ecological explanations for this variation. To our knowledge, this work is the first detailed account of this kind of anatomical variation in twigs across a broad sample of angiosperm species.

### The scope of anatomical variation

First main aim of this study was to describe the degree of anatomical variation in species within a limited density range ( $0.38\text{--}0.62\text{ g cm}^{-3}$ ). Ziemińska et al. (Chapter 3) showed that the variation in anatomical structures was the widest among medium-density species ( $0.5\text{--}0.75\text{ g cm}^{-3}$ ), leaving open however the possibility of high anatomical variation in lower-density species ( $<0.5\text{ g cm}^{-3}$ ). Here, we examined a larger number of lower-density species to address this issue.

There was wide variation in total parenchyma and total fibre fractions (Figs. 4-2 and 4-4a, b), and a strong trade-off was present between the two traits (Fig. 4-3). This trade-off has been observed previously across angiosperm species with more diverse densities (42 species from rainforest, 111 species from a wide variety of vegetation types within the tropical zone, and 24 species from tropical and temperate woodlands and forests; Poorter et al. 2010; Fichtler & Worbes 2012; Ziemińska et al. Chapter 3). Here we found that the scope of this trade-off increased towards low-density species. Additionally, within a given total fibre fraction, fibres could be composed of various proportions of wall relative to lumen (Fig. 4-4a, b). This further broadened the possible anatomical options, which are illustrated in Figure 4-4b (modified from Chapter 3). Examples of wood cross-sections of low-density species are shown in photographs in Figure 4-5. Species with densities lower than  $0.38\text{ g cm}^{-3}$  (not sampled in this study) would plausibly have even lower fibre wall fraction than it was found here (the minimum fibre wall fraction reported here is 0.15).

Anatomical structure in the lowest-density species studied here spanned a continuum from large total parenchyma with small total fibre and fibre lumen fractions to little total parenchyma with large total fibre and fibre lumen fractions. This result

confirmed earlier suggestions that lowest-density species could be composed of large parenchyma fraction and small fibre fraction (Ziemińska et al. Chapter 3). We propose that this fibre-parenchyma trade-off (meaning total fibre versus total parenchyma fractions) contributes an axis of variation that is largely orthogonal to wood density and that spans wider amplitude towards lower wood density. This implies that there may be substantial ecological strategy variation independent from wood density, especially among lower-density species.

A wider range of ecological strategies in lower-density species does not correspond to spanning a wider range of physical environments. On the contrary, in our study, species with density below  $0.5 \text{ g cm}^{-3}$  were abundant in the rainforest, but not in the two other sites that had lower rainfall or temperature. Also at global scale, lower density species are in a minority ("Global Wood Density Database" 2009). This suggests that strategies involving large parenchyma or fibre lumen fractions are not viable or not competitive in certain environments, for example, in the tropical woodland or the temperate forest sampled here.

Besides the general observation that similar density species could have diverse structure, we also recorded contrary instances. Species with similar anatomies could vary in their densities (Figures 4-2 and 4-4a). As indicated previously in the results, different densities could have stemmed from diverse starch content potentially influencing overall wood density. Variation in other tissue properties such as parenchyma wall fraction or conduits<sub>15µm</sub> wall fraction could also have played a role.

Vessel area has been shown to vary independently from wood density or with weak negative correlation across a large number of species (Jacobsen et al. 2007; Mitchell et al. 2008; Martínez-Cabrera et al. 2009, 2011; Fan et al. 2012). Was vessel area related to the fibre-parenchyma dimension? In our study, vessel area varied weakly or independently of parenchyma fraction (total, axial, and ray). Similarly, these properties were also unrelated in main stems across a set of species with wider density range (Martínez-Cabrera et al. 2009; Poorter et al. 2010; Fichtler & Worbes 2012; 61, 42, and 111 species, respectively, from tropical and subtropical zones). Thus, there seem to be at least three substantially orthogonal axes of variation: wood density, fibre-parenchyma, and vessel area.

## Site comparisons

### Parenchyma

Axial and ray parenchyma fractions had different patterns of variation across the sites. The spread of axial parenchyma fractions at the tropical forest encompassed the spread found at the tropical woodland and temperate forest. Ray parenchyma, in contrast, was shifted towards higher values at the tropical forest with the absolute range being similar between the sites. Across 81 species of *Ilex* (mainly mature stems), it has been noted that axial parenchyma is more abundant in the tropics than in the temperate regions (this finding is very likely to be valid, however, the methods of assessing parenchyma abundance or the statistical analysis used are not clear; Baas 1973). In addition, a global wood anatomy dataset indicates that the incidence of species with more abundant axial parenchyma is higher in the tropics (the abundance is inferred from axial parenchyma patterns rather than measured directly; Wheeler et al. 2007). This pattern has not been rigorously quantified and it is difficult to draw comparisons. Tentatively we can say that in twigs, similar to the mature stems analysed by Wheeler, Baas & Rodgers (2007), it is rather the incidence of species with abundant axial parenchyma that increases towards the tropics, while species with little axial parenchyma continue to be present. We did not find a trade-off between axial and ray parenchyma as was found in mature wood of shrubs and trees from tropical and subtropical climates (Martínez-Cabrera et al. 2009; Zheng & Martínez-Cabrera 2013; total more than 800 species studied).

Our second question was concerned with parenchyma trends across the precipitation gradient, and we hypothesized higher parenchyma fractions at the drier, more seasonal site. In fact, we did not find any difference in axial parenchyma fractions between the tropical rainforest and the tropical woodland, but ray parenchyma occupied larger fractions in the wet site (the tropical rainforest) than in the dry one (the tropical woodland). In a dataset of 61 species spanning across South and North Americas, axial parenchyma tended to be more abundant in warmer and drier sites but the correlations were weak (Martínez-Cabrera et al. 2009).

So far, as we are aware, there are no reports that convincingly demonstrate a function for a large fraction of parenchyma in the stem. If the fraction of parenchyma

indicates a capacity to store carbohydrates then there is the possibility, that parenchyma fraction is related to species ecological strategies. For example, starch storage has been shown to link with water stress (Wang, Quebedeaux & Stutte 1995; Naschitz et al. 2010), light stress (Myers & Kitajima 2007), with fruiting processes and climate seasonality (Chapin, Schulze & Mooney 1990; Landhäusser & Lieffers 2003; Naschitz et al. 2010), and with fire regimes (Pate et al. 1990; Bell, Pate & Dixon 1996). However, storage processes can differ between species (Barbaroux, Bréda & Dufrêne 2003; Körner 2003), and we do not yet have a quantitative analysis and a clear understanding of storage strategies on a global scale. Since stored carbohydrates are being withheld from commitment to growth, storage is not self-evidently an advantage; rather its benefits and costs need to be considered in particular climate contexts. Alternatively, in some instances parenchyma may serve as a storage compartment for water rather than for carbohydrates. This has been shown for several species (Holbrook 1995; Chapotin et al. 2006), but since most of them were cacti, Crassulaceae or baobabs, it remains uncertain how this might apply to more lignified stems with normal wood cylinder.

### **Water transport: vessels and tracheids**

Within the tropics, we expected smaller vessels in the dry site (the tropical woodland) than in the wet site (the tropical rainforest). The dry site had four times lower precipitation during the wettest month and a much longer dry season (Table 4-1). These conditions would be expected to lead to lower stem water potentials, carrying higher risk of incurring drought-induced embolism. It has been suggested that small vessels may be less prone to drought-induced embolism (Hargrave et al. 1994). Surprisingly though, there was no difference in average vessel area in our study. Moreover, variation in vessel area between species was wider at the wet site than at the dry one. Possibly the vessel size traits of species at the dry site can be thought of as adapted to take advantage of the short wet season, and the plants use different strategies to protect themselves during the dry season (Gleason et al. 2013; Crivellaro et al. 2012). Other relevant strategies include fine-scale modifications of intervessel pitting (Jansen et al. 2004; Choat, Cobb & Jansen 2008). Jansen et al. (2004) found the highest incidence of

species with vestured pits in deserts and in tropical seasonal woodlands. Vestured pits have been suggested to participate in embolism resistance (Zweypfenning 1978; Jansen et al. 2003; Choat et al. 2004,) and potentially they could play a role in the dry site. The other strategy might be related to the fraction of very small conduits (here: conduits narrower than 15  $\mu\text{m}$  called 'conduits<sub>15 $\mu\text{m}$</sub> ', which could be small vessels or tracheids). They were relatively abundant at the dry site, but not at the wet one. These conduits can potentially safely transport or store water (Sano et al. 2011) and have been implied to play a significant role in water stressed regions (Carlquist 1985, 2012; Carlquist & Hoekman 1985).

The fraction of conduits<sub>15 $\mu\text{m}$</sub>  was also relatively high in the temperate forest where temperatures below 0°C occur in winter. These climatic conditions are prone to freeze-thaw embolism. It has been shown that the smaller the vessels the more resistant they are to this type of embolism (Davis et al. 1999; Pittermann & Sperry 2003). Indeed, in our study, species from the temperate forest had significantly smaller vessels than in the tropical sites, as well as a larger fraction of conduits<sub>15 $\mu\text{m}$</sub> . These both would be expected to reduce the risk of freeze-thaw embolism.

## **Trait comparisons**

### **Water transport and parenchyma**

We hypothesized that species with vessels more prone to embolism might have more parenchyma tissues to participate in embolism repair, especially in sites with longer and/or more severe stress periods (dry seasons or low temperature seasons). It has been suggested that either or both of axial and ray parenchyma may facilitate embolism repair and vessel refilling (Salleo et al. 2004; Améglio et al. 2004; Secchi & Zwieniecki 2011; Nardini et al. 2011). However, we found only weak relationships between vessel properties (vessel area, hydraulically weighted diameter, vessel area to number ratio) and parenchyma properties (total parenchyma, axial and ray parenchyma), either across sites or within individual sites (Fig. 4-7a, b and c). Perhaps the presence of parenchyma itself rather than the fraction of cross-section contributed might be more important in embolism repair, and even a small proportion of

parenchyma tissues might be enough to refill vessels. Alternatively, large vesseled species, although in principle more prone to embolism, might in fact avoid it by having sufficient access to water (for example, via deeper roots). An additional explanation could be that vessel refilling under negative pressure might not be in fact as common as it has been thought (Sperry 2013, Wheeler et al. 2013).

## **Height and parenchyma**

We hypothesised that shade tolerant species in the rainforest, a site with a strong light gradient, would have higher parenchyma fraction. This hypothesis is based on the proposition that more parenchyma represents higher capacity to store metabolites. This hypothesis was also inspired by the finding that higher storage tended to increase seedling survival during light stress in seven rainforest species (Myers & Kitajima 2007). We found the opposite trend than predicted, axial parenchyma fraction tended to increase with maximum height within the rainforest ( $r = 0.34$ ,  $P < 0.05$ ), while ray parenchyma varied independently. What might be causing this contradiction? Firstly, the premise that high parenchyma fraction corresponds to high metabolites storage may not be valid in some cases. For example, in baobabs large proportions of parenchyma seem to be related to water storage rather than metabolites (however, this water may be used mainly for mechanical purposes than transpirational; Chapotin et al. 2006). We are not aware of studies that directly link parenchyma fractions with metabolites storage in adult woody species; certainly such studies would be of great help in interpreting variation of parenchyma fractions. Secondly, tall species may need parenchyma for different purpose than short species. For example, taller species being more exposed to winds might build more elastic twigs. Here, tall species in the rainforest site tended to have lower MOE (the lower MOE the more elastic wood is;  $r = -0.62$ ,  $P < 0.001$ ). This lower MOE could be partly due to higher axial parenchyma fraction ( $r = -0.35$ ,  $P < 0.05$ ).

## **Modulus of elasticity and parenchyma**



Finally, we asked whether parenchyma fractions, especially ray parenchyma, might affect modulus of elasticity (MOE; high MOE corresponds to stiffer wood). It has been shown across large datasets that wood density is positively correlated with MOE (Chave et al. 2009). However, there is also substantial variation in mechanical properties that is independent of wood density (Hepworth et al. 2002; Onoda, Richards & Westoby 2010) and likely is influenced by anatomical structure (Hepworth et al. 2002). Ray fraction was positively correlated with MOE in twigs across five species of *Acer* with wood density from 0.47 to 0.72 g cm<sup>-3</sup> (Woodrum et al. 2003). However, we found no such trend across species sampled here, and it was rather fibre wall plus vessel wall fraction that positively influenced MOE variation across all species. The lack of trend between ray fraction and MOE across all sampled here species might have several origins. Firstly, the influence of rays on mechanical properties might depend on the proportion of ray wall within ray fraction. Presumably, higher ray wall fraction would increase overall twig stiffness. Secondly, other ray properties, such as width, height, and number of rays per mm may play a more important role in stiffness than ray fraction. Thirdly, the variation in fibre wall and vessel wall fractions might have obscured the effects of ray fraction on MOE.

## Density of cell wall material

Density of swollen cell wall material has been shown to be around 1 g cm<sup>-3</sup> and relatively consistent across species (Kellogg & Wangaard 1969 and literature cited therein). However, among our dataset, the calculated swollen cell wall density varied from 1.09 to 2.66 g cm<sup>-3</sup> (stack bar graph in Fig. 4-11). There are several reasons for this discrepancy as discussed below.

Swollen cell wall material density can be calculated as overall wood density divided by total cell wall fraction (Kellogg & Wangaard 1969). And the overall wood density is measured as the ratio of dry wood mass to green or soaked volume (as it is done in many ecological studies; e.g. Martínez-Cabrera et al. 2009; Poorter et al. 2010; Fan et al. 2012; Fortunel et al. 2013), and cell wall fraction is calculated on swollen material (e.g. soaked material). Here, we did not use total cell wall fraction, but fibre wall fraction plus vessel wall fraction, as they were the only wall fractions we had measures

for (the remaining ones being parenchyma and conduits<sub>15µm</sub> walls). So that cell wall material density was calculated as follows: overall wood density / (vessel wall fraction + fibre wall fraction). The results are presented on a stack bar graph in Figure 4-11.

A first reason for the discrepancy might be that we did not use the total wall fraction. If we had, the calculated values of cell wall material would have been lower, and hence more in concordance with previous studies (Kellogg & Wangaard 1969 and literature cited therein). A second reason might be that our overall density measurements were overestimated for species which contained substances such as, for example, starch or mucilage (starch density is approximately  $1.5 \text{ g cm}^{-3}$ , Gordon 1987; Rodriguez-Perez et al. 2011). If those substances had not contributed to the overall density, this density would have been lower, and the estimated density of cell wall material would similarly have been lower. A third reason is related to the method of cell wall fraction measurements. Kellogg and Wangaard (1969) have shown that there is discrepancy in wall and lumen fraction estimations from blocks of wood vs. from cross-sections. This is because a cut block of wood and a cross-section undergo different levels of deformation (swelling or shrinkage of walls and lumens) during the preparation process. We did not estimate fractions from a twig, but only from a cross-section and have no reference point to estimate the error. Nevertheless, for example, in Kellogg & Wangaard (1969), the wall proportion estimated from a wet section varied from 4.4% lower to 9.7% higher relative to the fraction estimated from a surface of a wet block (across five angiosperms and two gymnosperms). Moreover, the authors have indicated that the error was bigger for lower density species. All those reasons were likely to have contributed to the relatively wide variation in the estimated density of swollen cell wall material.

In this study we draw attention to substantial variation in anatomical structure that is largely independent of wood density and, to our knowledge, has not been discussed widely in the trait ecology literature so far. Over recent years, functional ecologists have paid increased attention towards wood anatomy, and are becoming more aware of anatomical variations. We suggest there are several dimensions of variation that are substantially independent of each other, meaning that all sorts of

combinations among them can be found: 1) wood density variation, mainly driven by fibre wall and lumen fractions (Ziemińska et al. Chapter 3; Fujiwara et al. 1991; Jacobsen et al. 2007; Rana et al. 2009; Martínez-Cabrera et al. 2009), 2) a fibre-parenchyma axis of variation, with the breadth of variation increasing towards lower density species, and 3) a vessel area dimension. Among these, the vessel area dimension is relatively well understood and indicates water conductive safety and efficiency strategies (Tyree & Zimmermann 2002).

In this work, we sought potential ecological correlates of the fibre-parenchyma dimension of variation. Nevertheless, this dimension proved to be very weakly or not at all correlated with the other traits measured, nor with the climate at different sites. Consequently, the ecological significance of fibre-parenchyma variation remains unclear. Nevertheless, this study has made a contribution by describing quantitatively this intriguing anatomical variation, and by rejecting some of the possible hypotheses about what it might be correlated with.

## **4.6 Acknowledgements**

Many people helped throughout this study. We thank national parks staff and Ross and Maxine Blennerhasset at the Goshen cattle station for hospitality and access to sites. Queensland and New South Wales Governments gave permission to carry out this work. We are very grateful to Stephanie A. Stuart for the wood density data of the rainforest species, which hugely helped to better target our species. Warm thanks to everyone who assisted in the field and the lab: Chris, Irone and Tim Malloy, Yvonne Chang, Julieta Rosell, Mark Olson, Andrew Ford, Alicia Cook, Claire Laws, and Tanja Lenz. We kindly thank CSIRO Atherton and Andrew Ford for access to the lab facilities and fieldwork equipment, as well as the Daintree Rainforest Observatory staff, Peter Byrnes and Andrew Thompson, for their hospitality and cooperation during our stay in the observatory. Warm thanks to Andrew Ford, Alison Downing, and staff from the National Herbarium of New South Wales (Andrew Orme, Peter Weston, and Barbara Wiecek) for superb help with plant identification. This study was sponsored by the Macquarie

University Research Excellence Scholarship for KZ and James Cook University student grant for work in the Daintree Rainforest Observatory awarded to KZ and Julieta Rosell.

## 4.7 Figures

Figure 4-1 Illustration of wood tissues (a) and image analysis method used (b).

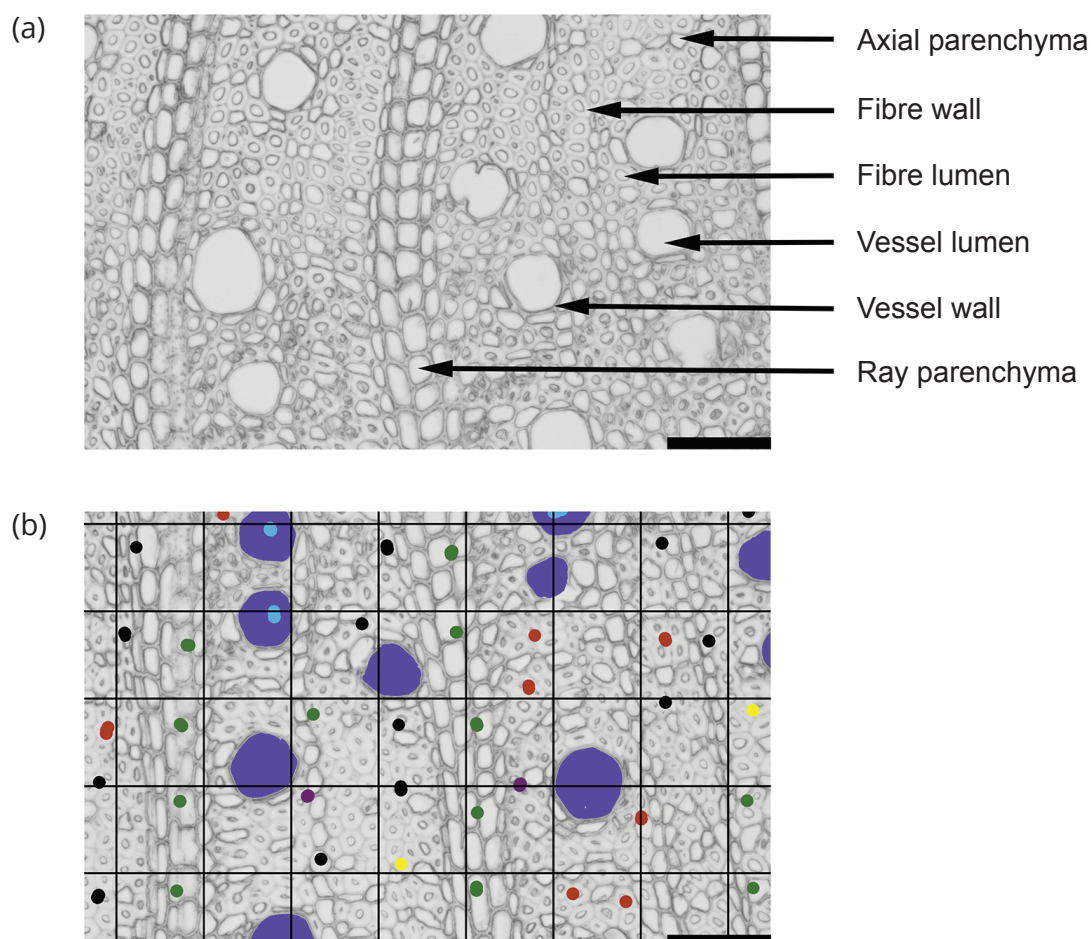


Figure 4-1 Illustration of wood tissues (a) and the image analysis method applied in this study (b), shown on an example of *Gomphandra australiana*, Icacinaceae. (b) Vessels were manually coloured (large circles in dark blue), and then the vessel areas were measured in image analysis software (see text for details). Grid method was used to estimate tissue fractions. Grid points were marked according to the tissue they fell in: light blue – vessel lumen, purple – vessel wall, red – axial parenchyma, green – ray parenchyma, yellow – fibre lumen and black – fibre wall. For clarity, the picture illustrates only a fragment of a larger, pie-shape area analysed. Scale bar corresponds to 100  $\mu\text{m}$ .

Figure 4-2     Stack bar graph of tissue fractions across 69 species. Each bar represents an individual species with the top bar representing fractions averaged across all species. Species are sorted in order of decreasing total parenchyma fraction (axial + ray). Numbers on the left side indicate wood density of a given species. Species name, family, and site are given on the right side of the graph. Site codes stand for: R – tropical rainforest (Cape Tribulation), W – tropical woodland (Blencoe Falls), and F – temperate forest (Thredbo).

Figure 4-2 Stack bar graph of tissue fractions across 69 species. See figure caption on opposite page.

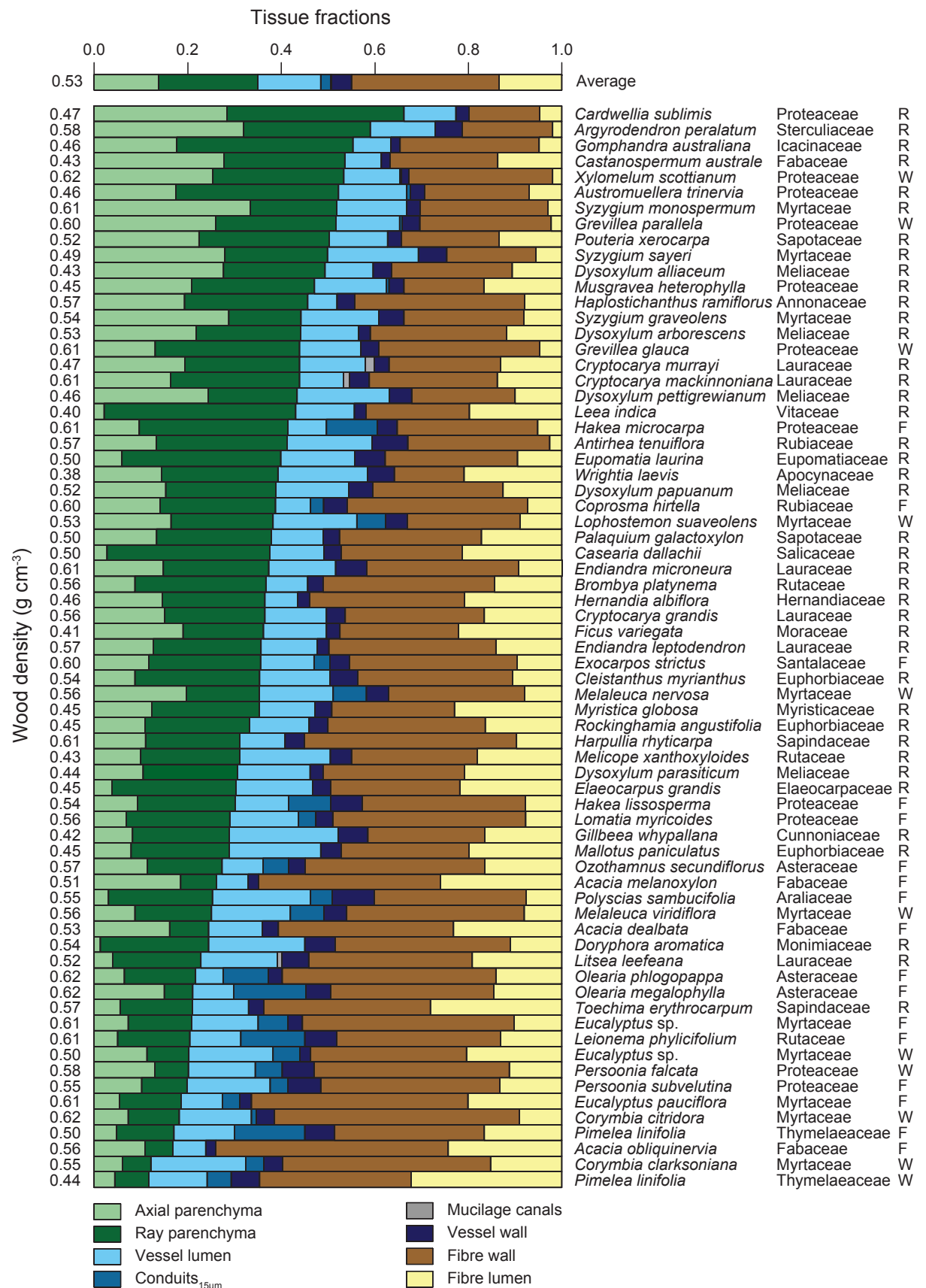






Figure 4-3 Relationship between total fibre fraction and total parenchyma fraction.  
\*\*\*  $P < 0.001$ .

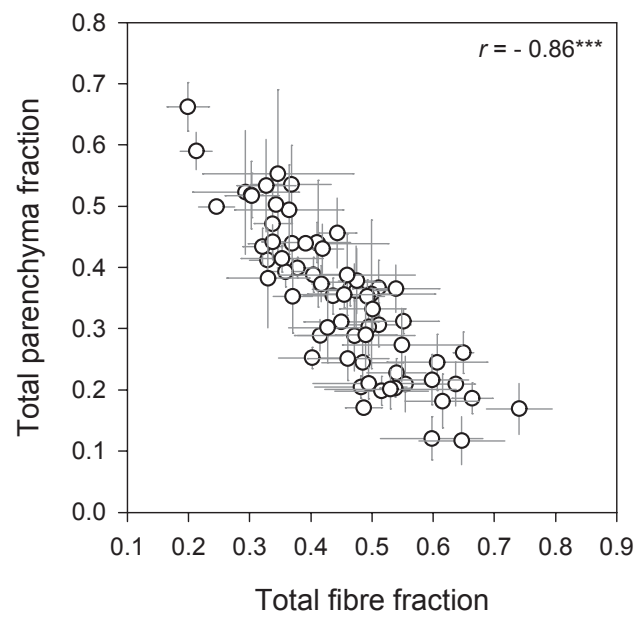


Figure 4-4 Relationship between fibre wall fraction and fibre lumen fraction across 93 species (69 from this study, black symbols, 24 from Chapter 3, grey symbols).

(a) Symbol diameter is proportional to species wood density, with the smallest diameter corresponding to the lowest density and the largest diameter to the highest density. Isolines indicate total fibre fraction increasing from bottom left corner towards the diagonal of the graph by a step of 0.1. Grey numbers above the X axis correspond to total fibre fraction indicated by a given isoline.

(b) A diagram illustrating Figure 4-4a flipped clockwise by 45° (flipped Figure 4-4a shown in upper left corner for comparison; diagram modified from Chapter 3). The six squares symbolize cross-sections through wood. Hexagons are fibres with fibre wall in brown and fibre lumen in bright yellow. Green is parenchyma (axial + ray). Vessel fraction did not show large variation across species and for simplicity was omitted in this diagram. Wood density increases towards the top of the diagram. Total fibre fraction (brown wall + yellow lumen) and total parenchyma fraction (green, includes axial + ray) covary negatively with each other and approximately orthogonally to wood density.

Figure 4-4 Relationship between fibre wall fraction and fibre lumen fraction across 93 species (a) and a diagram illustrating this relationship (b).

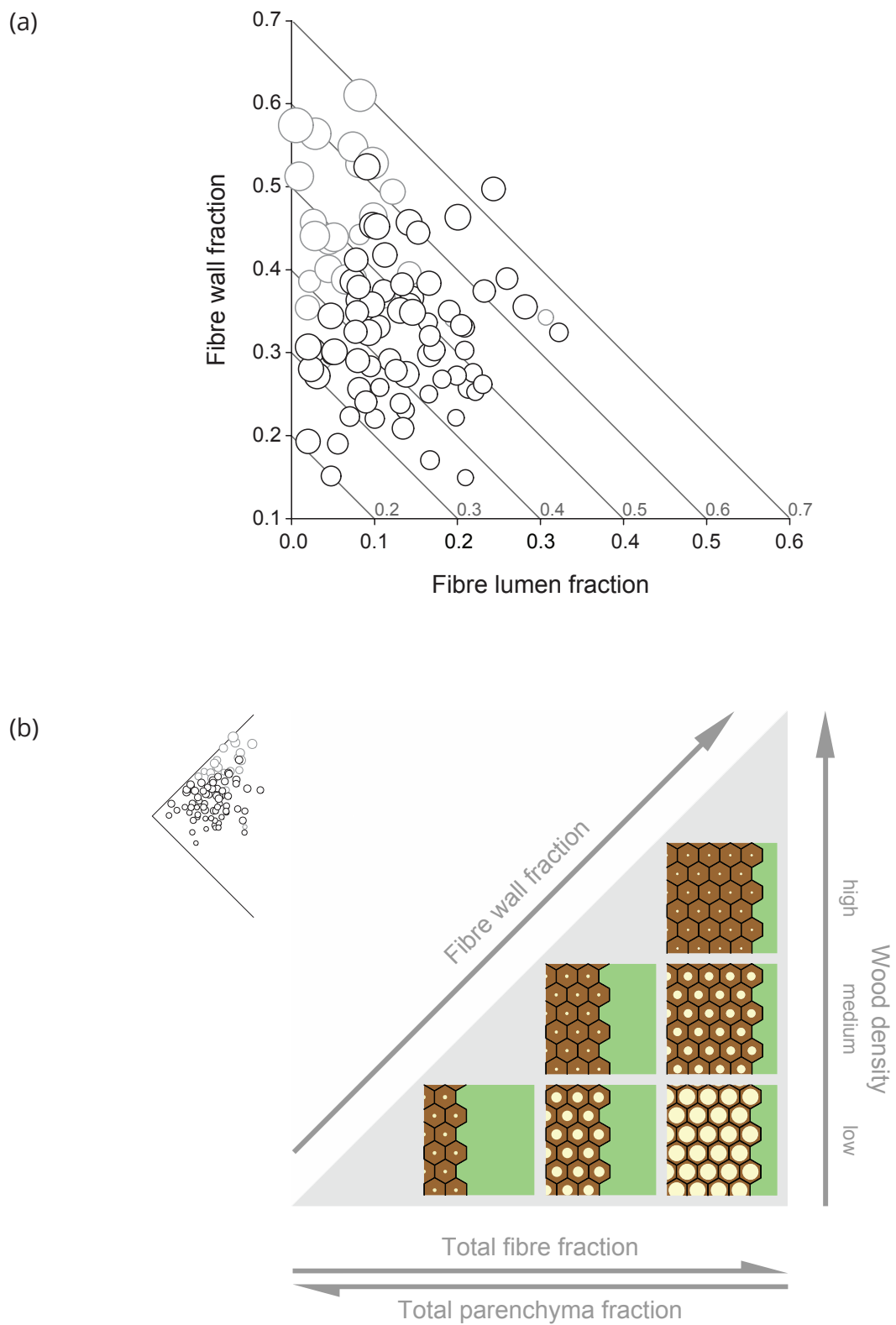




Figure 4-5 Cross-sections through twigs of three low-density species.

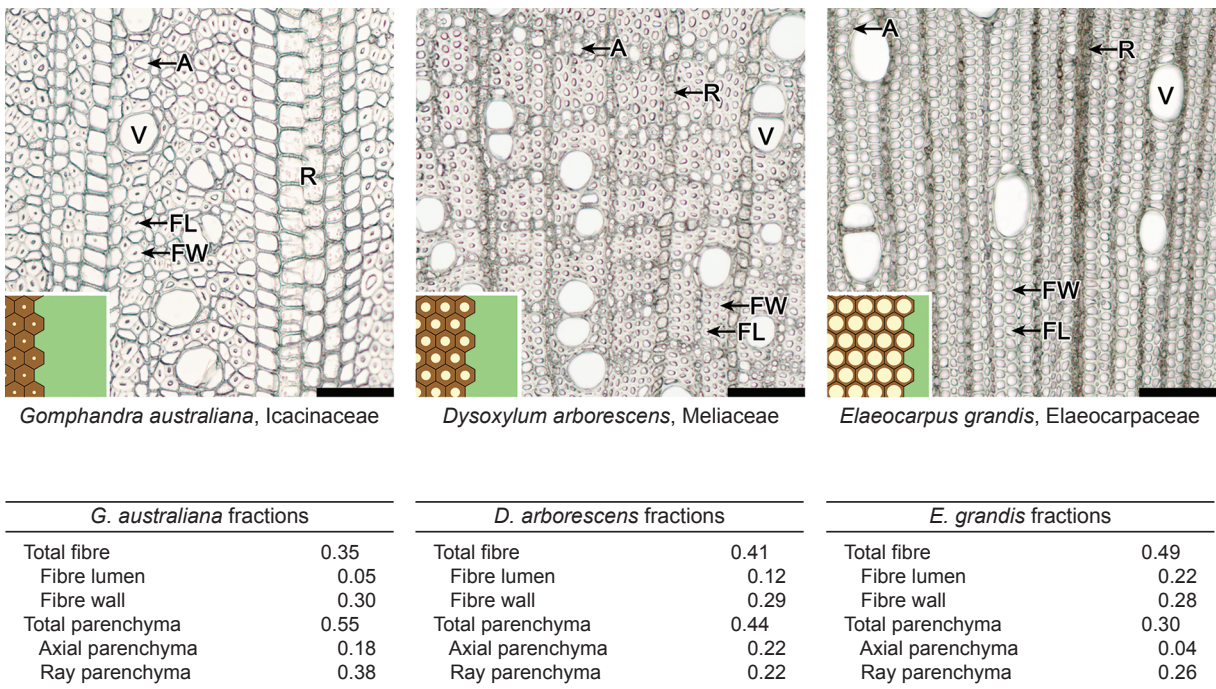


Figure 4-5 Cross-sections through twigs of three low-density species. These approximately correspond to the three low-density anatomies in Figure 4-4b (diagrams repeated here below images). *Gomphandra australiana* to the left (wood density  $0.47 \text{ g cm}^{-3}$ ), *Dysoxylum arborescens* in the middle (wood density  $0.53 \text{ g cm}^{-3}$ ), and *Elaeocarpus grandis* to the right (wood density  $0.44 \text{ g cm}^{-3}$ ). All three species were sampled in the tropical rainforest (Cape Tribulation). V – vessel, FL – fibre lumen, FW – fibre wall, A – axial parenchyma, R – ray parenchyma. Axial parenchyma in *E. grandis* is hardly discernible at this resolution (but higher resolution photos were used for image analysis, and axial parenchyma was possible to identify). Tissue fractions of each corresponding species are listed below the images. Scale bar corresponds to  $100 \mu\text{m}$ .



Figure 4-6 Box plots showing differences in anatomical traits and wood density between sites (and vegetation types).

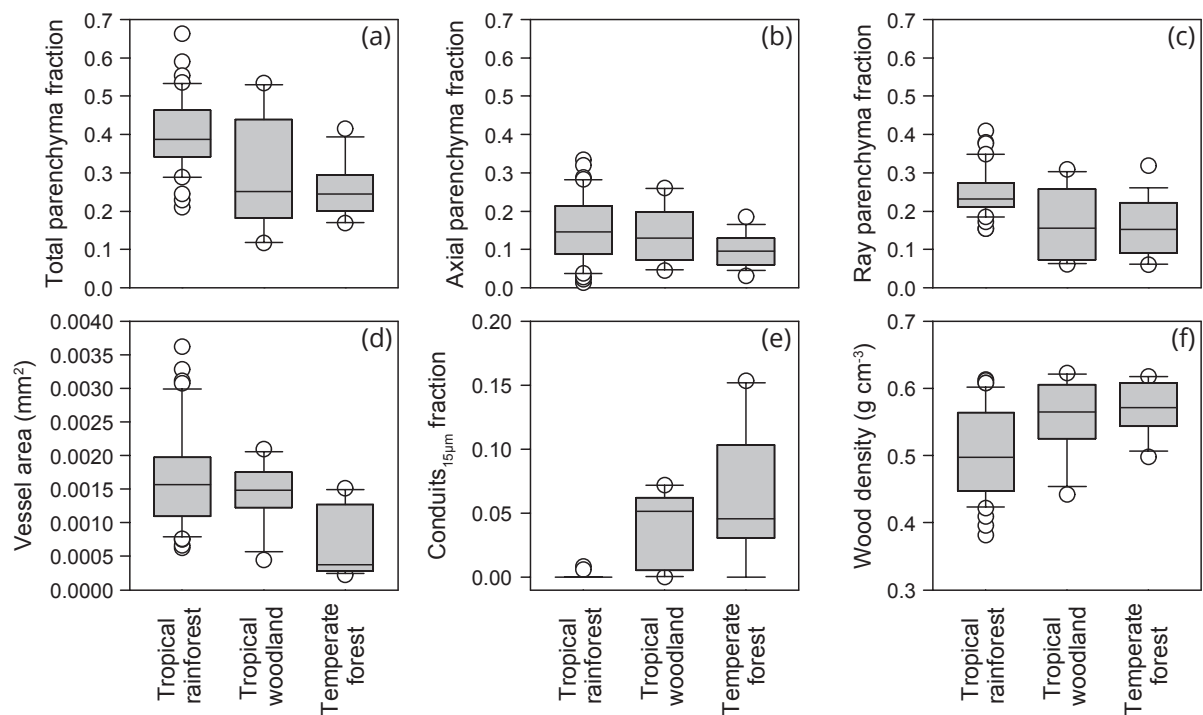


Figure 4-6 Box plots showing differences in anatomical traits and wood density between sites (and vegetation types): tropical rainforest (warm and wet site), tropical woodland (warm and dry site), and temperate forest (cool and wet site). The black line inside the grey box is a median. The box top and bottom boundaries indicate upper and lower quartile, and the whiskers are highest and lowest values excluding outliers. Circles are outliers, and each circle corresponds to an individual species.

Figure 4-7 Relationships between parenchyma traits and vessel area (i.e. cross-sectional average area). Each symbol corresponds to an individual species. Symbol type represents site of collection: green circles – tropical rainforest (warm and wet site), red squares – tropical woodland (warm and dry site), and blue triangles – temperate forest (cool and wet site). \*  $P < 0.05$ , \*\* $P < 0.01$ , ns – not significant.



Figure 4-7 Relationships between parenchyma traits and vessel area. See figure caption on opposite page.

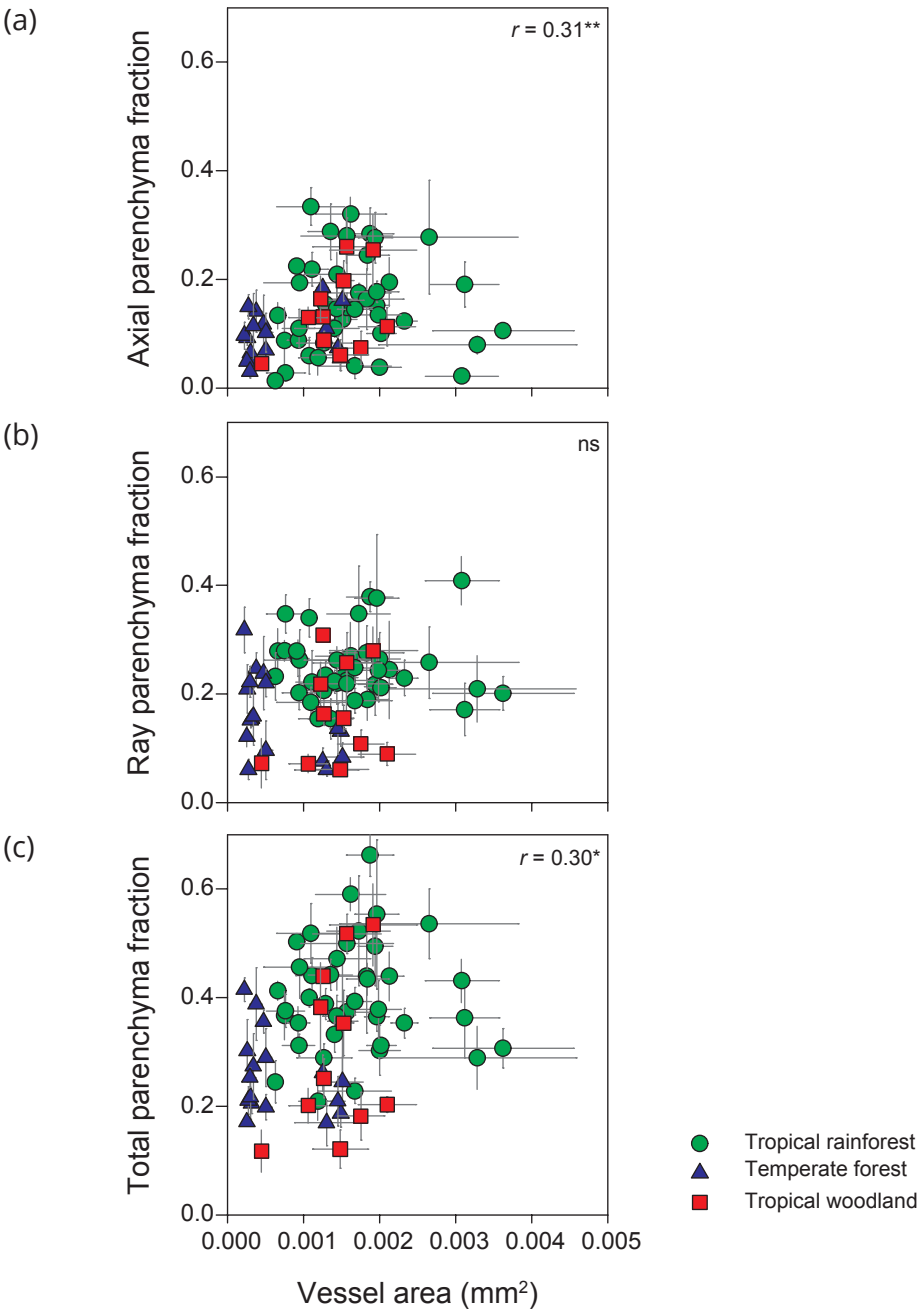


Figure 4-8 Relationships between parenchyma traits, fibre wall fraction plus vessel wall fraction and modulus of elasticity. Each symbol is an individual species. Symbol type represents site of collection: green circles – tropical rainforest (warm and wet site), red squares – tropical woodland (warm and dry site), and blue triangles – temperate forest (cool and wet site). \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ .

Figure 4-8 Relationships between parenchyma traits, fibre wall fraction plus vessel wall fraction and modulus of elasticity. See figure caption on opposite page.

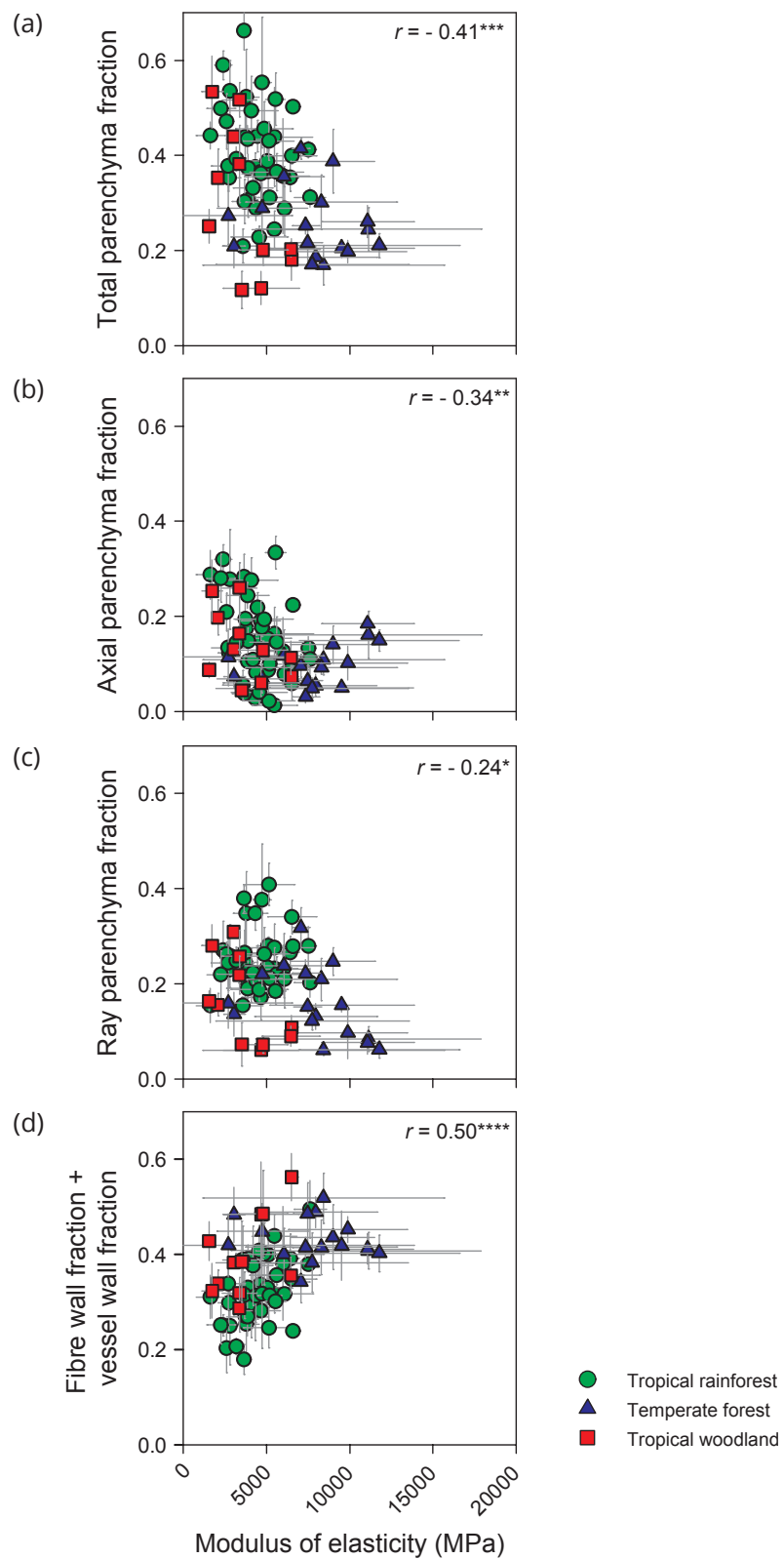




Figure 4-9 Relationship between ray parenchyma fraction and pith area.

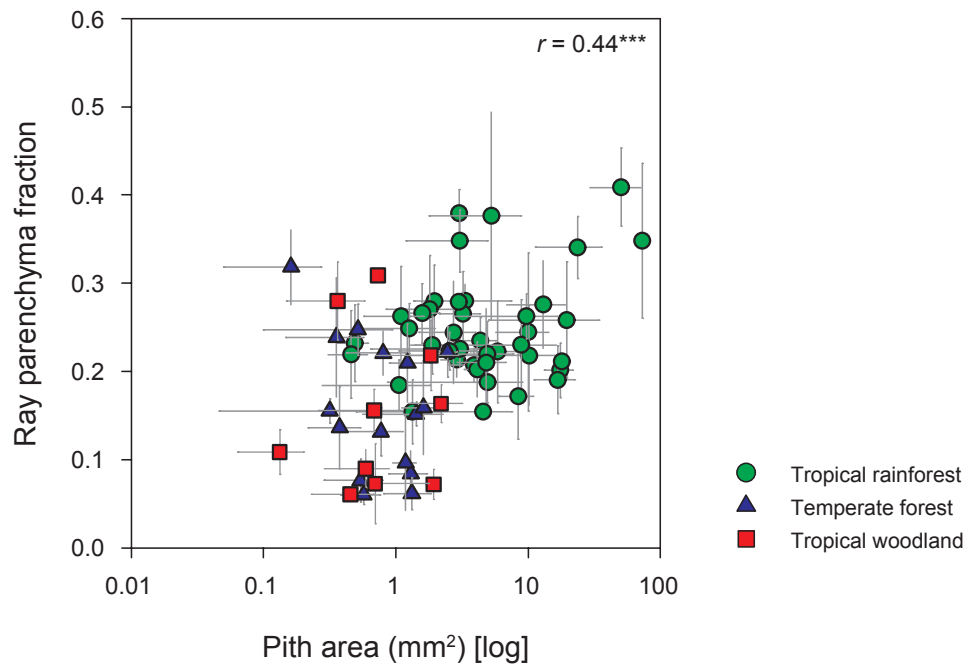


Figure 4-9 Relationship between ray parenchyma fraction and pith area (log-transformed). Symbol type represents site of collection: green circles – tropical rainforest (warm and wet site), red squares – tropical woodland (warm and dry site), and blue triangles – temperate forest (cool and wet site). \*\*\*  $P < 0.001$ .



Figure 4-10 Site and family comparisons for the 21 species from Myrtaceae and Proteaceae.

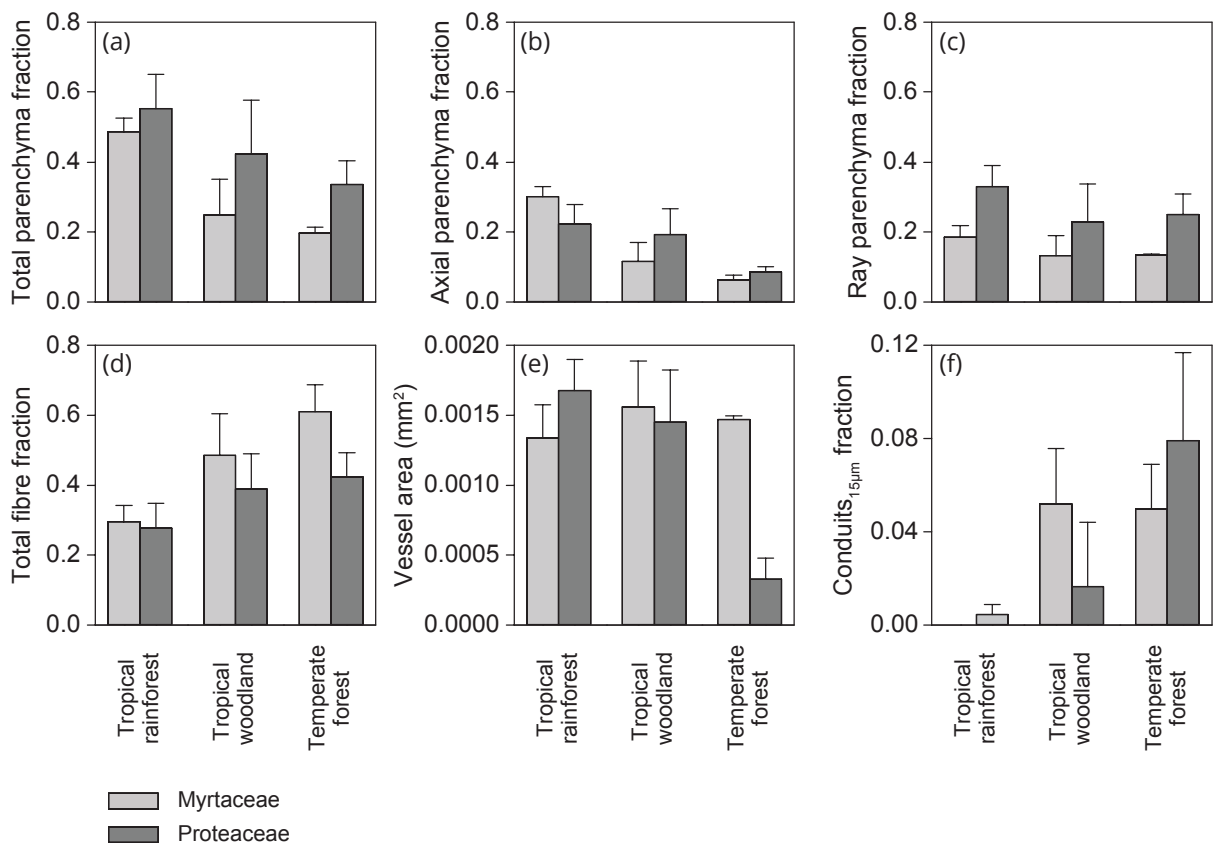
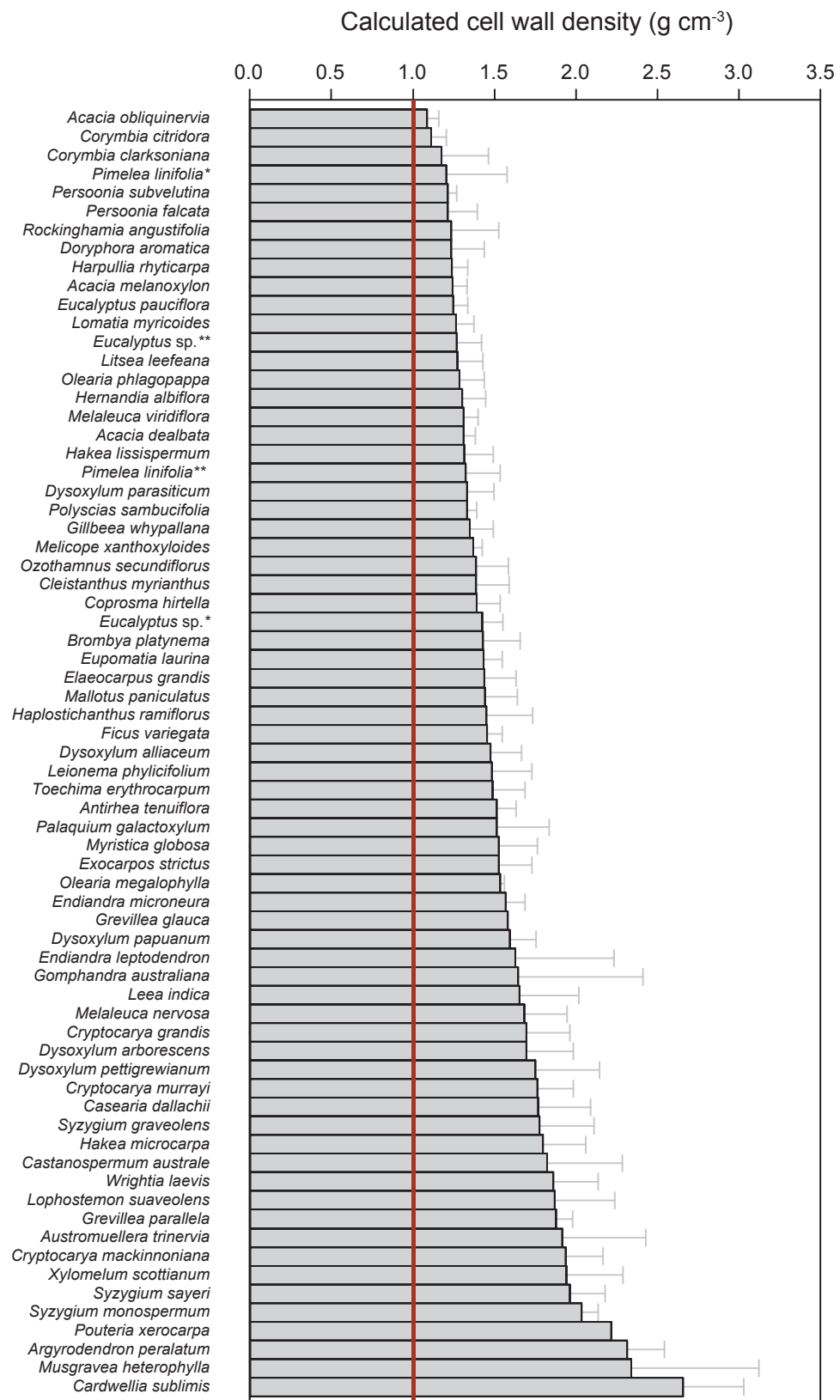


Figure 4-10 Site and family comparisons for the 21 species from Myrtaceae and Proteaceae. Bars indicate trait averages and whiskers indicate standard deviation. Light bars – Myrtaceae, dark bars – Proteaceae. Tropical rainforest is warm and wet site, tropical woodland is warm and dry site, and temperate forest is cool and wet site.

Figure 4-11 Bar graph representing estimated density of swollen cell wall material across 69 studied species. Each bar corresponds to one species and the bars are ordered from the lowest to the highest density. Values calculated for three replicates per species (with five exceptions mentioned in 'Materials and Methods' section. Whiskers indicate standard deviation. Red line indicates the density of  $1 \text{ g cm}^{-3}$ , which is approximately the density of swollen cell wall material measured by Kellogg and Wangaard (1969). \* Species from the tropical woodland, \*\* Species from the temperate forest.



Figure 4-11 Bar graph representing estimated density of swollen cell wall material across 69 studied species.





## 4.8 Tables

Table 4-1 Site details.

	Cape Tribulation, Daintree NP	Blencoe Falls, Girringun NP	Thredbo, Kosciuszko NP
Vegetation	Tropical rainforest	Tropical woodland	Temperate forest
Coordinates	16.104°S 145.449°E	18.162°S 145.490°E	36.482°S 148.350°E
Altitude (m)	50	650	1300
Sampling time	2012 May	2012 Oct	2012 Mar
Mean annual temperature (°C)	22.2	22.4	7.2
Maximum temperature of the warmest month (°C)	29.1 Dec, Jan	31.2 Dec	20.5 Jan
Maximum temperature of the coldest month (°C)	14.8 Jul	11.6 Jul	-1.6 Jul
Annual precipitation (mm)	4229	995	1834
Precipitation of the wettest month (mm)	817 Mar	197 Feb	210 Oct
Precipitation of the driest month (mm)	71 Oct	18 Sep	86 Feb
Number of months with precipitation below 100mm	3 Aug-Oct	9 Apr-Dec	1 Feb
Number of months with minimum temperature below 0°C	0	0	5 May-Sep

Notes: Temperature based on gridded 5km resolution data for 1961-2007. Precipitation data collected at the local weather stations (Cape Tribulation Store, Goshen, and Thredbo Village stations) within 5 km from the sites for 1971-1990. All data obtained from Australian Bureau of Meteorology. NP – national park.

Table 4-2 Traits overview.

Trait	Abbrev.	Units	Range of traits			n-fold variation
			Low	High	Average	
Wood density	–	g cm <sup>-3</sup>	0.38	0.62	0.53	1.6
Height	–	m	0.7	33.6	12.2	48.0
Maximum height	–	m	1	40	18.1	40
Modulus of elasticity	MOE	MPa	1555	11778	5200	7.6
Leaf area / sapwood area	–	cm <sup>2</sup> cm <sup>-2</sup>	838	24904	7884	29.7
Pith area	–	mm <sup>2</sup>	0.13*	72.6	5.6	547.8
Vessel area	A	mm <sup>2</sup>	0.0002	0.0036	0.0014	16.5
Vessel number per area	N	mm <sup>-2</sup>	34	730.7	148.7	21.5
Vessel area to number ratio	S	mm <sup>4</sup>	0.0000004	0.00011	0.00002	257.7
Hydraulically weighted diameter	D <sub>H</sub>	mm	0.02	0.08	0.05	4.4
Vessel lumen fraction	–	unitless	0.06	0.23	0.13	3.9
Vessel wall fraction	–	"	0.02	0.09	0.04	5.4
Total vessel fraction (lumen + wall)	–	"	0.09	0.3	0.18	3.4
Fraction of conduits with maximum lumen diameter smaller than 15 µm	conduits <sub>15µm</sub> fraction	"	0.002*	0.15	0.06	63.4
Axial parenchyma fraction	–	"	0.01	0.33	0.14	26.3
Ray parenchyma fraction	–	"	0.06	0.41	0.21	6.8
Total parenchyma fraction (axial + ray)	–	"	0.12	0.66	0.35	5.7
Fibre lumen fraction	–	"	0.02	0.32	0.13	16.2
Fibre wall fraction	–	"	0.15	0.52	0.32	3.5
Total fibre fraction (lumen + wall)	–	"	0.2	0.74	0.45	3.7
Mucilage canals fraction	–	"	0.01*	0.02	0.014	2.2
Axial parenchyma proportion relative to total parenchyma fraction	–	"	0.05	0.71	0.39	13.9
Fibre wall proportion relative to total fibre fraction	–	"	0.42	0.94	0.71	2.2

Notes: \* - this trait's minimum value was 0, the value reported here is the lowest value different from 0, which was used to calculate n-fold variation and mean values.

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## 4.10 Appendix

Table A4-1 Species and families.

Cape Tribulation, tropical rainforest		Blencoe Falls, tropical woodland	
Family	Species	Family	Species
Annonaceae	<i>Haplostichanthus ramiflorus</i>	Myrtaceae	<i>Corymbia citriodora</i>
Apocynaceae	<i>Wrightia laevis</i>		<i>Corymbia clarksoniana</i>
Cunnoniaceae	<i>Gillbeea whypallana</i>		<i>Eucalyptus</i> sp.
Elaeocarpaceae	<i>Elaeocarpus grandis</i>		<i>Lophostemon suaveolens</i>
Euphorbiaceae	<i>Cleistanthus myrianthus</i>		<i>Melaleuca nervosa</i>
	<i>Mallotus paniculatus</i>		<i>Melaleuca viridiflora</i>
	<i>Rockinghamia angustifolia</i>	Proteaceae	<i>Grevillea glauca</i>
Eupomatiaceae	<i>Eupomatia laurina</i>		<i>Grevillea parallela</i>
Fabaceae	<i>Castanospermum australe</i>		<i>Persoonia falcata</i>
Hernandiaceae	<i>Hernandia albiflora</i>		<i>Xylomelum scottianum</i>
Icaciniaceae	<i>Gomphandra australiana</i>	Thymelaeaceae	<i>Pimelea linifolia</i>
Lauraceae	<i>Cryptocarya grandis</i>	Thredbo, temperate forest	
	<i>Cryptocarya mackinnoniana</i>	Family	Species
	<i>Cryptocarya murrayi</i>	Araliaceae	<i>Polyscias sambucifolia</i>
	<i>Endiandra leptodendron</i>	Asteraceae	<i>Olearia megalophylla</i>
	<i>Endiandra microneura</i>		<i>Olearia phlogopappa</i>
	<i>Litsea leefeana</i>		<i>Ozothamnus secundiflorus</i>
Meliaceae	<i>Dysoxylum alliaceum</i>	Fabaceae	<i>Acacia dealbata</i>
	<i>Dysoxylum arborescens</i>		<i>Acacia melanoxylon</i>
	<i>Dysoxylum papuanum</i>		<i>Acacia obliquinervia</i>
	<i>Dysoxylum parasiticum</i>	Myrtaceae	<i>Eucalyptus pauciflora</i>
	<i>Dysoxylum pettigrewianum</i>		<i>Eucalyptus</i> sp.
Monimiaceae	<i>Doryphora aromatica</i>	Proteaceae	<i>Hakea lissosperma</i>
Moraceae	<i>Ficus variegata</i>		<i>Hakea microcarpa</i>
Myristicaceae	<i>Myristica globosa</i>		<i>Lomatia myricoides</i>
Myrtaceae	<i>Syzygium graveolens</i>		<i>Persoonia subvelutina</i>
	<i>Syzygium monospermum</i>	Rubiaceae	<i>Coprosma hirtella</i>
	<i>Syzygium sayeri</i>	Rutaceae	<i>Leionema phylicifolium</i>
Proteaceae	<i>Austromuellera trinervia</i>	Santalaceae	<i>Exocarpos strictus</i>
	<i>Cardwellia sublimis</i>	Thymelaeaceae	<i>Pimelea linifolia</i>
	<i>Musgravea heterophylla</i>		
Rubiaceae	<i>Antirhea tenuiflora</i>		
Rutaceae	<i>Brombya platynema</i>		
	<i>Melicope xanthoxyloides</i>		
Salicaceae	<i>Casearia dallachii</i>		
Sapindaceae	<i>Harpullia rhyticarpa</i>		
	<i>Toeckima erythrocarpum</i>		
Sapotaceae	<i>Palaquium galactoxylum</i>		
	<i>Pouteria xerocarpa</i>		
Sterculiaceae	<i>Argyrodendron peralatum</i>		
Vitaceae	<i>Leea indica</i>		

Table A4-2 Tissue fractions: total parenchyma, axial parenchyma, ray parenchyma, total fibre, fibre wall, fibre lumen.

Species	Total parenchyma		Axial parenchyma		Ray parenchyma		Total fibre		Fibre wall		Fibre lumen	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Tropical rainforest												
<i>Antirhea tenuiflora</i>	0.41	0.02	0.13	0.02	0.28	0.04	0.33	0.03	0.30	0.03	0.03	0.01
<i>Argyrodendron peralatum</i>	0.59	0.03	0.32	0.03	0.27	0.06	0.21	0.03	0.19	0.02	0.02	0.00
<i>Austromuellera trinervia</i>	0.52	0.10	0.17	0.02	0.35	0.09	0.29	0.09	0.22	0.06	0.07	0.05
<i>Brombya platynema</i>	0.37	0.04	0.09	0.06	0.28	0.02	0.51	0.04	0.37	0.05	0.14	0.02
<i>Cardwellia sublimis</i>	0.66	0.04	0.28	0.05	0.38	0.03	0.20	0.03	0.15	0.03	0.05	0.01
<i>Casearia dallachii</i>	0.38	0.03	0.03	0.01	0.35	0.04	0.47	0.04	0.26	0.07	0.21	0.03
<i>Castanospermum australe</i>	0.54	0.06	0.28	0.10	0.26	0.07	0.37	0.07	0.23	0.08	0.14	0.02
<i>Cleistanthus myrianthus</i>	0.35	0.03	0.09	0.01	0.27	0.03	0.44	0.05	0.33	0.05	0.11	0.01
<i>Cryptocarya grandis</i>	0.36	0.03	0.15	0.01	0.21	0.02	0.46	0.06	0.30	0.05	0.17	0.02
<i>Cryptocarya mackinnoniana</i>	0.44	0.10	0.16	0.06	0.28	0.05	0.41	0.11	0.27	0.06	0.14	0.06
<i>Cryptocarya murrayi</i>	0.44	0.05	0.19	0.05	0.24	0.09	0.37	0.01	0.24	0.01	0.13	0.02
<i>Doryphora aromatica</i>	0.24	0.04	0.01	0.01	0.23	0.04	0.48	0.07	0.37	0.05	0.11	0.02
<i>Dysoxylum alliaceum</i>	0.49	0.07	0.28	0.05	0.22	0.06	0.36	0.09	0.26	0.07	0.11	0.02
<i>Dysoxylum arborescens</i>	0.44	0.03	0.22	0.03	0.22	0.06	0.41	0.05	0.29	0.05	0.12	0.04
<i>Dysoxylum papuanum</i>	0.39	0.03	0.15	0.01	0.23	0.03	0.40	0.05	0.28	0.04	0.13	0.01
<i>Dysoxylum parasiticum***</i>	0.31	0.04	0.11	0.01	0.20	0.03	0.51	0.05	0.30	0.00	0.21	0.05
<i>Dysoxylum pettigrewianum***</i>	0.43	0.03	0.24	0.03	0.19	0.04	0.32	0.03	0.22	0.04	0.10	0.04
<i>Elaeocarpus grandis</i>	0.30	0.05	0.04	0.00	0.26	0.05	0.49	0.01	0.28	0.05	0.22	0.04
<i>Endiandra leptodendron</i>	0.36	0.12	0.13	0.07	0.23	0.05	0.50	0.10	0.36	0.12	0.14	0.01
<i>Endiandra microneura</i>	0.37	0.03	0.15	0.03	0.23	0.02	0.42	0.02	0.32	0.02	0.09	0.01
<i>Eupomatia laurina</i>	0.40	0.02	0.06	0.03	0.34	0.04	0.38	0.03	0.28	0.02	0.09	0.02
<i>Ficus variegata</i>	0.36	0.07	0.19	0.04	0.17	0.05	0.47	0.06	0.25	0.00	0.22	0.06
<i>Gillibea whypallana</i>	0.29	0.03	0.08	0.02	0.21	0.02	0.42	0.04	0.25	0.06	0.17	0.02
<i>Gomphandra australiana</i>	0.55	0.14	0.18	0.02	0.38	0.12	0.35	0.12	0.30	0.11	0.05	0.01
<i>Haplostichanthus ramiflorus</i>	0.46	0.06	0.19	0.00	0.26	0.06	0.44	0.03	0.36	0.05	0.08	0.03
<i>Harpullia rhyticarpa</i>	0.31	0.02	0.11	0.04	0.20	0.03	0.55	0.06	0.45	0.07	0.10	0.02
<i>Hernandia albiflora</i>	0.37	0.04	0.15	0.05	0.22	0.04	0.54	0.07	0.33	0.01	0.21	0.08
<i>Leea indica</i>	0.43	0.04	0.02	0.01	0.41	0.04	0.42	0.03	0.22	0.04	0.20	0.02
<i>Litsea leefeana</i>	0.23	0.02	0.04	0.02	0.19	0.02	0.54	0.05	0.35	0.05	0.19	0.04
<i>Mallotus paniculatus</i>	0.29	0.06	0.08	0.02	0.21	0.06	0.47	0.10	0.27	0.03	0.20	0.07
<i>Melicope xanthoxyloides</i>	0.31	0.01	0.10	0.01	0.21	0.01	0.45	0.06	0.27	0.02	0.18	0.04
<i>Musgravea heterophylla</i>	0.47	0.06	0.21	0.04	0.26	0.03	0.34	0.03	0.17	0.05	0.17	0.02
<i>Myristica globosa</i>	0.35	0.03	0.12	0.01	0.23	0.03	0.49	0.03	0.26	0.05	0.23	0.07
<i>Palaquium galactoxylum**</i>	0.38	0.05	0.13	0.01	0.24	0.06	0.48	0.03	0.30	0.07	0.17	0.04
<i>Pouteria xerocarpa*</i>	0.50	na	0.22	na	0.28	na	0.34	na	0.21	na	0.13	na
<i>Rockinghamia angustifolia</i>	0.33	0.03	0.11	0.02	0.22	0.02	0.50	0.05	0.34	0.08	0.16	0.03
<i>Syzygium graveolens</i>	0.44	0.03	0.29	0.05	0.15	0.04	0.34	0.02	0.26	0.04	0.08	0.03
<i>Syzygium monospermum</i>	0.52	0.06	0.33	0.03	0.18	0.03	0.30	0.02	0.27	0.02	0.03	0.00
<i>Syzygium sayeri</i>	0.50	0.00	0.28	0.05	0.22	0.05	0.25	0.03	0.19	0.03	0.06	0.03
<i>Toechima erythrocarpum</i>	0.21	0.04	0.06	0.03	0.15	0.01	0.64	0.03	0.36	0.06	0.28	0.04
<i>Wrightia laevis</i>	0.39	0.03	0.14	0.00	0.25	0.03	0.36	0.03	0.15	0.02	0.21	0.02

Species	Total parenchyma		Axial parenchyma		Ray parenchyma		Total fibre		Fibre wall		Fibre lumen	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Tropical woodland												
<i>Corymbia citridora</i>	0.18	0.04	0.07	0.03	0.11	0.03	0.61	0.06	0.52	0.06	0.09	0.01
<i>Corymbia clarksoniana</i>	0.12	0.04	0.06	0.03	0.06	0.01	0.60	0.08	0.44	0.10	0.15	0.02
<i>Eucalyptus</i> sp.	0.20	0.01	0.11	0.04	0.09	0.02	0.54	0.02	0.33	0.04	0.20	0.01
<i>Grevillea glauca</i> *	0.44	na	0.13	na	0.31	na	0.39	na	0.34	na	0.05	na
<i>Grevillea parallela</i>	0.52	0.04	0.26	0.05	0.26	0.06	0.30	0.04	0.28	0.03	0.02	0.01
<i>Lophostemon suaveolens</i>	0.38	0.08	0.16	0.04	0.22	0.04	0.33	0.07	0.24	0.05	0.09	0.02
<i>Melaleuca nervosa</i>	0.35	0.06	0.20	0.04	0.16	0.02	0.37	0.03	0.29	0.01	0.08	0.02
<i>Melaleuca viridiflora</i>	0.25	0.04	0.09	0.02	0.16	0.02	0.46	0.07	0.38	0.07	0.08	0.02
<i>Persoonia falcata</i>	0.20	0.03	0.13	0.02	0.07	0.02	0.53	0.11	0.42	0.11	0.11	0.01
<i>Pimelea linifolia</i>	0.12	0.04	0.04	0.01	0.07	0.05	0.65	0.07	0.32	0.07	0.32	0.02
<i>Xylomelum scottianum</i>	0.53	0.08	0.25	0.06	0.28	0.04	0.33	0.05	0.31	0.05	0.02	0.01
Temperate forest												
<i>Acacia dealbata</i>	0.24	0.05	0.16	0.05	0.08	0.03	0.61	0.08	0.37	0.03	0.23	0.08
<i>Acacia melanoxylon</i>	0.26	0.03	0.18	0.01	0.08	0.02	0.65	0.02	0.39	0.03	0.26	0.04
<i>Acacia obliquinervia</i>	0.17	0.04	0.11	0.03	0.06	0.01	0.74	0.05	0.50	0.05	0.24	0.09
<i>Coprosma hirtella</i>	0.39	0.07	0.14	0.04	0.25	0.03	0.46	0.11	0.39	0.09	0.07	0.03
<i>Eucalyptus pauciflora</i>	0.19	0.03	0.05	0.02	0.13	0.03	0.66	0.03	0.46	0.03	0.20	0.02
<i>Eucalyptus</i> sp.	0.21	0.05	0.07	0.02	0.14	0.05	0.55	0.05	0.45	0.07	0.10	0.02
<i>Exocarpos strictus</i>	0.36	0.02	0.12	0.05	0.24	0.07	0.45	0.08	0.36	0.05	0.10	0.05
<i>Hakea lissosperma</i>	0.30	0.06	0.09	0.01	0.21	0.04	0.43	0.06	0.35	0.06	0.08	0.01
<i>Hakea microcarpa</i>	0.41	0.02	0.10	0.02	0.32	0.04	0.35	0.07	0.30	0.05	0.05	0.02
<i>Leionema phyllifolium</i>	0.20	0.02	0.05	0.01	0.15	0.01	0.48	0.08	0.35	0.08	0.13	0.03
<i>Lomatia myricoides</i>	0.29	0.05	0.07	0.03	0.22	0.03	0.49	0.05	0.41	0.07	0.08	0.02
<i>Olearia megalophylla</i>	0.21	0.03	0.15	0.02	0.06	0.02	0.49	0.09	0.35	0.05	0.15	0.04
<i>Olearia phlogopappa</i>	0.22	0.04	0.06	0.03	0.15	0.01	0.60	0.06	0.46	0.07	0.14	0.06
<i>Ozothamnus secundiflorus</i>	0.27	0.06	0.11	0.06	0.16	0.05	0.55	0.10	0.38	0.06	0.17	0.04
<i>Persoonia subvelutina</i>	0.20	0.02	0.10	0.04	0.10	0.05	0.52	0.08	0.38	0.02	0.13	0.06
<i>Pimelea linifolia</i>	0.17	0.00	0.05	0.02	0.12	0.02	0.49	0.03	0.32	0.05	0.17	0.03
<i>Polyscias sambucifolia</i>	0.25	0.02	0.03	0.01	0.22	0.03	0.40	0.05	0.33	0.05	0.08	0.01

Notes: All species values averaged across three samples except for: \* – one sample, \*\* – two samples, \*\*\* – four samples, '-' indicates that given tissue did not occur in the studied species. AV – species average, SD – standard deviation.

Table A4-3 Tissue fractions: total vessel, vessel lumen, vessel wall, conduits<sub>15µm</sub>, mucilage canals.

Species	Total vessel		Vessel lumen		Vessel wall		Conduits <sub>15µm</sub>		Mucilage canals	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Tropical rainforest										
<i>Antirhea tenuiflora</i>	0.26	0.04	0.18	0.02	0.08	0.02	-	na	-	na
<i>Argyrodendron peralatum</i>	0.20	0.03	0.14	0.04	0.06	0.01	-	na	-	na
<i>Austromuellera trinervia</i>	0.18	0.02	0.15	0.01	0.03	0.01	0.008	0.006	-	na
<i>Brombya platynema</i>	0.12	0.02	0.09	0.02	0.03	0.00	-	na	-	na
<i>Cardwellia sublimis</i>	0.14	0.02	0.11	0.01	0.03	0.00	-	na	-	na
<i>Casearia dallachii</i>	0.15	0.02	0.12	0.02	0.04	0.01	-	na	-	na
<i>Castanospermum australe</i>	0.10	0.01	0.08	0.01	0.02	0.00	-	na	-	na
<i>Cleistanthus myrianthus</i>	0.21	0.05	0.15	0.05	0.06	0.01	-	na	-	na
<i>Cryptocarya grandis</i>	0.17	0.04	0.13	0.02	0.04	0.01	-	na	-	na
<i>Cryptocarya mackinnoniana</i>	0.14	0.02	0.09	0.03	0.04	0.02	-	na	0.013	0.021
<i>Cryptocarya murrayi</i>	0.17	0.04	0.14	0.03	0.03	0.01	-	na	0.019	0.020
<i>Doryphora aromatica</i>	0.27	0.04	0.21	0.02	0.06	0.02	-	na	-	na
<i>Dysoxylum alliaceum</i>	0.14	0.02	0.10	0.02	0.04	0.00	-	na	-	na
<i>Dysoxylum arborescens</i>	0.15	0.06	0.12	0.06	0.03	0.00	-	na	-	na
<i>Dysoxylum papuanum</i>	0.21	0.02	0.16	0.02	0.05	0.01	-	na	-	na
<i>Dysoxylum parasiticum***</i>	0.18	0.02	0.16	0.02	0.03	0.01	-	na	-	na
<i>Dysoxylum pettigrewianum***</i>	0.25	0.06	0.20	0.06	0.05	0.01	-	na	-	na
<i>Elaeocarpus grandis</i>	0.20	0.05	0.16	0.05	0.04	0.00	-	na	-	na
<i>Endiandra leptodendron</i>	0.15	0.02	0.12	0.02	0.02	0.00	-	na	-	na
<i>Endiandra microneura</i>	0.21	0.03	0.14	0.04	0.07	0.01	-	na	-	na
<i>Eupomatia laurina</i>	0.22	0.02	0.16	0.01	0.06	0.01	-	na	-	na
<i>Ficus variegata</i>	0.16	0.03	0.13	0.03	0.03	0.01	-	na	-	na
<i>Gillbeea whypallana</i>	0.30	0.01	0.23	0.01	0.06	0.00	-	na	-	na
<i>Gomphandra australiana</i>	0.10	0.03	0.08	0.02	0.02	0.01	-	na	-	na
<i>Haplostichanthus ramiflorus</i>	0.10	0.03	0.06	0.01	0.04	0.02	-	na	-	na
<i>Harpullia rhyticarpa</i>	0.14	0.04	0.10	0.03	0.04	0.01	-	na	-	na
<i>Hernandia albiflora</i>	0.10	0.03	0.07	0.02	0.03	0.02	-	na	-	na
<i>Leea indica</i>	0.15	0.01	0.13	0.02	0.02	0.02	-	na	-	na
<i>Litsea leefeana</i>	0.22	0.06	0.16	0.05	0.06	0.01	-	na	0.009	0.015
<i>Mallotus paniculatus</i>	0.24	0.04	0.20	0.02	0.04	0.02	-	na	-	na
<i>Melicope xanthoxyloides</i>	0.24	0.06	0.19	0.04	0.05	0.02	-	na	-	na
<i>Musgravea heterophylla</i>	0.19	0.03	0.15	0.03	0.03	0.01	0.006	0.002	-	na
<i>Myristica globosa</i>	0.16	0.02	0.12	0.01	0.04	0.01	-	na	-	na
<i>Palaquium galactoxylum**</i>	0.15	0.02	0.11	0.04	0.04	0.02	-	na	-	na
<i>Pouteria xerocarpa*</i>	0.15	na	0.12	na	0.03	na	-	na	-	na
<i>Rockinghamia angustifolia</i>	0.17	0.04	0.13	0.03	0.04	0.01	-	na	-	na
<i>Syzygium graveolens</i>	0.22	0.01	0.17	0.00	0.05	0.01	-	na	-	na
<i>Syzygium monospermum</i>	0.18	0.04	0.15	0.05	0.03	0.01	-	na	-	na
<i>Syzygium sayeri</i>	0.26	0.03	0.19	0.02	0.06	0.02	-	na	-	na
<i>Toeckima erythrocarpum</i>	0.15	0.01	0.12	0.00	0.03	0.01	-	na	-	na
<i>Wrightia laevis</i>	0.25	0.04	0.19	0.04	0.06	0.01	-	na	-	na



Species	Total vessel		Vessel lumen		Vessel wall		Conduits <sub>15µm</sub>		Mucilage canals	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Tropical woodland										
<i>Corymbia citridora</i>	0.19	0.04	0.15	0.03	0.04	0.01	0.010	0.014	-	na
<i>Corymbia clarksoniana</i>	0.24	0.04	0.20	0.04	0.04	0.02	0.039	0.020	-	na
<i>Eucalyptus</i> sp.	0.20	0.01	0.18	0.00	0.02	0.00	0.057	0.038	-	na
<i>Grevillea glauca</i> *	0.17	na	0.13	na	0.04	na	-	na	-	na
<i>Grevillea parallela</i>	0.17	0.03	0.14	0.02	0.04	0.02	0.005	0.002	-	na
<i>Lophostemon suaveolens</i>	0.23	0.03	0.18	0.02	0.05	0.01	0.062	0.011	-	na
<i>Melaleuca nervosa</i>	0.21	0.05	0.16	0.02	0.05	0.03	0.071	0.051	-	na
<i>Melaleuca viridiflora</i>	0.22	0.07	0.17	0.04	0.05	0.03	0.072	0.005	-	na
<i>Persoonia falcata</i>	0.21	0.05	0.14	0.04	0.07	0.03	0.058	0.025	-	na
<i>Pimelea linifolia</i>	0.19	0.06	0.12	0.05	0.06	0.03	0.051	0.022	-	na
<i>Xylomelum scottianum</i>	0.14	0.05	0.12	0.04	0.02	0.01	0.002	0.002	-	na
Temperate forest										
<i>Acacia dealbata</i>	0.15	0.04	0.12	0.02	0.03	0.02	-	na	-	na
<i>Acacia melanoxylon</i>	0.09	0.05	0.07	0.05	0.02	0.01	-	na	-	na
<i>Acacia obliquinervia</i>	0.09	0.02	0.07	0.02	0.02	0.01	-	na	-	na
<i>Coprosma hirtella</i>	0.13	0.06	0.07	0.04	0.05	0.02	0.028	0.016	-	na
<i>Eucalyptus pauciflora</i>	0.11	0.04	0.09	0.03	0.03	0.01	0.036	0.027	-	na
<i>Eucalyptus</i> sp.	0.17	0.07	0.14	0.06	0.03	0.01	0.063	0.026	-	na
<i>Exocarpos strictus</i>	0.16	0.06	0.11	0.04	0.04	0.02	0.033	0.037	-	na
<i>Hakea lissosperma</i>	0.18	0.02	0.11	0.02	0.07	0.01	0.091	0.017	-	na
<i>Hakea microcarpa</i>	0.12	0.02	0.08	0.01	0.04	0.01	0.110	0.041	-	na
<i>Leionema phyllifolium</i>	0.18	0.01	0.11	0.01	0.07	0.02	0.137	0.062	-	na
<i>Lomatia myricoides</i>	0.18	0.05	0.15	0.03	0.04	0.01	0.037	0.025	-	na
<i>Olearia megalophylla</i>	0.14	0.04	0.09	0.02	0.05	0.02	0.154	0.053	-	na
<i>Olearia phlogopappa</i>	0.09	0.01	0.06	0.01	0.03	0.01	0.097	0.033	-	na
<i>Ozothamnus secundiflorus</i>	0.12	0.03	0.09	0.03	0.04	0.00	0.054	0.030	-	na
<i>Persoonia subvelutina</i>	0.25	0.04	0.18	0.01	0.07	0.03	0.039	0.019	-	na
<i>Pimelea linifolia</i>	0.19	0.01	0.13	0.01	0.06	0.01	0.151	0.036	-	na
<i>Polyscias sambucifolia</i>	0.30	0.05	0.21	0.04	0.09	0.04	0.046	0.033	-	na

Notes: All species values averaged across three samples except for: \* – one sample, \*\* – two samples, \*\*\* – four samples, '-' indicates that given tissue did not occur in the studied species. AV – species average, SD – standard deviation.

Table A4-4 Anatomical traits: axial parenchyma relative to total parenchyma, fibre wall relative to total fibre, pith area relative to total fibre, pith area.

Species	Axial relative to total parenchyma		Fibre wall relative to total fibre		Pith area (mm <sup>2</sup> )	
	AV	SD	AV	SD	AV	SD
Tropical rainforest						
<i>Antirhea tenuiflora</i>	0.32	0.07	0.92	0.03	2.0	0.6
<i>Argyrodendron peralatum</i>	0.54	0.08	0.91	0.01	1.8	1.0
<i>Austromuellera trinervia</i>	0.34	0.05	0.77	0.10	72.6	5.2
<i>Brombya platynema</i>	0.23	0.13	0.71	0.05	3.3	4.1
<i>Cardwellia sublimis</i>	0.43	0.05	0.76	0.03	3.0	0.2
<i>Casearia dallachii</i>	0.07	0.04	0.54	0.11	3.0	1.9
<i>Castanospermum australe</i>	0.51	0.15	0.61	0.11	19.6	14.6
<i>Cleistanthus myrianthus</i>	0.25	0.04	0.76	0.04	1.6	0.5
<i>Cryptocarya grandis</i>	0.42	0.02	0.64	0.04	2.9	0.7
<i>Cryptocarya mackinnoniana</i>	0.36	0.05	0.67	0.06	13.0	6.1
<i>Cryptocarya murrayi</i>	0.45	0.14	0.65	0.03	10.0	4.4
<i>Doryphora aromatica</i>	0.05	0.03	0.77	0.01	0.5	0.1
<i>Dysoxylum alliaceum</i>	0.56	0.07	0.71	0.02	10.2	9.0
<i>Dysoxylum arborescens</i>	0.50	0.10	0.71	0.10	5.9	2.3
<i>Dysoxylum papuanum</i>	0.40	0.03	0.69	0.02	4.4	1.3
<i>Dysoxylum parasiticum***</i>	0.34	0.04	0.60	0.05	17.4	4.2
<i>Dysoxylum pettigrewianum***</i>	0.56	0.07	0.69	0.11	16.8	5.6
<i>Elaeocarpus grandis</i>	0.13	0.02	0.56	0.09	3.2	1.1
<i>Endiandra leptodendron</i>	0.34	0.09	0.70	0.10	8.9	7.6
<i>Endiandra microneura</i>	0.39	0.06	0.78	0.03	3.1	2.4
<i>Eupomatia laurina</i>	0.15	0.09	0.75	0.04	23.6	12.2
<i>Ficus variegata</i>	0.53	0.07	0.54	0.07	8.4	2.4
<i>Gillbeea whypallana</i>	0.28	0.07	0.60	0.09	3.9	1.4
<i>Gomphandra australiana</i>	0.33	0.04	0.86	0.02	5.3	3.5
<i>Haplostichanthus ramiflorus</i>	0.43	0.05	0.82	0.07	1.1	0.5
<i>Harpullia rhyticarpa</i>	0.35	0.10	0.82	0.04	4.1	0.8
<i>Hernandia albiflora</i>	0.40	0.12	0.62	0.10	4.9	2.7
<i>Leea indica</i>	0.05	0.03	0.53	0.07	50.3	21.0
<i>Litsea leefeana</i>	0.17	0.10	0.65	0.07	4.9	4.1
<i>Mallotus paniculatus</i>	0.28	0.07	0.59	0.06	4.8	1.9
<i>Melicope xanthoxyloides</i>	0.32	0.03	0.60	0.04	18.0	1.5
<i>Musgravea heterophylla</i>	0.44	0.04	0.50	0.10	9.7	7.0
<i>Myristica globosa</i>	0.35	0.05	0.53	0.12	1.9	0.5
<i>Palaquium galactoxylum**</i>	0.36	0.06	0.63	0.11	2.7	1.2
<i>Pouteria xerocarpa*</i>	0.45	na	0.61	na	3.0	na
<i>Rockinghamia angustifolia</i>	0.33	0.02	0.66	0.10	2.5	1.5
<i>Syzygium graveolens</i>	0.65	0.09	0.76	0.10	1.3	0.8
<i>Syzygium monospermum</i>	0.64	0.03	0.90	0.02	1.1	0.8
<i>Syzygium sayeri</i>	0.56	0.10	0.78	0.10	0.5	0.2
<i>Toeckima erythrocarpum</i>	0.25	0.10	0.56	0.08	4.6	2.9
<i>Wrightia laevis</i>	0.37	0.03	0.42	0.04	1.3	0.7

Species	Axial relative to total parenchyma		Fibre wall relative to total fibre		Pith area (mm <sup>2</sup> )	
	AV	SD	AV	SD	AV	SD
Tropical woodland						
<i>Corymbia citridora</i>	0.40	0.10	0.85	0.02	0.1	0.1
<i>Corymbia clarksoniana</i>	0.48	0.08	0.74	0.07	0.5	0.2
<i>Eucalyptus</i> sp.	0.55	0.14	0.62	0.04	0.6	0.3
<i>Grevillea glauca</i> *	0.30	na	0.88	na	0.7	na
<i>Grevillea parallela</i>	0.50	0.10	0.93	0.03	0.0	0.0
<i>Lophostemon suaveolens</i>	0.43	0.02	0.73	0.03	1.8	1.1
<i>Melaleuca nervosa</i>	0.56	0.01	0.79	0.05	0.7	0.4
<i>Melaleuca viridiflora</i>	0.35	0.03	0.82	0.06	2.2	1.0
<i>Persoonia falcata</i>	0.65	0.04	0.78	0.06	1.9	0.2
<i>Pimelea linifolia</i>	0.42	0.18	0.50	0.06	0.7	1.1
<i>Xylomelum scottianum</i>	0.47	0.07	0.94	0.03	0.4	0.2
Temperate forest						
<i>Acacia dealbata</i>	0.65	0.12	0.62	0.08	1.3	0.4
<i>Acacia melanoxylon</i>	0.71	0.06	0.60	0.06	0.5	0.3
<i>Acacia obliquinervia</i>	0.64	0.04	0.68	0.10	0.6	0.2
<i>Coprosma hirtella</i>	0.36	0.04	0.84	0.05	0.5	0.4
<i>Eucalyptus pauciflora</i>	0.29	0.11	0.70	0.03	0.8	0.3
<i>Eucalyptus</i> sp.	0.36	0.12	0.81	0.05	0.4	0.2
<i>Exocarpos strictus</i>	0.33	0.16	0.79	0.08	0.4	0.2
<i>Hakea lissosperma</i>	0.31	0.02	0.81	0.02	1.2	0.3
<i>Hakea microcarpa</i>	0.23	0.07	0.86	0.03	0.2	0.1
<i>Leionema phyllicifolium</i>	0.24	0.02	0.72	0.08	0.3	0.3
<i>Lomatia myricoides</i>	0.23	0.07	0.84	0.06	0.8	0.4
<i>Olearia megalophylla</i>	0.71	0.08	0.71	0.03	1.3	0.5
<i>Olearia phlogopappa</i>	0.29	0.10	0.76	0.10	1.4	0.9
<i>Ozothamnus secundiflorus</i>	0.41	0.16	0.70	0.03	1.6	0.5
<i>Persoonia subvelutina</i>	0.53	0.23	0.75	0.07	1.2	0.2
<i>Pimelea linifolia</i>	0.28	0.12	0.66	0.07	0.0	0.0
<i>Polyscias sambucifolia</i>	0.12	0.06	0.81	0.02	2.5	0.5

Notes: All species values averaged across three samples except for: \* – one sample, \*\* – two samples, \*\*\* – four samples, '-' indicates that given tissue did not occur in the studied species. AV – species average, SD – standard deviation.

Table A4-5 Vessel traits.

Species	Vessel area (mm <sup>2</sup> )		Vessel number per area (mm <sup>-2</sup> )		Vessel area to number ratio (mm <sup>4</sup> )		Hydraulically weighted diameter (mm)	
	AV	SD	AV	SD	AV	SD	AV	SD
Tropical rainforest								
<i>Antirhea tenuiflora</i>	0.00066	0.00004	278	48	0.0000024	0.0000005	0.034	0.009
<i>Argyrodendron peralatum</i>	0.00162	0.00046	89	29	0.0000203	0.0000118	0.064	0.061
<i>Austromuellera trinervia</i>	0.00172	0.00042	87	18	0.0000212	0.0000103	0.058	0.033
<i>Brombya platynema</i>	0.00075	0.00010	119	23	0.0000065	0.0000016	0.038	0.028
<i>Cardwellia sublimis</i>	0.00187	0.00031	61	14	0.0000330	0.0000146	0.056	0.022
<i>Casearia dallachii</i>	0.00076	0.00025	160	28	0.0000051	0.0000027	0.036	0.049
<i>Castanospermum australe</i>	0.00265	0.00117	34	16	0.0001064	0.0000959	0.070	0.173
<i>Cleistanthus myrianthus</i>	0.00093	0.00015	159	31	0.0000059	0.0000006	0.039	0.029
<i>Cryptocarya grandis</i>	0.00196	0.00016	69	17	0.0000300	0.0000092	0.066	0.043
<i>Cryptocarya mackinnoniana</i>	0.00183	0.00048	51	4	0.0000358	0.0000069	0.062	0.061
<i>Cryptocarya murrayi</i>	0.00213	0.00008	66	14	0.0000333	0.0000085	0.067	0.060
<i>Doryphora aromatica</i>	0.00063	0.00009	336	71	0.0000020	0.0000008	0.032	0.029
<i>Dysoxylum alliaceum</i>	0.00194	0.00022	52	5	0.0000370	0.0000006	0.064	0.040
<i>Dysoxylum arborescens</i>	0.00111	0.00021	110	34	0.0000106	0.0000036	0.046	0.040
<i>Dysoxylum papuanum</i>	0.00129	0.00003	121	21	0.0000108	0.0000020	0.049	0.029
<i>Dysoxylum parasiticum***</i>	0.00362	0.00093	44	8	0.0000864	0.0000364	0.083	0.059
<i>Dysoxylum pettigrewianum***</i>	0.00184	0.00028	113	52	0.0000204	0.0000126	0.058	0.055
<i>Elaeocarpus grandis</i>	0.00200	0.00028	81	16	0.0000250	0.0000028	0.066	0.051
<i>Endiandra leptodendron</i>	0.00151	0.00019	80	12	0.0000192	0.0000046	0.055	0.026
<i>Endiandra microneura</i>	0.00157	0.00034	92	22	0.0000179	0.0000068	0.053	0.034
<i>Eupomatia laurina</i>	0.00107	0.00002	147	10	0.0000073	0.0000006	0.043	0.012
<i>Ficus variegata</i>	0.00311	0.00045	45	15	0.0000789	0.0000413	0.077	0.051
<i>Gillbeea whypallana</i>	0.00127	0.00036	194	56	0.0000072	0.0000036	0.044	0.064
<i>Gomphandra australiana</i>	0.00196	0.00029	42	7	0.0000478	0.0000109	0.057	0.062
<i>Haplostichanthus ramiflorus</i>	0.00095	0.00047	84	59	0.0000169	0.0000126	0.043	0.134
<i>Harpullia rhyticarpa</i>	0.00094	0.00021	102	9	0.0000092	0.0000018	0.044	0.042
<i>Hernandia albiflora</i>	0.00144	0.00019	49	15	0.0000322	0.0000156	0.057	0.069
<i>Leea indica</i>	0.00308	0.00048	42	7	0.0000766	0.0000243	0.075	0.060
<i>Litsea leefeana</i>	0.00167	0.00049	106	56	0.0000194	0.0000108	0.054	0.055
<i>Mallotus paniculatus</i>	0.00328	0.00130	68	32	0.0000653	0.0000570	0.082	0.130
<i>Melicope xanthoxyloides</i>	0.00202	0.00019	96	18	0.0000215	0.0000042	0.062	0.049
<i>Musgravea heterophylla</i>	0.00144	0.00044	110	21	0.0000139	0.0000067	0.052	0.079
<i>Myristica globosa</i>	0.00232	0.00018	52	8	0.0000461	0.0000100	0.065	0.040
<i>Palaquium galactoxylum**</i>	0.00198	0.00029	55	13	0.0000362	0.0000031	0.063	0.086
<i>Pouteria xerocarpa*</i>	0.00091	na	137	na	0.0000066	na	0.049	na
<i>Rockinghamia angustifolia</i>	0.00140	0.00004	90	19	0.0000160	0.0000031	0.056	0.024
<i>Syzygium graveolens</i>	0.00135	0.00029	126	23	0.0000113	0.0000046	0.050	0.029
<i>Syzygium monospermum</i>	0.00109	0.00045	148	55	0.0000091	0.0000075	0.044	0.081
<i>Syzygium sayeri</i>	0.00157	0.00061	135	48	0.0000134	0.0000082	0.052	0.086
<i>Toeckima erythrocarpum</i>	0.00119	0.00025	104	21	0.0000120	0.0000044	0.052	0.088
<i>Wrightia laevis</i>	0.00167	0.00023	114	6	0.0000146	0.0000013	0.053	0.029

Species	Vessel area (mm <sup>2</sup> )		Vessel number per area (mm <sup>-2</sup> )		Vessel area to number ratio (mm <sup>4</sup> )		Hydraulically weighted diameter (mm)	
	AV	SD	AV	SD	AV	SD	AV	SD
Tropical woodland								
<i>Corymbia citridora</i>	0.00175	0.00031	88	3	0.0000199	0.0000029	0.066	0.025
<i>Corymbia clarksoniana</i>	0.00148	0.00036	144	48	0.0000119	0.0000077	0.068	0.050
<i>Eucalyptus</i> sp.	0.00210	0.00038	88	15	0.0000250	0.0000093	0.068	0.066
<i>Grevillea glauca</i> *	0.00126	na	104	na	0.0000121	na	0.057	na
<i>Grevillea parallela</i>	0.00157	0.00045	95	46	0.0000202	0.0000114	0.068	0.051
<i>Lophostemon suaveolens</i>	0.00122	0.00008	146	6	0.0000084	0.0000004	0.053	0.046
<i>Melaleuca nervosa</i>	0.00153	0.00013	103	9	0.0000148	0.0000019	0.060	0.019
<i>Melaleuca viridiflora</i>	0.00126	0.00007	133	30	0.0000098	0.0000020	0.055	0.003
<i>Persoonia falcata</i>	0.00106	0.00025	135	16	0.0000079	0.0000022	0.054	0.082
<i>Pimelea linifolia</i>	0.00045	0.00003	275	100	0.0000017	0.0000005	0.029	0.021
<i>Xylomelum scottianum</i>	0.00191	0.00057	63	14	0.0000312	0.0000101	0.065	0.095
Temperate forest								
<i>Acacia dealbata</i>	0.00151	0.00028	79	25	0.0000217	0.0000125	0.056	0.044
<i>Acacia melanoxylon</i>	0.00125	0.00007	54	34	0.0000290	0.0000151	0.052	0.040
<i>Acacia obliquinervia</i>	0.00130	0.00042	59	27	0.0000310	0.0000290	0.051	0.054
<i>Coprosma hirtella</i>	0.00037	0.00008	196	67	0.0000020	0.0000007	0.026	0.037
<i>Eucalyptus pauciflora</i>	0.00149	0.00005	60	22	0.0000269	0.0000083	0.052	0.015
<i>Eucalyptus</i> sp.	0.00145	0.00010	98	39	0.0000172	0.0000089	0.055	0.018
<i>Exocarpos strictus</i>	0.00047	0.00003	242	83	0.0000021	0.0000009	0.029	0.011
<i>Hakea lissosperma</i>	0.00026	0.00003	438	42	0.0000006	0.0000000	0.022	0.012
<i>Hakea microcarpa</i>	0.00022	0.00002	372	22	0.0000006	0.0000001	0.019	0.018
<i>Leionema phyllicifolium</i>	0.00031	0.00005	348	24	0.0000009	0.0000002	0.023	0.019
<i>Lomatia myricoides</i>	0.00050	0.00007	296	80	0.0000018	0.0000005	0.032	0.023
<i>Olearia megalophylla</i>	0.00027	0.00003	326	97	0.0000009	0.0000004	0.023	0.014
<i>Olearia phlogopappa</i>	0.00030	0.00004	195	12	0.0000015	0.0000001	0.024	0.021
<i>Ozothamnus secundiflorus</i>	0.00034	0.00003	266	101	0.0000014	0.0000006	0.025	0.013
<i>Persoonia subvelutina</i>	0.00050	0.00008	361	69	0.0000015	0.0000006	0.031	0.024
<i>Pimelea linifolia</i>	0.00025	0.00005	516	70	0.0000005	0.0000002	0.020	0.028
<i>Polyscias sambucifolia</i>	0.00029	0.00007	731	133	0.0000004	0.0000001	0.023	0.030

Notes: All species values averaged across three samples except for: \* – one sample, \*\* – two samples, \*\*\* – four samples, '-' indicates that given tissue did not occur in the studied species. AV – species average, SD – standard deviation.

Table A4-6 Non-anatomical traits.

Species	Wood density (g cm <sup>-3</sup> )		Height (m)		Maximum height (m)		Modulus of elasticity (MPa)		Leaf area to sapwood area ratio (cm <sup>2</sup> cm <sup>-2</sup> )	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Tropical rainforest										
<i>Antirhea tenuiflora</i>	0.57	0.03	12.9	1.9	13	na	7526	415	7328	2354
<i>Argyrodendron peralatum</i>	0.58	0.00	33.6	2.6	34	na	2384	495	14026	3351
<i>Austromuellera trinervia</i>	0.46	0.01	18.7	2.1	19	na	3782	783	15561	946
<i>Brombya platynema</i>	0.56	0.03	9.4	2.0	9	na	5106	716	9587	4664
<i>Cardwellia sublimis</i>	0.47	0.03	27.3	2.2	30	na	3646	158	7397	1567
<i>Casearia dallachii</i>	0.50	0.06	2.7	0.2	8	na	4311	737	15242	6477
<i>Castanospermum australe</i>	0.43	0.04	30.8	2.0	40	na	2790	393	11539	3348
<i>Cleistanthus myrianthus</i>	0.54	0.05	17.5	4.0	17	na	6413	508	7497	2591
<i>Cryptocarya grandis</i>	0.56	0.03	24.7	1.5	35	na	4624	680	11426	3803
<i>Cryptocarya mackinnoniana</i>	0.61	0.02	20.3	0.8	25	na	5484	2287	4543	602
<i>Cryptocarya murrayi</i>	0.47	0.04	19.0	2.8	30	na	3725	690	9398	2784
<i>Doryphora aromatica</i>	0.54	0.03	19.7	4.1	40	na	5474	1351	10216	3635
<i>Dysoxylum alliaceum</i>	0.43	0.05	19.8	0.4	38	na	4079	1571	11001	1728
<i>Dysoxylum arborescens</i>	0.53	0.03	16.7	3.6	30	na	4455	362	10666	351
<i>Dysoxylum papuanum</i>	0.52	0.07	30.6	1.4	31	na	5040	1431	9265	1310
<i>Dysoxylum parasiticum***</i>	0.44	0.06	12.6	2.2	20	na	3882	535	22164	8604
<i>Dysoxylum pettigrewianum***</i>	0.46	0.04	24.5	0.8	25	na	3864	1251	17811	8020
<i>Elaeocarpus grandis</i>	0.45	0.02	30.0	2.9	33	na	3674	727	8643	759
<i>Endiandra leptodendron</i>	0.57	0.02	15.2	2.4	18	na	5995	1549	13768	3782
<i>Endiandra microneura</i>	0.61	0.05	25.5	1.4	30	na	3894	224	8443	4895
<i>Eupomatia laurina</i>	0.50	0.02	7.2	3.4	8	na	6523	1446	11976	5183
<i>Ficus variegata</i>	0.41	0.01	16.1	1.7	20	na	4655	1138	6640	2537
<i>Gillbeea whypallana</i>	0.42	0.08	19.1	4.3	20	na	4376	271	6930	2805
<i>Gomphandra australiana</i>	0.46	0.02	10.0	7.1	20	na	4722	521	9030	2796
<i>Haplostichanthus ramiflorus</i>	0.57	0.01	2.5	0.2	4	na	4865	1714	8636	2901
<i>Harpullia rhyticarpa</i>	0.61	0.02	5.0	3.1	6	na	7625	945	16659	3773
<i>Hernandia albiflora</i>	0.46	0.05	5.2	0.8	15	na	5606	1631	11604	3420
<i>Leea indica</i>	0.40	0.02	3.8	1.0	4	na	5142	1515	24904	20077
<i>Litsea leefeana</i>	0.52	0.03	23.0	1.7	30	na	4567	1700	13299	4383
<i>Mallotus paniculatus</i>	0.45	0.05	20.0	3.3	20	na	6076	246	10457	2512
<i>Melicope xanthoxyloides</i>	0.43	0.01	11.3	4.5	11	na	5182	718	17138	11173
<i>Musgravea heterophylla</i>	0.45	0.05	24.5	2.6	30	na	2587	374	5856	2099
<i>Myristica globosa</i>	0.45	0.03	23.0	6.3	25	na	2746	664	6343	1644
<i>Palaquium galactoxylum**</i>	0.50	0.03	23.7	7.7	24	na	2688	990	10373	2043
<i>Pouteria xerocarpa*</i>	0.52	na	6.0	na	7	na	6597	na	7179	na
<i>Rockinghamia angustifolia</i>	0.45	0.01	16.6	1.8	17	na	4183	1180	7694	1296
<i>Syzygium graveolens</i>	0.54	0.05	30.1	2.1	30	na	1634	854	7622	2388
<i>Syzygium monospermum</i>	0.61	0.01	9.6	3.7	17	na	5533	595	7546	3598
<i>Syzygium sayeri</i>	0.49	0.04	33.0	1.8	35	na	2263	841	5351	2545
<i>Toeckima erythrocarpum</i>	0.57	0.03	15.9	1.0	20	na	3581	1219	6901	2865
<i>Wrightia laevis</i>	0.38	0.04	19.9	1.8	40	na	3180	411	6696	2183
Tropical woodland										
<i>Corymbia citridora</i>	0.62	0.05	7.5	1.9	35	na	6509	358	10830	770
<i>Corymbia clarksoniana</i>	0.55	0.04	4.4	2.1	25	na	4674	2261	8996	3679
<i>Eucalyptus</i> sp.	0.50	0.05	3.4	1.1	25	na	6475	1732	7782	1306

Species	Wood density (g cm <sup>-3</sup> )		Height (m)		Maximum height (m)		Modulus of elasticity (MPa)		Leaf area to sapwood area ratio (cm <sup>2</sup> cm <sup>-2</sup> )	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
<i>Grevillea glauca</i> *	0.61	na	3.6	na	10	na	3017	na	5369	na
<i>Grevillea parallela</i>	0.60	0.04	5.2	0.7	15	na	3380	1040	5413	256
<i>Lophostemon suaveolens</i>	0.53	0.02	2.2	0.3	20	na	3366	222	3700	1274
<i>Melaleuca nervosa</i>	0.56	0.05	3.5	0.8	10	na	2075	178	5400	1675
<i>Melaleuca viridiflora</i>	0.56	0.01	3.0	0.2	10	na	1555	214	4942	2251
<i>Persoonia falcata</i>	0.58	0.02	4.0	0.3	9	na	4791	1468	4093	1892
<i>Pimelea linifolia</i>	0.44	0.05	0.7	0.1	2	na	3511*	na	1922	620
<i>Xylomelum scottianum</i>	0.62	0.04	3.8	0.5	10	na	1735	636	4301	497

#### Temperate forest

<i>Acacia dealbata</i>	0.53	0.03	4.3	0.6	30	na	11131	6765	5318	1724
<i>Acacia melanoxylon</i>	0.51	0.00	3.1	0.6	30	na	11073	2760	3636	1686
<i>Acacia obliquinervia</i>	0.56	0.05	4.4	0.6	15	na	8412	7246	3926	550
<i>Coprosma hirtella</i>	0.60	0.05	1.4	0.1	2	na	8995	2518	1640	377
<i>Eucalyptus pauciflora</i>	0.61	0.02	7.8	1.5	20	na	7964	3659	2704	565
<i>Eucalyptus</i> sp.	0.61	0.03	22.0	6.6	25	na	3049	628	5413	1500
<i>Exocarpos strictus</i>	0.60	0.03	1.2	0.2	4	na	6043	2373	2591	534
<i>Hakea lissosperma</i>	0.54	0.01	2.0	0.2	5	na	8292	4540	995	216
<i>Hakea microcarpa</i>	0.61	0.02	1.7	0.3	2	na	7066	523	838	313
<i>Leionema phyllicifolium</i>	0.61	0.02	1.3	0.4	1	na	9521	4329	1994	388
<i>Lomatia myricoides</i>	0.56	0.03	2.5	0.3	6	na	4752	2711	3310	1132
<i>Olearia megalophylla</i>	0.62	0.05	1.2	0.2	2	na	11778	4811	1820	320
<i>Olearia phlogopappa</i>	0.62	0.01	1.4	0.2	1	na	7473	847	2357	539
<i>Ozothamnus secundiflorus</i>	0.57	0.01	1.8	0.2	2	na	2712	3829	1994	1240
<i>Persoonia subvelutina</i>	0.55	0.02	2.1	0.1	2	na	9886	3556	2224	289
<i>Pimelea linifolia</i>	0.50	0.01	1.8	0.4	2	na	7736**	5785	1702	516
<i>Polyscias sambucifolia</i>	0.55	0.00	2.7	0.3	5	na	7353	792	4397	512

Notes: All species values averaged across three samples except for\* – one sample, \*\* – two samples, \*\*\* – four samples, '-' indicates that given tissue did not occur in the studied species. AV – species average, SD – standard deviation.





## Chapter 5

# **DISCUSSION**



I set out to quantify the wood anatomy of twigs and its relation to wood density, and to contribute a firm anatomical basis to our understanding of plant functions and ecological strategies. Before this thesis, we were largely lacking quantitative data on twig wood anatomy across a broad range of angiosperm species and had limited knowledge on anatomical underpinnings of wood density variation in twigs. Here, I discuss how my work fills these gaps and how it may lead on to new research directions.

## **5.1 Axes of variation**

One main result has been that there are relatively few ways to achieve very high-density wood, but there are many alternative ways to achieve low-density wood. A visual summary of this finding is illustrated in Figure 4-4b. The two-dimensional layout of this figure implies two main anatomical dimensions, which are approximately orthogonal to each other. Both are complex. One is anatomical variation driving wood density, and the other is anatomical variation independent of density. With hindsight, these two dimensions can be discerned for main-stem wood from graphs presented by Martínez-Cabrera et al. (2009) and Poorter et al. (2010), but previous work on twigs (Jacobsen et al. 2007a) was not presented in such a way that this issue could be assessed. To my knowledge, the existence of these two axes of variation has not received prior attention nor has it been systematically analysed and described. Next, I discuss these two axes and their potential biological meaning.

### **5.1.1 Wood density dimension**

Across 24 angiosperm species from 4 sites in eastern Australia (Chapter 3) variation in twig wood density was mainly driven by fibre wall and lumen fractions, in concordance with other studies on stems of over 100 species (Fujiwara et al. 1991; Martínez-Cabrera et al. 2009) and twigs of 17 species (Jacobsen et al. 2007a). Wood density has been shown to strongly correlate with mechanical strength (MOR) and stiffness (MOE) across over 500 angiosperm species (Chave et al. 2009; Onoda, Richards & Westoby 2010). The mechanism linking wood density to mechanical traits is well established and relatively straightforward. Because fibre wall fraction was reported to

be on average the most abundant tissue and to be higher in higher-density species (Ziemińska et al. Chapter 3; Fujiwara et al. 1991; Jacobsen et al. 2007a; Martínez-Cabrera et al. 2009), we can infer that fibres are likely to be the decisive contributors to MOR and MOE. This is congruent with a common perspective that the primary role of fibres is mechanical support (Evert 2006), and with reports of a positive relationship between fibre wall fraction and MOR (Jacobsen et al. 2007a). There are also other anatomical factors contributing to mechanical properties of wood, for example, microfibril angle in fibre walls (Evans & Ilic 2001; Yang & Evans 2003; Barnett & Bonham 2004) or distribution and size of vessels (Beery, Ifju & McLain 1983; Hepworth et al. 2002). Although anatomical structure explains the mechanical behaviour of wood as a material, in order to understand the mechanical strategy of an organ or a whole plant other additional factors need to be taken into account. For example, the diameter of a shoot influences the whole-shoot mechanical properties, and the leaves and shoot geometry affect branch behaviour during wind stress (Wainwright et al. 1982; Vogel 1989; Gartner 1991; Niklas & Speck 2001; Read & Stokes 2006; van Gelder et al. 2006; Butler et al. 2011). In fact, Butler et al. (2011) showed that shoot geometry had a much stronger effect on shoot mechanical behaviour than wood density, the wood material property. Vegetation structure and climate potentially also affects mechanical behaviour of plants. For example, under the dense canopy of rainforest, pioneers may take advantage of a gap opening by growing fast and building mechanically risky stems (Read & Stokes 2006). Certainly, multiple evidence needs to be consulted (anatomy, morphology, environmental context) to fully understand plant mechanical strategies; wood density and fibre structure are informative and contributory elements.

Wood density has also been shown to correlate with hydraulic traits such as minimum water potential and water storage, especially capacitance (a measure of stored water released per water potential change). Minimum water potential tended to be more negative in higher-density species (Santiago et al. 2004; Ackerly 2004; Bucci et al. 2004; Jacobsen et al. 2007b; Gotsch et al. 2010), and capacitance tended to increase towards lower-density species (Meinzer et al. 2003, 2008; Scholz et al. 2007; Pratt et al. 2007). There is no direct mechanism linking fibre wall fraction to minimum water potential, so presumably there is something about the selective environment that leads

to low minimum water potential that also favours high wood density. A more direct mechanism possibly exists between density and capacitance. Higher fibre lumen fraction in low-density species can in principle store more water than the smaller fibre lumen fraction typical of higher-density species. Indeed, it was found that capacitance correlated positively with fibre plus vessel lumen fraction across nine species (Pratt et al. 2007). However, in this thesis, I showed that lower-density species had either high fibre lumen fraction or high parenchyma fraction, or somewhere in between – these two tissue fractions trading off against one another. Presumably, both tissues can contribute to capacitance, but it is unclear what would be the ecological difference between parenchyma capacitance and fibre lumen capacitance. This issue is discussed in the following section.

### **5.1.2 Anatomical variation independent of density**

The dimension of anatomical variation mostly independent of wood density was characterised across 69 angiosperm species analysed in Chapter 4. This dimension stretched along a fibre-parenchyma spectrum from high fibre fraction and low parenchyma fraction at one end to high parenchyma fraction and low fibre fraction at the other. This spectrum was relatively narrow in species with high density (top of diagram in Figure 4-4b), but it noticeably widened towards lower wood densities (bottom of diagram in Figure 4-4b). This was because high-parenchyma species necessarily have relatively low density. Also, high fibre fraction in low-density species consisted of a substantial share of fibre lumen relative to wall. What is the functional meaning of this continuum? What are the benefits and costs of having higher fibre fraction (with abundant fibre lumen relative to wall) versus higher parenchyma fraction?

Fibres are usually dead cells (whereas parenchyma cells are alive), and thus once fully developed they do not incur respiratory maintenance costs. This suggests that a strategy of higher fibre fraction relative to parenchyma would incur lower ongoing carbon costs per given wood volume. Would lower ongoing costs then allow photosynthesized carbon to contribute to faster plant growth instead of to maintaining current tissues (as in species with large parenchyma fraction)? One could hypothesise that, per given wood density, species with more fibre (and fibre lumen) fraction would

grow faster than species with high parenchyma fraction. Growth rates were not measured in the present study, but examining relationships between growth rates and wood density might be of help. Figure 5 in Chave et al. (2009) depicts wood density in relation to relative growth rate in saplings from across a broad set from tropical forests. Although negatively correlated, it is notable that the data points create an approximately triangle shape, where low-density species exhibit approximately twice the variation in relative growth rate than do high-density species. This difference might be partly explained by anatomical variation along the parenchyma-fibre spectrum in low-density species. Growth rate may be influenced by many factors (e.g. plant age, light, nutrient, or water limitations), and anatomical structure could be yet another possibility.

Large parenchyma fraction may be costly in maintenance, but presumably it has other advantages. Parenchyma transports and stores carbohydrates, and large carbohydrate storage is presumably most advantageous under certain conditions (e.g. leafing at the outset of growth season in deciduous trees or after major disturbance like fire or storm, discussed in more detail in Chapter 2). Although in principle the link between parenchyma fraction and carbohydrate storage sounds straightforward, as far as I am aware, it has not been systematically studied. This suggests a potentially interesting new research direction. One question that could be asked is: does parenchyma have other important functions besides storing carbohydrates, and under what conditions would these other functions be most beneficial?

Possible additional (or alternative) functions for parenchyma are water storage (Holbrook 1995; Chapotin, Razanameharizaka & Holbrook 2006) and vessel refilling (Zwieniecki & Holbrook 2009; Nardini, Lo Gullo & Salleo 2011). Higher water storage has been linked with abundant parenchyma in succulent species (cacti, baobabs), but its mechanisms and significance in woody angiosperms are not well understood. Indirect evidence of a relationship between parenchyma and water storage comes from studies linking wood density with capacitance, which have shown that lower-density species had higher capacitance (Meinzer et al. 2003, 2008; Scholz et al. 2007; Pratt et al. 2007). This capacitance could have resulted from either high parenchyma fraction or high fibre lumen fraction, as both anatomies are possible in lower-density species (Chapter 3 and 4). Possibly, there may be differences in the mechanisms of water release from the two

tissue types. In two conifers and one *Acer* species, it has been shown that extracellular capacitance, located in dead cell lumens including fibres, is used up at the outset of diurnal transpiration (Tyree & Yang 1990). Water release from parenchyma cells might be more difficult because it would require the whole wood volume to adjust (Holbrook 1995). The other function of parenchyma is embolism refilling. Increasing evidence suggests that carbohydrates released from parenchyma participate in this process (Salleo et al. 2004; Améglio et al. 2004; Salleo, Trifilò & Lo Gullo 2006; Secchi & Zwieniecki 2011). However, the refilling process has been studied on a relatively small number of species and it is not clear how it could be linked with broad variation in parenchyma fraction across a larger number of species. Is it all parenchyma within a given volume of wood that participates in refilling or is it only parenchyma in closest vicinity to vessels? Possibly, parenchyma cells that are in direct contact with vessels (contact cells, also called vessel-associated cells) play a major role in the mechanism which triggers vessel refilling (Czaninski 1977; Salleo et al. 2004; Améglio et al. 2004; Secchi & Zwieniecki 2011). The remaining parenchyma fraction could store water necessary to refill vessels (if not involved in any other function, for example, carbohydrate storage). The role of parenchyma in refilling is not well understood, and, in fact, even the refilling process has recently been questioned. The debate casts doubts on whether refilling under negative pressure actually happens and how common it is (Sperry 2013; Wheeler et al. 2013). A careful analysis of wood anatomy, capacitance, embolism, and refilling processes could perhaps help to disentangle the role of parenchyma in plant function and strategies.

Another tissue that has not been researched in detail is tracheids. These are conduits similar to vessels in their wall pitting but, devoid of perforation plates and having diameter similar to the diameter of fibres. Tracheids are believed to play supportive role in conducting water and have been suggested to function as safe bypass for water transport in water stressed habitats (Carlquist 1984, 1985; Carlquist & Hoekman 1985). In Chapter 4, the proportion of conduits smaller than 15 µm in lumen diameter has been measured (called conduits<sub>15µm</sub>). These conduits potentially encompass tracheids and/or small vessels. Within the two warm sites, the drier one had larger proportion of these conduits; in fact, only 5% of species from the wet site had conduits<sub>15µm</sub> in contrast with more than 90% and 80% of species from the warm dry site

and the cold wet site, respectively. These results support the hypothesis that tracheids or small vessels may be important as auxiliary, safe pathways for water transport in water stressed environments. However, the exact mechanisms and the significance of these cells for the overall safety strategies remain to be tested.

## **5.2 Limitations of this study and possible paths forward**

No study is perfect, but the limitations can inspire future research. Here, the labour-intensive and time-consuming anatomical quantifications constituted the largest practical challenge. The time constraint imposed unfortunate restrictions on the sampled growth form (self-supporting trees and shrubs only), number of studied species and sampled locations, and the level of anatomical detail.

I concentrated on twigs as they have largely been overlooked in anatomical studies, yet they are the subjects of many physiological and ecological works. It is important to note that some twigs were sampled just a metre above the ground (in shrubs), yet others were sampled from heights of up to 30 m (in trees, accessed via a canopy crane). Potentially, some functional processes might differ between twigs found at these heights. For example, the longer conductive pathway in tall trees can impose higher water flow resistivity (Gleason et al. 2012). Yet that effect could potentially be overcome by having larger vessels at lower heights within the tree (Sperry et al. 2007). In any case, sampling shrub twigs at lower heights than tree twigs is not itself a limitation but, still, the potential for this to affect the results should be borne in mind (e.g. in relationships, or lack thereof, between anatomical traits and plant height).

Vines and lianas, i.e. non-self-supporting growth forms, were not included in this study. I concentrated on self-supporting species, as they are the main component of the sampled vegetation types. However, caution should be taken in applying these findings to non-self-supporting species. For example, in this work, vessel lumen fraction did not vary very widely (4-fold; from 0.6 to 0.23) and contributed little to overall density variation. However, lianas are well known to have large vessel size and vessel number per area, likely resulting in relatively large vessel fractions (Bamber 1984; ter Welle 1985;



Gartner 1991). Vessel lumen fraction may play a more important role in explaining wood density variation across a wider variety of growth forms.

The number of species studied here, although relatively large, is by no means representative for the entire world flora. A similar critique applies to the climate range of sampled sites. And, of course, some very common plant functional types were not included; e.g. the deciduous species of temperate forests and evergreen conifers, both so common in the Northern Hemisphere.

I focused on quantifying tissue proportions as they were the most relevant in explaining wood density variation. Furthermore, tissue proportions are the most basic and functionally indicative features of wood. Nevertheless, other anatomical traits are potentially interesting too, for example, the structure of pits between parenchyma and vessels may add a clue to how water or carbohydrates can be released from parenchyma to transpiration stream.

The findings presented in this thesis highlighted some intriguing gaps in our knowledge. Up to the present, most literature on functional wood anatomy of angiosperms has focused on vessels, paying far less attention to parenchyma and fibres. Perhaps the most striking gap is our lack of understanding of parenchyma tissues. Parenchyma can occupy from 6 to over 60% of wood (Table 2-1) or in extreme cases to over 80% (e.g. in baobabs; Chapotin et al. 2006), yet we barely understand the functional meaning of this diversity, nor its relationship with climate. It is possible that the primary role of parenchyma varies among species, and climate zones, and even, potentially, among seasons: from chiefly being a site of carbohydrate storage via participating in embolism refilling to being a water reservoir. Another main result was the quantification of a trade-off between parenchyma and fibre fraction, variation on this axis widening towards lower-density species. We investigated this variation in relation to climate and functional traits (height, leaf area to sapwood area ratio, and modulus of elasticity). Nevertheless, these attempts were not successful in elucidating the functional meaning of this trade-off. It remains unclear what are the benefits of large parenchyma fraction versus large fibre fraction (with a significant fibre lumen component). Consequently, the next steps in understanding wood functional strategies via detailed anatomy could include:

- Measuring carbohydrate storage dynamics with reference to parenchyma (or living fibres). Probably this would help to explain large variation in parenchyma fraction across species and assess the significance of parenchyma as carbohydrate reservoir in various seasons and climates.

- Testing the mechanisms of water release and functional difference between fibre lumen capacitance and parenchyma capacitance. Such investigation would presumably elucidate benefits of large parenchyma fraction versus fibre lumen fraction.

- Exploring vessel-refilling mechanisms across species with diverse parenchyma fractions could potentially disentangle the role of parenchyma in refilling and shed some light on refilling mechanism itself.

### **5.3 Thesis context and significance**

Wood density has been suggested to be a key functional trait (Chave et al. 2009). It has frequently been measured and has correlated with various traits (mechanical, hydraulic, life history; Tables 2-3, 2-5, and 2-7). Yet wood density is only one value representing a complex and multifunctional wood and, in itself, has not been fully understood. Anatomical structure directly determines density, but anatomical underpinnings of density variation in twigs have not been quantified in a systematic way. The role of this thesis has been:

- To gather quantitative and organized knowledge of anatomical basis for wood density variation in twigs across a relatively broad range of angiosperm species.

- To show that there is a substantial fibre-parenchyma dimension independent from wood density dimension and also from vessel size dimension.

- To indicate that the width of fibre-parenchyma dimension increases towards lower-density species.

- This suggests that wood density might not be a straightforward indicator of plant functions (in contrast to arguments made by Chave et al. 2009 and others), with this especially the case among lower-density species.

- To test several hypotheses about what fibre-parenchyma spectrum might be correlated with; however, I did not succeed in identification of correlates of this spectrum, whose biological meaning continues to be opened for future research.

In this thesis, I compiled a broad dataset of anatomical variation in twigs and elucidated the anatomical basis of wood density variation. Anatomical evidence, together with physiological and ecological studies, can be an insightful tool in deciphering plant curious functions and strategies.

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