

DEBORA COELHO DA SILVA

**EFEITO DA LUZ NA RESISTÊNCIA E ESTRUTURA DO XILEMA AO EMBOLISMO
EM ESPÉCIES AMAZÔNICAS**

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**EFEITO DA LUZ NA RESISTÊNCIA E ESTRUTURA DO XILEMA AO EMBOLISMO
EM ESPÉCIES AMAZÔNICAS**

Tese de Doutorado apresentada à
Faculdade de Ciências Agronômicas da
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Orientadora: Dra. Carmen Regina Marcati

Coorientador: Dr. Marciel José Ferreira

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Impacto potencial desta pesquisa

Os resultados desta pesquisa fornecem subsídios importantes para compreender as características fisiológicas e anatômicas que podem ser utilizadas na seleção de espécies para áreas de regeneração de florestas secundárias. A variação nas estratégias hidráulicas entre espécies pioneiras e não-pioneiras demonstra que a plasticidade no uso da água e na resistência ao embolismo deve ser levada em consideração ao planejar esforços de restauração ecológica. Espécies com maior capacidade de ajuste às condições de luz apresentam vantagens em ambientes de transição, onde a disponibilidade de recursos pode ser altamente variável.

Além disso, os achados sobre a composição celular do xilema evidenciam que a resistência ao embolismo não pode ser atribuída a um único traço anatômico, mas sim à interação entre diferentes características estruturais. Isso reforça a necessidade de integrar abordagens anatômicas e fisiológicas na escolha de espécies para plantios em áreas de regeneração. Indivíduos juvenis com maior plasticidade anatômica e eficiência hidráulica têm mais chances de chegar à maturidade e cumprir sua função de recomposição da floresta, contribuindo para o estabelecimento e crescimento de comunidades florestais mais resilientes.

Dessa forma, este estudo não apenas amplia o conhecimento sobre os mecanismos fisiológicos e anatômicos das espécies arbóreas em regeneração, mas também fornece informações aplicáveis ao manejo florestal. A seleção de espécies baseada em suas estratégias hidráulicas pode otimizar a recuperação de florestas secundárias, promovendo a estabilidade dos ecossistemas e garantindo a manutenção dos serviços ecossistêmicos associados ao funcionamento da vegetação nativa.

Potential Research Impact

The findings of this study provide important insights into the physiological and anatomical characteristics that can be considered in species selection for regenerating secondary forests. The variation in hydraulic strategies between pioneer and non-pioneer species demonstrates that plasticity in water use and embolism resistance should be considered when planning ecological restoration efforts. Species with greater capacity to adjust to light conditions have advantages in transitional environments, where resource availability can be highly variable.

Additionally, the findings on xylem cellular composition indicate that embolism resistance cannot be attributed to a single anatomical trait but rather to the interaction among different structural characteristics. Juvenile individuals with greater anatomical plasticity and hydraulic efficiency are more likely to reach maturity and fulfill their role in forest recovery, contributing to the establishment and growth of more resilient forest communities.

Thus, this study not only expands knowledge on the physiological and anatomical mechanisms of regenerating tree species but also provides practical information for forest management. Species selection based on their hydraulic strategies can optimize the recovery of secondary forests, promoting ecosystem stability and ensuring the maintenance of ecosystem services associated with native vegetation function.

CERTIFICADO DE APROVAÇÃO

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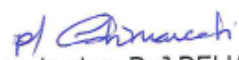
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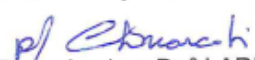
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Botucatu, 05 de dezembro de 2024

*Ao meu filho, Enzo, que foi a razão de eu
escolher ser pesquisadora,*

Aos meus pais e irmãos, suporte diário,

dedico.

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À Fazenda Experimental da UFAM (FAEXP) e todos os ajudantes de campo e funcionários. Sem o apoio de vocês nada disso seria viável.

A todo corpo docente e funcionários da FCA (UFAM e UNESP), por contribuírem na minha formação profissional.

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*“Palavras são, na minha não tão humilde
opinião, nossa inesgotável fonte de magia.*

Albus Dumbledore”

Harry Potter e as relíquias da morte –
parte 2. Direção: David Yates. [S.l.]:
Warner Bros. Pictures, 2011.

RESUMO

O crescimento de árvores em florestas secundárias é essencial para manutenção de futuras florestas maduras após distúrbios antrópicos, cada vez mais extensos e frequentes. A diferença no ritmo de crescimento das espécies entre grupos ecológicos contrastantes (pioneiras e não-pioneiras) é ecologicamente esperada. No entanto, períodos de secas naturais afetam o sucesso da regeneração natural de diferentes maneiras, dependendo das estratégias utilizadas pelos diferentes grupos ecológicos para lidar com esses eventos. Para verificar a resiliência de indivíduos juvenis nesse tipo de formação, investigamos como os traços hidráulicos, fisiológicos e anatômicos de cinco espécies arbóreas de grupos sucessionais contrastantes variam em função de um gradiente de luz em uma floresta secundária de 22 anos na Amazônia Central, Brasil. A tese está dividida em dois capítulos, o primeiro aborda a resistência do xilema ao embolismo, considerando três condições de luz criadas artificialmente (sub-bosque, intermediária e clareira). Medimos parâmetros microclimáticos, incluindo a transmitância da luz e o déficit de pressão de vapor (DPV), no auge dos períodos seco e úmido. Avaliamos o potencial hídrico foliar ao amanhecer (ψ_{pd}) e ao meio-dia (ψ_{md}), a resistência do xilema ao embolismo (P_{50}), margem de segurança hidráulica (HSM), densidade da madeira e o crescimento. No segundo capítulo abordamos a anatomia comparada do xilema e características de trocas gasosas para entender o desempenho das espécies amazônicas em relação a um gradiente de luz explorando a relação entre tipos celulares, fisiologia foliar e a resistência do xilema ao embolismo. Medimos diâmetro (μm) e densidade de vasos (n° de vasos/ mm^2) e o percentual de fração celular do xilema. Nos mesmos indivíduos, foram mensurados a fotossíntese, condutância estomática, transpiração, fotorrespiração e calculados a eficiência no uso da água e a eficiência intrínseca no uso da água. No primeiro capítulo, verificamos que o gradiente de luz afetou significativamente os parâmetros microclimáticos e consequentemente o DPV, porém apenas no período mais seco. O potencial hídrico foliar variou com a espécie, condição de luz e o período, destacando respostas específicas ao estresse hídrico sazonal, especialmente no período seco. P_{50} revelou padrões espécie-específicos dependentes do gradiente de luz, com *Carapa guianensis* mais resistente na condição de luz intermediária e *Tabebuia rosea* menos resistente em todas as condições de luz. A densidade da madeira foi influenciada pela condição de luz e espécie, exceto para *T. rosea*. HSM, variou com as espécies e a condição de luz no período seco, indicando que os diferentes níveis de segurança hidráulica variam sazonalmente. No período úmido, as condições de luz influenciaram mais fortemente a HSM. No segundo capítulo encontramos que o diâmetro hidráulico e densidade de vasos variaram significativamente entre as condições de luz e espécies. O aumento na disponibilidade de luz influenciou tamanho e a distribuição dos tipos celulares em todas as espécies, mas não de maneira linear entre as condições de luz. Já a redução na disponibilidade de luz produziu um grande percentual de parênquima para todas as espécies. Encontramos uma relação positiva entre a condutância estomática e o diâmetro hidráulico para todos os indivíduos na condição de clareira, enquanto no sub-bosque não se verificou relação. Essas descobertas demonstram que o uso de diferentes estratégias pelas espécies depende dos fatores microclimáticos, independente do grupo ecológico e são essenciais para o planejamento de condução de áreas em regeneração na região amazônica.

Palavras-chave: embolismo; P_{50} ; densidade da madeira; anatomia ecológica; plantio de enriquecimento.

ABSTRACT

Tree growth in secondary forests is essential for the maintenance of future mature forests after increased and frequent anthropogenic disturbance. Differences in growth rates between species from contrasting ecological groups (pioneers and non-pioneers) are ecologically expected. However, natural droughts affect the success of forest regeneration in different ways, depending on the strategies used by different ecological groups to cope with these events. To assess the resilience of juvenile individuals in such formations, we investigated how the hydraulic, physiological and anatomical traits of five tree species from contrasting successional groups vary along a light gradient in a 22-year-old secondary forest in Central Amazonia, Brazil. This thesis is divided into two chapters. The first chapter addresses xylem resistance to embolism under three artificially created light conditions (understory, intermediate, and gap). We measured microclimatic data, including light transmittance and vapor pressure deficit (VPD), at the peak of both the drier and wetter seasons. We evaluated predawn (ψ_{pd}) and midday (ψ_{md}) leaf water potential, xylem resistance to embolism (P_{50}), hydraulic safety margin (HSM), wood density, and growth. The second chapter focuses on the comparative anatomy of the xylem and gas exchange traits to understand the performance of Amazonian species along a light gradient, exploring the relationship between cellular composition, leaf physiology, and xylem resistance to embolism. We measured vessel diameter (μm), vessel density (number of vessels/ mm^2), and the percentage of xylem cellular fractions. In the same individuals, we measured photosynthesis, stomatal conductance, transpiration, photorespiration, and calculated water use efficiency and intrinsic water use efficiency. In the first chapter, we found that the light gradient significantly affected microclimatic parameters and, consequently, VPD, but only during the driest period. Leaf water potential varied with species, light conditions, and periods, highlighting specific responses to seasonal water stress, particularly in the drier season. P_{50} revealed species-specific patterns affected by light gradient, with *Carapa guianensis* being the most resistant in the intermediate condition and *Tabebuia rosea* the least in all light conditions. Wood density was influenced by light conditions and species, except for *T. rosea*. HSM varied with species and light conditions in the drier period, indicating that different levels of hydraulic safety fluctuate seasonally. In the wetter period, light conditions influenced HSM, particularly in the intermediate condition. In the second chapter, we found that hydraulic diameter and vessel density varied significantly among light conditions and species. Increased light availability affected the size and distribution of cell types in all species, but not in a linear pattern between light conditions. Reduced light availability resulted in a higher percentage of parenchyma in all species. We found a positive relationship between stomatal conductance and hydraulic diameter for all individuals in the gap condition, but no relationship in the understory. These results show that the use of different strategies by species depends on microclimatic factors, regardless of ecological group, and are essential for planning the management of regeneration areas in the Amazon region.

Key words: embolism; P_{50} ; wood density; ecological anatomy; enrichment planting.

LISTA DE ILUSTRAÇÕES

CAPÍTULO 1 – TRAÇOS HIDRÁULICOS E ESTRUTURAIS DE ÁRVORES AO LONGO DE UM GRADIENTE DE LUZ EM UMA FLORESTA SECUNDÁRIA AMAZÔNICA

Figure 1.1 – Location of the study area, in a secondary forest (FS) in the Central Amazon region. In the upper left corner is the perimeter of the State of Amazonas in blue. In the lower left corner, Manaus city is highlighted with the yellow perimeter and the experimental site in red. In the right is the experimental area in detail, with the layout of the five experimental blocks.

68

Figure 1.2 – Transmittance along a light gradient. The values are means ($n = 5$). The letters represent Tukey's *post hoc* test, comparing the differences across light conditions. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition.

69

Figure 1.3 – Microclimate variables in drier and wetter periods along a light gradient. The values are means ($n = 5$). a) vapor pressure deficit (kPa), b) relative humidity (%), c) soil moisture (%) d) temperature (°C), e) dew point (°C). The asterisks represent the differences between drier and wetter periods in each light condition compared by paired *t*-test. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a period. (** $P < 0.01$; *** $P < 0.001$).

70

Figure 1.4 – Total height growth (cm) and diameter (mm) five years after planting five Amazonian forest species. Values are means $\pm$ standard deviation ($n = 5$). Each color represents a light condition. The letters represent Tukey's *post hoc* test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light

condition. *C. fissilis* = *Cedrela fissilis*; *T. rosea* = *Tabebuia rosea*; *B. excelsa* = *Bertholletia excelsa*; *C. guianensis* = *Carapa guianensis*; *H. courbaril* = *Hymenaea courbaril*. (ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). _____ 71

Figure 1.5 – Predawn and midday leaf water potential in the drier and wetter period in five Amazonian tree species of different ecological groups. a) Predawn in drier period, b) Predawn in wetter period, c) Midday in drier period, d) Midday in wetter period. Values are means ($n = 5$). The letters represent Tukey's post hoc test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition. *Cf* = *Cedrela fissilis*; *Tr* = *Tabebuia rosea*; *Be* = *Bertholletia excelsa*; *Cg* = *Carapa guianensis*; *Hc* = *Hymenaea courbaril*. (ns = not significant; * $P < 0.05$; *** $P < 0.001$). _____ 72

Figure 1.6 – Xylem resistance to embolism (P_{50}) and wood density in five Amazonian tree species of different ecological groups along a light gradient. a) P_{50} , b) Wood density. Values are means ($n = 5$). The letters represent Tukey's post hoc test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition. (** $P < 0.01$; *** $P < 0.001$). _____ 73

Figure 1.7 – Hydraulic Safety Margins (HSM) in five Amazonian tree species of different ecological groups. a) HSM in the drier period, b) HSM in the wetter period. The dashed red line indicates HSM equal to zero. Negative values represent operation at more negative water potential than P_{50} . Values are means ($n = 5$). The letters represent Tukey's post hoc test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as

horizontal black lines and x, respectively. Each color represents a light condition. (ns = not significant; *P<0.05 *** P<0.001)._____74

CAPÍTULO 2 – A LUZ ALTERA AS CARACTERÍSTICAS ANATÔMICAS DO XILEMA ASSOCIADAS ÀS PROPRIEDADES HIDRÁULICAS

Figure 2.1 – Location of the study area within a secondary forest (FS) in the Central Amazon. The upper left inset shows the State of Amazonas outlined in blue, while the lower left inset highlights Manaus city within a yellow perimeter and the experimental site in red. The right side presents a detailed layout of the experimental area, showing the five experimental blocks. _____131

Figure 2.2 – Illustration of *Hymenaea courbaril* wood tissue used for cell fraction measurements. (A) identification of the cell types within the sample. (B) 1mm² area analyzed for cell type quantification. The inset details the grid spacing (C), with colors and numbers indicating different cell types: 1 (blue) - ray parenchyma, 2 (cyan blue) - vessel wall, 3 (green) - vessel lumen, 4 (pink) – banded parenchyma, 5 (orange) - paratracheal parenchyma, 6 (baby pink) - fiber lumen, 7 (red) - fiber. Scale bar: 200µm _____132

Figure 2.3 – Hydraulic diameter and vessel density across light conditions. Boxplots illustrate the effect of three light conditions—gap (orange), intermediate (green), and understory (blue)—on (A) hydraulic diameter (µm) and (B) vessel density (mm²), for five tree species (*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*). Letters indicate Tukey's post hoc test results comparing light conditions within each species. Boxplots display upper and lower quartiles, whiskers represent the 10th and 90th percentiles, and the median and mean values are marked by black horizontal lines and an “x”, respectively. (ns = P<0.05; **P<0.001; ***P<0.0001). _____1333

Figure 2.4 – Radar plots illustrating the effect of (A) light conditions and (B) ecological groups on wood cellular composition. Measured variables include the proportion of fiber lumen, fiber wall, vessel lumen, vessel wall, paratracheal parenchyma, rays, and axial parenchyma in lines or bands. Each light condition (gap in orange, intermediate in green, and understory

in blue) and ecological group (pioneer in orange, non-pioneer in blue) is color-coded. Lines represent the relative percentage of each cellular component across conditions. _____1344

Figure 2.5 – Transverse sections of the five studied species (*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*) (A-O) under three light conditions. The images illustrate that vessels are larger under higher light availability (gap) compared to lower light availability (understory). Additionally, a greater amount of axial parenchyma is observed in the understory conditions. _____135

Figure 2.6 – Vulnerability index and conductive area across light conditions. Boxplots depict the effect of three light conditions—gap (orange), intermediate (green), and understory (blue)—on (A) the vulnerability index and (B) the conductive area for five tree species (*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*). Letters indicate Tukey's post hoc test comparisons across light conditions within each species. Boxplots show upper and lower quartiles, whiskers represent the 10th and 90th percentiles, and median and mean values are marked by black horizontal lines and an "x", respectively. (ns = $P < 0.05$; *** $P < 0.0001$). _____136

Figure 2.7 – Relationships between hydraulic diameter and stomatal conductance (g_s) across light conditions. Each color represents a light condition: gap (red), intermediate (green), and understory (blue). An ANCOVA analysis was performed, with regression lines showing the trend for each light condition _____137

LISTA DE TABELAS

CAPÍTULO 1 – TRAÇOS HIDRÁULICOS E ESTRUTURAIS DE ÁRVORES AO LONGO DE UM GRADIENTE DE LUZ EM UMA FLORESTA SECUNDÁRIA AMAZÔNICA

- Table S 1.1. Maximum, average and minimum values of the six microclimate variables (n = 4). The values calculated refer to the daily variation between 5am and 7pm, taken between two and six days per block in each seasonal period (drier and wetter periods). Transmittance was only calculated in the drier period. _____ 84
- Table S 1.2. Results of two-way anova (*F values*, *R - adjust*), for hydraulic traits of five tree species (*B. excelsa*, *C. guianensis*, *c. fissilis*, *H. courbaril*, *T. rosea*) planted in different light conditions (gap, intermediate and understory). Each value is mean $\pm$ sd (n = 5). The slope values (s) are the median $\pm$ sd. Different letters indicate a difference between the light conditions for each species tested by tukey *post hoc* test. Bold letters indicate difference between light conditions. _____ 85
- Table S 1.3. Results of paired *t*-test (*t values*), for height (cm) and diameter (mm) of two sucessional groups (pioneer and non-pioneer) planted in different light conditions (gap, intermediate and understory). Each value is mean $\pm$ sd (n = 5). Different letters indicate differences between sucessional groups in each light conditions. Bold letters indicate difference across light conditions. (ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). ____ 87
- Table S 1.4. Results of two-way ANOVA (*f values*, *r2 - adjust*), for P_{50} of five tree species (*B. excelsa*, *C. guianensis*, *C. fissilis*, *H. courbaril*, *T. rosea*) planted in different light conditions (gap, intermediate and understory). Each value is mean $\pm$ sd (n = 5). Different letters indicate a difference between the light conditions for each species tested by tukey *post hoc* test. Bold letters indicate difference between light conditions. The individuals excluded from these analyses were: be: id31, 62; cg: id33, 73,74, 14,37; tr: 46,52, 57; hc: 60; cf: 39. _____ 88

CAPÍTULO 2 – A LUZ ALTERA AS CARATERÍSTICAS ANATÔMICAS DO XILEMA ASSOCIADAS ÀS PROPRIEDADES HIDRÁULICAS

Table 2.1 – Scientific and common names, ecological groups and key anatomical features of the five species planted in the secondary forest enrichment system. _____ 129

Table 2.2 – Percentage of cell fractions across a light gradient (gap, intermediate, and understory) in successional groups (pioneer and non-pioneer) and comparisons among the five studied species—*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*—in the Central Amazon. _____ 130

Table S 2.1 – Results of two-way ANOVA (*F* values, *R* - *adjust*), for hydraulic traits of five tree species (*B. excelsa*, *C. guianensis*, *C. fissilis*, *H. courbaril*, *T. rosea*) planted in different light conditions (gap, intermediate and understory). Each value is mean $\pm$ SD (*n* = 5). The slope values (*S*) are the median $\pm$ SD. Different letters indicate a difference between the light conditions for each species tested by Tukey *post hoc* test. Bold letters indicate difference between light conditions. _____ 138

SUMÁRIO

INTRODUÇÃO GERAL	25
CAPÍTULO 1 – TRAÇOS HIDRÁULICOS E ESTRUTURAIS DE ÁRVORES AO LONGO DE UM GRADIENTE DE LUZ EM UMA FLORESTA SECUNDÁRIA AMAZÔNICA	31
1.1 ABSTRACT.....	32
1.2 INTRODUCTION.....	33
1.3 MATERIAL AND METHODS.....	38
1.3.1 Study Area and Species	38
1.3.2 Experimental Design.....	38
1.3.3 Microclimate during drier and wetter periods	39
1.3.4 Height and diameter.....	40
1.3.5 Leaf water potential.....	40
1.3.6 Vulnerability curves and hydraulic safety margins	40
1.3.7 Wood density	42
1.4 STATISTICS.....	43
1.5 RESULTS	44
1.5.1 Microclimate in the experimental plots	44
1.5.2 Growth	45
1.5.3 Leaf Water Potential	46
1.5.4 Xylem embolism resistance (P_{50}).....	47
1.5.5 Wood density	47
1.5.6 Hydraulic safety margins.....	48
1.6 DISCUSSION.....	49
1.6.1 Leaf water potential as an indicator of stress	49
1.6.2 Light affects xylem embolism resistance and wood density	52
1.6.3 Hydraulic resilience across periods.....	55
1.7 CONCLUSIONS.....	57
1.8 ACKNOWLEDGMENTS.....	59
1.9 AUTHORS' CONTRIBUTIONS.....	59
1.10 FUNDING	59
1.11 CONFLICT OF INTEREST	59
1.12 DATA AVAILABILITY.....	60

1.13	REFERENCES.....	61
1.14	SUPPLEMENTARY DATA.....	75

CAPÍTULO 2 – A LUZ ALTERA AS CARACTERÍSTICAS ANATÔMICAS DO XILEMA ASSOCIADAS ÀS PROPRIEDADES HIDRÁULICAS 89

2.1	ABSTRACT.....	90
2.2	INTRODUCTION.....	92
2.3	MATERIAL AND METHODS.....	98
2.3.1	Study site.....	98
2.3.2	History of land use and seedling production.....	98
2.3.3	Implementation and experimental design.....	99
2.3.4	Xylem anatomy.....	100
2.3.5	Gas Exchange.....	101
2.4	STATISTICS.....	101
2.5	RESULTS.....	103
2.5.1	Xylem anatomical traits	103
2.5.2	Cell fraction	104
2.5.3	Anatomical features.....	106
2.5.4	Vulnerability index and percentage of conductive area	107
2.5.5	Relationships between leaf physiology and hydraulic diameter.....	108
2.6	DISCUSSION.....	110
2.6.1	The effect of light on the anatomical structure of the xylem and its hydraulic properties	110
2.6.2	Influence of light on cell fraction and its functional implications.....	112
2.6.3	Coordination between leaf physiology and hydraulic diameter.....	114
2.7	CONCLUSION.....	117
2.8	ACKNOWLEDGMENTS.....	118
2.9	AUTHORS' CONTRIBUTIONS.....	118
2.10	FUNDING.....	118
2.11	CONFLICT OF INTEREST.....	118
2.12	REFERÊNCIAS.....	119
2.13	SUPPLEMENTARY MATERIAL.....	138
	CONSIDERAÇÕES FINAIS.....	142
	REFERÊNCIAS	145

INTRODUÇÃO GERAL

A dinâmica das florestas tropicais é amplamente moldada por estratégias ecológicas de uso e aquisição de recursos, que refletem a adaptação das plantas aos gradientes ambientais, como luz e disponibilidade hídrica (Reich 2014; Guimarães *et al.*, 2018). Em termos hidráulicos, as espécies florestais exibem diferentes estratégias de transporte de água, que variam desde alta eficiência hidráulica até maior resistência ao embolismo, sendo estas estratégias críticas para a sobrevivência em condições de estresse hídrico (Santiago *et al.*, 2018; Ribeiro-Júnior *et al.*, 2023). Além disso, a segregação de nicho hidrológico, ou seja, a capacidade de diferentes espécies explorarem fontes hídricas distintas no solo, desempenha um papel fundamental na coexistência de espécies em florestas tropicais (Silvertown *et al.*, 2015; Brum *et al.*, 2019; Souza *et al.*, 2020).

O desempenho de espécies tropicais em relação a disponibilidade hídrica é um tópico de grande discussão nos últimos anos, em decorrência do aumento dos extremos climáticos (Campbell *et al.*, 2018; Costa *et al.*, 2020; Macieira *et al.*, 2021). No entanto, em florestas tropicais úmidas, o efeito da disponibilidade de luz é o fator determinante para o crescimento de espécies lenhosas (Poorter; Arets, 2003). Considerando que dentro das florestas existem diferentes nichos de disponibilidade de luz, as características funcionais das espécies podem ter algum nível de plasticidade, dependendo do grupo sucessional ao qual pertençam.

Para espécies não-pioneiras, ou aquelas crescendo em ambientes de sub-bosque, a principal característica é o ritmo de crescimento mais lento, que é acompanhado por características funcionais mais convergentes com o ritmo de crescimento (Poorter *et al.*, 2018; Santos; Ferreira, 2020). Os indivíduos apresentam características como a menor condutância estomática, menor área foliar específica, e

menor crescimento gerando um menor acúmulo de nutrientes que é ocasionado pela baixa capacidade fotossintética (Kardiman; Ræbild, 2018; Santos; Ferreira, 2020). Anatomicamente, as folhas podem apresentar menor densidade estomática, já que a transpiração nos sub-bosques das florestas tropicais úmidas costuma ser menor (Kardiman; Ræbild, 2018). Além disso, os órgãos da planta, podem atuar de forma coordenada para lidar com a demanda conflitante de disponibilidade dos recursos limitantes da produtividade (Manzone *et al.*, 2014). Por exemplo, fatores como a baixa densidade da madeira, associada a espécies de ritmo de crescimento mais lento (não-pioneiras), podem contribuir para a construção de tecidos de xilema secundário menos resistentes (Castillo-Figueroa *et al.*, 2023), o que levaria a menor resistência a formação de embolismos.

Por outro lado, as espécies pioneiras ou pioneiras de vida longa são adaptadas à ambientes de pleno sol (Swaine; Whitmore, 1988; Chazdon, 2014), portanto seu desempenho em crescimento é favorecido quando há a abertura de áreas de clareira (Santos *et al.*, 2020). Esse desempenho pode ser associado tanto a características foliares (Santos; Ferreira, 2020; Modolo *et al.*, 2021) como do xilema secundário (Campbell *et al.*, 2018). Espécies crescendo em ambientes com alta disponibilidade de luz em geral possuem alta densidade estomática, índice estomático, capacidade fotossintética e condutância estomática e, conseqüentemente, maior acúmulo de carboidratos (Guimarães *et al.*, 2018; Poorter *et al.*, 2019). No xilema secundário, os vasos tendem a apresentar características que resultam em maior eficiência hidráulica no transporte de água (Evert; Esau 2013). Essa característica é crucial para espécies pioneiras que crescem em ambientes com alta disponibilidade de luz, onde a demanda hídrica é elevada devido ao aumento do déficit de pressão de vapor, o que intensifica a transpiração foliar nesses locais (Baas *et al.*, 2004). Apesar de todas essas

adaptações fornecerem suporte para um rápido crescimento, a eficiência no transporte de água está diretamente relacionada ao tamanho diâmetro dos vasos.

De acordo com a equação postulada por Hagen-Poiseuille, a condutividade no lúmen dos vasos aumenta à quarta potência de seu diâmetro (Sperry *et al.*, 1988). Isso significa que pequenas variações no diâmetro dos vasos podem levar a grandes mudanças na condutividade hidráulica das plantas. Espécies pioneiras, que geralmente têm vasos maiores para maximizar a eficiência do transporte de água (Carlquist 2013), poderiam ser particularmente vulneráveis à eventos de seca extrema. Isso porque vasos maiores também são mais suscetíveis à formação de embolismos, um bloqueio que ocorre quando bolhas de ar se formam nos vasos devido ao estado metaestável da água sob pressão negativa, interrompendo o fluxo de água (Schenk *et al.*, 2015). Portanto, em condições de seca, onde o risco de embolismo é maior, espécies pioneiras devem sofrer maior redução significativa na capacidade de transporte de água, tornando-se mais vulneráveis à seca (Oliveira *et al.*, 2019). De maneira oposta, espécies não-pioneiras já tem sua fisiologia e anatomia adaptada para lidar com pouca luz por boa parte de sua vida, o que a tornam mais conservativas (Apgaua *et al.*, 2017), bem como apresentam xilema secundário com transporte de água com características de segurança hidráulica (ISLAM *et al.*, 2019). Isso implica em vasos com diâmetros menores, porém em maior quantidade por área (Choat *et al.*, 2007). Essas características favorecem plantas crescendo no sub-bosque a lidar melhor com eventos de embolia causados por eventos de seca.

Globalmente, há uma tendência de lenta recuperação da vegetação após eventos extremos de seca (de até quatro anos) (Anderegg *et al.*, 2015) bem como a indução à mortalidade, que são influenciados também pelos traços hidráulicos das espécies (Anderegg *et al.*, 2015, 2016). Neste contexto, o principal tecido condutor de

água é o xilema secundário, estudado na anatomia ecológica em relação à suas mudanças estruturais em respostas às pressões abióticas (Carlquist 1975; Bass *et al.*, 2004; Olson *et al.*, 2020). O transporte de água ocorre sob pressão negativa (ie. tensão), e essa tensão pode ser fortemente aumentada quando há aumento do déficit de pressão de vapor (DPV) associado a baixa umidade do solo. Essa condição favorece a ocorrência de eventos de cavitação, que são as formações de bolhas de ar nos condutos do xilema sob forte pressão negativa (Tyree; Sperry, 1988). Esse processo pode ser agravado com a passagem de nanobolhas de ar para conduítes vizinhos (*air seedling*), que se expandem no vaso, causando embolismo no sistema hidráulico, impedindo o transporte de água do solo até as folhas (SCHENK *et al.*, 2015). Porém, as plantas possuem diversos mecanismos para contornar a embolia.

As aclimações para lidar com o embolismo no xilema são espécie-específicas e podem ser influenciadas pela condição de luz, clima, grupo sucessional (Lens *et al.*, 2013; Poorter *et al.*, 2010). No xilema secundário, a diminuição no diâmetro do lúmen dos vasos, agrupamento dos vasos, aumento da espessura da parede celular são reportadas em florestas tropicais secas e também para espécies de crescimento lento ou crescendo em sub-bosques em florestas tropicais úmidas (Alves; Angyaglossy-Afonso, 2000; Levionnois *et al.*, 2020; Poorter *et al.*, 2010). Diante disso, poderíamos inferir que, sendo pouco afetadas pela ausência de precipitação ao longo de toda sua evolução, a vegetação crescendo sob condições moderadas a intensas de precipitação tenderiam a não investir em características funcionais hidráulicas para lidar com o estresse hídrico, já que isso não seria um fator limitante para o crescimento. Entretanto, diversos estudos apontam que plantas crescendo nestas condições desenvolvem uma grande quantidade de atributos hidráulicos de resistência à seca (Barros *et al.*, 2019; Fontes *et al.*, 2020; Bittencourt *et al.*, 2020;

Brum *et al.*, 2020; Penha *et al.*, 2024). Na Amazônia, a hidráulica de plantas tem sido investigada em várias escalas, incluindo o perfil vertical da floresta e os efeitos do gradiente de luz e seca sobre os fluxos de água, conforme evidenciado por estudos em florestas de Caixuanã, Tapajós e Manaus (Stark *et al.*, 2012; Giles *et al.*, 2022; Sousa *et al.*, 2020; Penha *et al.*, 2024). Esses estudos destacam a interação entre a luz e a disponibilidade hídrica, fatores frequentemente correlacionados no ambiente natural. Neste cenário, o entendimento da diversidade de estratégias anatômicas do transporte de água em florestas tropicais úmidas, em plantas crescendo dentro de um gradiente de luz, é crucial para o entendimento das possíveis respostas à seca quando variações microclimáticas são consideradas.

Os serviços das florestas na regulação do clima e do estoque de carbono mundial é tema de debate central na fronteira da ciência, em especial para florestas de regiões tropicais (Gatti *et al.*, 2021; Köhl *et al.*, 2017; Baumbach *et al.*, 2023; IPCC 2023). Florestas tropicais são responsáveis pelo maior estoque de madeira nativa do mundo, sendo o Brasil o segundo país em cobertura vegetal (FAO 2020). Estas florestas são responsáveis por inúmeros serviços ambientais que incluem desde o fornecimento de recursos madeireiros até o fornecimento de informações cruciais para modelos climáticos e vegetacionais (Chazdon *et al.*, 2016; Franklin *et al.*, 2020). Em florestas tropicais secas, a respostas de crescimento em árvores expostas à ausência de água é bastante estudada (Marcelo-Peña *et al.*, 2019; Rungwattana; Hietz, 2017). Por outro lado, espécies que crescem em florestas mais úmidas, como no caso da Amazônia Central, o comportamento de florestas que crescem sem restrição hídrica ainda é pouco compreendido, ainda que exista um período mais seco na região, com precipitação abaixo de 100 mm ao mês (Macieira *et al.*, 2021; Tavares *et al.*, 2023).

Para a fitofisionomia de florestas de crescimento secundário, ou florestas secundárias, essas são informações praticamente inexistentes.

Florestas secundárias são de extrema relevância, pois já representam cerca de 23% das áreas da Amazônia Legal (INPE 2016; MAPBIOMAS 2024). Além disso, áreas de floresta jovem (> 20 anos) são capazes de capturar 20 vezes mais carbono que florestas maduras (Heinrich *et al.*, 2021). Estudos que permitam identificar e comparar o nível de vulnerabilidade à seca de árvores crescendo nestas florestas com base em suas características anatômicas e fisiológicas podem contribuir com informações essenciais ao manejo e conservação dessas áreas, pela indicação de espécies arbóreas adequadas a mudanças no regime de precipitação, além de respostas dessas florestas à eventos de seca extrema ou estiagem prolongada. Apesar de a resistência do xilema ao embolismo ser frequentemente investigado em ambientes secos característicos, o presente trabalho busca investigar a luz como fator modulador de traços anatômicos e hidráulicos, reforçando a necessidade de separar e compreender os efeitos dessas covariáveis nos gradientes ambientais. Diante deste cenário o objetivo geral do trabalho foi investigar como um gradiente de luz afeta a anatomia do xilema e a resistência ao embolismo espécies tropicais em seus anos iniciais (5 anos). Nossa hipótese principal é que as espécies irão modificar sua estrutura anatômica, refletindo estratégias para lidar com os diferentes níveis de luz. Essa modificação será mediada pela maior disponibilidade de luz, com as maiores diferenças observadas (resistência ao embolismo e características anatômicas) na condição de clareira.

CAPÍTULO 1

TRAÇOS HIDRÁULICOS E ESTRUTURAIS DE ÁRVORES AO LONGO DE UM GRADIENTE DE LUZ EM UMA FLORESTA SECUNDÁRIA AMAZÔNICA

¹Hydraulic and structural traits of trees across light gradients in the Amazon secondary forest

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1.1 Abstract

Amazonian species are generally unable to adapt to long drought periods, indicating a low capacity to adjust their hydraulic traits. Secondary forests account for 20% of forest cover in the Amazon, making natural regeneration species crucial under climate change scenarios. In this study, we compared the hydraulic traits of five species, including non-pioneers (*Bertholletia excelsa* Bonpl., *Carapa guianensis* Aubl., *Hymenaea courbaril* L.) and pioneers (*Cedrela fissilis* Vell., *Tabebuia rosea* (Bertol.) Bertero ex A.DC.), across light conditions (understory, intermediate, gap) in a 22-year-old secondary forest in Central Amazon, Brazil. Twenty-five saplings were planted and monitored in 3 plots $\times$ 5 blocks. Five years after the plantation, we assessed growth, wood density, leaf water potential at predawn and midday, xylem embolism resistance (P_{50}), and hydraulic safety margins (HSM). Leaf water potential ranged from -2.9 to 0 MPa. The non-pioneer species *C. guianensis* and *H. courbaril* exhibited the lowest P_{50} (-4.06 MPa), indicating higher embolism resistance, whereas the pioneer *T. rosea* had the highest P_{50} (-1.25 MPa), indicating lower resistance. HSM varied from -1.60 to 3.26 MPa, with lower values in gap conditions during the dry period (-1.60 MPa), especially affecting pioneer species. Wood density was influenced by both light and species type, with non-pioneers showing generally higher density, with *H. courbaril* reached 0.75 g cm⁻³ in the understory while the pioneer *T. rosea* showed the lowest density (0.27 g cm⁻³). These results highlight that light conditions affect hydraulic traits differently across species strategies, especially during early growth. Non-pioneer, slow-growing native species appear more resilient to light variation, making them suitable for future plantations aimed at climate adaptation in secondary forests.

Key words: xylem embolism resistance, leaf water potential, hydraulic safety margins, wood density, ecological groups, enrichment planting

1.2 *Introduction*

The biodiversity in the tropics, the region with the majority of known angiosperms on Earth, faces threats from climate change, particularly because of the reduced precipitation and increased water demand of trees submitted to warmer conditions (Bauman et al. 2022). Angiosperms often adopt risky strategies to cope with drought, especially fast-growing species, which operate close to their limit of hydraulic resistance (Choat et al. 2012; Tavares et al. 2023). The Amazon includes regions with different rainfall levels (Tavares et al. 2023). Although the central Amazon is characterized by high rainfall throughout the year, the reduction in precipitation during the drier period can affect the mesoclimate of localized areas. Understanding the hydraulic strategies of tropical species is key to understanding how trees cope with natural water stress and how they can respond to longer dry periods or deal with reductions in water availability.

The most critical and well documented factor for the survival and growth of regeneration in the moist tropical forest is light (Fox 1976, Chazdon and Fletcher 1984, Mesquita 2000, Zhou et al. 2023). Radiation shapes the fast-slow continuum observed in tropical forests, with dominant and taller trees often being more vulnerable to drought (Oliveira et al. 2021; Guillemot et al. 2022; Araújo et al. 2024). At structural level, conditions of high light availability can affect the leaf traits, i.e. decreasing the area, increasing the ratio between mass and area (Hertel et al. 2021), increasing the size and density of stomata (Contin et al. 2020), and increasing the venation (Sack and Scoffoni 2013) also xylem anatomical characteristics can be modified by light, especially vessel lumen diameter and fiber thickness (Apgaua et al. 2017; Echeverría et al. 2021). These light-related anatomical adjustments are reflected in the physiology of trees.

There is a wide literature on the effects of light on various physiological processes, such as the increase in photosynthesis and stomatal conductance in the intermediate and full light conditions (Kitajima 1994; Lusk and Pozo; 2002; Santos and Ferreira 2020a), the decrease in specific leaf area (Contin et al. 2020) and the increase in photochemical efficiency (Contin et al. 2020; Modolo et al. 2017). Quantifying the effects of light on plant hydraulics at structural and physiologic levels requires measurements of a number of functional traits that can provide mechanistic explanations of the underlying processes (Violle et al. 2007).

Measurements of leaf water potential are widely used to describe the water status for its sensitivity to microclimatic variations (Wu et al. 2018; Mas et al. 2024). During the hottest period of the day, leaf water potential reflects the maximum water stress tolerated by plants. Such a measurement is closely associated with the weather conditions occurring during data collection (Mas et al. 2024). However, the magnitude of this variation between the seasons reflects the species plasticity to water stress, and can be used as a strong proxy to assess the vulnerability of leaves to natural drought events (Skelton et al. 2017; Avila et al. 2021). The tensions created in the leaf are responsible for water ascending through the xylem, which plays a major role in the hydraulic processes of trees.

Xylem embolism resistance (P_{50}), measured in terms of the xylem sap pressure inducing 50% loss of water conductivity, stands out as an essential mechanism to describe the adaptive strategies of trees (Lens et al. 2013; Brodribb 2017) as it reflects a clear physiological trait linked to mortality, indicating the risk of hydraulic failure (Baas et al. 2004; Oliveira et al. 2019). Hydraulic failure is characterized by a loss of xylem hydraulic conductance. This process is initiated by cavitation, the formation of air bubbles within the water-conducting system during drought events (Venturas et al.

2017). Although variation in P_{50} is known as an answer to natural or induced water stress (Choat et al. 2012; Bittencourt et al. 2019), the effects of the variation in light availability, which is common in tropical ecosystems, is less investigated.

Another essential hydraulic trait, the hydraulic safety margin (HSM), has gained prominence in recent years for understanding drought resistance limits and mortality (Tavares et al. 2023). The HSM represents the strategy relative to a critical threshold, typically P_{50} . It indicates that plants with small (or negative) safety margins are subjected to substantial embolism and, consequently, a higher risk of hydraulic failure (Choat et al. 2012, Uri et al. 2013). Trees with lower safety margins are more prone to hydraulic failure, particularly during drought periods. During drier periods, the physiological change in leaf water potential could then affect the hydraulic safety margin. Variations in these traits are closely associated with xylem cell structure, especially wood density (Baas et al. 2004), although some studies point out that interconnectivity and pit ultrastructure can drive embolism, despite a lack of direct links with wood density (Kaack et al. 2019).

Wood density plays a crucial role throughout a tree's lifespan, influencing its survival rate and ecological characteristics. Trees with a higher wood density and lower growth rates tend to have higher survival rates (Eller et al. 2018; Oliveira et al. 2021). Instead, fast-growing species with a lower wood density typically exhibit lower resistance to drought (Oliveira et al. 2021). However, light availability emerges as a significant factor driving these patterns, especially in tropical forests (Kallarackal and Ramírez 2024).

In the context of climate change, hydraulic traits are fundamental to assess the survival and growth of trees, and finally predict the future evolution of forest ecosystems (Brodribb et al. 2020). The current literature points to a lack of data on the plasticity of hydraulic characteristics, especially P_{50} , at an intraspecific level (Choat et al. 2018),

leading plants to employ alternative strategies, such as partial leaf loss, to withstand stress events or even hydraulic segmentation (Avila et al. 2021).

Recent findings indicate that phenotypic plasticity can delay hydraulic failure in populations of tropical species like *Corymbia calophylla* inhabiting environments with contrasting precipitation levels (Challis et al. 2022). However, how such adaptive responses occur under the light gradients in tropical regions still require answers. Understanding the physiological processes underlying the mortality of fast-growing species during drought events remains an ecological challenge.

Research on how native Amazon species, including those of commercial interest, cope with water stress provides valuable insights for planning the best forest management strategies and adaptation of the tropical ecosystems to the future changing climate (Brodribb et al. 2020). Forest management in the Amazon must consider both the current market demand and the resilience and resistance of trees to environmental stresses. Current research on hydraulic traits primarily focuses on adult individuals, leaving the plasticity of these traits largely unexplored for seedlings and saplings, a crucial phase for survival of the regeneration (Avila et al. 2021, Tavares et al. 2023, Araújo et al. 2024). In this study, we selected five Amazonian species present in an enrichment planting (see details in Santos and Ferreira 2020b) with high commercial interest for timber and non-timber production belonging to two ecological groups (pioneers and non-pioneers). We aimed to investigate (i) how the hydraulic and structural traits in leaves (leaf water potential) and xylem (P_{50} , WD, HSM) change under a light gradient and between seasonal periods in the Amazon. We tested the hypotheses that (1) light exposure reduces leaf water potential and wood density; and that these changes (2) increase the sensitivity to drought, mainly during the drier period. We expect the variation in light conditions in our experiment to be a strong

factor in initially changing the physiological parameters and, as a consequence, the hydraulic parameters, specifically the P_{50} between light conditions.

1.3 Material and Methods

1.3.1 Study Area and Species

The experiment was conducted in a 22-year-old secondary forest at the Experimental Farm of the Federal University of Amazonas, Manaus, Brazil (Fig. 1). According to the Koppen-Geiger system, the climate is Af (Beck et al. 2018), with an annual temperature of 27 °C and total rainfall of 2408 mm. The drier period (monthly rainfall <100 mm) occurs between August and September (Tanaka et al. 2014). The relative humidity calculated at a monthly resolution reaches a minimum of 75% in August and a maximum of 92% in April, during the wetter season (Araújo et al. 2002). The soils are acidic and contain clay (pH = 4.2), with low levels of P ($1.4 \pm 0.76 \text{ mg kg}^{-1}$), Ca ($0.035 \pm 0.03 \text{ cmol}_c \text{ kg}^{-1}$) and Mg ($0.084 \pm 0.01 \text{ cmol}_c \text{ kg}^{-1}$) (for more details see Santos and Ferreira 2020b).

1.3.2 Experimental Design

We planned five randomized blocks, including three plots of 270 m² with different light conditions and forest densities (understory, intermediate and gap). To create gaps, the natural vegetation was removed by thinning through felling of canopy and subcanopy trees (DBH ≥ 5 cm) and cutting of understory vegetation (DBH < 5 cm) (see details in Santos et al. 2020). Seedlings of five species were grown in a forest nursery for six months, and planted in the field in March 2017. A total of 25 saplings per plot were planted, five per species, at a spacing of 3 m x 3 m and in pits of 30 cm x 30 cm x 30 cm. The species belong to two ecological groups: non-pioneer [*Bertholletia excelsa* Bonpl., *Carapa guianensis* Aubl., *Hymenaea courbaril* L.] and pioneer species [*Cedrela fissilis* Vell. and *Tabebuia rosea* (Bertol.) Bertero ex A.DC.].

A total of 375 seedlings were planted. In each plot, we selected one individual per species (5 blocks x 3 light conditions x 5 species, for a sampling size of 75). We preferred individuals with size close to the average of the plot, and the selection criterion was based on the average height and diameter values of the five individuals of each species per plot, consistently avoiding those individuals at the extremes of these parameters, i.e., the tallest or shortest individuals in the plot. The species were chosen for their importance for timber and non-timber production in the Amazon.

1.3.3 Microclimate during drier and wetter periods

The light conditions were characterized by Photosynthetic Photon Flux Density (PPFD) measurements obtained using a line sensor (MQ-301: Line quantum with 10 sensors and handheld meter, Apogee Instruments Inc., UT) at six moments during the day (7 a.m., 9 a.m., 11 a.m., 1 p.m., 3 p.m. and 5 p.m.). Each measurement was replicated six times, and the total daily irradiance in each plot was calculated as a product of daily averages and photoperiod (Chazdon and Fetcher 1984). We recorded the irradiance above each planted seedling. One sensor was installed outside the experimental plots to measure the PPFD in full sun using a sensor MQS B/ULM-500 logger (Heinz Walz, Germany) installed in a forest gap located 100 m from the study area, and collecting data every 30 s from 6 am to 6 pm. The light transmittance of each subplot was calculated by the ratio between the PPFD within the subplots and in full sun (Gendron et al 1998).

Temperature, relative humidity and dew point were collected during the drier (September-October 2021) and wetter (February-March 2022) periods. We installed one sensor protected with a solar radiation shield and connected to a data logger

HOBO (U23-002, Sigma Sensors, CA) in each plot for three to six days. We calculated the hourly Vapor Pressure Deficit (VPD) from 5 am to 6 pm.

Soil moisture was measured during the drier (September 2021) and the wetter (February 2022) period. Composite samples, consisting of three subsamples collected from each subplot at a depth of 20 cm, were weighed to determine the soil wet mass, and dried until reaching a constant mass (soil dry mass). The water mass content was calculated as a difference between the wet soil mass and the dry soil mass, divided by the dry soil mass (Gardner 1986).

1.3.4 Height and diameter

The individuals were periodically monitored at intervals of two to three months. Measurements included tree height, and the root collar diameter at 5 cm above the ground. This diameter was used because not all the individuals were tall enough to collect the diameter at breast height when this study was carried out.

1.3.5 Leaf water potential

The data was collected five years after planting. We measured predawn water potential (Ψ_{pd}), one hour before sunrise, and midday water potential (Ψ_{md}), from 12 to 2 pm. The measurements were taken in the field, in both drier and wetter periods with a Scholander chamber (Model 1505D, PMS Instrument, OR). Two leaves from the upper third of the canopy of each individual were used.

1.3.6 Vulnerability curves and hydraulic safety margins

Xylem embolism resistance (P_{50}) was measured in the wetter period. We used one plant of each species per light condition, for a total of 75 individuals (3 light

conditions $\times$ 5 species $\times$ 5 blocks). The branch was chosen according to the size of the tree. In trees taller than 2 m, we used a branch located in the upper third of the canopy, while smaller plants were used as a whole due to the low number of leaves available. To ensure that the effect of plant size was minimized, the apical meristem was used as a reference point to guarantee that all the branches had the same ontogenetic age. We used the average height of the trees in the understory (± 120 cm), and used this value to select the place to collect the branches on larger individuals, starting from the apical meristem downwards. This guaranteed that the cambium was sampled at the same physiological age irrespective of the total tree height. Before the measurements, we evaluated the maximum length of the vessel of each species (three branches per species) to ensure adequate branches for the measurements, using the air injection method (Greenidge 1952). The branches were collected in the predawn, placed in plastic bags, and taken to the laboratory. Immediately upon arrival, the branches were placed in buckets of water for about two hours to ensure a complete hydration before the measurements. The branches were cut again with a sharp blade, to avoid occlusion of the vessels, connected to a vacuum system (pneumatron, Pereira et al. 2020) sealed with brand cable ties and plastic film. The equipment generates air discharge values measured every 15 minutes. The changes in air pressure inside the vessel were measured and converted to volume using the Ideal Gas Law. The xylem water potential was estimated from the leaf water potential. Parallel to the air discharge measurements, we measured leaf water potential in a Scholander chamber (Model 1505D, PMS Instrument, OR) during branch desiccation. We attempted to collect the leaf water potential every -1 MPa during the curves. To ensure that the leaf potential was in equilibrium with the branch potential, we placed the entire branch in a plastic bag for 30 minutes before each measurement. After complete desiccation of the

branch (lasting from three to eight days), we calculated the number of moles of air discharged from the branch. We estimated the vulnerability curves using the Weibull function fitted with the *fitplc* package version. Given the limitations of the manual method, such as its restriction to intermittent measurements, we initially ran the analysis using only curves with a characteristic sigmoidal shape. However, we chose to include all individuals in the main analysis for a more comprehensive assessment. Additionally, we extracted values for the curve's slope (% MPa⁻¹), which indicates the rate at which embolisms occur in the trunk (Trueba et al. 2017).

The hydraulic safety margin (HSM) was calculated as the difference between Ψ_{md} and P_{50} (Choat et al. 2012). In order to understand how the microclimatic conditions, affect Ψ_{md} in each period (drier and wetter), we considered the use of the values observed in the field to be the most appropriate way for describing the water balance.

1.3.7 Wood density

We used the water displacement method to determine wood density, calculated as a ratio between the mass of dry wood and volume of saturated wood (USDA Forest Service, 1956). The samples used were taken from the same branches used for the vulnerability curves. We submerged the samples in water until complete saturation and calculated the saturated volume of wood by water displacement. To determine the dry mass, the samples were dried at 105° until reaching three consecutive measurements with constant weight (between three to five days).

1.4 Statistics

The data were submitted to tests of normality (Shapiro-Wilk) and homogeneity of variances (Levene), and transformed when the assumptions of normality and homoscedasticity were not met. We performed a paired t-test to check the variation in microclimate data between the drier and wetter periods for each light condition. A two-way ANOVA was performed to compare the functional traits between light conditions and species. A one-way ANOVA was carried out to compare the light transmittance between the lighting conditions. Tukey test was used for multiple comparisons. All the analyses and graphs were performed using the R software version 4.3.0.

1.5 Results

1.5.1 Microclimate in the experimental plots

Measurements of transmittance were collected in the wetter period, corresponding to the periods when the physiological variables, especially P_{50} , were collected. The mean values were significantly different between the light conditions ($F = 338.3$, $P < 0.001$) (Fig. 2; Table S1). Among the light conditions, the minimum transmittance ranged from 7.2% in the understory to 56.36% in the gap. The maximum transmittance ranged from 9.3% in the understory to 100% in the gap.

In general, the drier period had the higher instantaneous vapor pressure deficit (VPD), which ranged from 0 to 2.39 kPa (Table S1), with the highest average being observed in the gap (0.49 kPa) (Fig. 3a). The same pattern was detected in the wetter period, with VPD ranging from 0 to 1.76 kPa (Table S1), and the highest average being observed in the gap (0.17 kPa). VPD differed significantly between the drier and wetter periods in all light conditions (t ranging from 5.82 to 10.68, $P < 0.01$). On average, relative humidity in the drier and wetter periods was > 85 and $> 95\%$ in all light conditions, respectively (Fig. 3b) and differed significantly between periods (t ranging from -8.25 to -4.68, $P < 0.01$).

The soil moisture in the drier period ranged from 27.1 to 34.0% (Table S1). In the wetter period, soil moisture ranged from 27.3 to 32.41%. Surprisingly, no variations in soil moisture were observed between the drier and the wetter periods (t ranging from -2.63 to -1.33, $P > 0.05$) (Fig. 3c). Air temperature in the drier period ranged from 21.0 to 34.0 °C (Table S1). In the wetter period, it ranged from 21.4 to 32.4 °C (Table S1). The temperature varied significantly between the drier and wetter periods just in the gap condition ($t = 3.17$, $P < 0.05$) (Fig. 3d). In the drier period, the dew point ranged from 20.6 to 28.4 °C. In the wetter period, the range was between 21.0 and 27.9 °C.

No variation in the dew point was observed between periods and light conditions (t ranging from 1.05 to 0.60, $P > 0.05$) (Fig. 3e).

1.5.2 Growth

The height of the individuals varied widely between the light conditions (Fig. 4), with the variation between light conditions varying depending on the species ($F = 13.83$, $P < 0.0001$). The average height ranged from 123.1 cm in the understory to 517.8 cm in the gap (Fig. 4a). The tallest individuals in the gap belonged to non-pioneers *B. excelsa*, with 659.8 cm, and *C. guianensis*, with 595.7 cm. In the understory, the smallest individuals were the pioneer *C. fissilis* with 93.2 cm and *B. excelsa* with 119.3 cm. The species with the greatest height response to canopy opening was the non-pioneer *H. courbaril*, although all species responded to the greater opening in the gap condition. For diameter (Fig. 4b), the variation across light conditions also depends on the species. ($F = 13.93$, $P < 0.0001$). In the understory, the average diameter was 17.84 mm, in the intermediate 40.43 mm and in the gap 93.55 mm (Fig. 4b). The largest individuals in the gap were the non-pioneers *B. excelsa*, with 114.08 mm and *H. courbaril*, with 102.79 mm. In the understory, the smallest individuals were also *B. excelsa* with 13.19 mm and *H. courbaril* with 13.34 cm. Additionally, a difference in height was observed between the successional groups (Table S3), but only in the gap conditions with the non-pioneers exhibiting the highest average height ($t = 4.29$, $P < 0.0001$). With regard to diameter, the successional groups demonstrated contrasting patterns in the gap and the understory. In the gap, the non-pioneers displayed a larger diameter ($t = 2.05$, $P < 0.05$, Table S3), whereas in the understory, the opposite was true, with the pioneers exhibiting a larger diameter ($t = -3.09$, $P < 0.001$).

1.5.3 Leaf Water Potential

Leaf water potential ranged from -1.05 to 0 MPa at predawn (Ψ_{pd}), and from -2.9 to -0.2 MPa at midday (Ψ_{md}) (Fig. 5, Table S2). Leaf water potential was species-specific in each light condition, except at midday in the wetter period. We observed a higher variation in Ψ_{pd} during the drier period (Fig. 5a), indicating that water stress affected the species differently depending on the light condition ($F = 12.08$, $P < 0.001$). Although Ψ_{pd} of all species in the gap indicated a sufficient hydration (similar averages and close to 0 MPa), we observed clear differences between species in the other light conditions. *B. excelsa* and *C. fissilis* had the most negative Ψ_{pd} in the intermediate and understory conditions, while *H. courbaril* reached low Ψ_{pd} only in the understory. *T. rosea* had the most negative Ψ_{pd} under intermediate light condition. In the drier period, the different levels of dehydration in the predawn do not remain the same at midday, except for *C. fissilis* ($F = 5.47$, $P < 0.001$). Individuals with dehydration in the Ψ_{pd} , e.g. *T. rosea* in the intermediate light condition (-0.9 MPa), remain with values close to those in the other light conditions at midday, and not more dehydrated (Fig. 5a, c). The exception was *C. fissilis*, which presented an opposite pattern to Ψ_{pd} , with lower Ψ_{md} in the gap and higher Ψ_{md} in the intermediate and understory (Fig. 5c).

During the wetter period, only two species were affected in the predawn according to the light condition: *C. guianensis* and *T. rosea* ($F = 5.24$, $P < 0.01$). *C. guianensis* had more negative Ψ_{pd} in the understory and gap, and *T. rosea* in the understory and intermediate conditions (Fig. 5b). No differences were observed at midday in the wetter period ($F = 1.24$, $P > 0.05$) (Fig. 5d). *C. guianensis* and *T. rosea* were excluded from some analyses due to the low number of leaves measured.

1.5.4 Xylem embolism resistance (P_{50})

The species studied exhibited a P_{50} ranging from -4.04 in *C. guianensis* to -1.25 MPa in *T. rosea* (Fig. 6a; Table S2, Fig. S2). We detected an interaction between the two factors studied, indicating that P_{50} varied across the light gradient depending on the species ($F = 7.09$, $P < 0.001$). This effect was mostly evident for *C. fissilis*, *C. guianensis* and *H. courbaril*. *C. guianensis* showed higher resistance in the intermediate light condition, while *C. fissilis* and *H. courbaril* showed lowest resistance in the understory. *B. excelsa* and *T. rosea* showed no variation in P_{50} between light conditions, but the variation of these species differed for *B. excelsa*, as shown by the interquartile range of the boxplots, mostly in the intermediate light condition. We also analyzed the interaction between factors using only the sigmoidal curves ($N = 49$), as the manual methodology does not allow for continuous data collection, leading to some curves showing a less sigmoidal shape ($N = 57$; see Fig. S2). This analysis detected an interaction between the two factors studied (Table S4), indicating that the P_{50} varied along the light gradient depending on the species ($F = 5.64$, $P < 0.05$).

1.5.5 Wood density

Wood density of the species ranged from 0.27 g cm⁻³ in *T. rosea* to 0.75 g cm⁻³ in *H. courbaril* (Fig. 6b; Table S2). Wood density was affected significantly by both factors and their interaction, indicating that there is an effect of the light condition depending on the species evaluated ($F = 18.06$, $P < 0.001$). All species were affected by the light condition, except *T. rosea*. This species was the one with the lowest density, with a global average of 0.38 g cm⁻³. *C. fissilis*, *C. guianensis* and *H. courbaril* showed the same pattern, with a higher wood density detected in the understory. *B. excelsa* showed the higher wood density in the intermediate light condition.

1.5.6 Hydraulic safety margins

The hydraulic safety margin ranged between -1.60 MPa in *B. excelsa* and 3.24 MPa in *H. courbaril* (Fig. 7a; Table S2). The effect of light conditions on the hydraulic safety margin differed among species only during the drier period ($F = 2.43$, $P < 0.01$). *C. fissilis* and *H. courbaril* showed opposite patterns (Fig 7a), as observed for P_{50} and wood density. The lowest values were observed in the gap for the *C. fissilis* and in the understory, for the *H. courbaril*, although this difference was more pronounced for *H. courbaril*. The hydraulic safety margin in the wetter period ranged between -0.41 MPa in *C. fissilis* and 3.06 MPa in *H. courbaril*. In the wetter period, light condition and species independently affected the hydraulic safety margin ($F = 4.34$, $P < 0.001$). The intermediate light condition had the higher hydraulic safety margin ($F = 7.79$, $P < 0.001$). When we analyzed only the species, no difference was detected ($F = 2.39$, $P > 0.05$). In the drier period, we were unable to collect *C. guianensis*. We excluded *T. rosea* from this analysis due to the low number of repetitions available during the wetter period.

1.6 Discussion

In this study, we assessed the hydraulic and structural traits of tree species under different light conditions during wetter and drier periods in the Amazon. We validate the first hypothesis that higher light conditions reduced wood density, but did not reduce leaf water potential, except for *C. fissilis* and *T. rosea*. Unlike our expectations, the higher light exposure did not decrease the P_{50} , except for *C. guianensis* and *H. courbaril*. We found differences in the hydraulic safety margin across drier and wetter periods only for *C. fissilis* and *H. courbaril* in the dry period, which confirmed partially our second hypothesis. Leaf water potential was species-specific and related to light conditions at predawn, while this interaction was observed only in Ψ_{md} in the drier period. The wood density was affected by both light conditions and species, indicating that the anatomical structure of the studied species, with the exception of the pioneer *T. rosea*, can adapt to different light conditions.

1.6.1 Leaf water potential as an indicator of stress

We note that all Ψ_{pd} of understory plants exhibited more negative potentials during the drier period, except *T. rosea*, which exhibits an intermediate state (Fig. 5a). In the wetter period, only *T. rosea* and *C. guianensis* Ψ_{pd} had different levels of hydration between the light conditions (Fig. 5b). We note that all Ψ_{pd} of understory plants exhibited more negative potentials during the drier period, except *T. rosea*, which exhibits an intermediate state (Fig. 5a). Soil water potential significantly influences various physiological processes in plants growing in dry areas (Venturas et al. 2017). However, this pattern may diverge under wetter conditions. Our results failed to demonstrate a variation in soil moisture across light conditions and the drier and wetter

periods (Fig. 3c), confirming the findings of other authors (Santos et al. 2020; Modolo et al. 2024, in press; Table S1), the differences observed between individuals can be attributed to other factors, like vapor pressure deficit (Binks et al. 2023), nighttime transpiration (Donovan et al. 2000), reduced hydraulic conductivity (Kangur et al. 2020), chronic stress (Niinemets 2010), their position along the continuum between isohydric and anisohydric behaviors and depth of the root (Klein 2014, Martínez-Vilalta and Garcia-Forner, 2017). These factors can affect plants individually or in combination.

VPD is a significant driver of leaf responses in tropical rainforests (Binks et al. 2023), a trend partially supported by our findings. VPD reaches its highest levels in the drier period, when we observe the most significant differences between light conditions, notably for the Ψ_{pd} . However, only *C. fissilis* exhibits differences in Ψ_{md} , while other species maintain their leaf water potential relatively constant across light exposures. Under wetter conditions, a balance between leaf and soil water potential is expected. Moisture content is a proxy of soil conditions, and the absence of differences between the drier and wetter periods (Fig.3c) suggests that the differences observed between species can be attributed to other factors. Our results support the conclusions of Donovan et al. (2000), suggesting that Ψ_{pd} may not always reflect soil water potential, even under well-watered conditions. This mismatch in Ψ_{pd} may be attributed to the reduced hydraulic conductivity associated with nighttime transpiration, and may contribute in terms of growth or survival, since water is available in soil (Donovan et al. 2000; Kangur et al. 2020). Nighttime temperatures can be high during the drier period in the Amazon, although the high nighttime humidity, which can often reach 100%, makes this relationship less likely. Despite the lack of differences in soil moisture at our site, high temperatures could lead to a greater decline in hydraulic conductivity

associated with stomatal opening, resulting in variations in Ψ_{pd} . It should be noted that the microclimate of a secondary forest can differ from that of a full sun environment. In addition, the short period of days (2 to 6) of collection in each block may not faithfully represent the entire period evaluated.

The interaction between light and species in the response of leaf water potential could involve chronic stress of trees (Niinemets 2010). *C. fissilis* and *T. rosea* are categorized as pioneers, the performance of which is affected by light intensity (Eller et al. 2018). However, light appears to affect all species only in Ψ_{pd} , as evidenced by the lowest Ψ_{pd} levels observed in the understory. Over time, a failure to recover water status during the night can lead to mortality. For instance, after successive extreme drought events, trees may struggle to recover their gas exchange if the leaves remain vulnerable to water deficits (Skelton et al. 2017). *B. excelsa* and *H. courbaril* appear to have less control over stomatal opening, i.e. the variation observed depends on the sensitivity of stomata to reductions in soil water potential (Martinez-Vilalta et al. 2014), also characterized by their low stomatal conductance rates (Santos and Ferreira 2020a). These strategies are closely related to xylem characteristics (Klein 2014; Tsuji et al. 2020), potentially explaining both the Ψ_{pd} imbalance and control of leaf water potential at midday, although the relationship between stomatal control and leaf water potential has not been found for many species (Martinez-Vilalta and Garcia-Forner, 2017). One factor that may be related to these results is root depth (Martinez-Vilalta and Garcia-Forner, 2017). Considering that root depth is also reflected in plant growth (Fig. 4), we can infer that there are differences in root size across the light conditions. Higher root depth may reflect a greater capacity for water uptake (Brum et al. 2019), and therefore the maintenance of leaf water potential close to zero for plants in the gap, whereas

plants in the understory would be limited to a small amount of water that their roots could intercept.

1.6.2 Light affects xylem embolism resistance and wood density

Understanding the plasticity in hydraulic traits remains a crucial issue related to the resistance to embolism in the tropics (Islam et al. 2018, Bittencourt et al. 2022). Recently, the term adaptive plasticity has emerged to characterize plant hydraulic adaptations to environmental conditions and microclimate (Challis et al. 2022). We observed specific adjustments related to the interaction between the light conditions and species, particularly evident in P_{50} and wood density, which confirm our hypothesis. *C. guianensis* and *H. courbaril* exhibit plastic adaptation in embolism resistance as a response to varying light conditions, while *C. fissilis*, exhibits a tendency to reduce P_{50} in intermediate light conditions. (Fig. 6a). Notably, certain species exhibit greater plasticity in wood density, adjusting this trait according to light conditions. This can also be attributed to some level of hydraulic segmentation (Johnson et al. 2016) although, we emphasize that segmentation does not follow a clear pattern between the different species (Li et al. 2020; Avila et al. 2021). This topic deserves further and deeper investigations. An intriguing pattern emerges when considering wood anatomy and P_{50} . For *H. courbaril*, we observe that higher wood density (in the understory) corresponds to lower resistance to embolism (Fig. 6b), while *C. fissilis* exhibited the opposite pattern, with the lowest resistance to embolism (in the gap) corresponding to the lowest density. *T. rosea* exhibits the highest P_{50} and lowest wood density in all light conditions. Despite the seedlings in the gaps being four times taller than those in the understory (Fig 4), our findings do not support the hypothesis that smaller plants are more resistant to xylem embolism (Oliveira et al. 2021;

Guillemot et al. 2022). It is crucial to determine whether these responses are linked to specific traits such as foliar (e.g. stomatal control), anatomical (e.g. vessel size and distribution) adjustments (Blackman et al. 2023) or because young individuals may show patterns different from adult trees to predict the responses to interactions among environmental stressors.

In previous studies involving the same individuals, the plasticity of a set of physiological leaf characteristics related to nutrient acquisition (nitrogen concentration), photochemical efficiency (chlorophyll pigments and chlorophyll *a* fluorescence) and biochemical efficiency (potassium use efficiency) were associated with greater growth capacity in the gaps (Modolo et al. 2021). Plasticity in these individuals seems to follow a similar pattern for P_{50} and wood density, supporting the hypothesis that plasticity converges in the gap, even for hydraulic traits. However, this response is species-specific. These findings suggest that the acquisitive-conservative path is not consistent throughout the entire plant. The trade-off between growth and xylem embolism resistance predicts that fast-growing plants prioritize tissue expansion, such as wider vessels, making them more susceptible to drought (Tsuji et al. 2020). This study contributes to sustaining this prediction, as we observe differences in the resistance to xylem embolism among ecological groups (Table S2), with pioneer species being more vulnerable to drought. Moreover, our findings indicate that resistance to xylem embolism is affected by light condition for two of the non-pioneer species investigated (*C. guianensis* and *H. courbaril*) and one pioneer species (*C. fissilis*) (Fig. 6a). This leads to the conclusion that, when growing under high light intensity, the slow-growing *H. courbaril* can adjust their hydraulic traits to promote growth without compromising hydraulic safety, indicating a high plasticity of the species. We also observed that the

intermediate light condition leads to greater resistance to embolism among the species investigated.

Different tree species exhibit distinct responses to drought, because of their hydraulic traits (Blackman et al. 2023). Notably, certain species, such as *C. fissilis*, *C. guianensis*, and *H. courbaril*, exhibit greater resilience under intermediate light conditions, while showing increased vulnerability in the understory. This underscores the species-specific nature of hydraulic trait adaptation, as exemplified by *B. excelsa*, which maintains a consistent P_{50} value across varying light environments, although wood density does not vary in the same direction. Although the range in P_{50} among species is consistent with expectations for tropical regions, the interspecific differences found among light conditions are an innovative aspect of our results.

Against our expectations, P_{50} did not decrease as the canopy opened, but showed the lowest values under intermediate light conditions for *C. fissilis* and *C. guianensis*, and in gap and intermediate conditions for *H. courbaril*. These tree species were able to alter the xylem resistance values, but for the light condition where they were probably better adapted. No differences in P_{50} among light conditions were observed for the other species. For *B. excelsa*, although the density of the wood changes with the light condition, there are no changes in the P_{50} , which indicates that there may be other adaptations at cellular level in the xylem. We suggest studies on how the cellular proportion in the wood (fibers, vessels and parenchyma), pit ultrastructure, and the contact area between parenchyma and vessel can explain this difference. Regardless of the ecological group, for *T. rosea* even if the individual is limited by light, there is no need to adopt an ecological strategy that can overcome the rapid growth and resistance to embolism trade-off. As a fast-growing species, *T. rosea* had a lower wood density, exhibited lower resistance to drought, and was not influenced by the light

conditions. Resistance to embolism emerges as another important functional trait for investigating species-specific adaptive plasticity related to microclimate and predicting mortality risks in our study species. This constitutes a significant discovery from a silvicultural perspective, as it underscores the importance of exploring the potential of slow-growing native species, which may exhibit greater resilience to shifts in light conditions, and may prove to be a reliable and effective choice for future plantations.

1.6.3 Hydraulic resilience across periods

A special pattern in Hydraulic Safety Margin (HSM) was detected in *H. courbaril*, *C. fissilis* and *T. rosea*, which displayed a clear risk strategy for xylem embolism operating beyond the critical limit. This underscores a high plasticity in hydraulic adjustments for *H. courbaril* and *C. fissilis*, because for these species this variation is dependent on the light condition. In contrast, *B. excelsa* exhibited a low variation in HSM across light conditions. These findings support previous results, particularly those by Islam et al. (2018), which show how hydraulic characteristics are modulated in response to interannual fluctuations in weather, especially precipitation. We recognize that the measurement of leaf water potential is sensitive to daily VPD, thus we took several precautions in the field to ensure that measurements were taken on similar days, never after rainy days and recording local temperature and humidity. Our experimental design should be more representative of the water balance experienced by the trees. This demonstrates an ability to adapt to changing environmental conditions.

These observed patterns provide valuable insights into the hydraulic strategies employed by our species to cope with changing light conditions. The flexible adjustment of hydraulic traits in response to light availability suggests a high degree of adaptability and resilience of *H. courbaril*. This adaptive plasticity may confer

advantages in terms of resource utilization and overall fitness, enabling *H. courbaril* to thrive in habitats with differing light conditions. Our results reject the theoretical hypothesis that only taller trees suffer hydraulic damage in soils with constant humidity because of their vulnerability (Trugman et al. 2021). In addition, for *T. rosea* the HSM is low for all light conditions, although the tendency is in the gap and intermediate conditions the HSM values are lowest.

B. excelsa exhibited a relatively stable HSM across light conditions and periods, indicating a different hydraulic strategy compared to the other species. Although *B. excelsa* does not show the same level of plasticity as *H. courbaril* and *C. fissilis*, its hydraulic traits seem to be well tuned to maintain a consistent performance in variable environmental contexts. This consistency in hydraulic function could be advantageous for *B. excelsa* in maintaining reliable water transport across different light conditions. However, under prolonged water stress, this species may face potential risks associated with mortality due to hydraulic failure.

1.7 Conclusions

Our study demonstrates that the physiological and structural traits of leaves and xylem in Amazonian trees vary under a light gradient and between periods. Our investigation confirmed the hypothesis that light exposure reduces wood density, except for *T. rosea*. However, leaf potential did not show a consistent pattern of reduction related to light conditions, especially at Ψ_{md} . Consistent with our expectations, P_{50} changes according to the light condition, although it is also dependent on the species. Additionally, we observed differences in hydraulic safety margins between the drier and wetter periods, supporting our second hypothesis that these changes increase drought sensitivity, particularly during the drier period. Wood density was influenced by both light conditions and species, indicating that the anatomical structure of our studied species, with the exception of *T. rosea*, can adapt to wide light conditions. We observed that the hydraulic safety margin of the species *C. fissilis*, *H. courbaril* and *T. rosea* operates at approximately -1 MPa in the drier period, with variations depending on light conditions for the first two species. This demonstrates the lower resistance to xylem embolism of seedlings in the Amazon, which could have serious consequences in the case of long dry periods, as predicted in the context of climate change. This reflects implications for species-level ecological distribution particularly for pioneer species, considering that light is a strong environmental filter for regenerating. Consequently, not only mature forests but also all local regeneration processes will be at risk. Our findings constitute a significant contribution to the knowledge on Amazon species, suggesting the importance of a careful selection of species based on their hydraulic characteristics and local conditions. The slow-growing native species, which may exhibit a greater resilience to changes in light, represent a reliable and effective

choice for future plantations. These seedlings growing in intermediate and gap conditions and which are able to adjust their hydraulic traits can be an excellent option for future plantations in secondary forests. Non-pioneer species may be able to maintain drought resistance during prolonged periods without rain compared to pioneers, since these species have shown a greater ability to adapt their hydraulic traits in response to microclimatic changes. In conclusion, native non-pioneer species can cope with natural drought events during the first phases after plantation, however pioneer species are much less prepared to adapt to possible longer periods of drought. The results of our study contribute to enhancing the sustainability and resilience of reforestation efforts in tropical regions.

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1.9 Authors' Contributions

Study conceptualization and design: DCS DSP, CRM, MJF; Methods: DCS, DSP, ZTMG, GDM; Data collection: DCS, ZTMG; Formal analysis/interpretation: DCS, DSP, ZTMG, GDM, SR; Writing - original draft: DCS; Supervision: CRM, MJF, SR; Funding acquisition: CRM, MJF, SR. All authors reviewed and approved the final manuscript.

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1.11 Conflict of interest

None.

1.12 Data availability

The data, software and scripts underlying this article are available in borealis, at <https://doi.org/10.5683/SP3/2SBTF8>.

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List of figure legends

Figure 1. Location of the study area, in a secondary forest (FS) in the Central Amazon region. In the upper left corner is the perimeter of the State of Amazonas in blue. In the lower left corner, Manaus city is highlighted with the yellow perimeter and the experimental site in red. In the right is the experimental area in detail, with the layout of the five experimental blocks.

Figure 2. Transmittance along a light gradient. The values are means ($n = 5$). The letters represent Tukey's *post hoc* test, comparing the differences across light conditions. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition.

Figure 3. Microclimate variables in drier and wetter periods along a light gradient. The values are means ($n = 5$). a) vapor pressure deficit (kPa), b) relative humidity (%), c) soil moisture (%) d) temperature ($^{\circ}\text{C}$), e) dew point ($^{\circ}\text{C}$). The asterisks represent the differences between drier and wetter periods in each light condition compared by paired *t*-test. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a period. (** $P < 0.01$; *** $P < 0.001$).

Figure 4. Total height growth (cm) and diameter (mm) five years after planting five Amazonian forest species. Values are means $\pm$ standard deviation ($n = 5$). Each color represents a light condition. The letters represent Tukey's *post hoc* test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition. *C. fissilis* = *Cedrela fissilis*; *T. rosea* = *Tabebuia rosea*; *B. excelsa* = *Bertholletia excelsa*; *C. guianensis* = *Carapa guianensis*; *H. courbaril* = *Hymenaea courbaril*. (ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Figure 5. Predawn and midday leaf water potential in the drier and wetter period in five Amazonian tree species of different ecological groups. a) Predawn in drier period, b) Predawn in wetter period, c) Midday in drier period, d) Midday in wetter period. Values are means ($n = 5$). The letters represent Tukey's *post hoc* test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition. *Cf* = *Cedrela fissilis*; *Tr* = *Tabebuia rosea*; *Be* = *Bertholletia excelsa*; *Cg* = *Carapa guianensis*; *Hc* = *Hymenaea courbaril*. (ns = not significant; * $P < 0.05$; *** $P < 0.001$).

Figure 6. Xylem resistance to embolism (P_{50}) and wood density in five Amazonian tree species of different ecological groups along a light gradient. a) P_{50} , b) Wood density. Values are means ($n = 5$). The letters represent Tukey's post hoc test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition. (** $P < 0.01$; *** $P < 0.001$).

Figure 7. Hydraulic Safety Margins (HSM) in five Amazonian tree species of different ecological groups. a) HSM in the drier period, b) HSM in the wetter period. The dashed red line indicates HSM equal to zero. Negative values represent operation at more negative water potential than P_{50} . Values are means ($n = 5$). The letters represent Tukey's post hoc test, comparing the differences across light conditions for each species. Boxplots represent upper and lower quartiles; whiskers achieve the 10th and 90th percentiles; the median and mean values are drawn as horizontal black lines and x, respectively. Each color represents a light condition. (ns = not significant; * $P < 0.05$ *** $P < 0.001$).

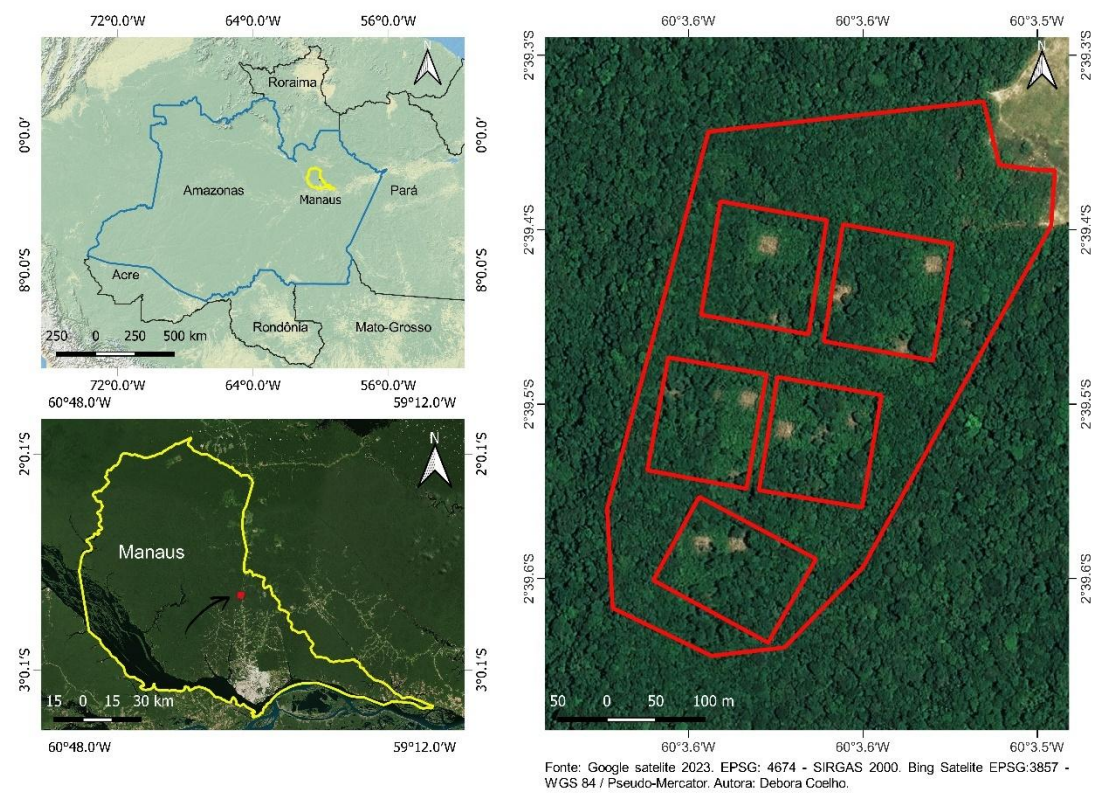


Figura 1.1

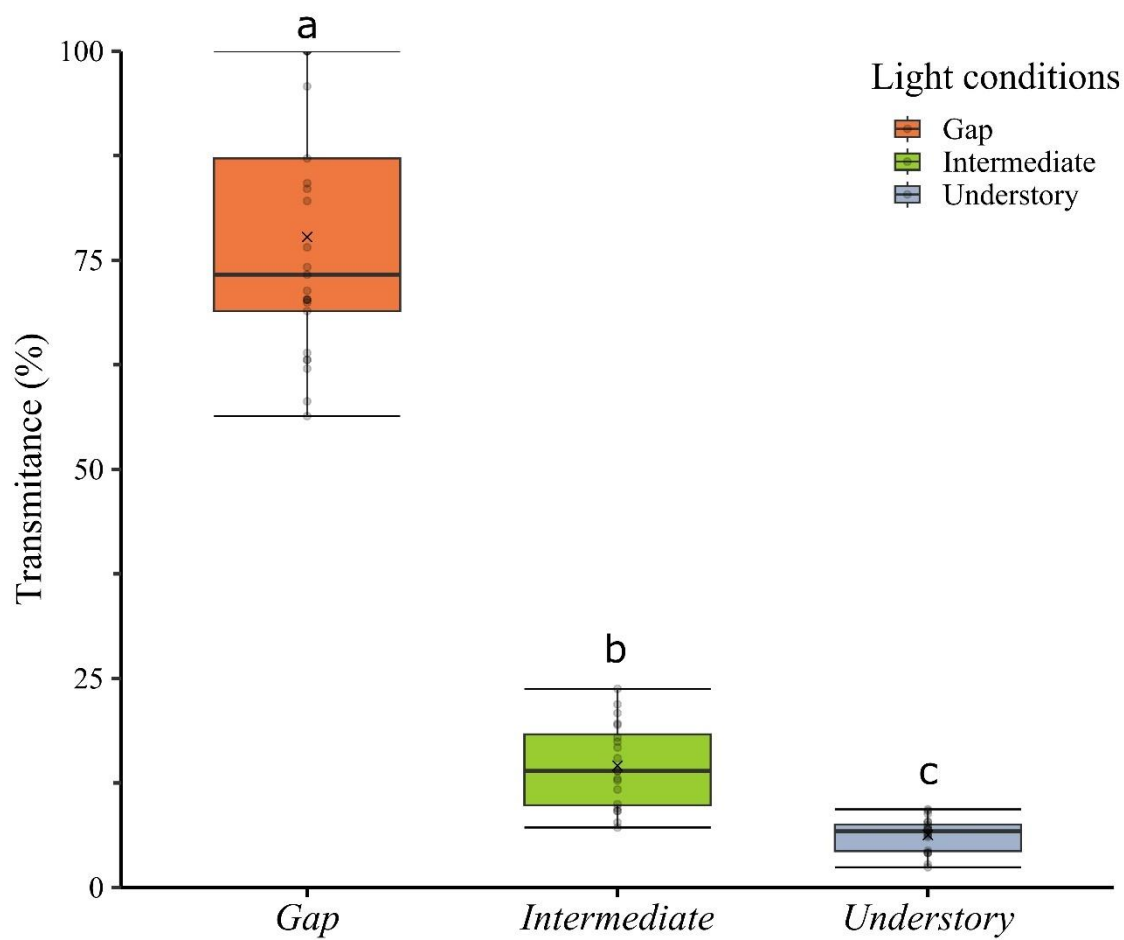


Figure 1.1

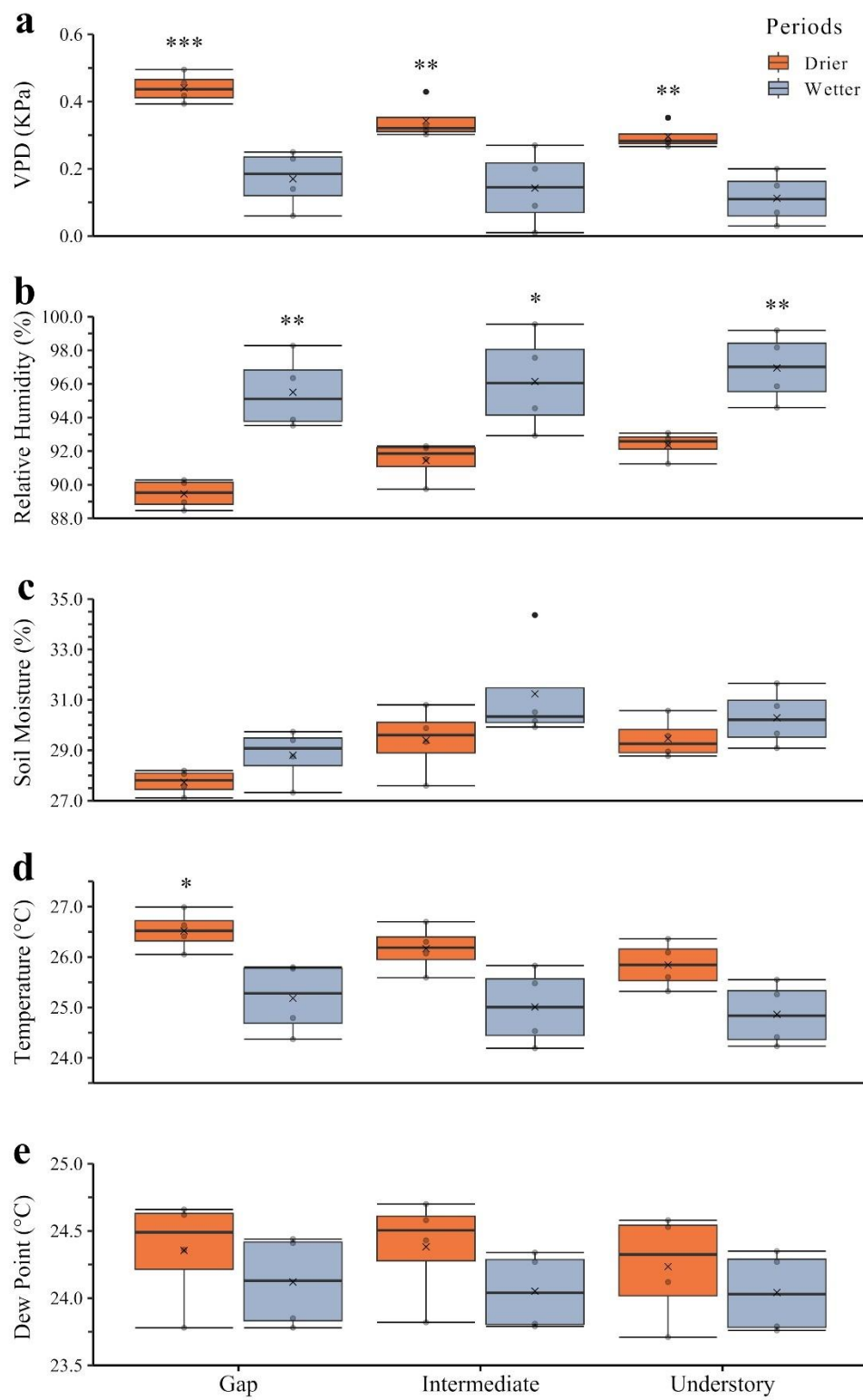


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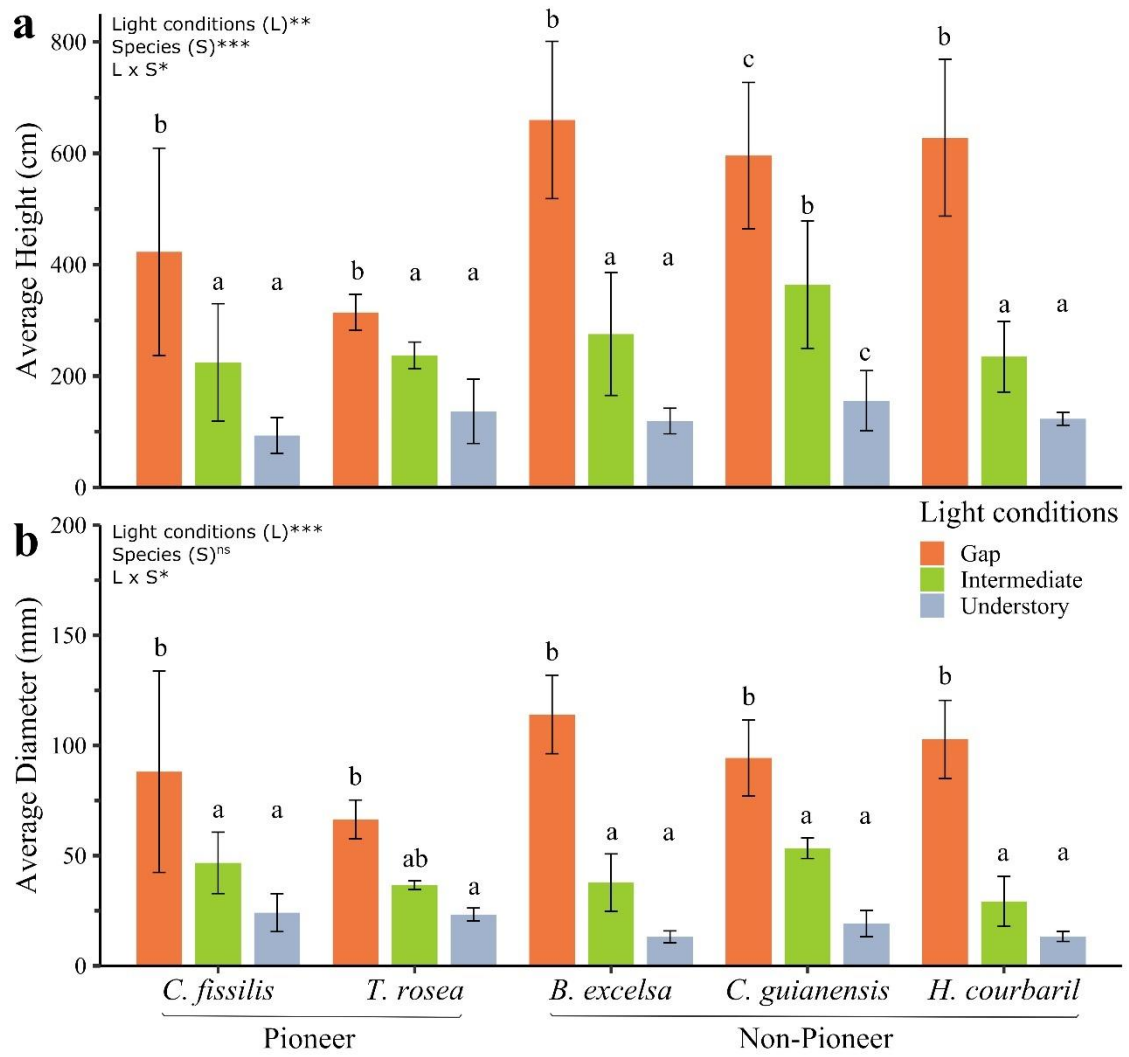


Figure 1.3

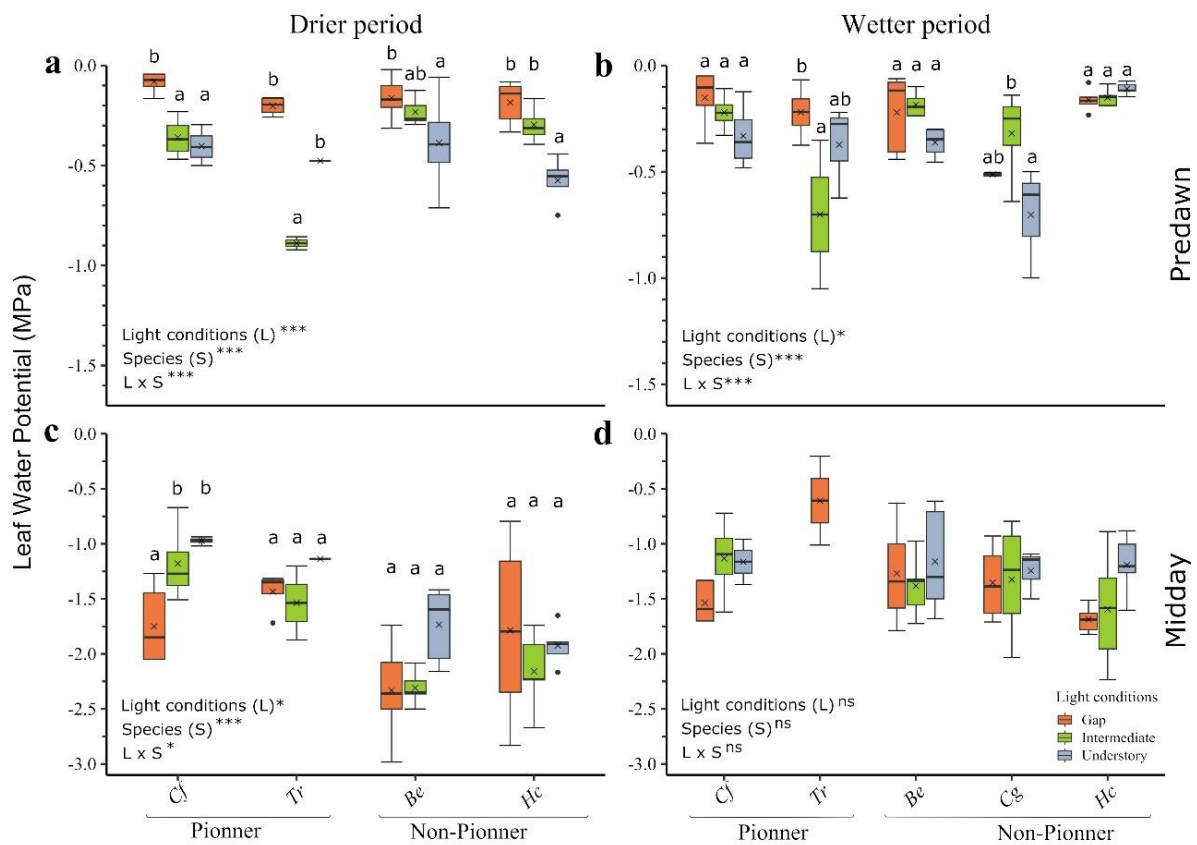


Figure 1.4

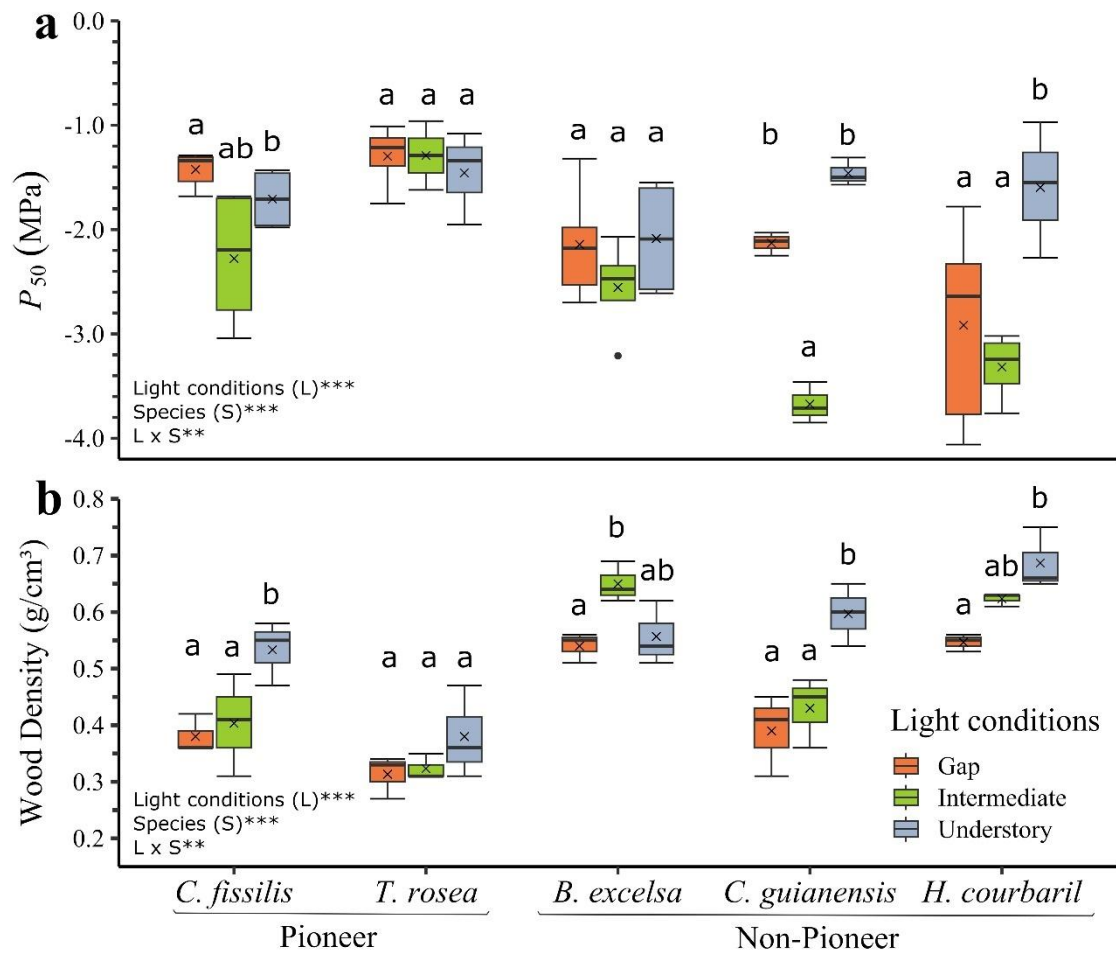


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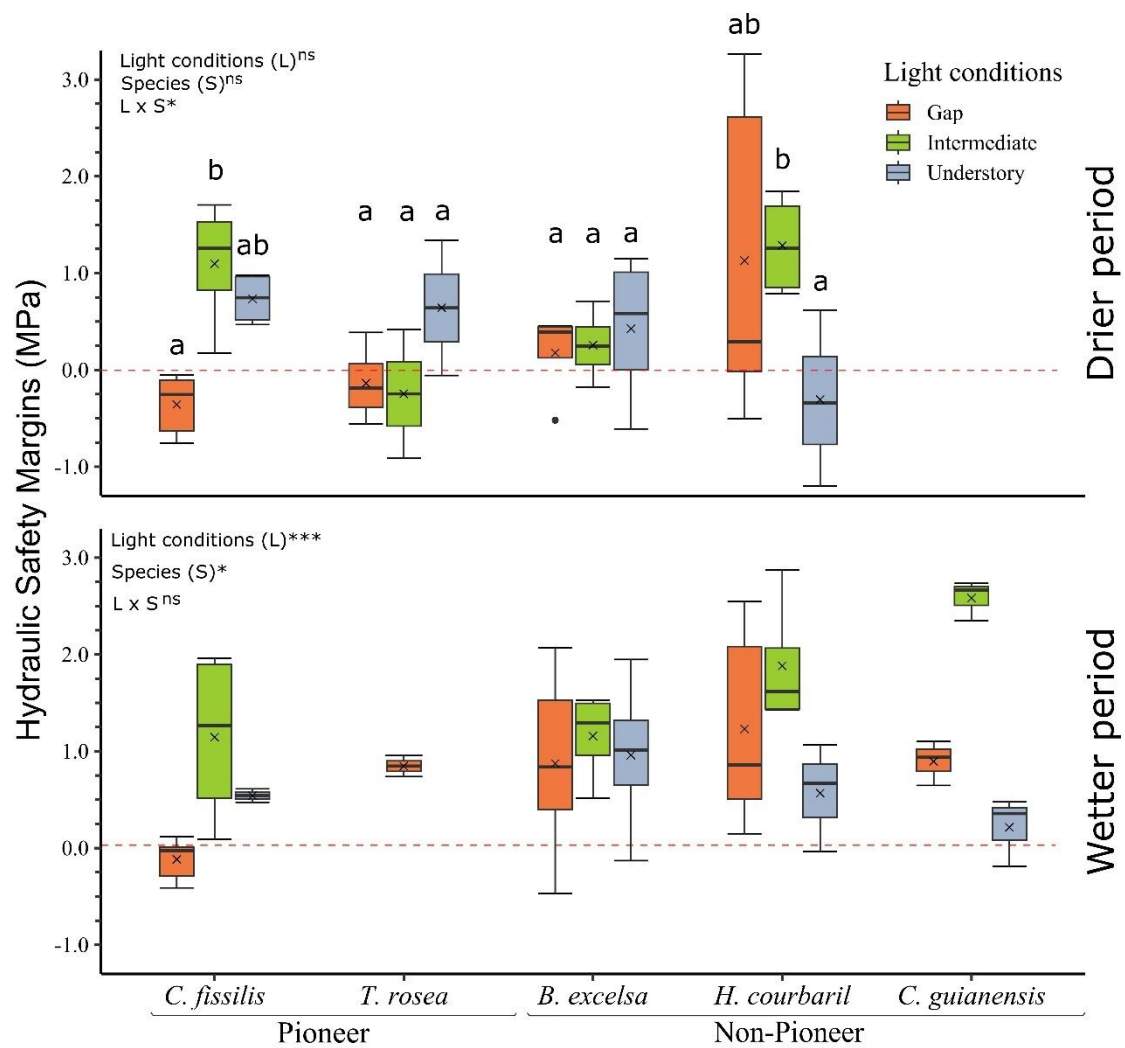


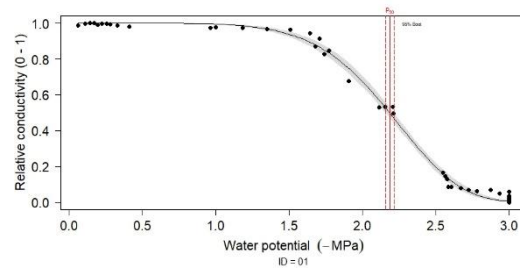
Figure 1.6

1.14 Supplementary data

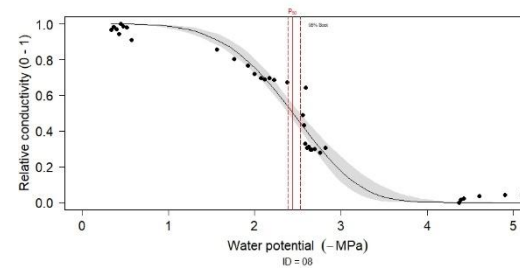
Figure S1. Individual Vulnerability Curves of five Amazonian forest species. Each column represents a light condition: gap, Intermediate and understory. The dashed lines indicate the P_{50} value. The solid lines indicate the 95% confidence interval in relation to the P_{50} value. Below each graph, the ID represents the number of each individual.

Bertholletia excelsa

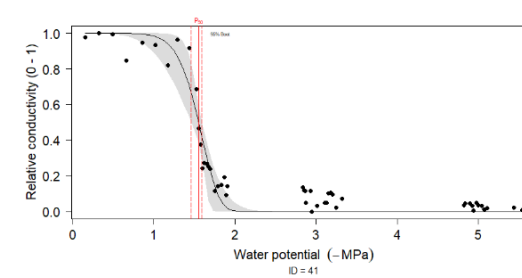
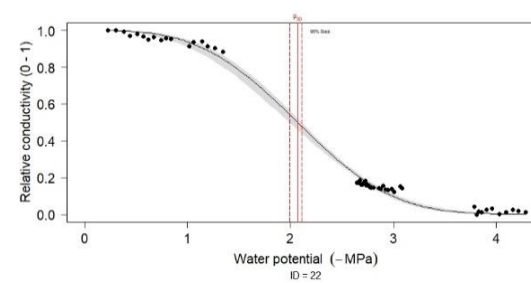
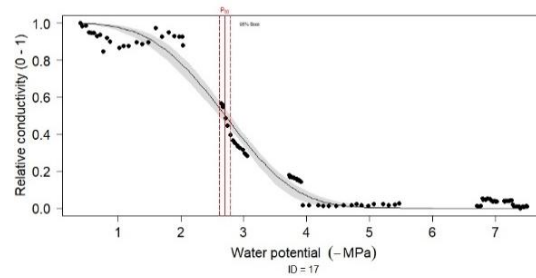
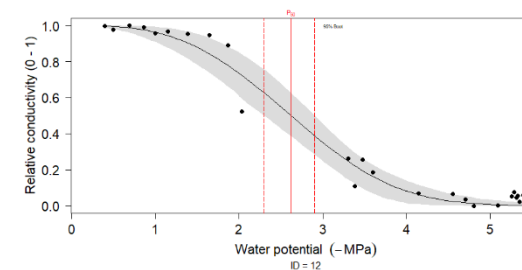
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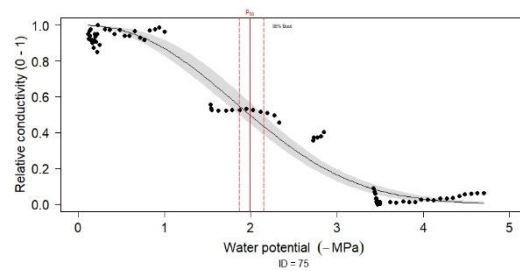
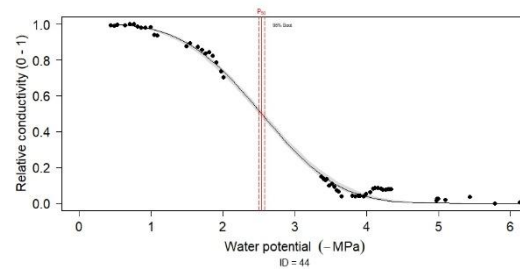
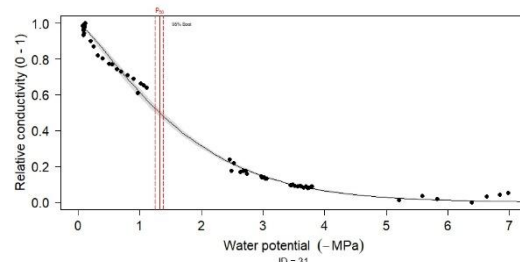
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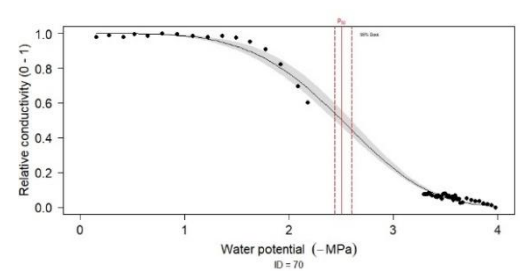
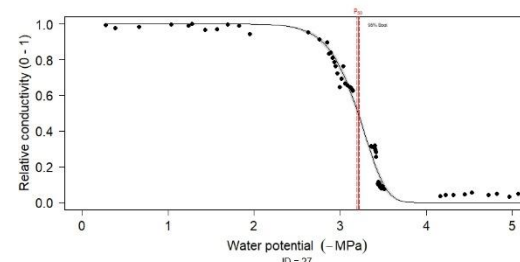
Understory



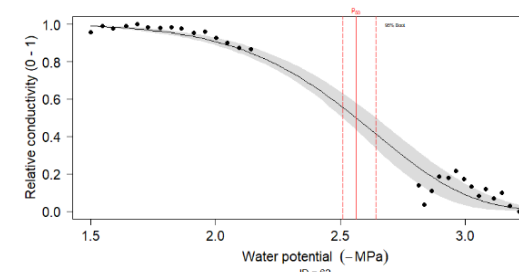
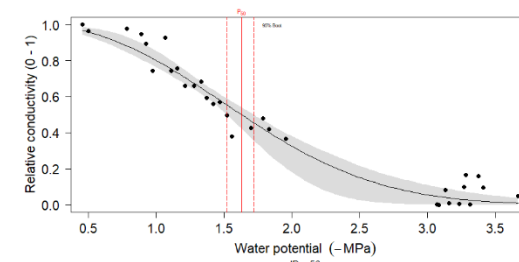
Gap



Intermediate



Understory

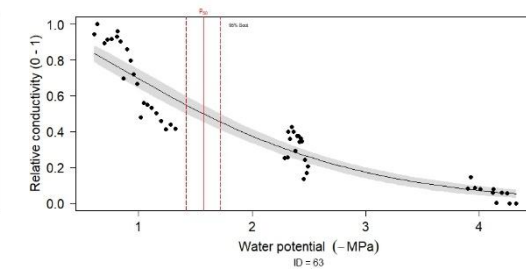
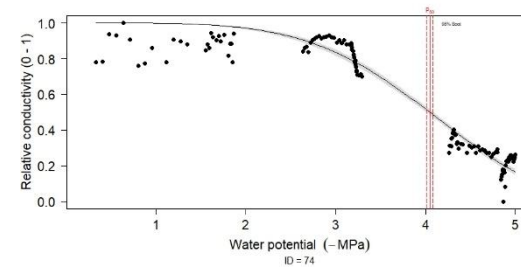
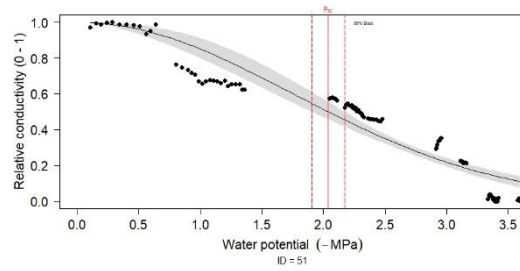
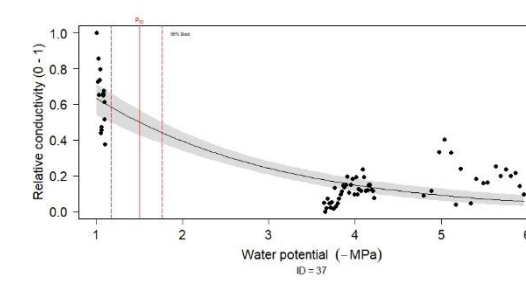
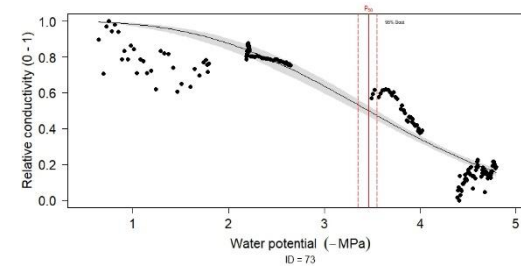
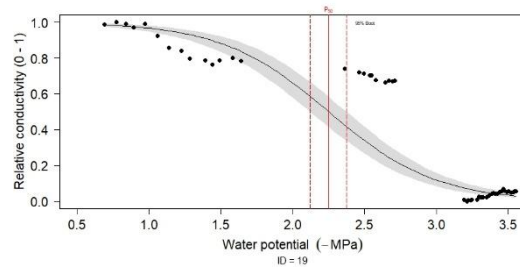
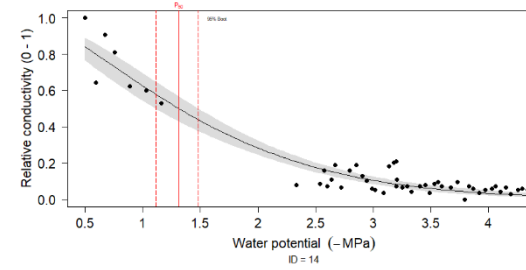
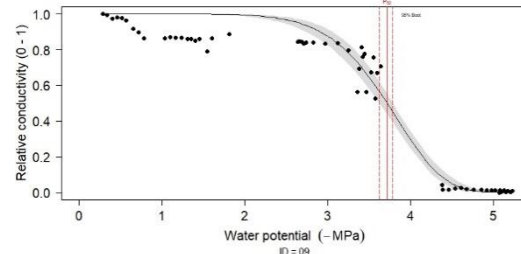
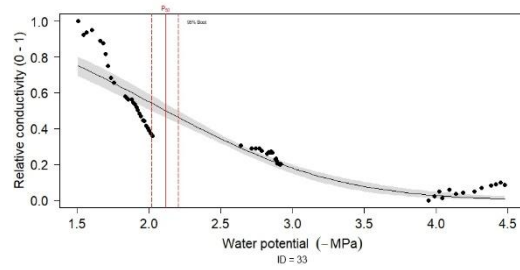


Carapa guianensis

Gap

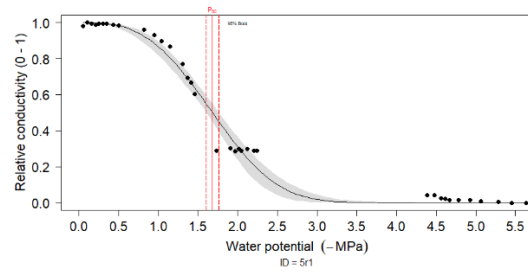
Intermediate

Understory

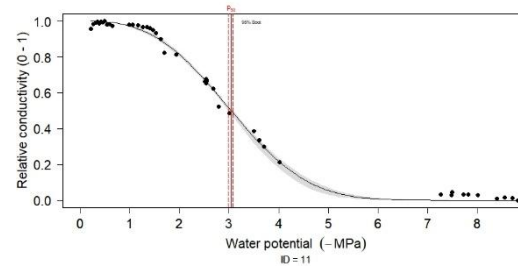


Cedrela fissilis

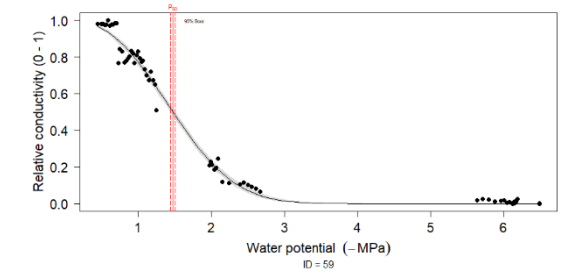
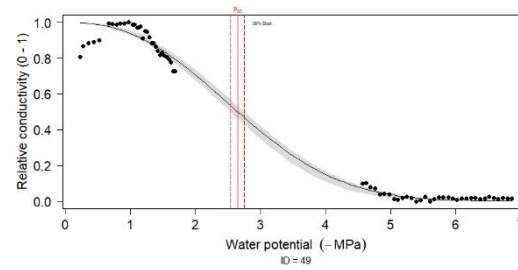
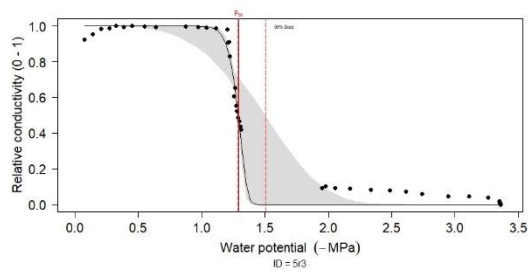
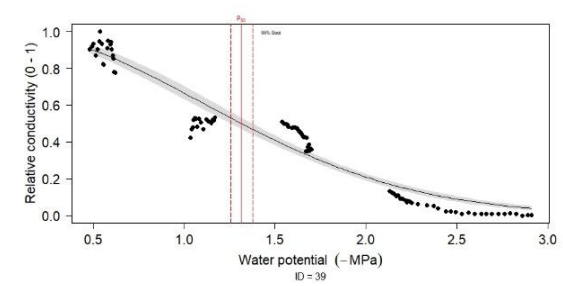
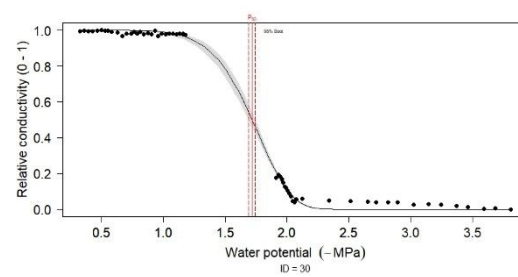
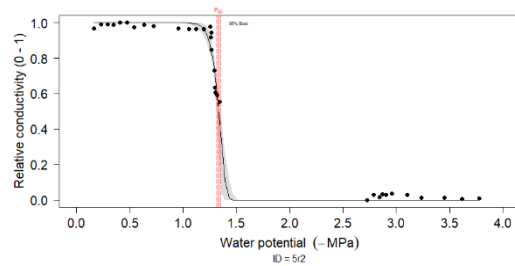
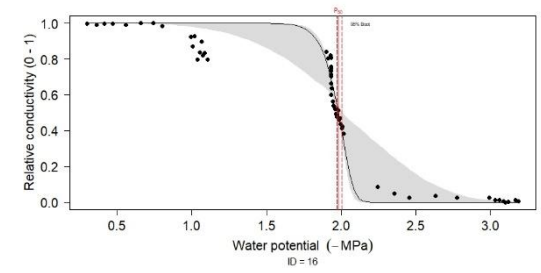
Gap



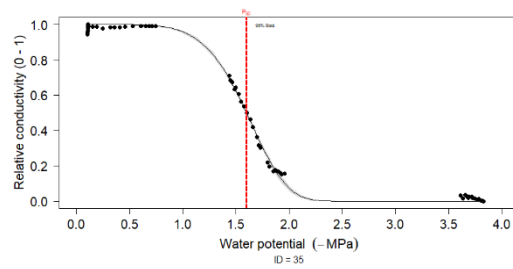
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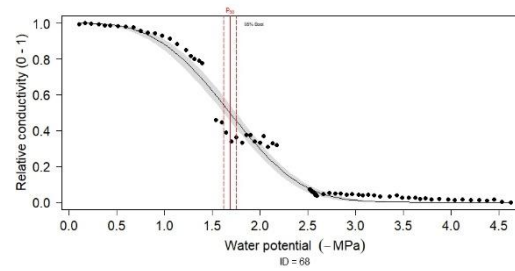
Understory



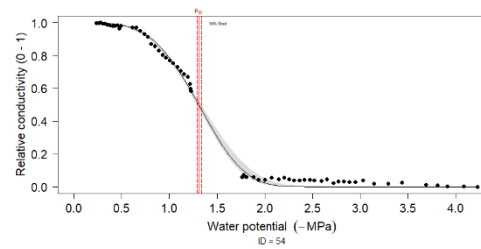
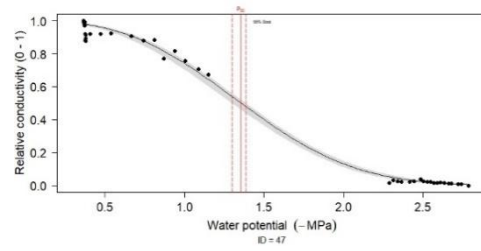
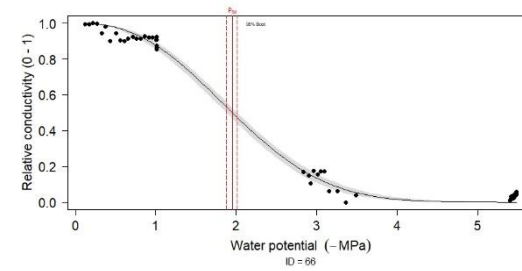
Gap



Intermediate



Understory

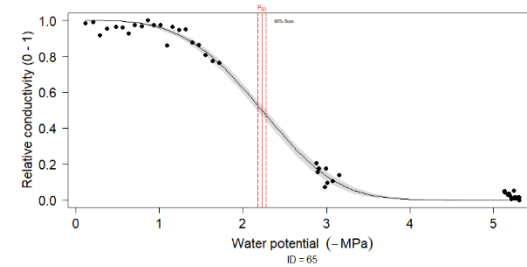
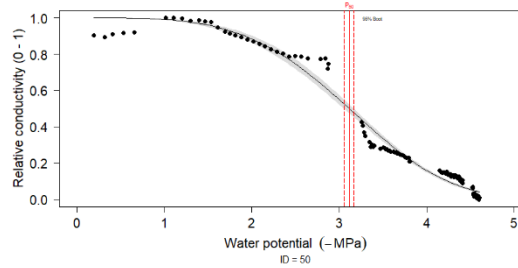
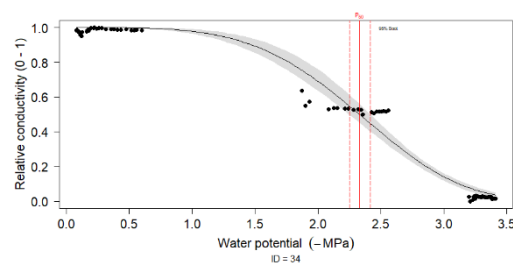
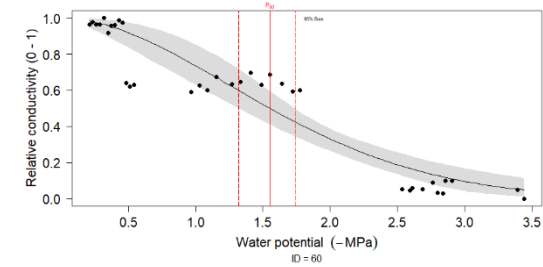
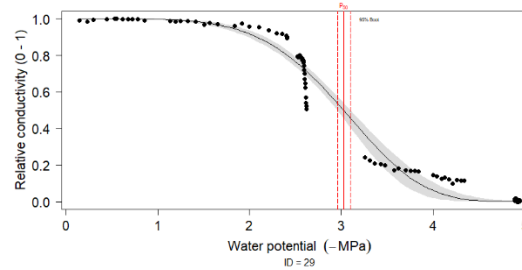
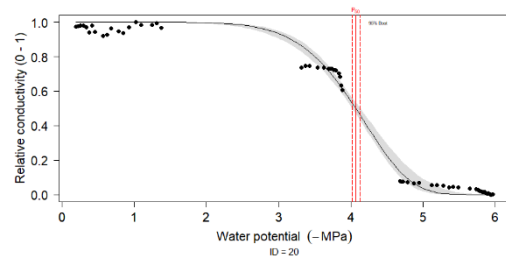
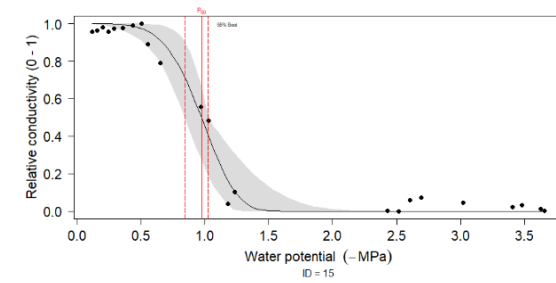
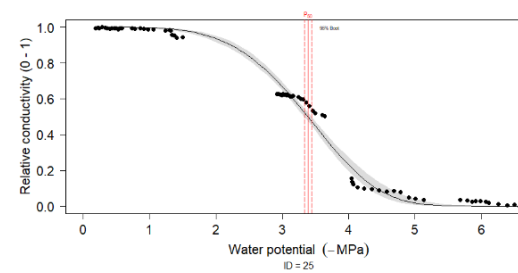
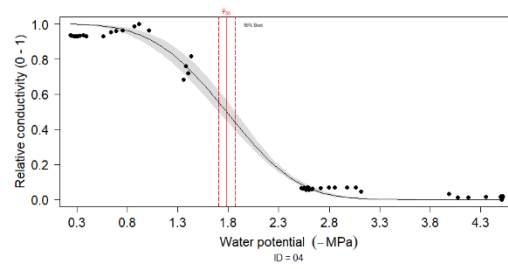


Hymenaea courbaril

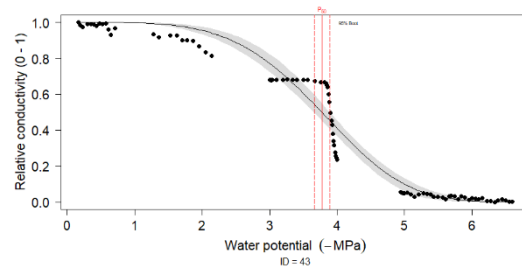
Gap

Intermediate

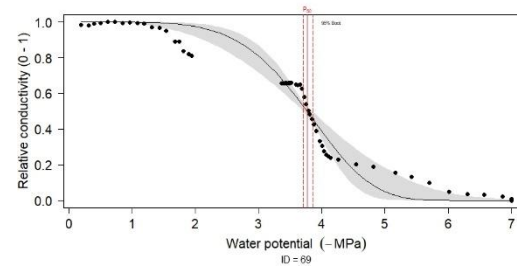
Understory



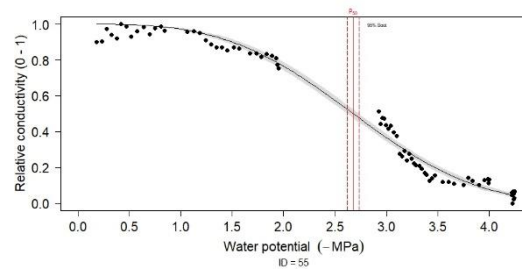
Gap



Intermediate

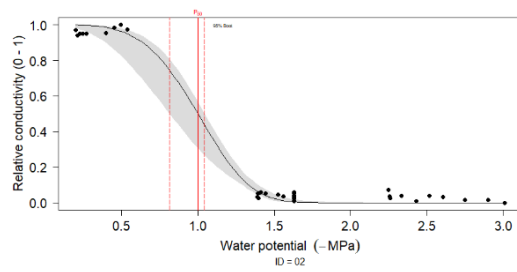


Understory

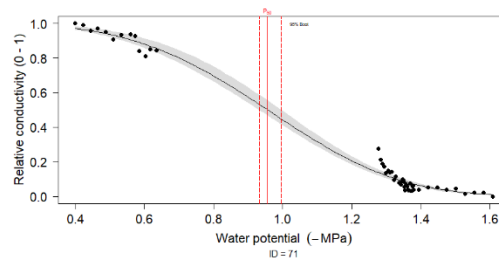


Tabebuia rosea

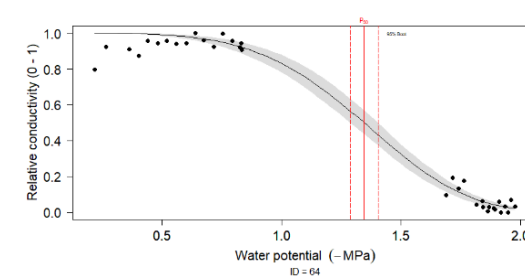
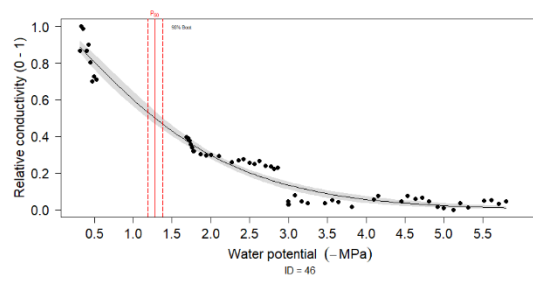
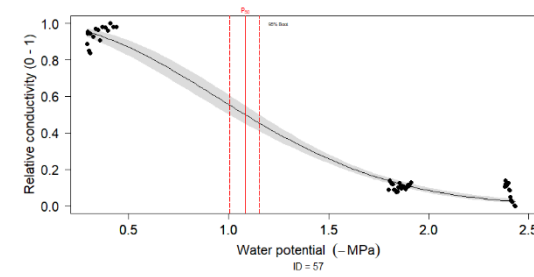
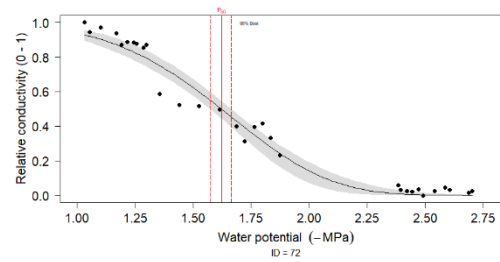
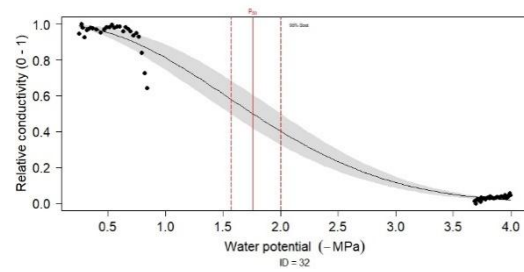
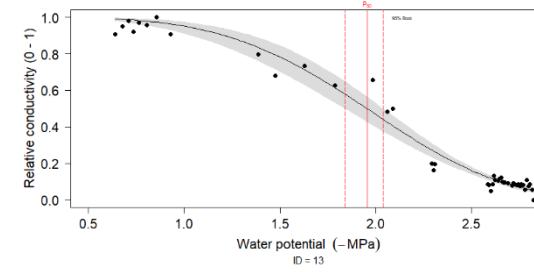
Gap



Intermediate



Understory



Gap

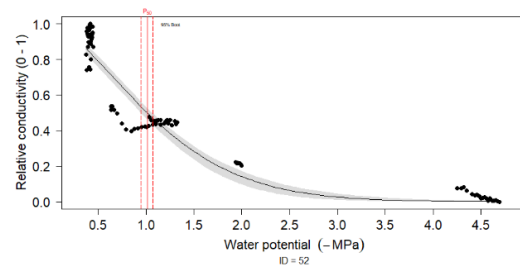
*Intermediate**Understory*

Table S 1.1. Maximum, average and minimum values of the six microclimate variables (n = 4). The values calculated refer to the daily variation between 5am and 7pm, taken between two and six days per block in each seasonal period (drier and wetter periods).

Transmittance was only calculated in the drier period.

	Light Conditions	VPD (Kpa)		Relative Humidity (%)		Soil Moisture (%)		Temperature (°C)		Dew Point (°C)		Transmittance (%)
		Drier	Wetter	Drier	Wetter	Drier	Wetter	Drier	Wetter	Drier	Wetter	Drier
Minimum	Gap	0.01	0.00	54.77	62.99	27.11	27.32	21.06	21.41	20.61	21.04	56.36
	Intermediate	0.00	0.00	55.59	67.04	27.59	29.92	21.22	21.51	20.62	21.10	7.17
	Understory	0.00	0.00	60.61	70.15	28.77	29.09	21.20	21.56	20.69	21.12	2.42
Average	Gap	0.51	0.20	88.14	94.78	27.73	28.80	26.86	25.31	24.43	24.12	77.77
	Intermediate	0.41	0.17	90.21	95.58	29.40	31.24	26.55	25.14	24.52	24.08	14.56
	Understory	0.35	0.14	91.10	96.15	29.47	30.29	26.15	24.96	24.31	24.00	6.28
Maximum	Gap	2.39	1.76	99.68	100.00	28.20	29.74	34.07	32.41	28.36	27.89	100.00
	Intermediate	2.30	1.49	100.00	100.00	30.80	34.37	33.50	31.59	28.22	27.19	23.75
	Understory	1.90	1.31	99.90	100.00	30.57	31.66	32.33	30.70	27.45	26.80	9.35

Table S 1.2. Results of two-way ANOVA (*F* values, *R* - adjust), for hydraulic traits of five tree species (*B. excelsa*, *C. guianensis*, *C. fissilis*, *H. courbaril*, *T. rosea*) planted in different light conditions (gap, intermediate and understory). Each value is mean $\pm$ SD (n = 5). The slope values (S) are the median $\pm$ SD. Different letters indicate a difference between the light conditions for each species tested by Tukey *post hoc* test. Bold letters indicate difference between light conditions.

Species		Light Conditions	P_{50} (MPa)	S value (%)	Wood density (g cm ³)	Drier Period			Wetter Period		
						Ψ_{pd} (MPa)	Ψ_{md} (MPa)	HMS (MPa)	Ψ_{pd} (MPa)	Ψ_{md} (MPa)	HMS (MPa)
Pioneer	<i>C. fissilis</i>	Gap	-1.43 a $\pm$ (0.17)	117.14 $\pm$ (296.7)	0.35 a $\pm$ (0.02)	-0.10 b $\pm$ (0.06)	-1.69 b $\pm$ (0.32)	0.24 a $\pm$ (0.28)	-0.17 a $\pm$ (0.13)	-1.42 $\pm$ (0.30)	0.001 $\pm$ 0.23
		Intermediate	-2.28 ab $\pm$ (0.69)	50.02 $\pm$ (57.9)	0.40 a $\pm$ (0.09)	-0.36 a $\pm$ (0.10)	-1.18 a $\pm$ (0.36)	1.11 b $\pm$ (0.67)	-0.22 a $\pm$ (0.09)	-1.13 $\pm$ (0.37)	1.16 $\pm$ 0.90
		Understory	-1.71 b $\pm$ (0.30)	58.16 $\pm$ (197.7)	0.53 b $\pm$ (0.06)	-0.40 a $\pm$ (0.09)	-0.97 a $\pm$ (0.03)	0.70 ab $\pm$ (0.32)	-0.33 a $\pm$ (0.16)	-1.18 $\pm$ (0.31)	0.48 $\pm$ 0.18
	<i>T. rosea</i>	Gap	-1.30 a $\pm$ (0.32)	56.56 $\pm$ (51.1)	0.31 a $\pm$ (0.04)	-0.20 b $\pm$ (0.05)	-1.43 a $\pm$ (0.19)	0.14 a $\pm$ (0.35)	-0.22 b $\pm$ (0.13)	-0.61 $\pm$ (0.57)	1.19 $\pm$ (0.27)
		Intermediate	-1.29 a $\pm$ (0.59)	118.625 $\pm$ (18.5)	0.32 a $\pm$ (0.02)	-0.89 a $\pm$ (0.05)	-1.54 a $\pm$ (0.48)	-0.29 a $\pm$ (1.06)	-0.70 a $\pm$ (0.49)	-	-
		Understory	-1.46 a $\pm$ (0.45)	69.61 $\pm$ (27.2)	0.38 a $\pm$ (0.08)	-0.48 b	-1.14 abc	0.70 a $\pm$ (0.91)	-0.37 ab $\pm$ (0.22)	-	-
Non-Pioneer	<i>B. excelsa</i>	Gap	-2.14 a $\pm$ (0.54)	44.59 $\pm$ (23.3)	0.54 a $\pm$ (0.03)	-0.16 b $\pm$ (0.11)	-2.33 a $\pm$ (0.47)	-0.20 a $\pm$ (0.88)	-0.22 a $\pm$ (0.19)	-1.27 $\pm$ (0.46)	0.86 $\pm$ 0.95
		Intermediate	-2.56 a $\pm$ (0.54)	61.445 $\pm$ (3.9)	0.65 b $\pm$ (0.04)	-0.23 ab $\pm$ (0.07)	-2.31 a $\pm$ (0.15)	-0.22 a $\pm$ (0.43)	-0.18 a $\pm$ (0.06)	-1.38 $\pm$ (0.28)	1.12 $\pm$ 0.44
		Understory	-2.09 a $\pm$ (0.58)	78.45 $\pm$ (70.3)	0.56 ab $\pm$ (0.06)	-0.39 a $\pm$ (0.24)	-1.73 a $\pm$ (0.34)	0.56 a $\pm$ (0.78)	-0.32 a $\pm$ (0.11)	-1.16 $\pm$ (0.48)	1.09 $\pm$ 0.84

	<i>C. guianensis</i>	Gap	-1.43 b ± (0.17)	42.66 ± (18.8)	0.43 a ± (0.03)	-	-	-	-0.41 ab ± (0.20)	-1.35 ± (0.37)	1.08 ± 0.03
		Intermediate	-2.28 a ± (0.69)	45.7 ± (21.0)	0.43 a ± (0.06)	-	-	-	-0.32 b ± (0.22)	-1.33 ± (0.56)	2.65 ± 0.10
		Understory	-1.71 b ± (0.30)	31.6 ± (6.9)	0.60 b ± (0.06)	-	-	-	-0.70 a ± (0.26)	-1.25 ± (0.22)	0.57 ± 0.74
	<i>H. courbaril</i>	Gap	-2.92 a ± (0.97)	61.09 ± (13.7)	0.55 a ± (0.02)	-0.19 b ± (0.11)	-1.79 a ± (0.83)	1.24 ab ± (1.58)	-0.16 a ± (0.06)	-1.69 ± (0.12)	1.34 ± 0.91
		Intermediate	-3.32 a ± (0.33)	46.90 ± (6.1)	0.62 ab ± (0.01)	-0.40 b ± (0.24)	-2.16 a ± (0.36)	1.43 b ± (0.59)	-0.15 a ± (0.04)	-1.59 ± (0.53)	2.03 ± 0.10
		Understory	-1.60 b ± (0.65)	55.51 ± (75.05)	0.69 b ± (0.06)	-0.50 a ± (0.21)	-1.92 a ± (0.19)	-0.41 a ± (0.69)	-0.11 a ± (0.03)	-1.19 ± (0.28)	0.46 ± 0.20
<i>F</i> _(species)		9.96***	-	46.67 ***	4.91**	14.99***	1.25 ^{ns}	3.60*	3.03*	3.95*	
<i>F</i> _(light)		15.31***	-	19.19***	23.41***	3.10	1.35 ^{ns}	0.93 ^{ns}	2.22 ^{ns}	10.67***	
<i>F</i> _(SxL)		3.58**	-	3.43**	2.99*	2.67*	2.41*	3.16*	0.50 ^{ns}	1.99 ^{ns}	
<i>R - Adjust</i>		0.63	-	0.84	0.56	0.48	0.23	0.29		0.42	

Table S 1.3. Results of paired t-test (t values), for height (cm) and diameter (mm) of two successional groups (pioneer and non-pioneer) planted in different light conditions (gap, intermediate and understory). Each value is mean $\pm$ SD (n = 5). Different letters indicate differences between successional groups in each light conditions. Bold letters indicate difference across light conditions. (ns = not significant; * P<0.05; ** P<0.01; *** P<0.001).

Light Conditions	<i>Height (cm)</i>		<i>t</i>	<i>Diameter (mm)</i>		<i>t</i>
	Pioneer	<i>Non-pioneer</i>		Pioneer	<i>Non-pioneer</i>	
Gap	386.75b $\pm$ (158.88)	630.14 a $\pm$ (130.14)	4.29***	80.88b $\pm$ (38.28)	104.41 a $\pm$ (18.17)	2.05*
Intermediate	228.67a $\pm$ (82.70)	286.29a $\pm$ (104.41)	1.19 ^{ns}	43.34a $\pm$ (12.03)	39.19a $\pm$ (14.06)	-0.62 ^{ns}
Understory	111.86a $\pm$ (46.54)	129.19a $\pm$ (30.60)	1.00 ^{ns}	23.76 a $\pm$ (6.35)	14.64b $\pm$ (4.12)	-3.09**

Table S 1.4. Results of two-way ANOVA (F values, R² - adjust), for P50 of five tree species (*B. excelsa*, *C. guianensis*, *C. fissilis*, *H. courbaril*, *T. rosea*) planted in different light conditions (gap, intermediate and understory). Each value is mean $\pm$ SD (n = 5). Different letters indicate a difference between the light conditions for each species tested by Tukey post hoc test. Bold letters indicate difference between light conditions. The individuals excluded from these analyses were: *Be*: ID31, 62; *Cg*: ID33, 73,74, 14,37; *Tr*: 46,52, 57; *Hc*: 60; *Cf*: 39.

Specie s		Light Conditions	P_{50} (MPa)
Pioneer	C. fissilis	Gap	-1.43 a ± (0.33)
		Intermediate	-2.28 ab ± (0.69)
		Understory	-1.80 b ± (0.29)
	T. rosea	Gap	-1.38 a ± (0.52)
		Intermediate	-1.29 a ± (0.47)
		Understory	-1.46 a ± (0.45)
Non-Pioneer	B. excelsa	Gap	-2.35 a ± (0.33)
		Intermediate	-2.56 a ± (0.48)
		Understory	-2.09 a ± (0.58)
	C. guianensis	Gap	-2.07 b ± (0.06)
		Intermediate	-3.59 a ± (0.33)
		Understory	-1.57 b ± (0.01)
	H. courbaril	Gap	-2.92 a ± (0.97)
		Intermediate	-3.32 a ± (0.33)
		Understory	-1.60 b ± (0.65)
$F_{(species)}$			7.41***
$F_{(light)}$			9.61***
$F_{(S \times L)}$			2.62*
$R^2 - Adjust$			0.57

CAPÍTULO 2

A LUZ ALTERA AS CARACTERÍSTICAS ANATÔMICAS DO XILEMA ASSOCIADAS ÀS PROPRIEDADES HIDRÁULICAS

²Light alters xylem anatomical features associated with hydraulic properties

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1.1 Abstract

The comparative anatomy of the xylem provides insight into the performance of Amazonian species in relation to water availability. We explore xylem anatomy, focusing on the relationship between different cell types and xylem resistance to embolism, alongside leaf photosynthesis, stomatal conductance, and transpiration. We investigated the anatomical responses to a light gradient in two successional groups (pioneers and non-pioneers) across five species. The experiment, conducted in a secondary forest in Central Amazonia, is part of a silvicultural enrichment system with artificially created canopy openness levels (understory, intermediate, and clearing). Quantitative anatomical analyses included vessel diameter, vessel density, and cell fraction. Based on these parameters, we calculated the hydraulic diameter, vessel grouping index, vulnerability index, and the percentage of conductive area and cell fraction. The physiological characteristics measured were photosynthesis (A_{sat}), stomatal conductance (g_s), and transpiration (E). Hydraulic diameter did not follow a clear pattern of increase with greater light availability ($F = 10.19$, $P < 0.001$), except in *H. courbaril*. Vessel density also exhibited a non-linear response to species and increasing light ($F = 6.26$, $P < 0.001$). *H. courbaril* was the only species that did not change vessel density across light conditions, indicating a specific anatomical strategy involving hydraulic diameter and vessel density. Cell fraction was significantly affected by light conditions, with the highest investment in fiber walls under gap conditions and in parenchyma (arranged in lines/bands) in the understory. We observed a direct effect of light conditions only on the relationship between hydraulic diameter and stomatal conductance ($F(2,54) = 3.88$, $P < 0.001$), while other relationships between hydraulic diameter and leaf physiological characteristics were independently influenced by light conditions or species. Our results highlight the strong influence of light gradients on

xylem anatomy and its potential impact on xylem resistance to embolism. Successional group did not strongly affect species' anatomical responses, suggesting that light is a dominant environmental filter for tropical species during early years of regeneration.

Keywords: light gradient, ecological anatomy, safety-efficiency trade-off, cell fraction, conductive tissues

1.2 Introduction

Light availability is a key environmental filter shaping the composition and structure of regenerating forests. In the Amazon, where rainfall remains relatively constant, light availability becomes a determining factor for plant growth and survival (Finegan 1992; Poorter and Bongers, 2006). Although important studies have identified diverse water-use strategies within this biome (Brum *et al.*, 2023; Penha *et al.*, 2024), light remains a dominant factor influencing forest dynamics. Light drives the distribution of plant species into ecological groups along a successional continuum, from pioneer species thriving in high-light environments to late-successional species adapted to the shaded understory (Swaine and Whitmore, 1988; Poorter and Bongers, 2006). These groups differ in hydraulic, physiological, and anatomical traits, with pioneers typically displaying greater phenotypic plasticity to optimize water transport and use, whereas late-successional species maintain traits favoring stability in low-light environments (Paradizo *et al.*, 2015; Poorter *et al.*, 2019). A key question that arises from this contrast is whether plant strategies can be consistently grouped by ecological classification and to what extent are modulated by the light gradient.

Phenotypic plasticity refers to an organism's ability to modify its morphological, physiological, or anatomical traits in response to environmental variability, enabling adaptation and survival under changing conditions (Bradshaw 1965; Schlichting and Smith, 2002). In tropical forests, phenotypic plasticity is particularly crucial for juvenile trees, as it influences how species respond to light by optimizing resource acquisition and water transport under varying conditions. For young trees in secondary forests, this capacity is essential for coping with heterogeneous light environments, where adjustments in wood density, vessel size, and hydraulic conductivity can determine survival and growth (Oliveira *et al.*, 2019).

Under high-light conditions, species tend to allocate more resources to rapid growth, often developing larger xylem vessels to maximize water transport efficiency. Conversely, low-light conditions favor traits that enhance survival, such as denser wood and smaller vessels that increase resistance to water stress (Gebauer *et al.*, 2014; De Mil *et al.*, 2018). However, the specific strategies and trade-offs underlying these adaptations may vary among species. The effects of light on the anatomical plasticity of young trees, particularly in secondary forests, remain poorly understood, highlighting the need for field experiments to capture fine-scale variations (Chazdon 2014; Brum *et al.*, 2023). Field studies in regenerating forests provide valuable insights into how environmental conditions, such as light availability, shape the structure and function of tree seedlings over time. By examining a controlled gradient of light — from shaded understory to more exposed gaps — such studies can reveal how species adjust their hydraulic architecture in response to different microclimatic conditions (McDowell *et al.*, 2018; Brum *et al.*, 2023).

Changes in hydraulic architecture, particularly hydraulic diameter, play a central role in regulating stomatal conductance and photosynthetic rates in plants (Brodribb and Feild, 2000; Brodribb and Holbrook, 2004). Larger hydraulic diameters enhance hydraulic conductance, improving water transport to the leaves, which in turn supports higher stomatal conductance and increases photosynthetic capacity (Sack and Holbrook, 2006; Brodribb and Jordan, 2008). This relationship underscores the coordination between a plant's hydraulic architecture and its gas exchange processes, which is critical for maintaining water balance and carbon assimilation (Brodribb *et al.*, 2005).

Variations in light availability can affect this coordination. Increased irradiance has been shown to enhance photosynthetic rates by improving water supply through

wider and more efficient conduits (Brodribb and Jordan, 2011). Conversely, under shaded conditions, reduced hydraulic efficiency can limit stomatal conductance and photosynthesis, thereby constraining carbon gain (Sack *et al.*, 2006). Understanding these interactions is essential for elucidating plant adaptive strategies, particularly in older secondary forests where species must adjust their hydraulic and physiological traits to optimize resource use along a light gradient (Brodribb and Feild, 2010). Recent studies in Amazonian forests have emphasized the significance of hydraulic traits in determining species' responses to varying precipitation regimes and drought events, reinforcing the need to consider these traits when assessing plant function and adaptation in tropical ecosystems (Oliveira *et al.*, 2019). Since hydraulic properties ultimately depend on xylem structure, anatomical traits play a fundamental role in balancing water transport efficiency and safety under different light conditions.

The efficiency and safety of water transport in plants are closely linked to xylem anatomy (Carlquist *et al.*, 1977; Hacke and Sperry 2014; Olson 2020). Xylem vessels, which conduct water from the roots to the aerial parts, are particularly vulnerable to cavitation—the formation of air bubbles that disrupt the water column—under drought conditions (Tyree and Sperry, 1988). The anatomical characteristics of these vessels, including their diameter, wall thickness, pit membrane porosity and membrane thickness, play a crucial role in regulating the trade-off between hydraulic efficiency and safety (Hacke *et al.*, 2006; Schenk *et al.*, 2015). Species with wider vessels generally exhibit higher hydraulic conductivity but are also more susceptible to embolism, particularly when pit membranes facilitate the passage of air bubbles from embolized to functional conduits (Pitterman *et al.*, 2010; Kaack *et al.*, 2021).

The spread of embolism in xylem vessels is primarily explained by two mechanisms. The widely accepted air-seeding hypothesis proposes that nano-bubbles

pass through pit membranes into adjacent vessels, leading to embolism propagation (Schenk *et al.*, 2015). The structural properties of pit membranes, particularly their porosity and thickness, significantly influence the likelihood of this process occurring, shaping species-specific responses to hydraulic stress (Hacke *et al.*, 2006; Pitterman *et al.*, 2010; Kaack *et al.*, 2021). A second proposed mechanism involves the phase transition of metastable water under extreme negative pressure. However, controlled studies suggest that the pressures required for spontaneous cavitation (-20 to -30 MPa at 20–30°C) are unlikely to be reached in natural environments (Caupin *et al.*, 2012). While these mechanisms provide insight into embolism formation, the processes governing its spread and the ecological adaptations observed in natural conditions remain only partially understood (Lens *et al.*, 2023; Olson *et al.*, 2023).

A vast body of botanical literature has explored the comparative anatomy of xylem, emphasizing the structure and function of its constituent cells as shaped by strong, non-linear natural selection (Carlquist 2001; Olson 2020; Anfodillo *et al.*, 2021;). These studies highlight the trade-off between vessel size and vulnerability to drought as a key adaptation balancing hydraulic efficiency and safety (Carlquist 1977; Olson *et al.*, 2014; Olson *et al.*, 2023). Height growth directly influences this relationship, with taller individuals generally predicted to be more vulnerable to drought, while smaller individuals—particularly those growing in understory conditions—tend to be more resistant (Olson *et al.*, 2021; Guillemont *et al.*, 2021, Brum *et al.*, 2023). However, height is ultimately a function of xylem tissue development, involving the production and enlargement of multiple cell types beyond vessels. A deeper understanding if these structural variations could provide more robust insights into plant hydraulic strategies.

In regions with minimal rainfall variation, such as the Central Amazon, adaptive differences are largely driven by the most limiting environmental filter—light availability (Poorter 1999; Modolo; Santos; Ferreira, 2021). Light strongly influences species distribution into ecological groups. While classification criteria may vary, we adopt the broadest contrast: pioneer and non-pioneer species (Swaine and Whitmore, 1988; Finegan 1992; Poorter and Bongers, 2006; Chazdon 2014). In comparative wood anatomy studies, the dependence of xylem cell dimensions on climate is well documented, particularly in relation to large climatic gradients such as latitude, altitude, and topography (Noshiro and Baas, 2000; Godfrey *et al.*, 2020). However, the influence of microclimatic light gradients on wood cell fractions—especially in the context of ecological groups—has been less explored (Gebauer *et al.*, 2014; De Mil *et al.*, 2018; Eller *et al.*, 2018). Much research has focused on the ability of species from both ecological groups to tolerate prolonged drought (Tng *et al.*, 2018; Brum *et al.*, 2018), but less attention has been given to these adaptations during early regeneration. The hydraulic architecture of plants growing in regenerating environments reflects diverse strategies for coping with water scarcity (Brum *et al.*, 2018; Penha *et al.*, 2018). Over time, these strategies may select for long-term functional and physiological traits that either enhance or limit water-use efficiency (McDowell *et al.*, 2018) potentially influenced by the ecological group.

Secondary forests—including degraded areas, selectively logged regions, and abandoned agricultural lands—account for approximately 23% of the forest landscape in the Brazilian Amazon, covering around 8.6 million hectares (INPE, 2016; MAPBIOMAS, 2024). These forests play a crucial role in ecological restoration due to their capacity for rapid natural regeneration, offering a cost-effective alternative to active restoration methods (Crouzeilles *et al.*, 2020). However, the absence of legal

protection, coupled with recurring land-use changes, threatens their resilience and the recovery of biodiversity, particularly in early successional stages (Nunes *et al.*, 2020). Understanding the dynamics of these forests, especially in their initial phases of regeneration, is essential for ensuring their long-term contribution to sustainable forest management.

In this scenario, we hypothesize that in juvenile trees, the anatomical plasticity of xylem and leaf physiological traits is influenced by light availability. Specifically, we aim to: i) investigate whether the effects of light gradients on xylem anatomical traits and hydraulic properties are species-dependent; and ii) analyze whether the relationships between leaf gas exchange and xylem hydraulic diameter are coordinated along a light gradient. We expect that under gap conditions, non-pioneer species will exhibit greater plasticity, while pioneer species in understory conditions will show a lower capacity to anatomical adjustment. The findings of this study will contribute to ecological discussions on successional groups based on anatomical characteristics and provide insights into hydraulic safety-efficiency trade-off.

1.3 Material and methods

1.3.1 Study site

The study was conducted in the Central Amazon, where the climate is classified as Af (tropical rainforest) according to the Köppen-Geiger classification system (Beck *et al.*, 2018). The region experiences an average annual temperature of 27 °C and an annual precipitation of 2,408 mm. The drier season, defined as months with rainfall below 100 mm, typically occurs between August and September (Tanaka *et al.*, 2014). Relative humidity varies throughout the year, reaching its lowest levels (75%) in August and peaking at 92% in April, coinciding with the rainy season (Araújo *et al.*, 2002). The soil in the study area is characterized by high acidity (pH of 4.2) and a clay-rich texture. It has low concentrations of phosphorus ($1.4 \pm 0.76 \text{ mg kg}^{-1}$), calcium ($0.035 \pm 0.03 \text{ cmolc kg}^{-1}$), and magnesium ($0.084 \pm 0.01 \text{ cmolc kg}^{-1}$) (for additional details, see Santos and Ferreira, 2020b).

1.3.2 History of land use and seedling production

The study was conducted in a 22-year-old secondary forest located at the Experimental Farm of the Federal University of Amazonas, in Manaus, Brazil (Fig. 1). Seedlings were produced at the forest nursery of the Federal University of Amazonas ($3^{\circ} 6'S$, $59^{\circ} 58'W$). All seedlings were uniformly fertilized and acclimatized to ensure similar conditions at the time of planting (SANTOS; FERREIRA, 2020).

1.3.3 Implementation and experimental design

The experiment was implemented as a silvicultural enrichment system, applying different levels of canopy opening in an area of 17 ha. Thinning of arboreal individuals from the secondary vegetation was carried out by applying a basal area removal treatment (or refinement) in main plots of 2,318m² (38 x 61 m) at levels of 0%, 20%, 40%, 60%, 80%, and 100%, thereby creating a gradient of canopy openness. In this study, the categories were defined as understory (0%), intermediate (40%), and gap (100%). Planting occurred between February 24 and March 8, 2017. Seedlings were planted at a spacing of 3 m x 3 m in 30 x 30 x 30 cm holes. All plants received similar fertilization at the time of planting, with a top dressing applied two months later. The fertilizations aimed to ensure that the growth variation of the plants was predominantly influenced by the gradient of canopy openness and not by differences in soil nutrient availability. For more details, see Santos and Ferreira (2020). The experimental design followed a randomized block layout with five replications. At the time of data collection, the seedlings were five years old.

Five forest species were selected based on criteria such as seed availability (minimum of five parent trees), economic potential, and ecological and social importance in the region. Information on the ecological groups and anatomical characteristics is presented in Table 1. The light levels were characterized according to the Photosynthetic Photon Flux Density (PPFD): low irradiance (understory [0%] $\approx$ PPFD = 2.59 mol.m².day⁻¹), moderate irradiance (intermediate [40%] $\approx$ PPFD = 10.87 mol.m².day⁻¹) and high irradiance (gap [100%] $\approx$ PPFD = 33.05 mol.m².day⁻¹). The non-pioneer species included *Bertholletia excelsa*, *Carapa guianensis* and *Hymenaea courbaril*, while the pioneer species were *Cedrela fissilis* and *Tabebuia rosea*.

1.3.4 Xylem anatomy

To ensure consistency in sampling, samples were collected at an equal distance from the apex of the plant across all three light conditions. For understory individuals, which were significantly smaller, samples were taken from the basal region of the stem. The distance from the apex to the base of the stem were used as a standard for collecting samples from individuals in intermediate and gap conditions. This approach was standardized to one individual per species per light condition, with five replications (species (5) x light condition (3) x block (5) = N = 75). The height of the plants ranged from 886 cm in gap conditions to 70 cm in the understory. For more details, see Coelho-Silva *et al.* (2024). After collection, the samples were fixed in 70% FAA and subsequently stored in vials containing 70% ethanol until permanent slides could be prepared.

To prepare the slides, samples were sectioned using a sliding microtome in the three conventional planes used for wood anatomy: transverse, tangential longitudinal, and radial longitudinal. The preparation of histological slides followed the techniques outlined by Johansen (1940) and Sass (1951). The histological sections were first clarified in 50% sodium hypochlorite and then washed in 1% acetic water. A double-staining process was employed using 1% aqueous safranin (Bukatsch, 1972) and 1% aqueous astra blue (Roeser, 1972) to enhance the contrast of cell structures. After staining, the sections were dehydrated using a series of ethanol solutions (30% - 50% - 70% - 90% - 95% - 100%) and finally embedded in butyl acetate. The permanent slides were mounted in a synthetic medium (Entellan®).

After mounting the slides, quantitative parameters were obtained using light microscopy with an Axio Cam MRC camera and a computer equipped with Axiovision software. The quantitative anatomical parameters included vessel area (μm^2) and

vessel density (number of vessels/mm²), and the percentage of cell fractions (%), as shown in Figure 2, following a protocol adapted from Zieminska and Wright (2015). Measurements and qualitative parameters were taken following the recommendations of the IAWA Committee (1989), Carlquist (1977, 2001) and Wheeler (1986). All images were analyzed using ImageJ software. After taking measurements, we calculated the hydraulic diameter (D_h), the percentage of conductive area, the vulnerability index (VI) (hydraulic diameter/vessel density), and the vessel grouping index (number of vessels in 1mm² area of xylem tissue/ total number of vessel groups within the same area).

1.3.5 Gas Exchange

Leaf gas exchange measurements were conducted during the wetter period of 2022, on one leaf per individual, between 8:00 a.m. and 11:00 a.m., using an infrared gas analyzer (LI-6400XT, LI-COR). Prior to data collection, tests were performed on at least three leaves per individual to ensure that the measurements were representative of the overall plant. The analyzer's chamber settings included an airflow rate of 400 $\mu\text{mol s}^{-1}$, a CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$, a leaf temperature maintained at 31 °C, and a photosynthetic photon flux density (PPFD) of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Key parameters measured included the light-saturated photosynthetic rate (A_{sat}), transpiration rate (E) and stomatal conductance (g_s). Water use efficiency (WUE) and intrinsic water use efficiency (WUE_i) were calculated as the ratio of A_{sat} to E and A_{sat} to g_s , respectively.

1.4 Statistics

A two-way ANOVA was performed to compare the anatomical traits across different light conditions and species. Tukey's test was used for multiple comparisons,

and the package estimated marginal means (EMMs) was employed. The percentage of cell fraction was calculated in 1mm^2 , with the total number of points measured considered as 100%. For the different inferences, we grouped the average percentage values by light condition, successional group, species, and light x species interaction. An ANCOVA was conducted to assess if hydraulic diameter (covariate) influences stomatal conductance (g_s) and how light conditions (categorical factor) affect this relationship. Additionally, a linear mixed-effects model was applied to assess whether hydraulic diameter influences gas exchange (A_{sat}) and how light conditions (gap, intermediate, and understory) modulate this process. The model included hydraulic diameter (continuous) and light condition (categorical) as fixed effects, as well as their interaction, to test if light availability affects the relationship between hydraulic diameter and gas exchange by influencing either the slope or the intercept. To account for interspecific variability, species was included as a random intercept. This analysis was based on the expectation of a positive linear association under increasing light availability. The assumptions of normality and homoscedasticity were verified using the Shapiro-Wilk and Levene's tests, respectively, and data were transformed when necessary. Additionally, residual diagnostics were performed to ensure that the assumptions of the linear mixed-effects model were met. All analyses and graphs were generated using R software version 4.3.0.

1.5 Results

1.5.1 Xylem anatomical traits

Hydraulic diameter exhibited a significant interaction between species and light conditions ($F = 10.19$, $P < 0.001$; Fig. 3A). Light availability generally promoted larger hydraulic diameters across all species, except for *Tabebuia rosea*. *Bertholletia excelsa* showed significantly larger hydraulic diameters in gap conditions (104.44 μm) compared to intermediate (59.45 μm) and understory conditions (62.35 μm), suggesting that higher light availability enhances hydraulic diameter in this species. For *Carapa guianensis*, hydraulic vessel diameter values were higher in gap (103.39 μm) and intermediate (103.33 μm) conditions, with the lowest value observed in the understory condition (53.05 μm). In the case of *Hymenaea courbaril*, significant differences were noted across all light conditions, with hydraulic diameter values progressively increasing from understory (59.21 μm) to intermediate (86.5 μm) and reaching the highest value in gap conditions (110.9). For *Cedrela fissilis*, hydraulic diameter was significantly higher under gap conditions (120.23) compared to understory conditions (105.28 μm). In contrast, *T. rosea* showed no significant differences among light conditions (gap = 80 μm , intermediate = 83.56 μm , understory = 79.86 μm), indicating a similar hydraulic diameter across varying light conditions.

The significant interaction in vessel density (vessels/ mm^2) suggests that species respond differently to variations in light conditions ($F = 6.27$, $P < 0.0001$, Fig. 3B). This interaction indicates that changes in vessel density under different light conditions are not consistent across species, highlighting the importance of species-specific responses to light gradients. For *B. excelsa*, vessel density was significantly higher under intermediate light conditions (36.96/ mm^2) compared to gap (14.31/ mm^2). In *C. guianensis*, a significant increase in vessel density was observed under low light

conditions (43.014/mm²) compared to intermediate (19.45/mm²) and gap (23.81/mm²). For *T. rosea*, vessel density was higher in gap conditions (53.51/mm²) compared to understory (26.78/mm²). In contrast, *H. courbaril* and *C. fissilis* exhibited similar vessel densities across different light treatments, with no significant differences observed between gap, understory, and intermediate conditions.

1.5.2 Cell fraction

We opted to use a radar chart to effectively illustrates the variation across multiple response variables simultaneously. The radar chart presents the variation of seven response variables across the three light conditions (understory, intermediate, and gap). Each axis represents a different variable, and values are standardized to enable direct comparisons among the light conditions. The shaded areas depict the range of variation for each light condition, highlighting overall trends. Data points positioned farther from the center indicate higher values for the respective variable. When analyzing the cell fraction under the light gradient (Fig. 4A; Table 2), the proportions of fiber wall and vessel wall were slightly higher in gap conditions (38.88% and 5.74%, respectively) compared to intermediate (36.88% and 5.22%, respectively) and understory conditions (28.69% and 3.76%, respectively). The fiber lumen exhibited a similar pattern, being larger in high light availability conditions, with a more pronounced difference between gap (6.80%) and intermediate (6.86%) compared to understory conditions (3.90%). The percentage of vessel lumen was also higher in gap conditions (14.26%) compared to intermediate (11.40%) and understory (6.04%) conditions, indicating a greater capacity for water transport under increased light availability. Conversely, a higher relative percentage of axial parenchyma in lines or bands was observed in understory (36.49 %) and intermediate (20.42%) conditions,

with only 14.65% in the gap conditions. This suggests an increased need for storage tissues under reduced light availability. Paratracheal parenchyma, which is associated with vessels, showed similar values across light conditions (gap = 4.25%, intermediate = 4.33%, understory = 3.51%), indicating that this may be a more genetically conserved trait. The percentage of rays, which play a role in lateral transport and storage, was notably higher in understory conditions (17.62%) compared to intermediate (14.88%) and gap (15.42%) conditions. This suggests an adaptation to limited light availability, with greater emphasis on radial transport and carbohydrate storage.

When comparing the cell fractions between successional groups (Fig. 4B; Table 2), non-pioneer species exhibited a marginally higher proportion of fiber walls (36.89%) than pioneer species (31.86%). This trend indicates that non-pioneer species in gap conditions allocate more resources to their cell walls. In contrast, vessel walls were slightly higher in pioneer species (5.60%) compared to non-pioneers. Additionally, the fiber lumen was larger in pioneer species (6.32 %) than in non-pioneers (5.63%), and the vessel lumen was also greater in pioneers (12.56 %) compared to non-pioneers (9.79%), suggesting enhanced water transport capacity in pioneer species. Pioneer species displayed a higher relative percentage of parenchyma arranged in lines or bands (26.01%) compared to non-pioneers (21.72%) (Fig. 4A). Notably, the values for paratracheal parenchyma were similar across successional groups (pioneer = 4.85%, non-pioneer = 3.55%), indicating that this may be a conserved trait. Finally, the percentage of rays was higher in non-pioneer species (17.84%) than in pioneer (12.80%), implying that each successional group allocates resources differently among various types of parenchyma (radial versus axial, in lines or bands).

1.5.3 Anatomical features

When assessing the investment patterns of the anatomical features for each species across different light conditions, notable variations were observed (Fig. 5, Table S1). The features most influenced by light conditions included fiber and vessel wall, fiber and vessel lumen, and axial parenchyma arranged in lines or bands. *Cedrela fissilis* exhibited a higher investment in fiber wall and fiber lumen under gap conditions (Fig. 5A), while a lower amount of axial parenchyma arranged in lines of bands was present. In the understory, however, the pattern was reversed, with more than half of the carbon investment allocated to reserve tissue (Fig. 5C), while a smaller proportion was dedicated to fiber wall and fiber lumen. *Carapa guianensis* showed a similar, though less pronounced, pattern. Notably, this species maintained fiber lumen size across light conditions (Fig. 5J, K and L), and there was a twofold increase in investment in axial parenchyma in lines/bands under understory conditions compared to gap conditions (Table S1). Vessel lumen sizes exhibited similar trends across all species, with larger lumen sizes observed in gap conditions (Fig. 5A, D, G, J, M). The greatest variation in vessel lumen area was observed in *Tabebuia rosea* (Fig. 5D, E, F), while the least variation was noted in *Bertholletia excelsa* (Fig. 5G, H, I). Notably, in *Hymenaea courbaril*, investment in vessel lumen was highly responsive to changes in light availability (Fig. 5M, N, O).

When comparing species differences (Fig. S1), *C. fissilis*, *B. excelsa*, and *H. courbaril* exhibited higher proportions of fiber lumen compared to other species (7.56 %, 7.42 % and 6.20 %, respectively). *B. excelsa* also showed the highest percentage of fiber wall (44.10 %), suggesting enhanced mechanical support. *H. courbaril* ranked second in fiber wall investment (39.24 %), followed by *C. fissilis* (34.24 %). The species with the lowest investment in this trait were *C. guianensis* (27.85 %) and *T. rosea*

(29.19 %). In terms of vessel lumen, *T. rosea* and *C. guianensis* had the largest vessel lumen areas (13.55% and 13.41 %, respectively), which could facilitate more efficient water transport. In contrast, *H. courbaril* and *B. excelsa* had the smallest vessel lumen areas (8.57 % and 7.21 %, respectively). Only two species showed the highest investment in rays: *C. guianensis* (21.38 %) and *B. excelsa* (19.58 %). For *C. fissilis*, *H. courbaril* and *T. rosea*, ray investment was relatively low (13.12%, 12.69 %, 12.42 %, respectively). Regarding axial parenchyma in lines/bands, *H. courbaril* and *T. rosea* exhibited the highest proportions (27% each), while *B. excelsa* showed the lowest investment in this feature (14.13 %).

1.5.4 Vulnerability index and percentage of conductive area

The vulnerability index revealed significant interactions between light conditions and species ($F = 8.65$, $P < 0.0001$, Fig. 6A). The R-squared value of 0.74, indicates that 73.6% of the variance in the response variable is explained by the model. Comparisons of means across light conditions for each species show significant differences for all non-pioneer species (Fig. 6A). For these species, the vulnerability index was higher under conditions of greater light availability. Specifically, *Bertholletia excelsa*, *Hymenaea courbaril* and *Carapa guianensis* had significantly higher vulnerability index values in gap conditions (6.63, 6.03 and 4.47, respectively) compared to intermediate (1.86, 4.78 and 5.51 respectively) and understory conditions (2.98 and 2.34 and 1.32 respectively), which did not differ significantly from each other. In contrast, no significant differences were observed among light conditions for the pioneer species. The vulnerability indices for *Cedrela fissilis* (gap = 4.55, intermediate = 4.78 and understory = 5.66) were twofold higher than those for *Tabebuia rosea* (gap = 1.75, intermediate = 2.37 and understory = 2.32).

The analysis of variance (two-way ANOVA) showed significant effects of both species and light condition on the conductive area, but no significant interaction between these factors ($F = 1.27$, $P > 0.05$, Fig. 6B). Both species ($F = 8.25$, $P < 0.0001$) and light condition ($F = 41.87$, $P < 0.0001$) independently significantly influenced the percentage of conductive area (Fig. 6B). When assessed additively, conductive area was significantly greater in gap and intermediate conditions compared to understory ($F = 16.65$, $P < 0.0001$). Notably, the most pronounced differences were observed between the species *B. excelsa* and *C. guianensis* ($F = 4.84$, $P < 0.01$).

1.5.5 Relationships between leaf physiology and hydraulic diameter

The interaction between hydraulic diameter (D_h) and light conditions was significant ($F(2,54) = 3.61$, $P < 0.05$, Fig. 7A), indicating that the relationship between D_h and stomatal conductance (g_s) varies across light conditions. The interaction between D_h and intermediate light conditions was significant ($F(1,54) = 9.72$, $P < 0.001$), with a negative relationship between D_h and g_s under intermediate light (slope = -0.00398). However, the interactions between D_h and both understory and gap light conditions were not significant. Light conditions had a significant overall effect on leaf photosynthesis ($Asat$) ($F(2,57) = 21.66$, $P < 0.0001$, Supplementary data S2-A), while the effect of hydraulic diameter was not significant ($F(1,58) = 35.24$, $P > 0.05$). Intermediate light conditions resulted in a significant reduction in $Asat$ compared to gap conditions ($F(1,57) = 27.03$, $P < 0.001$), and $Asat$ was further reduced under understory conditions ($F(1,57) = 40.34$, $P < 0.001$).

The interaction between D_h and light conditions on transpiration (E) was not significant ($F(2,54) = 2.34$, $P > 0.05$, Supplementary data S2-B). However, the interaction between D_h and intermediate light conditions was significant ($F(1,54) =$

4.49, $P < 0.05$), with a negative relationship between D_h and E in these conditions (slope = -0.0264). The main effect of D_h on E was significant ($F(1,54) = 16.45$, $P < 0.001$), as was the main effect of light conditions ($F(2,54) = 15.83$, $P < 0.001$). The interaction between D_h and intermediate light conditions was significant ($F(1,54) = 4.49$, $P < 0.05$), while the interaction between D_h and understory conditions was not significant ($F(1,54) = 0.26$, $P > 0.05$). The interaction between D_h and light conditions on water-use efficiency (WUE) was not significant ($F(2,54) = 0.10$, $P > 0.05$, Supplementary data S2-C). The effect of D_h on WUE was not significant ($F(1,54) = 2.42$, $P > 0.05$, Supplementary data S2-B), while light conditions had a significant effect on WUE ($F(2,54) = 4.93$, $P < 0.05$). Similarly, the interaction between D_h and light conditions on intrinsic water-use efficiency (WUEi) was not significant ($F(2,54) = 1.97$, $P > 0.05$, Supplementary data S2-D). The effect of D_h on WUEi was not significant ($F(1,54) = 1.83$, $P > 0.05$), and light conditions also had no significant effect on WUEi ($F(2,54) = 0.54$, $P > 0.05$).

1.6 Discussion

In this study we investigated how a light gradient influenced the anatomical features of five tree species in the juvenile stage and how these changes affect physiological and hydraulic responses. Based on our results, we partially support our hypothesis. We found that xylem anatomical traits, such as hydraulic diameter, vessel density, and percentage of conductive area, were dependent on light conditions, aligning with the expectation that species exhibit plasticity in response to a light gradient (Fig. 3, 4). However, the interaction between hydraulic diameter and light conditions only influenced hydraulic conductance (Fig. 7). This suggests that while anatomical adjustments occurred, the expected divergence in plasticity between gap and understory species was not fully supported in physiological traits, even though light conditions strongly influenced these parameters (Supplementary data S2). The species exhibited higher variation in their anatomical characteristics across the light conditions except to *Tabebuia rosea*, reflecting a lower capacity to adjust to this environmental filter. The results demonstrate that a light gradient can influence hydraulic architecture differently across species. Significant increases in hydraulic diameter were observed in species under higher light conditions, except to *T. rosea*.

1.6.1 *The effect of light on the anatomical structure of the xylem and its hydraulic properties*

The non-pioneer species, such as *Carapa guianensis* and *Hymenaea courbaril*, exhibited larger hydraulic diameters under higher light conditions (gap). This could be interpreted as an adaptive response to maximize water transport in environments with higher light availability, as evidenced by the highly light-responsive conductive area in these species (Fig 6B). However, this increased size may also heighten susceptibility

to hydraulic failure under stress conditions. Larger vessel diameters are more vulnerable to embolism (Olson *et al.*, 2020), as indicated by the high vulnerability index in the highest light conditions for both species (Fig 6A). Despite this, both species showed higher P_{50} values compared to the understory conditions (Coelho-Silva *et al.*, 2024), suggesting that other anatomical features or physiological strategies may account for their different levels of xylem resistance to embolism. Conversely, species like *Tabebuia rosea* showed less plasticity in hydraulic diameter across light conditions. This may reflect a strategy to cope with light gradients, as evidenced by the significant reduction in vessel density under understory conditions (Fig. 3B), indicating that species with lower plasticity may rely on vessel density adjustments than on changes in vessel hydraulic diameter.

Both *Bertholletia excelsa* and *Carapa guianensis* showed significant differences in vessel density across light conditions, but in distinct ways. For *B. excelsa*, the highest vessel density was observed in the intermediate light conditions, while for *C. guianensis*, it was in the understory, highlighting the species-specific influence of light on vessel density. In contrast, *Cedrela fissilis* and *Hymenaea courbaril* showed no significant differences between treatments, consistent with the lack of variation in hydraulic diameter for these species across light conditions (Fig. 3A). These results suggest that the effect of light availability on vessel density is highly species-dependent. While species like *B. excelsa*, *C. guianensis*, and *T. rosea* show marked changes in vessel density under different light conditions, others, such as *C. fissilis* and *H. courbaril*, maintain relatively consistent values. Different adaptations of Amazonian species to water regimes have been previously demonstrated, such as the influence of root distance from the water table on forest structure (Brum *et al.*, 2018), and the finding that the average diameter, weighted by species abundance, increases

in the wetter Amazon (Central Amazon), while vessel density increases in the drier Amazon (Eastern Amazon) (Barros *et al.*, 2019). Our study introduces another important ecological filter, highlighting the complexity of how species adjust their wood structure in response to light variation. This has significant implications for understanding their ecological strategies and adaptability to changes in light availability, especially in the face of shifting forest dynamics due to climate change and land-use alterations.

1.6.2 Influence of light on cell fraction and its functional implications

The radar plot illustrates distinct variations in cellular composition based on light conditions and ecological group. Gap conditions favor larger vessel lumen areas, which likely enhance water conduction under greater photosynthetic activity. Conversely, under understory conditions plants are characterized by increased allocation to axial parenchyma and ray tissues, indicating a shift towards storage-oriented structures. In other words, there is a higher investment in reserve tissues (i.e. starch) in response to reduced light availability. These results demonstrate the significant influence of light conditions on the anatomical composition of cellular fractions. In gap conditions, the higher proportions of fiber and vessel walls suggest a thickening of these structures, likely providing enhanced mechanical support and improved hydraulic maintenance. This response can be associated with a high production of lignin, which generally occurs under greater light availability (Cabane, 2012). The larger vessel lumen under increased light availability indicates a greater capacity for water transport, an adaptation to meet the higher transpiration demand in these environments. These findings reinforce the notion that the vulnerability-diameter link (Olson *et al.*, 2023) is influenced by both ecological factors (successional group)

and microclimate conditions (light). In contrast, the higher percentage of axial parenchyma in lines or bands, as well as the elevated ray fraction under understory conditions, reflect a shift towards resource storage and lateral transport (Dalling *et al.*, 2024).

When comparing successional groups, pioneer species exhibited larger fiber and vessel lumen, consistent with their strategy of rapid growth and enhanced hydraulic conductivity in high-light environments (Pratt and Jacobsen, 2017). In contrast, non-pioneer species showed greater investment in cell walls (Supplementary data Table S1), which may enhance structural resilience. Additionally, the greater fraction of rays observed in non-pioneer species under gap conditions could facilitate the radial translocation of photosynthates from the phloem (Nadak *et al.*, 2022), potentially leading to increased cell wall production. These structural adaptations in response to light availability not only support efficient resource allocation but also contribute to the overall growth and resilience of these species. Consequently, the interplay between light conditions and anatomical features plays a crucial role in determining the physiological performance of non-pioneer species within their ecosystems.

The similar allocation to paratracheal axial parenchyma across light conditions and successional groups suggests that this trait is more genetically conserved and less influenced by microclimate factors. Few studies have investigated the relationship between the arrangement of different types of axial parenchyma and the xylem resistance to embolism (Lourenço Jr *et al.*, 2022; Placová *et al.*, 2024). Although certain landscapes, such as altitude differences, seem to affect the distribution of paratracheal axial parenchyma (Placová *et al.*, 2024), the microclimatic conditions

investigated here did not significantly alter axial parenchyma directly connected to the vessels.

The differences in resource allocation between cell wall, lumen size, and parenchyma proportions across light conditions and successional groups highlight distinct adaptive strategies. Pioneer species prioritize rapid hydraulic transport, while non-pioneers focus on structural integrity. However, both groups show significant investment in reserve tissue, especially in low-light environments. These findings underscore the role of light availability and ecological strategy in shaping the anatomical traits of regenerating seedlings in secondary forests. In seedlings, this response may be related to a defense-growth trade-off. Pioneers tend to invest in rapid growth strategies (e.g., low wood density, less fiber wall, and wider conduits), whereas non-pioneers focus on defense strategies (e.g., higher wood density, thicker fiber walls, and smaller fiber lumens) (Gonzales-Melo 2022). Notably, greater herbivory in the understory contributes to higher soluble sugars in the leaves of these species (Coelho *et al.*, 2020). This could also explain the higher parenchyma fraction observed in both successional groups under these light conditions (Fig. 4).

1.6.3 Coordination between leaf physiology and hydraulic diameter

For most of the physiological aspects investigated, light strongly influences leaf physiological performance, consistent with previous findings for the same species at two years of age (Santos and Ferreira, 2020). Specifically, we observed that the relationship between stomatal conductance (g_s) and hydraulic diameter depends on light conditions, suggesting a coordinated response between these traits, with light acting as the main driver.

The significant interaction between light conditions and g_s in relation to hydraulic diameter highlights the influence of the light gradient on water transport in seedlings. This may be linked to the inherent trade-offs in angiosperm xylem functioning (Pratt and Jacobsen, 2017). In high-light environments, growth occurs with the necessary anatomical and physiological adjustments; in contrast, in the understory, this relationship remains unchanged across species. This reaffirms light as a critical environmental filter for tropical species during their early establishment, particularly in secondary forests (Matsuo *et al.*, 2024). Under gap conditions, where water transport demands are higher (Chen *et al.*, 2021, Flo *et al.*, 2022), g_s increases in correlation with a larger hydraulic diameter (Fig 3). This suggests that seedlings in light-abundant environments enhance their hydraulic pathways to support increased photosynthetic activity (Asaf), as seen in Supplementary Data (S2-A). The larger hydraulic diameters observed in high-light plants may reflect a structural adjustment to facilitate efficient water transport, aligning with previous findings in moist forest species (Liu *et al.*, 2019). This adaptation helps maintain hydration and physiological function under high evaporative demand.

In understory conditions, however, the weaker interaction between g_s and hydraulic diameter suggests moderated water transport needs, potentially due to lower g_s values. Plants in shaded environments may prioritize water conservation over maximizing hydraulic efficiency (Wang and Wang, 2023). Consequently, smaller hydraulic diameters in low-light conditions could represent an adaptive strategy to conserve resources where water demand is lower. These findings emphasize the role of light in shaping hydraulic architecture, where conductance acts as a key modulator of hydraulic diameter adjustments, optimizing water transport based on environmental demands (Cruziat *et al.*, 2002).

The variation in vapor pressure deficit (VPD) across light conditions, with notably higher VPD in gaps, may explain the lack of significant interaction between transpiration and light in relation to hydraulic diameter. Under gap conditions, elevated VPD creates a strong driving force for transpiration, primarily regulated by atmospheric demand (Chen *et al.*, 2021), rather than by anatomical adjustments in hydraulic diameter. This suggests that while hydraulic diameter may respond to g_s to optimize water transport, transpiration in high-light, high-VPD environments is mainly controlled by external evaporative demand (Flo *et al.*, 2020). Consequently, plants in gaps may rely on transpiration to regulate leaf temperature, reducing the need for direct structural adjustments to transpiration in response to light availability. In contrast, under low VPD conditions, plants can limit transpiration (Koehler *et al.*, 2024), as observed in the understory. This suggests that VPD is a dominant factor in transpiration regulation across light conditions, diminishing the effect of light on the transpiration-hydraulic diameter relationship. Notably, *Hymenaea courbaril* demonstrated high responsiveness to variations in light, particularly through increased hydraulic diameter in more open environments.

1.7 Conclusion

Our findings have important ecological implications, demonstrating that hydraulic adaptations in plants vary along a light gradient. This variation highlights the functional plasticity of tropical species, as different light levels influence their investment in hydraulic traits, allowing them to adjust water transport to meet specific water and light demands. These structural and functional differences underscore the complexity of species' responses to light availability and the significant role of light in shaping their hydraulic architecture. From a silvicultural perspective, understanding these adaptive responses is crucial for managing secondary forests, where species regeneration is closely tied to microclimatic conditions. Knowledge of species-specific responses to light enables more strategic decisions in species selection and placement, ultimately improving forest resilience. By optimizing light conditions in managed forests, we can promote the establishment of more resilient plant communities, enhancing forest structure and function in recovering ecosystems.

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1.9 Authors' Contributions

Study conceptualization and design: DCS DSP, CRM, MJF; Methods: DCS, DSP, ZTMG, GDM; Data collection: DCS, ZTMG; Formal analysis/interpretation: DCS, DSP, ZTMG, GDM, SR; Writing - original draft: DCS; Supervision: CRM, MJF, SR; Funding acquisition: CRM, MJF, SR. All authors reviewed and approved the final manuscript.

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1.11 Conflict of interest

None.

1.12 Referências

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List of Tables

Table 1 – Scientific and common names, ecological groups and key anatomical features of the five species planted in the secondary forest enrichment system.

Table 2 – Percentage of cell fractions across a light gradient (gap, intermediate, and understory) in successional groups (pioneer and non-pioneer) and comparisons among the five studied species—*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*—in the Central Amazon.

List of figure legends

Figure 1 - Location of the study area within a secondary forest (FS) in the Central Amazon. The upper left inset shows the State of Amazonas outlined in blue, while the lower left inset highlights Manaus city within a yellow perimeter and the experimental site in red. The right side presents a detailed layout of the experimental area, showing the five experimental blocks.

Figure 2. Illustration of *Hymenaea courbaril* wood tissue used for cell fraction measurements. (A) identification of the cell types within the sample. (B) 1mm² area analyzed for cell type quantification. The inset details the grid spacing (C), with colors and numbers indicating different cell types: 1 (blue) - ray parenchyma, 2 (cyan blue) - vessel wall, 3 (green) - vessel lumen, 4 (pink) – banded parenchyma, 5 (orange) - paratracheal parenchyma, 6 (baby pink) - fiber lumen, 7 (red) - fiber. Scale bar: 200µm

Figure 3. Hydraulic diameter and vessel density across light conditions. Boxplots illustrate the effect of three light conditions—gap (orange), intermediate (green), and understory (blue)—on (A) hydraulic diameter (µm) and (B) vessel density (mm²), for five tree species (*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*). Letters indicate Tukey's post hoc test results comparing light conditions within each species. Boxplots display upper and lower quartiles, whiskers represent the 10th and 90th percentiles, and the median and mean values are marked by black horizontal lines and an “x”, respectively. (ns = $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$).

Figure 4. Radar plots illustrating the effect of (A) light conditions and (B) ecological groups on wood cellular composition. Measured variables include the proportion of

fiber lumen, fiber wall, vessel lumen, vessel wall, paratracheal parenchyma, rays, and axial parenchyma in lines or bands. Each light condition (gap in orange, intermediate in green, and understory in blue) and ecological group (pioneer in orange, non-pioneer in blue) is color-coded. Lines represent the relative percentage of each cellular component across conditions.

Figure 5. Transverse sections of the five studied species (*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*) (A-O) under three light conditions. The images illustrate that vessels are larger under higher light availability (gap) compared to lower light availability (understory). Additionally, a greater amount of axial parenchyma is observed in the understory conditions.

Figure 6. Vulnerability index and conductive area across light conditions. Boxplots depict the effect of three light conditions—gap (orange), intermediate (green), and understory (blue)—on (A) the vulnerability index and (B) the conductive area for five tree species (*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*). Letters indicate Tukey's post hoc test comparisons across light conditions within each species. Boxplots show upper and lower quartiles, whiskers represent the 10th and 90th percentiles, and median and mean values are marked by black horizontal lines and an “x”, respectively. (ns = $P < 0.05$; *** $P < 0.0001$).

Fig. 7. Relationships between hydraulic diameter and stomatal conductance (g_s) across light conditions. Each color represents a light condition: gap (red), intermediate (green), and understory (blue). An ANCOVA analysis was performed, with regression lines showing the trend for each light condition.

Table 1.1

Species	Common name	Ecological Group ¹	Porosity ² / Vessel/mm ²	Axial parenchyma ²	Ray width ²
<i>Bertholletia excelsa</i> Bonpl.	Castanheira da Amazônia	Non-Pioneer (PST)	Diffuse-porous (≤ 5 - 20)	Reticulate, marginal or in seemingly marginal bands	Width 1 to 3 cells; Larger rays commonly 4- to 10-seriate
<i>Carapa guianensis</i> Aubl.	Andiroba	Non-Pioneer (PST)	Diffuse-porous (≤ 5 - 20)	Scanty paratracheal, vasicentric; marginal or in seemingly marginal bands	Larger rays commonly 4- to 10-seriate
<i>Cedrela fissilis</i> Vell.	Cedro	Pioneer (LLP)	Semi-ring-porous (≤ 5 - 20)	Apotracheal diffuse; Scanty paratracheal, vasicentric; marginal or in seemingly marginal bands	Width 1 to 3 cells; Larger rays commonly 4- to 10-seriate
<i>Hymenaea courbaril</i> L.	Jatobá	Non-Pioneer (PST)	Diffuse-porous (≤ 5 - 20)	Scanty paratracheal, vasicentric, lozenge-aliform, confluent, marginal or in seemingly marginal bands	Width 1 to 3 cells; Larger rays commonly 4- to 10-seriate
<i>Tabebuia rosea</i> (Bertol.) Bertero ex A.DC.	Ipê rosa	Pioneer (LLP)	Diffuse-porous (5 - 40)	Scanty paratracheal, vasicentric, lozenge-aliform, confluent, marginal or in seemingly marginal bands	Width 1 to 3 cells

PST – Partially Shade Tolerant; LLP - Long-lived Pioneer (> 30 years).

¹ Ecological group as defined by: Swaine e Whitmore, 1988; Finegan, 1992; Poorter; Bongers, 2006; Chazdon, 2014.

² Anatomical features of the species according to the Inside Wood website, 2024.

Table 1.2

	Gap	Intermediate	Understory	<i>Pioneer</i>	<i>Non-pioneer</i>	<i>C. fissilis</i>	<i>T. rosea</i>	<i>B. excelsa</i>	<i>C. guianensis</i>	<i>H. courbaril</i>
Fiber wall	38.88 ± (8.22)	36.88 ± (11.32)	28.69 ± (12.8)	31.86 ± (12.74)	36.86 ± (10.19)	34.12 ± (16.34)	29.19 ± (6.22)	44.10 ± (7.54)	27.85 ± (7.94)	39.24 ± (7.62)
Vessel wall	5.74 ± (2.50)	5.22 ± (1.71)	3.37 ± (1.54)	5.60 ± (1.64)	4.58 ± (1.79)	4.22 ± (1.72)	6.99 ± (2.91)	4.06 ± (1.71)	5.68 ± (1.75)	3.95 ± (1.36)
Fiber lumen	6.81 ± (4.00)	6.86 ± (3.66)	3.90 ± (2.22)	6.32 ± (4.28)	5.23 ± (3.13)	7.56 ± (5.33)	4.85 ± (2.35)	7.42 ± (4.15)	3.39 ± (1.57)	6.20 ± (1.91)
Vessel lumen	14.26 ± (4.16)	11.40 ± (5.73)	6.04 ± (3.16)	12.56 ± (6.14)	9.76 ± (5.01)	11.71 ± (2.78)	13.55 ± (5.95)	7.21 ± (2.87)	13.41 ± (5.72)	8.57 ± (3.85)
Paratracheal parenchyma	4.25 ± (1.90)	4.33 ± (3.14)	3.51 ± (1.29)	4.85 ± (2.77)	3.55 ± (1.56)	4.01 ± (2.68)	5.85 ± (2.66)	3.50 ± (1.20)	4.93 ± (1.20)	2.23 ± (0.71)
Rays	15.42 ± (5.11)	14.88 ± (5.06)	17.62 ± (6.70)	12.80 ± (4.04)	17.84 ± (5.73)	13.12 ± (4.05)	12.42 ± (4.19)	19.58 ± (3.85)	21.38 ± (5.68)	12.69 ± (3.19)
Line/bands parenchyma	14.65 ± (7.66)	20.2 ± (10.68)	36.49 ± (13.57)	26.01 ± (17.3)	21.72 ± (11.88)	25.05 ± (21.75)	27.15 ± (11.03)	14.13 ± (11.01)	23.36 ± (11.76)	27.12 ± (9.75)

Figures

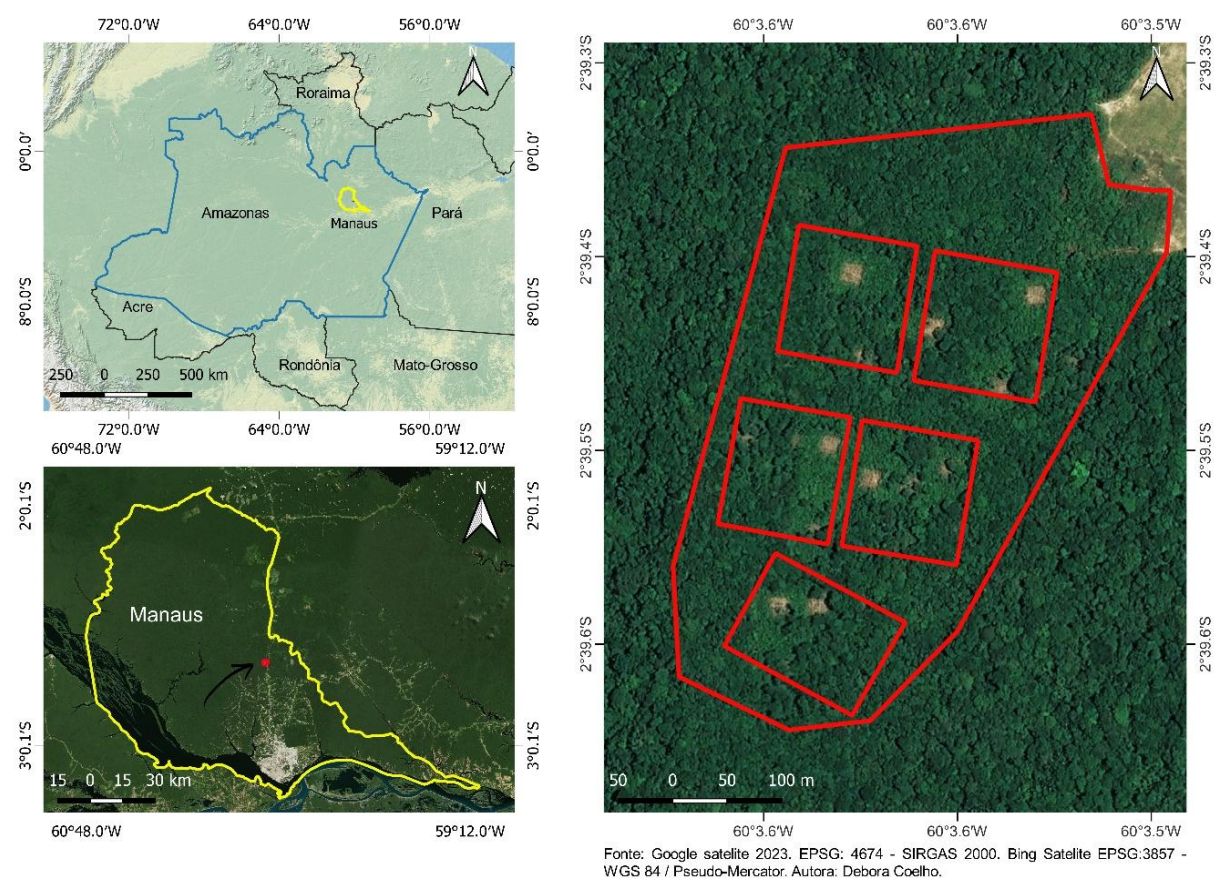


Figure 1.7

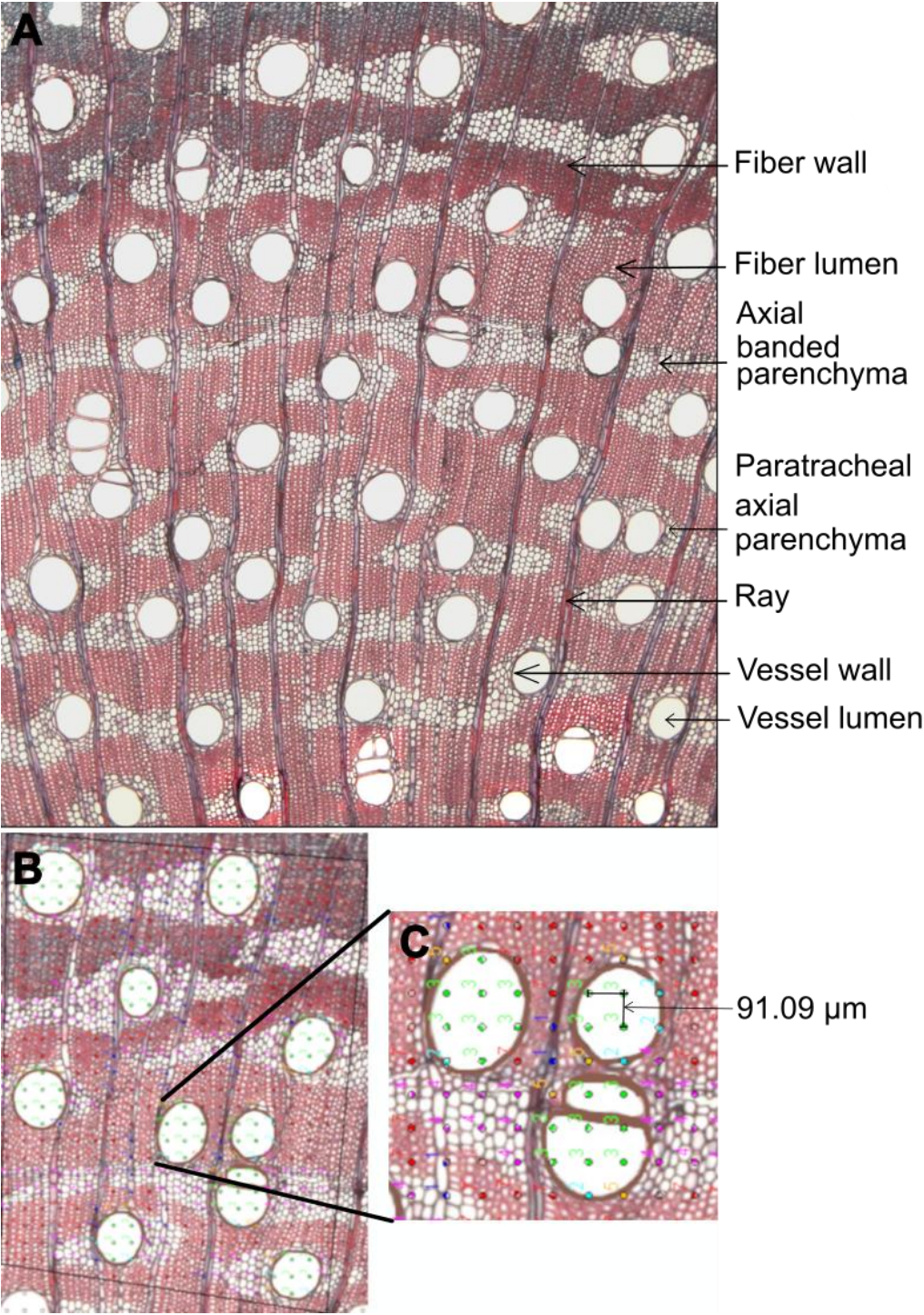


Figura 1.2

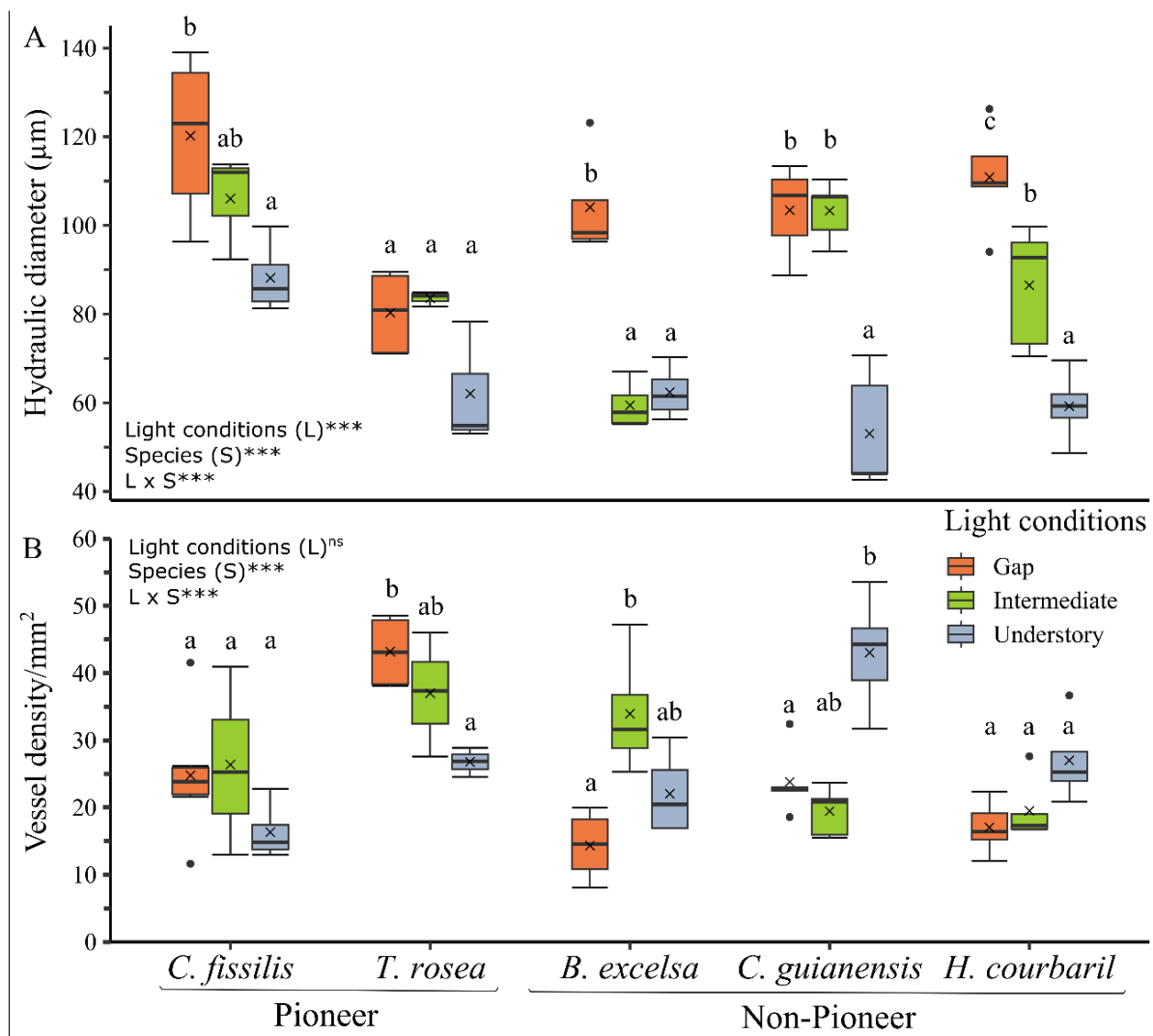


Figure 1.8

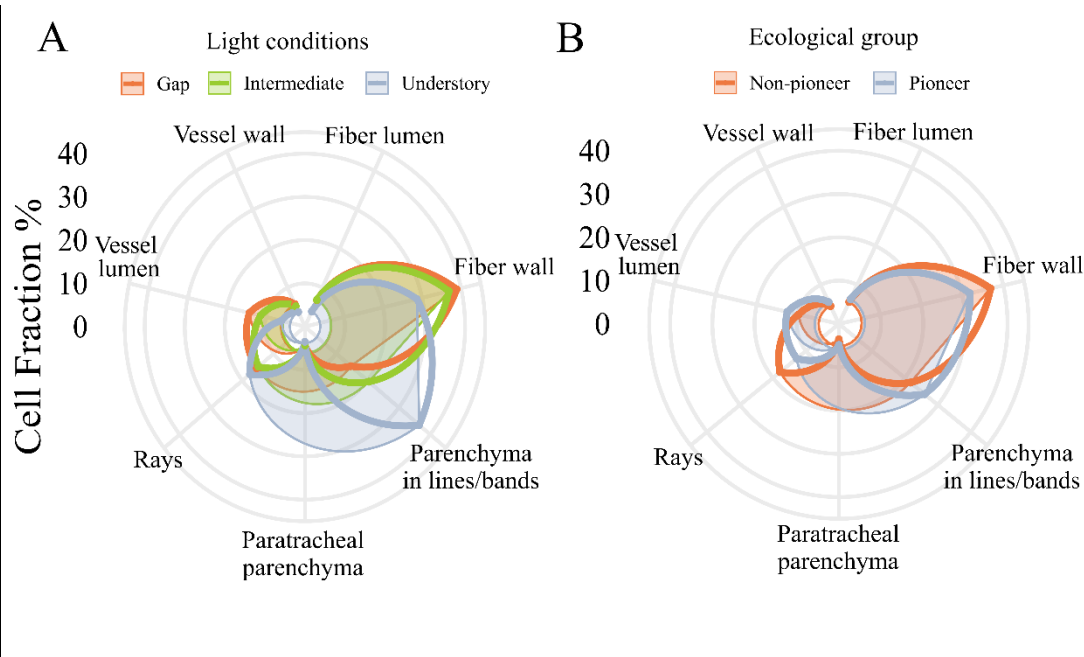


Figure 1.9

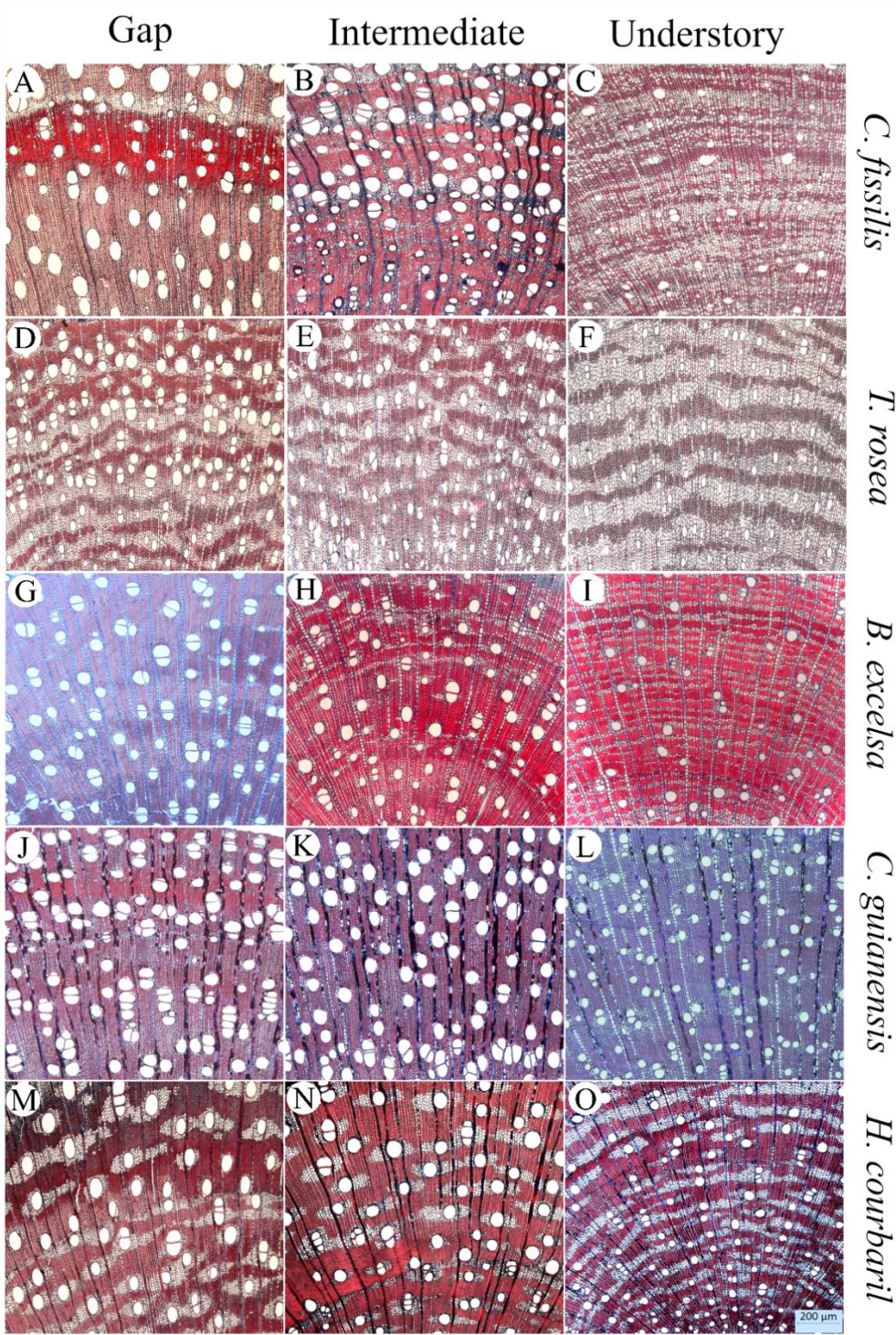


Figure 1.10

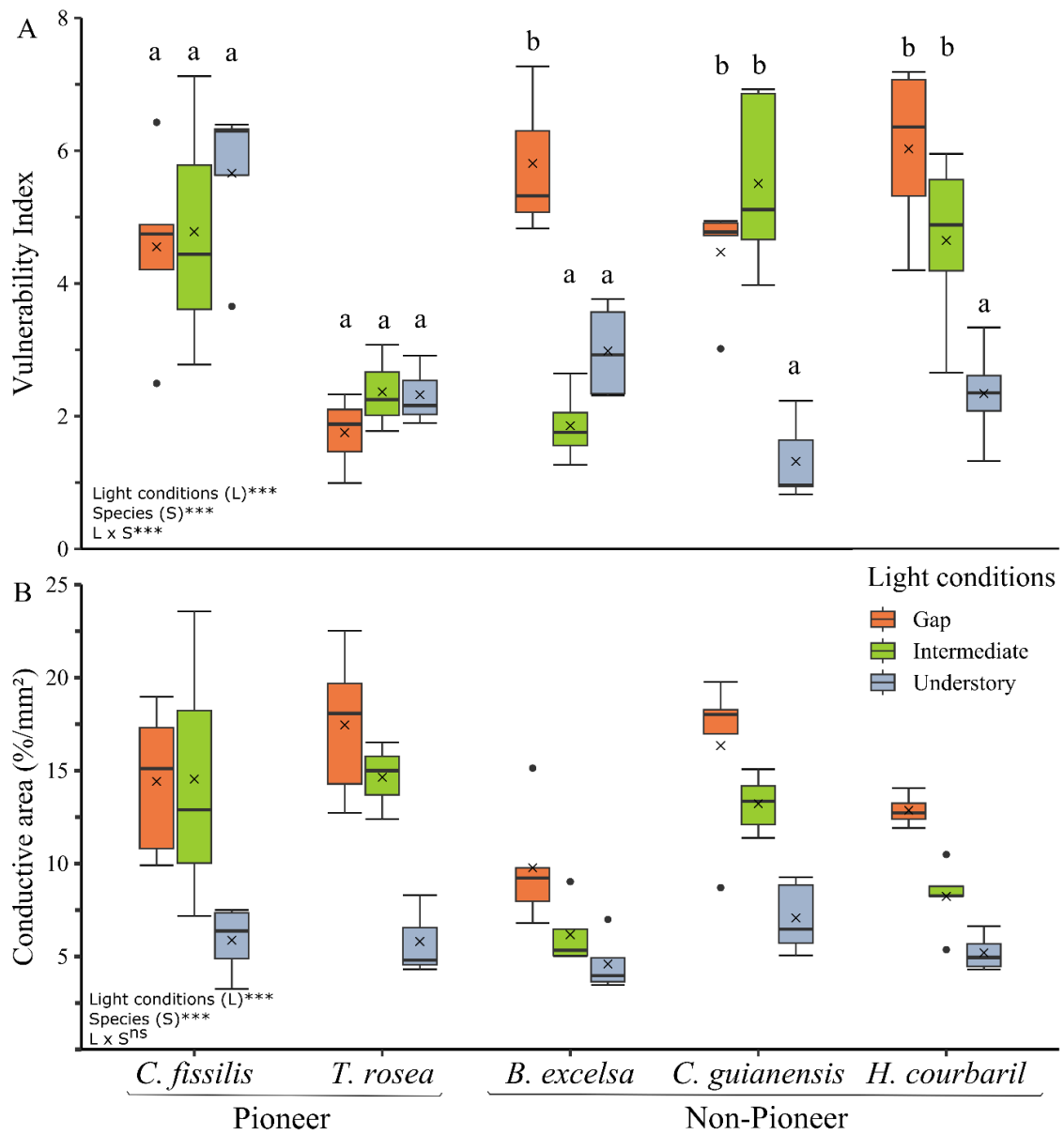


Figure 1.11

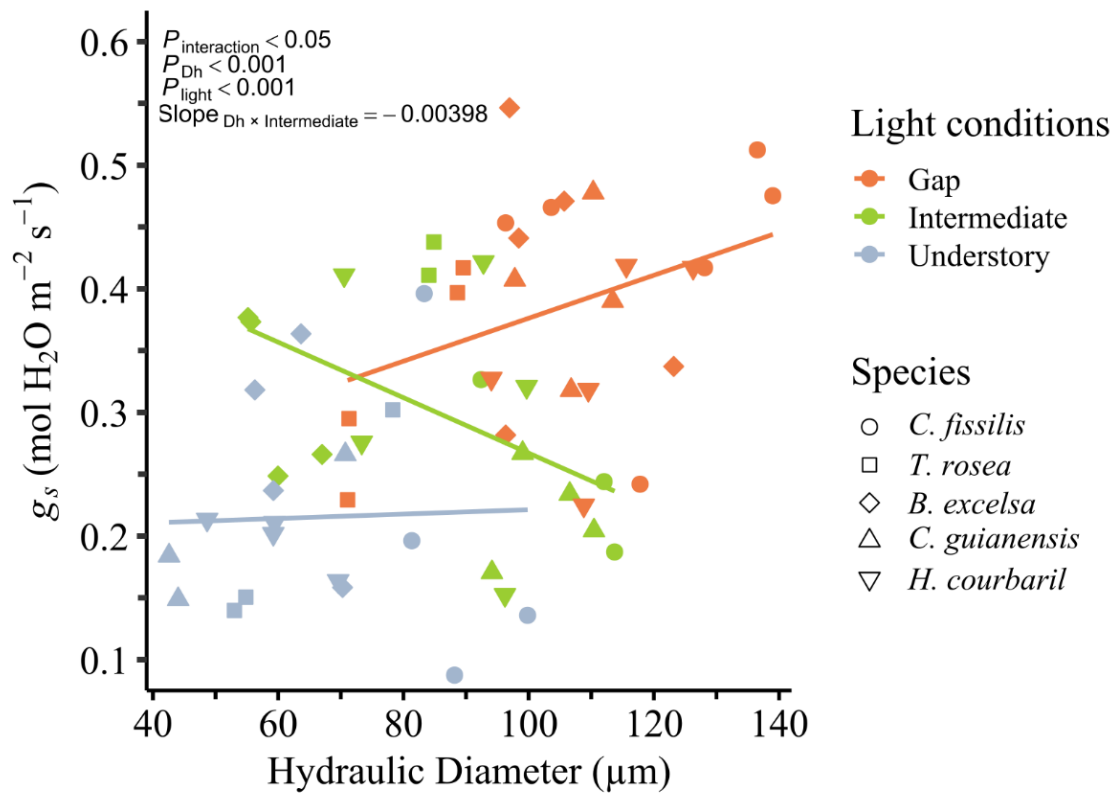


Figure 1.12

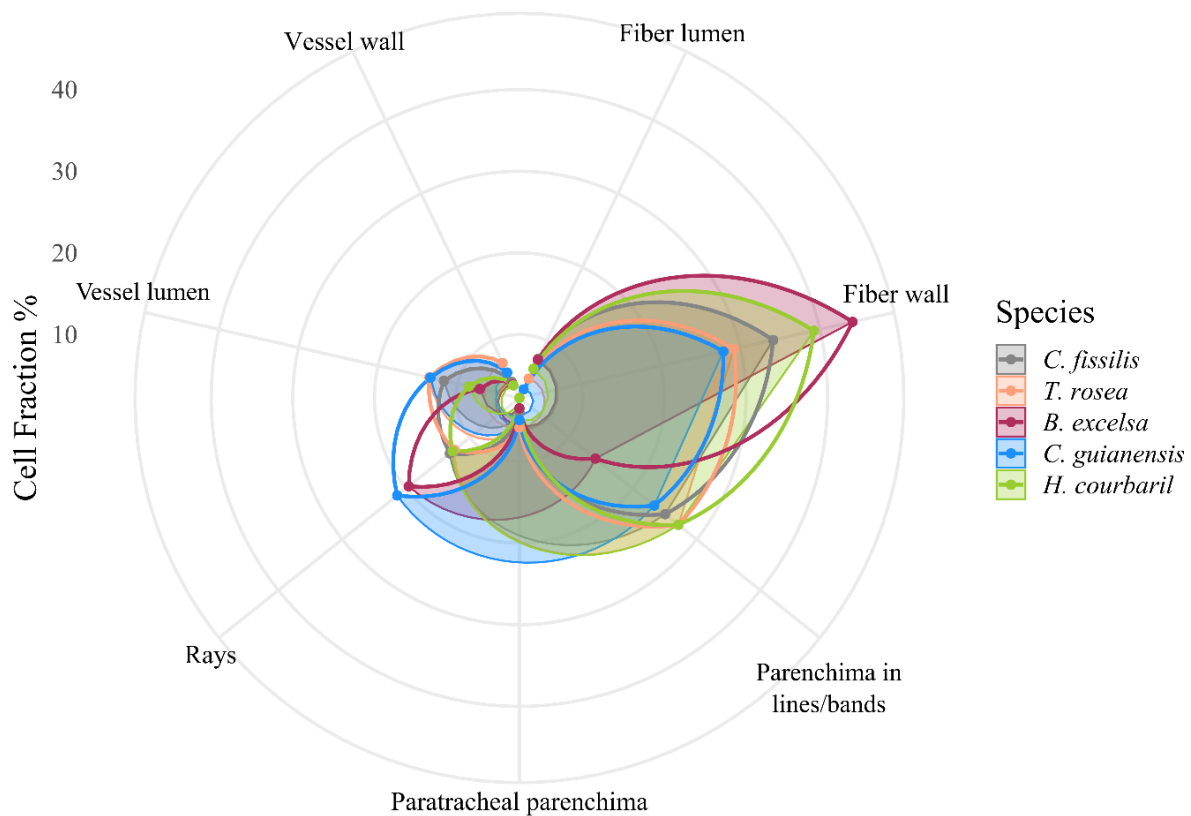
1.13 Supplementary material

Table S 1.5 – Percentage of cell fractions in five commercial species (*Cedrela fissilis*, *Tabebuia rosea*, *Bertholletia excelsa*, *Carapa guianensis*, and *Hymenaea courbaril*) along a light gradient in the central Amazon (N = 5 ± SD).

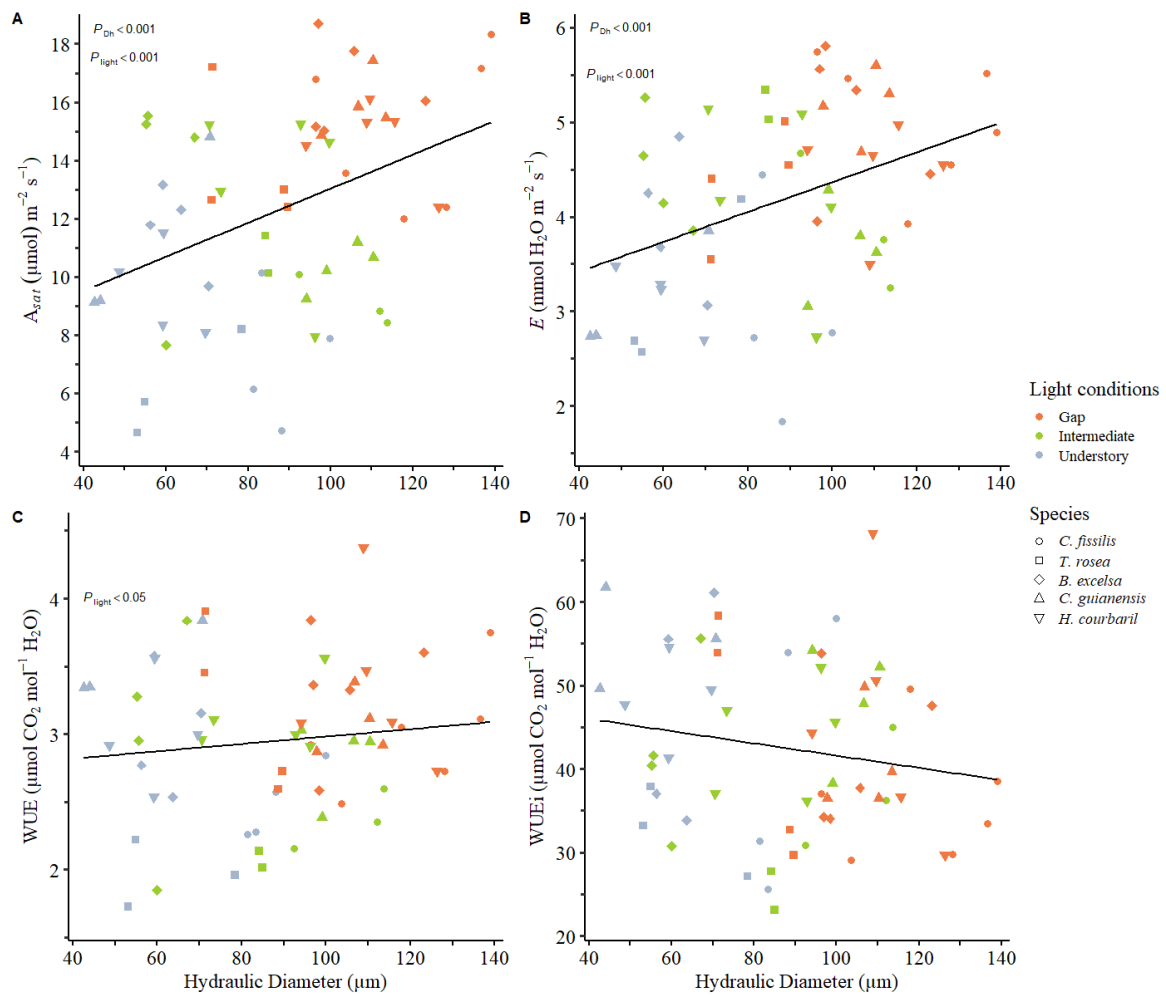
Species		Light Conditions	Fiber wall	Vessel wall	Fiber lumen	Vessel lumen	Paratracheal parenchyma	Rays	Line/bands parenchyma
			%						
Pioneer	<i>C. fissilis</i>	Gap	45.00 ± (6.70)	4.88 ± (1.31)	10.51 ± (2.64)	14.00 ± (4.24)	3.37 ± (1.62)	12.25 ± (2.47)	9.68 ± (6.59)
		Intermediate	36.94 ± (16.37)	5.23 ± (1.56)	9.66 ± (6.85)	15.17 ± (9.09)	6.53 ± (4.82)	10.45 ± (2.03)	16.29 ± (5.06)
		Understory	15.68 ± (10.87)	3.33 ± (0.46)	1.56 ± (1.07)	5.69 ± (2.82)	3.08 ± (0.68)	15.97 ± (5.84)	54.68 ± (9.09)
	<i>T. rosea</i>	Gap	30.42 ± (4.13)	8.78 ± (2.18)	3.51 ± (0.91)	17.51 ± (3.08)	5.76 ± (1.84)	13.26 ± (3.99)	20.76 ± (3.42)
		Intermediate	30.32 ± (3.96)	7.15 ± (0.68)	8.07 ± (2.31)	18.80 ± (0.65)	9.58 ± (3.88)	7.54 ± (6.10)	22.56 ± (2.47)
		Understory	27.08 ± (9.62)	4.67 ± (3.04)	4.92 ± (2.38)	7.98 ± (6.06)	4.11 ± (0.80)	13.82 ± (2.28)	37.43 ± (12.85)
Non-Pioneer	<i>B. excelsa</i>	Gap	44.35 ± (6.97)	3.93 ± (2.10)	9.02 ± (5.56)	9.65 ± (2.10)	3.95 ± (1.44)	21.27 ± (3.95)	7.88 ± (5.39)
		Intermediate	49.81 ± (6.18)	5.23 ± (1.56)	8.13 ± (1.76)	6.85 ± (1.42)	3.46 ± (1.42)	18.99 ± (2.55)	7.55 ± (4.05)
		Understory	38.07 ± (5.69)	3.07 ± (0.46)	4.14 ± (3.13)	4.52 ± (1.82)	2.98 ± (0.51)	18.06 ± (2.55)	28.59 ± (5.20)
	<i>C. guianensis</i>	Gap	33.00 ± (6.60)	6.72 ± (2.24)	3.70 ± (2.16)	17.42 ± (3.70)	5.88 ± (1.23)	19.24 ± (4.17)	14.05 ± (6.63)
		Intermediate	36.94 ± (16.37)	6.20 ± (0.20)	2.87 ± (1.48)	16.40 ± (3.42)	3.58 ± (0.76)	18.72 ± (3.55)	25.99 ± (13.44)
		Understory	15.68 ± (10.84)	4.24 ± (0.86)	3.51 ± (1.15)	7.01 ± (1.95)	5.05 ± (0.93)	25.65 ± (6.50)	30.55 ± (9.66)
	<i>H. courbaril</i>	Gap	40.39	4.55	6.57	12.75	2.84	11.36	21.89

			$\pm (0.97)$	$\pm (1.73)$	$\pm (1.63)$	$\pm (1.77)$	$\pm (0.99)$	$\pm (14.11)$	$\pm (4.84)$
		Intermediate	37.63 $\pm (4.37)$	3.83 $\pm (1.31)$	6.87 $\pm (1.97)$	7.43 $\pm (2.42)$	-2.22 $\pm (0.54)$	14.11 $\pm (3.06)$	27.90 $\pm (6.41)$
		Understory	27.08 $\pm (9.62)$	3.35 $\pm (0.80)$	4.89 $\pm (1.95)$	4.77 $\pm (1.24)$	1.93 $\pm (0.49)$	12.57 $\pm (3.93)$	32.69 $\pm (14.66)$

S1 Radar plot of measured variables, including the proportion of fiber lumen, fiber wall, vessel lumen, vessel wall, paratracheal axial parenchyma, rays, and axial parenchyma in lines or bands. Each species is represented by a different color (*Cedrela fissilis*: gray, *Tabebuia rosea*: salmon, *Bertholletia excelsa*: pink, *Carapa guianensis*: blue, *Hymenaea courbaril*: green), with each line tracing the relative percentage of each cellular component per species.



S2 – Analysis of transpiration, and water use efficiency using Analysis of Covariance (ANCOVA) and Photosynthesis (Asat) using Linear Mixed Model (LMM). ANCOVA models included hydraulic diameter (Dh) and light conditions (gap, intermediate, and understory) as fixed factors for transpiration (E), water use efficiency (WUE), and intrinsic water use efficiency (WUEi). The LMM for Photosynthesis (Asat) included species as a random factor (random intercept), and Dh and light conditions as fixed factors. (A) Photosynthesis (Asat). (B) Transpiration (E). (C) Water Use Efficiency (WUE). (D) Intrinsic Water Use Efficiency (WUEi). Colors represent light conditions: orange (gap), green (intermediate), blue (understory). Symbols represent species: diamond (*C. fissilis* - *Cedrela fissilis*), circle (*T. rosea* - *Tabebuia rosea*), upward triangle (*B. excelsa* - *Bertholletia excelsa*), downward triangle (*C. guianensis* - *Carapa guianensis*), left-pointing triangle (*H. courbaril* - *Hymenaea courbaril*).



CONSIDERAÇÕES FINAIS

Este estudo fornece novas contribuições sobre a resistência do xilema ao embolismo em indivíduos juvenis de cinco espécies arbóreas pertencentes a dois grupos ecológicos distintos em uma floresta secundária. Diferente da maioria dos estudos, que se concentram em florestas maduras, esta pesquisa destaca as estratégias hidráulicas de árvores jovens (cinco anos de idade) crescendo sob diferentes condições de luz. Os resultados demonstram claramente que a resistência do xilema ao embolismo (P_{50}) varia significativamente em função da interação entre espécies e condições de luz. As espécies não-pioneiras apresentaram valores mais negativos de P_{50} e demonstraram maior capacidade de ajuste desse parâmetro à disponibilidade de condições de luz. Durante o período mais seco, o maior déficit de pressão de vapor (VPD) foi um fator determinante para as diferenças fisiológicas observadas, refletidas em variações significativas no potencial hídrico foliar e na margem de segurança hidráulica entre as estações seca e chuvosa. A densidade da madeira também apresentou variações substanciais, com uma forte interação entre condições de luz e espécies, reforçando que as estratégias hidráulicas diferem entre as espécies e o gradiente de condições de luz. Nesse contexto, as espécies não-pioneiras exibiram maior plasticidade em resposta ao gradiente de condições de luz em comparação com as pioneiras.

O segundo capítulo abordou os traços anatômicos dessas espécies e sua correlação com os parâmetros de troca gasosa, incluindo fotossíntese, condutância estomática e eficiência no uso da água. Foram encontradas interações significativas entre o diâmetro hidráulico e a densidade de vasos entre as espécies e as condições de luz, evidenciando que o diâmetro hidráulico não aumenta linearmente com a disponibilidade de condições de luz para todas as espécies. A composição do tecido

do xilema (proporção de fibras, vasos e parênquima) variou consideravelmente entre as condições de luz e as espécies. Todas as espécies acumularam mais parênquima na condição de sub-bosque, enquanto uma maior proporção de fibras foi observada na condição de clareira. A baixa plasticidade anatômica de *Tabebuia rosea* está diretamente relacionada à sua menor resistência ao embolismo, conforme observado no primeiro capítulo. Entre os traços fisiológicos, apenas a condutância estomática foi explicada pela interação entre o diâmetro hidráulico e as condições de luz. O principal achado deste capítulo é que o diâmetro hidráulico, isoladamente, não determina a resistência do xilema ao embolismo. A composição celular do xilema deve ser considerada para compreender as estratégias das espécies na condução de água.

Ao integrar perspectivas fisiológicas e anatômicas, este estudo confirma a influência das diferenças interespecíficas e das condições de luz na determinação da função do xilema. Os resultados evidenciam que árvores jovens em florestas secundárias adotam diferentes estratégias hidráulicas, com espécies não-pioneiras demonstrando maior plasticidade em resposta à disponibilidade de condições de luz. Esses achados têm implicações diretas para o manejo de florestas secundárias, destacando a importância de considerar a diferença entre os grupos sucessionais ao selecionar espécies para áreas similares. A escolha de espécies com maior plasticidade hidráulica pode ser determinante para o sucesso da regeneração e resiliência dessas áreas, auxiliando na restauração ecológica e na manutenção da funcionalidade do ecossistema.

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