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Jehová Lourenço Junior

Ecologia funcional de comunidades arbóreas ambientalmente diversas : acessando drivers fitofisionômicos, fingerprints de mudanças climáticas e nicho ecológico

Functional Ecology of environmentally diverse tropical tree communities: Drivers of forest physiognomy, Trait-based driver theory, and ecological niche

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Area of concentration: Plant Physiology.

Advisor: Prof.ª. Dr.ª Camilla Rozindo Dias MIlanez

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Trait-based Ecology of environmentally diverse tropical plant communities: Drivers of forest physiognomy, Trait-based driver theory, and wood anatomy

JEHOVÁ LOURENÇO JUNIOR

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ATA DE DEFESA DE TESE DO CURSO DE DOUTORADO EM BIOLOGIA VEGETAL DO PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL DO CENTRO DE CIÊNCIAS HUMANAS E NATURAIS DA UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO - ATA Nº 016/2018 - 24/08/2018

Aos vinte e quatro dias do mês de agosto de dois mil e dezoito, em sessão pública, realizada na **na sala de web conferência do NTI, Campus Goiabeiras,** na Universidade Federal do Espírito Santo, procedeu-se à avaliação da tese do aluno **Jehová Lourenço Junior**. Às treze horas, a Profª. Drª. Camila Rozindo Dias Milanez, UFES (Orientadora e Presidente da Comissão) Examinadora de Defesa de Tese, deu início aos trabalhos, convidando a tomarem assento à mesa os demais integrantes da Comissão, os professores Dr. José Aires Ventura e Dr. Geraldo Rogério Faustini Cuzzuol - examinadores internos, e os professores: Prof. Dr. Brian Joseph Enquis- USA (Co-orientador estrangeiro), Dr. Scott Saleska, University of Arizona - USA e Dra. Erica Newman, University of Arizona - USA - examinadores externos. A seguir, a presidente solicitou ao doutorando que fizesse uma explanação de seu trabalho, intitulado **"Ecologia funcional de comunidades arbóreas ambientalmente diversas: Acessando drivers fitofisionômicos, fingerprints de mudança climática e nicho ecológico".** Finda a apresentação, a presidente passou a palavra aos examinadores, que procederam a arguição do candidato. Ao final, a Comissão em sessão reservada deliberou pela **APROVAÇÃO** da referida tese nos termos do Regimento Interno do Programa de Pós-Graduação em Biologia Vegetal e a presidente da sessão alertou que o aprovado somente terá direito ao título de Doutor após entrega da versão final de sua tese, em papel e meio digital, à Secretaria do Programa. Encerrada a sessão, eu, Profª. Drª. Camila Rozindo Dias Milanez, presidente da Comissão Examinadora, lavrei a presente ata que vai assinada por mim, pelos demais componentes da Comissão e pelo doutorando.

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DEDICATION

I dedicate this thesis in memory of my father-in-law, Orlandino Eduardo Pin, whom passed away in the first week of my sandwich Ph.D. at the University of Arizona. You will be in our hearts forever!

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> *Equipped with his five senses, man explores the universe around him and calls the adventure Science*.
>
> *Edwin Powell Hubble*

ABSTRACT

Recognized by its high environmental diversity, the Paulo Cesar Vinha State Park (PEPCV) is a model system to the study of environmental gradients effect over plant communities, providing the assess to timely ecological questions related to the coexistence theories, the impact of climate change in forests, and to assess several hypotheses related to plant hydraulics. The use of novel ecological theories recently created (Trait-based driver theory and the multidimensional hypervolume) may provide a better understanding about the impact of climate change over the biodiversity and the assembly mechanisms structuring the biodiversity in forests. We setup 42 plots (5 to 25 meters) in a patch of *restinga* from PEPCV to describe the taxonomic composition of the study area. From January to May 2016 we collected leaves and branches to perform the trait-based ecology survey. We collected several soil samples of each plot to quantify several physicochemical parameters and the soil humidity. Moreover, the water table depth was measured. All the environmental parameters were used to understand the *restinga* forest functioning. We collected: specific leaf area, leaf dry matter content, stomatal density, leaf thickness, plant height and diameter at the breast height, xylem density, mean xylem area, potential hydraulic conductivity (Ks), bending resistance (bend), vessel grouping index, solitary vessel fraction, and proportions of fibers, parenchyma and conductive area. The dataset was analyzed through novel ecological tools and theory – hypervolume analysis and the trait-based driver theory. The outcomes show that the water table depth (WT) is the main driver of *restinga* species distribution, and soil coarseness and WT are the main drivers of *restinga* forest physiognomy. Aluminum had a negative effect on forest biomass. We suggest that salinity exerts an important role in the *restinga* forest functioning. The moments of trait distribution show a transition in the functional composition the plant communities, ranging from setup of traits of conservative strategies (drier site) to acquisitive traits setup (floodable communities). The forests wood anatomy composition variation along the water availability gradient is consistent to the safety-efficiency tradeoff proposed by the coercion-tension theory. We suggested a new model explain the Ks variance, which is based on Bittencourt et al. (2016) model. We conclude that *restinga* is a model system to assess the effect of environmental gradients and the impact of climate change on the forest functioning. *Restinga* of PEPCV is an unique natural laboratory to assess timely ecological questions.

Key-words: Climate change. Plant community ecology. Trait-based driver theory. Wood anatomy. Trait-based ecology.

RESUMO

Reconhecido pela sua ampla diversidade ambiental e fitofisionômica, sugere-se que o parque estadual Paulo Cesar Vinha (PEPCV) seja um ambiente modelo para se avaliar o impacto das mudanças climáticas sobre a biodiversidade, bem como testar as teorias da coexistência (ex. teoria de nichos e teoria neutra). O uso de ferramentas recentemente criadas na ciência ecológica, como a teoria de drivers de características e o hipervolume multidimensional, podem contribuir para a melhor compreensão do impacto das mudanças climáticas sobre a biodiversidade e as forças estruturantes da biodiversidade. Foram motadas 42 parcelas para se realizar o levantamento florístico e fitossociológico de um trecho da *restinga* do parque PEPCV para a decrição da composição taxonômica da área de estudo. Durante os mêses de Janeiro a maio de 2016 foram feitas coletas de ramos e folhas para os estudos de ecologia funcional. Foram coletadas amostras de solo para a quantificação de diversos parâmetros físico-químicos e quantificação da umidade. Além disso, o lençol freático foi medido. Todos esses parâmetros ambientais foram ultilizados para se compreender o funcionamento das florestas estudadas. Os seguintes atributos funcionais foram coletados: área foliar específica, conteúdo de matéria seca da folha, densidade estomática, espessura da folha, altura e diâmetro a altura do peito, densidade do xilema, área média do xylema, conductividade hidráulica potencial (Ks), resistência ao envergamento (*bend*), indice de agrupamento de vasos, fração de vasos solitários, proporção de parênquima, fibras e de área condutiva. O conjunto de dados foi analisado a partir de ferramentas e teorias recentes na ciência ecológica – análises hipervolumes e a teoria de *drivers* de características funcionais. Os resultados revelaram que a profundidade do lençol freático é o fator determinante da distribuição das espécies de *restinga* e que os principais *drivers* da fitofisionomia das florestas de *restinga* são a granulometria e o lençol freático. O alumínio tem impacto negativo sobre a biomassa das comunidades estudadas. Sugerimos que a salinidade exerça um papel importante no funcionamento do bioma *restinga*. Os resultados dos momentos estatísticos mostram uma transição na composição funcional das comunidades de plantas estudadas, variando de estratégias conservativas (regiões mais secas) a estratégias aquisitivas (regiões mais úmidas). A análise da composição de caracteres anatômicos da madeira ao longo do gradiente de umidade seguiu com muita precisão o tradeoff entre segurança e eficiência no transporte de água proposto pela teoria da coesão-tensão. Sugerimos um novo modelo teórico para a explicação da variação do Ks, modificando o modelo de configurações de espaços de uso da madeira proposto por Bittencourt et al (2016). Concluímos que as comunidades de *restinga* são ambientes modelos para o estudo de gradientes ambientais e do efeito das mudanças climáticas sobre o funcionamento das florestas. Trata-se de um laboratório natural único para se acessar questões oportunas na ciência ecologia.

Palavras-chave: Mudanças climáticas. Ecologia de comunidades. Trait-based driver theory. Anatomia da madeira. Ecologia funcional.

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INTRODUCTION

The term *restinga* has been differently defined in the scientific literature, where we can find the geological description of the physic environment (SUGUIO; MARTIN, 1990) and the ecological definition adopted in this study, which considers *restinga* as the "*coastal plant communities marginally localized in the Atlantic forest domain"* (ARAUJO, 2000; SCARANO, 2009), exhibiting the highest taxonomic similarity to the core Atlantic forest ecosystem in comparison to other habitats (NEVES et al., 2017).

The *restinga* forests are largely distributed along the Brazilian East coast. Its geological origin is recent (Quaternary) and is remarkable for the strong marine transgression and accumulation of crystalline sediments, which created a conspicuous soil mosaicism along a slightly waved relief (SUGUIO; MARTIN, 1990). Such a high soil heterogeneity underlies a high diversity of plant communities closely distributed, showing several types physiognomies ranging from herbaceous plants to forest trees over than 20 meters height, which create an interesting complex of mosaic vegetation (ULE, 1901).

Such an intriguing diversity of plant communities is related to a high species substitution associated to steep environmental gradients, as firstly observed by Ule (1901) in the coast of Rio de Janeiro State. *Restinga* is a unique system for studies related to the effect of environmental gradients in plant communities, which potentially can provide important insights for new hypothesis and persistent ecological questions, as those related to the coexistence theories, ecosystem functioning, mechanisms of community assembly, diversity maintenance, and the impact of the climate change in forests.

Thereby, this study has the main goal of address questions related to fundamental theories in ecology, bringing new insights about ecological processes occurring at fine spatial by the assess of timely ecological theories and approaches, as the trait-based driver theory (ENQUIST et al., 2015) and n-dimensional hypervolumes (BLONDER et al., 2014a).

This doctorate project is structured into four main axes: 1) Forest inventory to identify the plant species and assess the taxonomic communities composition along the soil gradients; 2) Soil analysis to determine the physicochemical soil parameters related to nutrients and water availability; 3) Trait-based ecology survey to describe the trait composition of the communities along the soil gradients and the traits distributions at community and species level; and 4) wood anatomy survey to assess the wood tissue variation along soil water availability gradient.

Several questions are addressed by each chapter:

Questions – Chapter 1

1) What the main environmental driver of species distribution shifts?
2) Does plant communities' trait composition changes along the environmental gradient?
3) Do species phenotypes shift in trait space to match the environmental conditions?
4) how wide-spread species shift along the multidimensional trait space?
5) What environmental drivers and ecological processes can be unveiled by the shape of the traits distribution? Are there evidences of climate change drivers in *restinga* plant communities?

Questions – Chapter 2

1) What the main environmental drivers of functional composition in *restinga* forests?
2) How the most important environmental drivers influence *restinga* forest physiognomy and functional composition?

Questions – Chapter 3

1) What hydraulic traits correlates with the variance in Ks?
2) How the water availability gradient affects the wood anatomy traits and the setups of wood space-use?
3) How the communities wood trait composition is affected by the ecological processes?

MAIN OBJECTIVE

Use the environmentally diverse *restinga* plant communities of the PEPCV to test several ecological hypotheses made by the trait-based driver theory, the coexistence theories and contribute to the current discussion about the safety-efficiency hydraulic tradeoff as an important mechanism in plant ecology.

SPECIFIC OBJECTIVES

- Determine the environmental drivers of species distribution and trait composition;
- Assess the shifting of trait composition along the water availability gradient;
- Assess the ecological processes structuring the *restinga* plant communities;
- Look for fingerprints of climate change or environmental gradient effect on trait distribution;
- Test the environmental-matching hypothesis by the intraspecific trait variability of *restinga* plant species;
- Test the Bittencourt *et al.* (2016) model based on hydraulic safety-efficiency tradeoff.

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CHAPTER 1 – Assessing the trait-based driver theory in environmentally diverse tropical plant communities

Running head: Shaping the trait distribution of tropical plant species and plant communities

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Keywords: Trait driver theory, trait distribution, plant assembly, species richness, water availability gradient, plant assembly, intraspecific variation, climate change.

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ABSTRACT

1. Despite of the many studies using trait-based approach to assess the impact of environmental gradients in forest trait composition, the relative role of (i) intraspecific variation in community assembly; (ii) microclimatic or fine scale abiotic variation in shaping local trait diversity remain poorly understood. To advance understanding we tested several assumptions and predictions of Trait Driver Theory (TDT). We quantified the shape of trait distributions related to tree carbon, nutrient economics and stem hydraulics across a small-scale but steep gradient of soil water availability.

2. We utilized a unique and steep environmental gradient in the Brazilian *restinga* Atlantic forest communities that spans a very short spatial distance (207 ±60 meters). We collected leaf and wood samples of tree species across 42 patches of *restinga* forest. Furthermore, to detect if species directionally shift in niche space, we analyzed species composition in multidimensional hypervolume space.

3. Despite short geographic distances, we observe large shifts in species replacement and intraspecific variation reflected by a directional shift in plant function. Consistent with TDT, we observe (i) decreasing mean and variance of several leaf carbon and nutrient economic traits as well as stem hydraulic traits; (ii) peaked trait distributions indicating strong functional convergence; and (iii) trait distributions that are also skewed in directions that are consistent with a shifting forest responding to recent hotter and drier conditions.

4. Synthesis. Observed species replacements along the water table gradient and interspecific measures of functional diversity (community kurtosis and skewness) are consistent with strong phenotype/environmental matching of plant carbon, nutrient, and hydraulic strategies. We observe environmental filtering in both extremities of the gradient, selecting for acquisitive (wet) to conservative (dry) setup of traits. Similarly, species that span the entire water availability gradient are characterized by directional intraspecific shifts in multi-trait space that mirror interspecific shifts. Strong environmental gradients across short spatial scales provide unique systems to accurately assess assembly processes and address long-held assumptions and timely hypothesis predicted by ecological theory.

INTRODUCTION

A central challenge in ecology is understanding and predicting the response of communities to changing climates, past, present, and future. The use of natural gradients to understand how biological diversity responses to climate has a long history (CLEMENTS; HANSON; WEAVER, 1929; GAUSE, 1932; HUTCHINSON, 1957). Observed ecological shifts along environmental gradients (such as temperature, water availability, etc.) can reflect evolutionary adaptation, plastic shifts in phenotypes, and responses to multiple stressors over a range of conditions (POLECHOVÁ; BARTON, 2015; RIESCH; PLATH; BIERBACH, 2018). Nonetheless, disentangling and deducing the mechanisms that drive shifts in biological diversity remains a fundamental problem (HUBBELL, 2001; MCGILL, 2003; ADLER; HILLERISLAMBERS; LEVINE, 2007).

In this paper, we assess two central concepts in trait-based ecology. First, we utilize recent theoretical advances to assess the importance of phenotype-environment matching and ecological filtering for optimal phenotypes on driving community composition (WESTOBY; WRIGHT, 2006). Second, we assess if the “fingerprints” of climatic changes associated with increased temperatures are revealed in community trait distributions. To make progress on this front, we focus on traits that are proposed to be linked to differences in ecological and evolutionary strategies. Specifically, the leaf-height-seed (LHS) strategy scheme has been proposed to capture the strategy of seed plant species through three trait-related independent dimensions of ecological variation (WESTOBY, 1998). Variation in vegetative growth, competitive ability and regenerative processes, can be assessed by specific leaf area (SLA; light-capturing area deployed per dry mass allocated), plant canopy height at maturity, and seed mass.

Trait-based ecology has mainly focused on trait-climate linkages by assessing how mean environmental conditions within communities (KRAFT et al., 2015) influence the mean community phenotype. This is considered the “mean-field” approach applied at a local scale. Trait-based ecology has largely treated observed community trait variation as reflecting internal niche partitioning driven by species interactions (KEDDY, 1992; WEIHER; KEDDY, 1995; WRIGHT; REICH;

WESTOBY, 2001). However, variation in environmental filtering has also been shown to operate within communities (ADLER et al., 2013). Increasingly, several studies have emphasized that a mean-field approach neglects the importance of intraspecific variation (ITV) within the community (VIOLLE et al., 2012), setting a limit on the generality of resulting ecological inferences. The importance of ITV in ecological studies is undeniable, due to its contribution to functional diversity maintenance (SIEFERT et al., 2015; ROSS et al., 2017) and community assembly processes (CORNWELL; ACKERLY, 2009; JUNG et al., 2010). Nonetheless, assessing the responses of both intra- and interspecific variation could help identify the strength and generality of core assumptions and predictions of trait-based theory for how communities assemble across gradients, and how global warming affects diversity and ecosystem functioning as well.

Recently, Enquist et al. (ENQUIST et al., 2015, 2017), building on the work of Norberg and colleagues (NORBERG et al., 2001), introduced Trait Driver Theory to link how variation in inter-and intraspecific variability in ecological communities can be linked to the performance of individuals across environmental gradients. Trait Driver Theory (or TDT) goes beyond the mean field limits (VIOLLE et al., 2012; ENQUIST et al., 2015) and proposes the investigations of the functional traits distribution by its decomposition into the first four central statistical moments - *mean, variance, skewness, and kurtosis* - to assess the drivers and dynamics of trait distribution in space and time.

Assessing several prominent assumptions and predictions of Trait Driver Theory (TDT)

According to TDT assumptions, the *mean* value allows us to understand how functional traits shift as the environment alters the optimal trait values. The *variance* provides information about ecological processes affecting trait space wideness. Decreasing variance can be caused by environmental filtering and/or competitive exclusion, whereas competitive niche displacement, immigration and/or temporal variation in the optimum trait value cause the increase of *variance*. *Kurtosis* measures the peakiness of the trait distribution, providing information about community-level ecological processes. TDT predicts that a more peaked and positive distribution reflects competitive exclusion or habitat filtering. Values close to -1.2 are related to a uniform trait distribution and even niche partitioning, while more negative values reflect the coexistence of contrasting ecological strategies.

Trait Driver Theory also makes a series of predictions concerning the dynamical response of communities to environmental change as viewed through the shape of trait distributions. Specifically, in a shifting community, TDT predicts an increase of *skewness* and/or the importance of rare species or rare traits advantage in local coexistence. For example, if phenotype/environment matching is strong, then climate change (e.g., via increases in temperature and/or drought) will cause community trait distributions to track the environment due to decreasing performance or fitness of the existing dominant phenotypes and increasing performance or fitness of some currently rare phenotypes (ENQUIST et al., 2015, 2017). As this shift occurs, an increase in the skewness is expected, reflecting a lag in time as individuals with newly maladaptive traits slowly decline in frequency. Thus, environmental change (e.g., climate warming/drought) will cause a trait distribution to shift upward or downward along the gradient, and the associated lagging tail will exhibit negative, or positive skewness relative to the initial distribution, respectively.

By analyzing the trait distribution, one can recast ecological theories that make differing hypotheses about what influences the shape of trait distributions and the ecosystem functioning. These hypotheses include phenotype–environment matching; abiotic filtering; strength of local biotic forces via trait variance and kurtosis; the competitive-ability hierarchy hypothesis; and others (ENQUIST et al., 2015). In a complementary test for the phenotype-environment matching hypothesis, we also assessed species location in a multidimensional trait space by hypervolume analysis (BLONDER et al., 2014a), which is a feasible way to collapse multi-trait datasets into a simpler framework, allowing us to detect possible shifting of species ecotypes in trait space.

We assessed TDT by quantifying species and community level trait distributions across a steep environmental gradient that spans a short geographic distance. This unique system allows us to assess several core assumptions and predictions of TDT. We tested species location and phenotypes shifting in multidimensional hypervolume space along the environmental gradient, to address the following questions: 1) What the main environmental driver of species distribution shifts? 2) Does plant communities' trait composition changes along the environmental gradient? 3) Do species phenotypes shift in trait space to match the environmental conditions? 4) how do widespread species shift along multidimensional trait space? 5) What environmental drivers and ecological processes can be unveiled by the shape of the trait distributions? 6) Is there evidence of climate change drivers in *restinga* plant communities?

MATERIAL AND METHODS

Study Area

Located between the coordinates 20°33'-20°38'S and 40°23'-40°26'W, Paulo Cesar Vinha State Park (PEPCV) in Espirito Santo State (Figs 1A and B) holds a prominent position between others *restinga* forests, for its high environmental heterogeneity, where 11 plant communities spread out side-by-side along narrow environmental gradients (PEREIRA, 1990). PEPCV is characterized by a conspicuous soils mosaicism and a relief slightly waved, which created conditions for the occurrence of lower sites with organic and shallow soils (floodable and permanently flooded areas) and upper sites (non-floodable or drier areas) where well-drained sandy soils predominate.

All this singular environmental variability makes *restinga* a model system to address timely questions and hypotheses from Ecology, as those related to community assembly processes (KEDDY, 1992; STUBBS; WILSON, 2004; CORNWELL; SCHWILK; ACKERLY, 2006) and the importance of intraspecific variation in a community level context (JUNG et al., 2010; BOLNICK et al., 2011; VIOLLE et al., 2012; VOLF et al., 2016), besides of providing an unique opportunity to test the coexistence theories (HUTCHINSON, 1957; MACARTHUR; LEVINS, 1967; HUBBELL, 2001) and the effect of the climate change in a wide diversity of environmentally contrasting plant communities, just observed at larger spatial scales (e.g. regional scale).

The study area (Fig. 1C) comprises a short flooding gradient transition, where we setup 42 plots of 5 x 25 meters across three types of forests: floodable, intermediate, and drier forests, where all trees with diameter at the breast high (dbh) ≥5cm were tagged and had height and dbh measured. As the *restinga* plant communities are closely distributed, the fine-scale distribution of the plots allowed us to precisely track the effect of the strong and continuous soil gradients (207.4 ± 60.7 meters) in forest taxonomy and trait composition. For simplicity, each plot was defined as a plant community. The maps were drawn by using the maptools (BIVAND; LEWIN-KOH, 2017) and raster package (HIJMANS, 2017) in the R Statistical Environment.

Soil analysis and variables selection

We collected five soil samples per community at 15 cm deep, which were homogenized in the field to produce one compound soil sample for the quantification of nutritional and physicochemical soil composition from the 42 communities. Several parameters were determined by the analysis, including coarseness (proportion of fine and coarse sand, silt, and clay); nutrients (P, K, Na, Ca, Mg, Al, H-Al [potential acidity], Zn, Mn, Cu and B); Organic Matter (OM); pH; Sodium Saturation Index (SSI), Cation exchange capacity (CEC) and base saturation (BS), following the Brazilian Agricultural Research Corporation protocol (DONAGEMA et al., 2011).

In addition, we calculated the soil water retention capacity or field capacity (FC) for each plant community. First, we collected two soil samples (15 cm deep) per community using flexible pipes to remove the soil layer in a way to preserve the integrity of its structure. Then, we sliced the pipes and left one pipe tip opened to introduce the water and installed a fine net in the other tip to retain the soil and allow the water flow during the lab experiment, which consisted in carefully introducing water within the pipe until the soil exceed its maximum water retention capacity. When the water stopped to leak, the samples were weighed in a balance, dried in an oven at 60 ºC, and weighted until reach out a constant weight. The FC was determined following equation: FC = (Wet soil – Dry soil)/Wet soil, according to Donagema et al. (2011).

The water table depth (WT) was directly measured in the floodable areas by digging shallow holes in the soil. Then, we measured the slope variance toward the intermediate and dry plots, taking the WT of the nearby floodable area as reference. This way, we estimated the WT of the intermediate and the dry plots according to the variance detected along the soil slope.

The fitting of the most important environmental variables was performed via generalized additive models (GAM) and generalized linear models (GLM) following the script provided by Neves et al. (2017), which comprises in 1) the exclusion of species found at a single site; 2) the Hellinger transformation of the binary presence/absence data (LEGENDRE; GALLAGHER, 2001); 3) a forward selection method of environmental variable for redundancy analysis (RDA); and 4) additional and progressive elimination of collinear variables based on their variance inflation factor (VIF) and ecological relevance, until maintaining only those with VIF<4 (QUINN; KEOUGH; PETRAITIS, 2002).

Plant species composition matrices were reduced to two dimensions using non-metric multidimensional scaling (NMDS). Ordinations were based on species relative abundance, following the single site species exclusion and Hellinger transformation, as previously described. Communities were compared by the Simpson similarity index, thus, the shifting across the NMDS axes is given to the dissimilarity caused by the species replacement. The analysis reached out 14.67% of stress. We analyzed the shifting in taxonomy and trait community composition across the WT gradient.

The GAM, GLM, NMDS, and ordination analysis were conducted in the R statistical environment, using the packages vegan (OKSANEN et al., 2018), and recluster (DAPPORTO et al., 2015).

Traits measurements

The sampling of the leaf and wood traits was performed in over than 80% of most abundant species, according to the species abundance table calculated from the forest inventory we performed within the study area (S.M. Table S1). To maintain the consistency of sampling effort, we kept the similar number of 5 individuals sampled per species, and per environment. Thus, if the species occurs in one, two or three sites, we collected 5, 10 or 15 individuals, to detect the fine-scale environmental influence on species functional traits composition.

We selected plant height (H), specific leaf area (SLA), leaf dry matter content (LDMC) and wood density (WD) because have been highlighted their link to major ecological strategy axes (WESTOBY, 1998; WRIGHT et al., 2004; CHAVE et al., 2009). Moreover, we also measured leaf thickness (LT) and stomatal density (SD), given their commonly related link with physiological responses to environmental stresses, as water shortage (EL-SHARKAWY; COCK; PILAR, 1985; LAMBERS; CHAPIN; PONS, 2008). All procedure followed the new handbook protocol for standardized trait measurements (PÉREZ-HARGUINDEGUY et al., 2013).

The leaf plant traits were based on three leaves per individual, and the same twigs were used to collect sample for WD measurements. Both leaf and wood samples were previously hydrated and kept in the fridge overnight for 12 hours. We measured leaf area, wet weight, and dry weight to calculate SLA and LDMC. The wood volume was calculated according to the water displacement method (CHAVE, 2005), with the support of a high precision balance. After that, the twigs were dried in an oven at 60 ºC and weighted to determine WD by wood dry mass per wood volume (g/cm^3).

The stomatal measurements were performed by the leaf imprinting method (WILSON; PUSEY; OTTO, 1981), which consist in pushing the leaf surface against a drop of cyanoacrylate adhesive, such a way to leave a thin layer where the leaf surface stay printed. In such printed layer is possible to account the number and measure the stomatal size or other structures on leaf surface. In this study, we analyzed both lower and upper leaves surfaces, but the accountings just were performed in the lower surface, as all species exhibited abaxial pattern of stomata location. We used a microscope with a camera coupled and took photos in 10 times of magnification, which were analyzed in the software TSView, version 6.1.3.2 (Tucsen Imaging Technology Co. Ltd., Fuzhou, Fujian, China).

Trait prediction procedure

As we measured the height of all individuals reported in the forest inventory, we used that information for the trait prediction procedure. Thus, for each species, we used the traits values collected in the same site of occurrence of the individuals under trait prediction (floodable, intermediate, and dry site). We took the advantage of the known correlations between the reference trait (e.g. height) and the predicted trait (e.g. SLA). The boundaries of trait variation under prediction were limited to the

95% of confidence interval informed by the linear regression. We compared the predicted trait mean-values with the traditional community-weighted mean trait values (Fig. 2), which is calculated by the species traits mean weighted by their abundances in each community. More detailed information and the R script used in trait predictions is provided in the supplemental material (S.M.), which is available in the online version of this article.

Principal components analysis (PCA) and species hypervolume centroid

The trait dataset (SLA, LDMC, WD, SD, and H values) was collapsed into the PCA space, following the score extraction of the PCA1 and PCA2, which were used to make the hypervolume analysis (BLONDER et al., 2014b). This procedure is suggested to multidimensional dataset to avoid issues related to the low number of observation per species, and to optimize the time spent int the hypervolume calculations, as well (BLONDER, 2016). We made the hypervolume analysis for each ecotype, that is, for each species in each site (floodable, intermediate, and dry), and then we extracted the hypervolume centroid, to find the species centroid position into the hypervolume space.

Moreover, the PCA was executed by centering and scaling the score values, as the traits entering the hypervolume analysis must be comparable and uncorrelated (BLONDER et al., 2014b). The hypervolume analysis were performed in the hypervolume package (BLONDER et al., 2014a).

RESULTS

We sampled 863 individuals from 95 species and 73 genera, distributed within 45 families. Myrtaceae (17), Fabaceae (7), Rubiaceae (5) and Sapotaceae (4) were the best represented families. A large fraction of the trees encountered were identified to genus and species level (96%), and a lower proportion were just identified at the family level (4%; see the species list provided in table SM: Appendix S2). Despite the close physical distance between the forest types (207 ±60 meters), we just found six species occurring in all three forest types (*Emmotum nitens*, *Protium icicariba*, *Pseudobombax grandiflora*, *Tapirira guianensis, Aspidosperma pyricollum*, and *Cynophalla flexuosa*).

The forward-selection procedure retained just four environmental variables (WT, B, Mg, coarse sand), explaining 18.05% of the variation in tree species composition, that is, 82% remained unexplained (Table 1). The shifting across the NMDS axes (Figs 1D and E) is given to the dissimilarity caused by the species replacement. Communities were mainly separated in NMDS space by water-related soil parameters (WT and coarse sand).

Table 1. Environmental variables selected after forward selection analysis.

Variable	adj. R^2 cum.	ΔAIC	F	VIF
Water table depth (wt)	0.10290	-12.393	5.70	1.63
Magnesium (Mg)	0.14111	-13.284	2.78	2.35
Boron (B)	0.16312	-13.466	2.02	2.13
Coarse sand	0.18057	-13.471	1.81	1.83

Goodness-of-fit of the predictor variables was assessed through adjusted coefficients of determination, Akaike information criterion (AIC), F-values and significance tests ($p < 0.01$ in all cases). VIF, variance inflation factor, obtained using the r-squared value of the regression of one variable against all other explanatory variables. adj. R^2 cum. = cumulative adjusted coefficient of correlation.

The floodable site has a lower species richness than dry site (S.M.: table S1) and there is a clear species replacement into the first three meters of WT variation (Fig. 1B), that is, communities in the extremities of the gradient are more distinct (Fig. 1A) and flooding exerts a stronger environmental filtering on communities' taxonomic diversity than do drier conditions. For simplicity, we choose the water table depth (WT) as the main environmental variable because it was the most explicative variable of species distribution and performed the best fitting regression models with all response variables evaluated in this study.

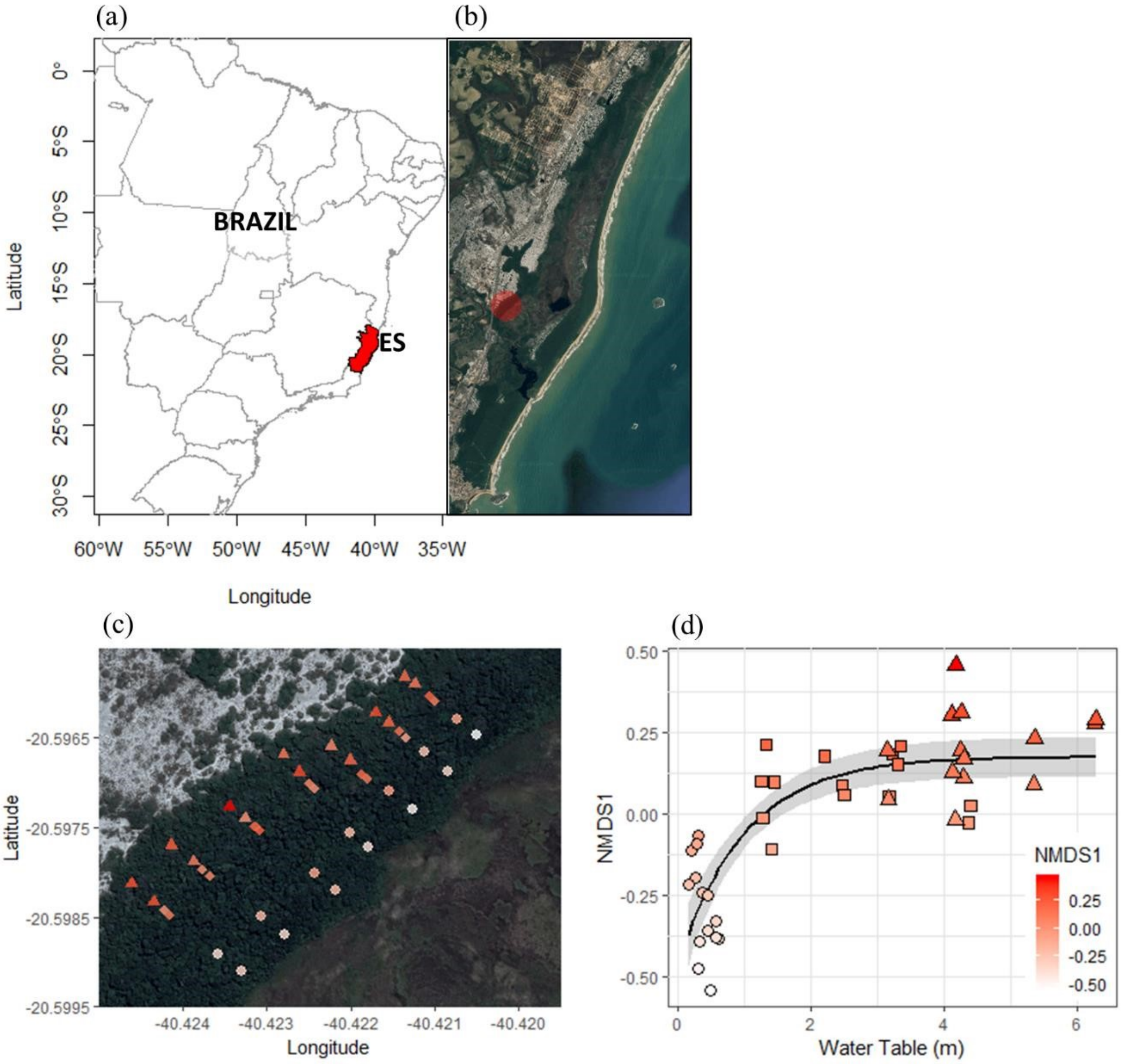
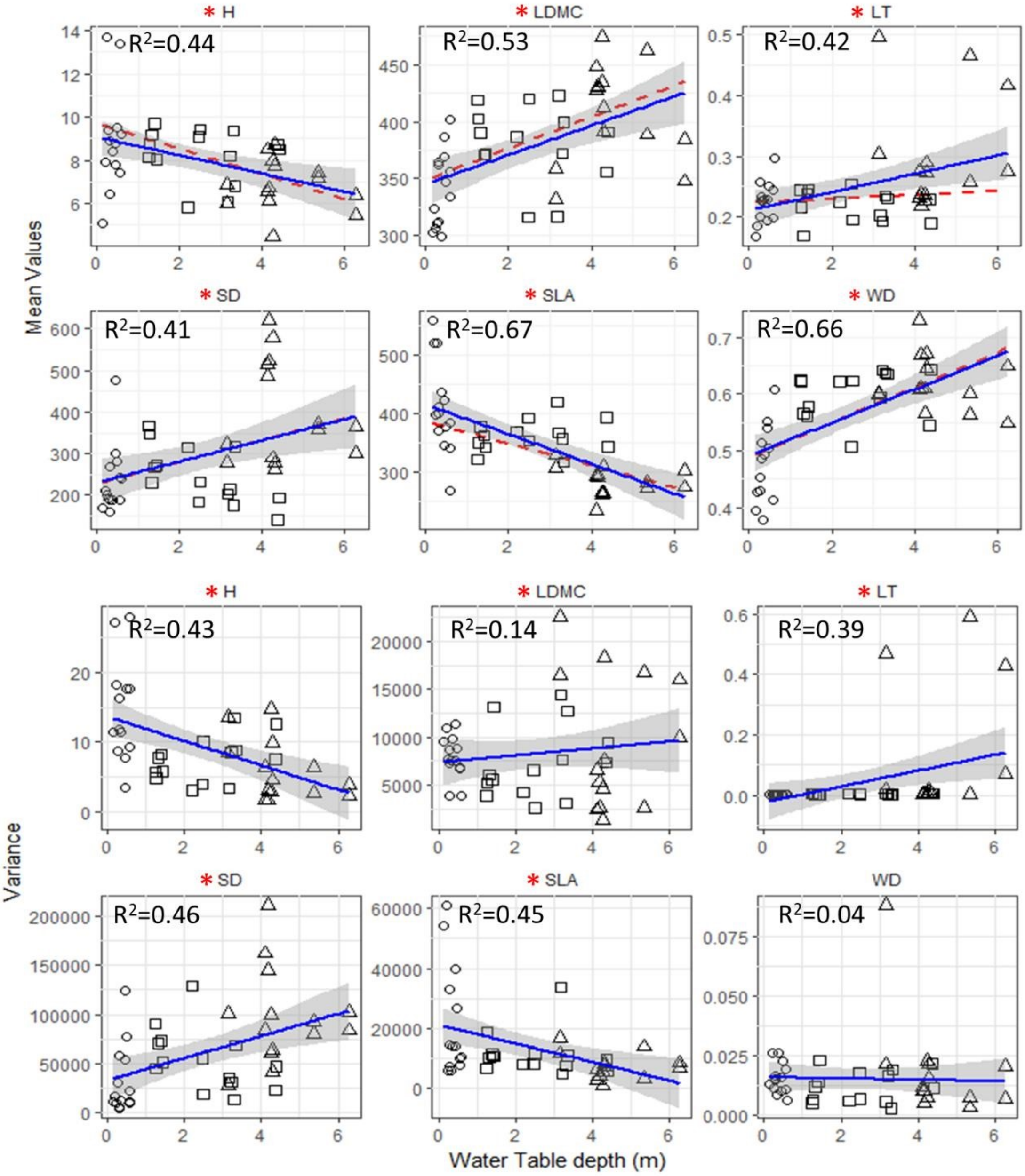


Figure 1. (a) Study site consisting of a steep hydrological gradient located in Espirito Santo State (ES) - Brazil. **(b)** Paulo Cesar Vinha State Park and study area location (red dot). (c) Detailed map of the study area, showing the 42 plots/communities that spans a gradient ~ 207 ± 60 meters from floodable forest (○), intermediate (□) and dry (Δ) forest communities. The variation in symbol color measures the magnitude of community shift in species composition along the NMDS axis (first component eigenvalues) due to the dissimilarity caused by the species replacement, calculated by Simpson index. **(d)** Communities taxonomic composition shifts across the hydrological gradient from the floodable to dry sites, unveiling a steep species replacement along the water table gradient, associated with the shifting of trait composition.

All mean trait values shifted from floodable to dry communities, exhibiting a trend of increase in LDMC, SD, WD and LT, and decrease in H and SLA (Fig. 2A). Except for LT, the community-weighted means (CWM) and mean predicted values fitted closely. The drier conditions caused the variance reduction of H and SLA, and the increasing of LDMC and SD variance (Fig. 2B). Few traits showed trend of changing skewness along the gradient (Fig. 3). Exceptions for LT, which tended to be more positively skewed; and the communities WD and LDMC values distribution had negative skewness. Both positive (SLA and H) and negative (LDMC and WD) skewness are consistent with the a hypothesis of directional shiftings of traits distribution toward drier environmental conditions owned to climate change (ENQUIST et al., 2017). Moreover, the kurtosis value for all traits were positive (Fig. 3), revealing that, in general, the distribution of trait values of all individuals within communities are clustered into a tiny fraction of available trait space. The more dry



communities tend to be more clustered with more positive kurtosis values for LT and WD values than those from floodable communities.

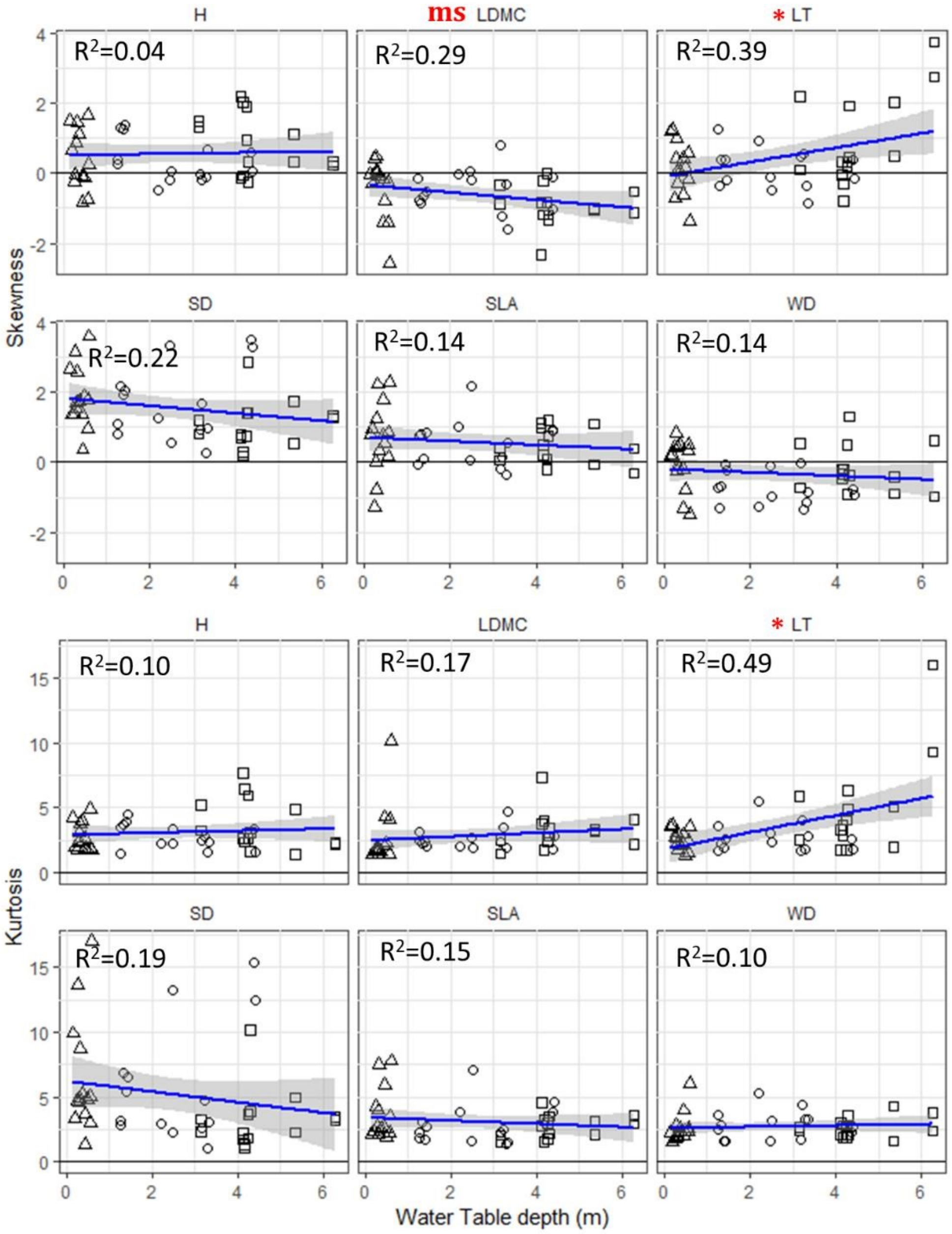


Figure 3. Tests of two assumptions of Trait Driver Theory. Skewness, and kurtosis of height, leaf dry matter content (LDMC), leaf thickness (LT), stomatal density (SD), specific leaf area (SLA), and wood density (WD) of 42 plant communities from floodable (○), intermediate (□), and dry (Δ) sites along the water table gradient. Statistically significant (*), p-value<0.05, or marginally significant (ms) [p-value=0.6]. If phenotype/environment matching is strong then environmental change (e.g., climate warming/drought) will cause a trait distribution to shift upward (or downward) across the gradient (Figure 2), then the mean trait value of the community will also shifts upward (or downward) and the associated lagging tail will exhibit negative (or positive) skewness relative to the initial distribution.

The first and second principal components (Fig. 4A) explained 68.5% of trait variation. This variation is mainly due to variation in LDMC (loadings -57.3) and WD (loadings -47.6%) exhibiting the most negative values, and SLA (loadings 55.0) expressing the most positive values. Height (95.2%) was the most positive and determinant loading trait along the PCA2, and stomatal density or SD expressing a negative loading (-27.3%). The traits values distribution of every single individual tree within the PCA space (Fig. 4A) highlights that individuals from the more floodable sites (black symbols) are shifted more in the SLA region of the PCA, whereas those individuals from the drier sites (white symbols) are mainly placed in the quadrant of the trait PCA space influenced more by variation in LDMC, LT, and SD. Individuals are also segregated in in PCA space by body size (PC2). The taller

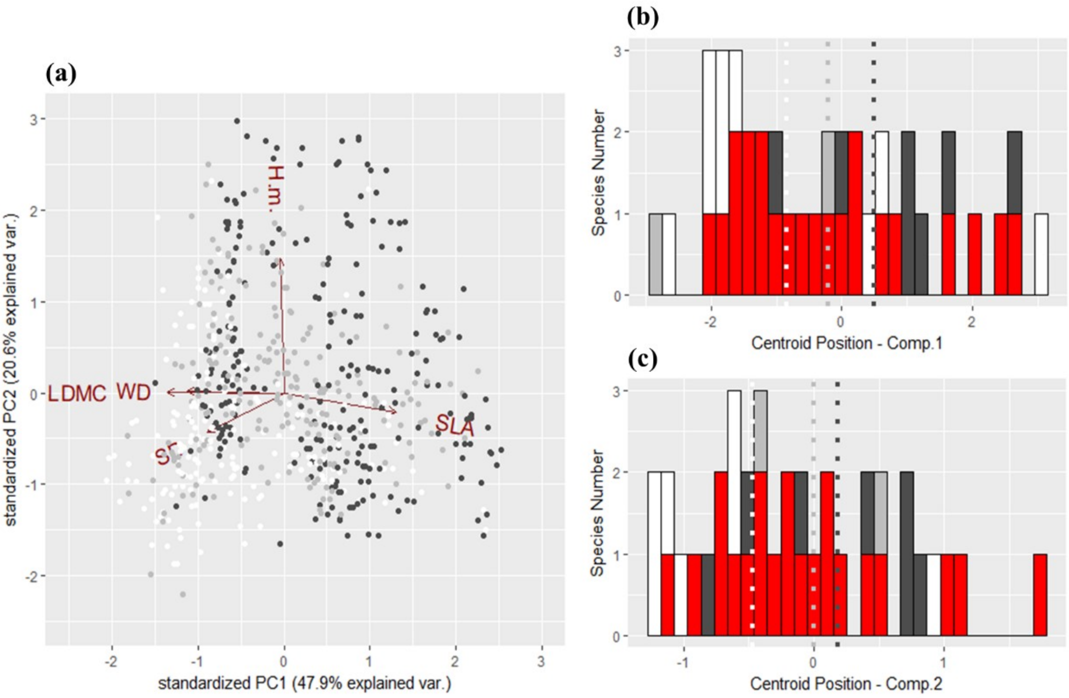
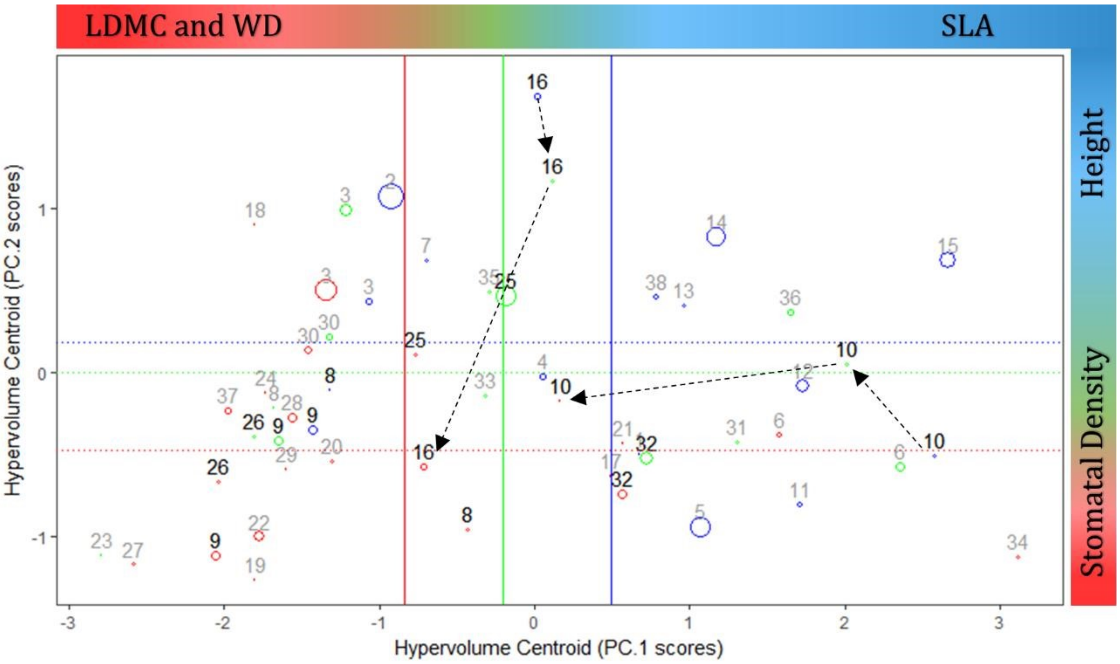


Figure 4. A - Principal components analysis (PCA) of specific leaf area (SLA); height (H); wood density (WD); leaf dry matter content (LDMC); and stomatal density (SD) from 626 individual trees within 38 species. **B** and **C** – Positions of species hypervolume centroid, showing specialist species from the floodable (dark gray), intermediate (gray) and dry (white) sites. Generalist species ecotypes (red) have a wider spatial distribution, occurring in two or three sites. Hypervolume centroids were extracted from hypervolume analysis using the PCA scores of the first and second principal components. Dotted lines indicate the mean hypervolume centroids from each site, exhibiting the directional shifting: floodable/intermediate/dry sites (dark gray/gray/white).

ones are mainly from floodable and intermediate sites, whereas smaller individuals are mostly from the dry site.

Species restricted to one or a few locations along the gradient appear to have well-defined niche space positions. For example, species restricted to either the floodable and dry sites tend to be found in the extremities of trait space, exhibiting positive and negative centroid values, respectively (Figs 4B and C). In contrast, widely-spread species (red bars) exhibit wider distribution along both centroid axis of PC1 and PC2. Such result highlights the role of intraspecific variation in the observed shift of trait composition at community level (Figs 2A and 5); (Fig. 1D) and



Box 1. Species distribution along the hypervolume space. Arrows indicate species ecotypes shifting along the two dimensions of the hypervolume, following the shift in the site mean centroid (solid and dotted lines) across floodable (blue), intermediate (green) and dry (red) sites. The first PCA component is related to LDMC, WD, and SLA, whereas the second component is related to H and SD (see Fig. 4A and S.M.: table S1). The number of individuals within the species is represented by the circle size and the species occurring in more than one site are identified by numbers in bold font. *1 – A. fraxinifolia; 2 – C. brasiliense; 3 – E. nitens; 4 – E. edulis; 5 – G. schotiana; 6 – G. opposite; 7 – I. laurina; 8 – P. heptaphyllum; 9 – P. icicariba; 10 – P. grandiflora; 11 – S. glandulatum; 12 – S. selloi; 13 – S. amara; 14 – S. globulifera; 15 – T. cassinoides; 16 – T. guianensis; 17 – C. arborescens; 18 – B. tetraphylla; 19 – C. guazumifolia; 20 – C. flexuosa; 21 – K. albopunctata; 22 – E. bahiensis; 23 – Eugenia.sp2; 24 – M. cestrifolia; 25 – M. venulosa; 26 – M. bergiana; 27 – M. floribunda; 28 – O. notata; 29 – Ocotea sp; 30 – P. coelomatica; 31 – R. grandiflora; 32 – R. reticulata; 33 – R. capixabensis; 34 – S. sycocapum; 35 – C. saligna; 36 – E. pentaphylla; 37 – Z. glabra; 38 – A. triplinervia*.

the community trait composition received a greater contribution of species ecotypes shifting in trait space. A bidimensional overview of species centroid position in hypervolume space is provided in the box 1, which shows species shifting in a multidimensional hypervolume space by the changing of specific traits or setups of traits.

DISCUSSION

The WT was revealed to be the most important driver of changing in communities' taxonomic composition of *restinga* (table 1). The sensitivity to WT was also observed in plant communities from Amazonian basin (SCHIETTI et al., 2014) and Magnago et al. (2013) previously reported the changing of *restinga* forests structure and diversity along flooding gradient. The species replacement (Fig. 1D) was followed by the shifting of the community trait composition. Toward the dry end of the gradient, communities revealed a trend of transition from acquisitive (floodable) to conservative (dry) strategies of resource use (WRIGHT et al., 2004; CHAVE et al., 2009), increasing LDMC, LT, and WD and decreasing SLA and H mean values (Fig. 2A). WD has also been related to soil water availability for its correlation to the wood hydraulic traits (HACKE et al., 2001; HACKE, 2015), as denser woods with small xylem vessels and high fiber content should be more mechanically resistant to implosion in strong negative water potentials found in drier environments (SWENSON; ZAMBRANO, 2017). Similarly, higher stomatal density (SD) seems to be related to the plant water use strategy by a finer control of the leaf transpiration (EL-SHARKAWY; COCK; PILAR, 1985). Interestingly, high SD was reported for xeric species (DUNLAP; STETTLER, 2001; PEARCE et al., 2005), and our results show the increasing of SD toward the dry communities (Fig. 2), which may contribute to the ongoing discussion about the SD role in saving or luxury water consumption by leaves (GALMÉS et al., 2007).

Several environmental drivers have been reported in the literature for constraining trait variance, as elevational gradients (SIDES et al., 2014; ASNER et al., 2017), high solar radiation, and the shortage of water and soil nutrients (CORNWELL; ACKERLY, 2009; KATABUCHI et al., 2012). Severe environmental conditions as those found in both extremities of the WT gradient produced filtering effects (KEDDY, 1992) on different traits (Fig. 2), reducing the variance in SLA and

H (drier plots) and LDMC and SD (wetter plots). Consequently, wet and dry communities have higher diversity of resource acquisition and conservative traits, respectively.

Such shift in trait composition setups with ecological strategies patterns in association to the species replacement (Fig. 2D) was earlier reported by Cornwell and Ackerly (2009) in light and water gradients, and by Katabuchi et al. (2012) in water and soil fertility gradients. The environmental constraints found into the study area seems to exert two majors effects in the *restinga* plant species distribution. First, confining the least plastic species, as the species only located in floodable or drier sites were mainly placed in the extremities of both hypervolume centroid axis (Fig. 4B). Second, widespread species are compelled to vary their setup of traits to fit the changes in the environmental conditions across the strong environmental gradient. Consequently, such species produce phenotypes which fill in a large range of the niche space (hypervolume axes, Fig. 4B, and C), shifting deeply along the multidimensional hypervolume space (Box 1).

These results highlight the importance of analyzing the intraspecific variation in a multi-trait approach, as the species seems to reach out their functional optimality by the variation of specific traits in a multivariate context (MUSCARELLA; URIARTE, 2016). In addition, the leaf and wood traits are not limited to the *economics spectrum* (LAUGHLIN, 2014) and provide a wide variety of ecological strategies by the combination of different setups of traits, which allow tree species to adjust to the environment, reflecting their spatial distribution across environmental gradients. The capacity of a species to produce multiple phenotypes is owed to the phenotypic plasticity (DEWITT; SIH; WILSON, 1998), which was also reflected in a directional shifting of the shape of species trait distribution, similar to the observed at community level (S.M. Fig. S4).

According to TDT, reflecting selection and ecological filtering for optimal phenotypes, local community trait distributions will tend toward a unimodal distribution and more “peaky” distributions, reflecting strong local sorting and convergence/dominance of optimal trait values (positive kurtosis) (ENQUIST et al., 2015, 2017). Our analyses indeed are consistent with such predictions with positive kurtosis being seen for all traits. Although these expectations partially fitted the TDT expectation of unimodality of trait distribution, as some communities presented bimodal shapes (S.M. Fig. S4), suggesting the selection of more than one strategy in the *restinga* communities. On the other hand, the directionality of the shifting in trait composition is consistent with the ecological filtering selection for optimal phenotypes (ENQUIST et al., 2017).

Similarly to Enquist et al. (2017), the shapes of community trait distribution were characterized by highly positive kurtosis (Fig. 3), indicating strong convergence of functional strategies within a given community with a high number of species or individuals share similar trait values within the communities (ENQUIST et al., 2015; GROSS et al., 2017). Such congested trait space may be the result of strong environmental filtering and/or competitive dominance of individuals with traits closer to the optimum value (ENQUIST et al., 2017). Interestingly, both LT and WD traits distribution presented a trend of becoming more peaked toward the dry end of the gradient, which ultimately highlights the importance of species finely adjust such traits in drier environments.

TDT makes two major assumptions about skewed trait distributions, assuming that asymmetries could be caused by 1) the most significative contribution of rare species or rare trait values to the community composition (ENQUIST et al., 2015); or 2) an ongoing or recent directional shifting in community trait composition due to the changing in an environmental driver (e.g. drought by climate change), resulting in either positive or negative skewed trait distribution depending on the direction that communities shift their mean traits values to track the environmental changing (ENQUIST et al., 2017). For instance, understory species as *Cyathea phalerata* (Fig. 5B) depicts the first skewness assumption, as this species contribute with extreme SLA values, which resulted in more positive skewed SLA distribution in floodable communities where this species is present (Fig. 5A).

Our results are consistent with another set of dynamical predictions for how communities respond to climate change. According to TDT if recent directional warming and/or increase in droughts is influencing the species composition of these forests then we would expect to see directional shift in species composition reflected in the trait distributions. In a shifting climate, community trait distributions will shift reflecting a shift in the optimal trait value, but the mean will lag behind the optimal phenotype. In the case where the mean community trait increases as distance to the water table also increase, we would expect that with warming due to climate change, trait distributions will be characterized by *negative* skewness as the community shifts to the new optimal trait value (See scheme in S.M. Fig. S5). In contrast, if the mean community trait is observed to decrease across the water table gradient then warming would lead to communities characterized by *positive* skewness. Thus, the skewness of the trait distribution can reflect shifting optima and past change. In sum, the higher trait moments of communities are expected to deviate in a direction that is dictated by the underlying relationships with access to water.

These forests appear to have experienced unprecedented drought and increased temperatures. Given the close matching between forest functional traits and the hydrological gradient, we would predict that recent directional shifts in climate (associated with water supply and drought) would directionally shift the functional composition of these forests. Indeed, just previous to the field sampling in 2016, this region experienced unprecedented drought and above average temperatures during 2014 and 2015. These were the driest years recorded in the past 50 years (Nobre et al. 2016).

Further, there has also been a long-term decadal increase/decrease in temperature and drought. ClimateWizard (GIRVETZ et al., 2009) shows that this area has experienced high rates of change in mean annual temperature over the last 50 years. Moreover, this region is considered a hotspot of climate change in South America (TORRES; MARENGO, 2014), which is expected to experience severe increases in temperature (above 4°C) and drought events in the upcoming decades (STOCKER et al., 2013; VAN OLDENBORGH et al., 2013; TORRES; MARENGO, 2014; MARENGO et al., 2017). Such observed directional shifts in climate associated with water supply would imply that these *restinga* forests may be currently responding.

Several traits do indeed exhibit patterns of relative mean and skewness that are consistent with trait distributions tracking recent climate warming (Fig. 2). This striking result is consistent with the hypothesis that recent climate change is currently causing forests to shift their entire trait distributions in the direction of shifting climates. For example, the observed skewed distributions for SLA and H (two traits whose mean community trait values are negatively associated with depth to the water table) exhibits positive skewness. In contrast, LDMC and WD (two traits that are positively associated with depth to the water table) exhibit negative skewness values. Such signatures in the higher moments of the trait distribution are consistent with the expectation that forests are currently directionally shifting in their trait composition in response to the decreasing of rainfall and the increasing of the temperature, possibly driven by the climate change. Notwithstanding that, a long-term study with periodic taxonomic census and trait collection is needed to test if such skewed patterns are consistent with the shifting of the communities' trait composition toward drier and warmer environmental conditions. Thus, all plants included in this study were tagged, with the intention of perform a long-term study to track the possible effect of climate-changing in trait community composition along the time.

Local-scale studies in highly diverse environments like *restinga* habitat are timely to accurately assess the microclimate drivers of trait diversity, whose understanding still remains superficial in Ecology (STARK et al., 2017). Implementing microscale studies to investigate the effect of environmental gradients and climate changing over diversity may provide a more feasible way to track climate changing and answer timely ecological questions regarding to diversity, besides of provide an accurate view of ecological processes and niche-based forces structuring ecological communities.

In this study, we used a strong local gradient as evidence that fine-scale processes can drive tree community functional composition and diversity. Our results provide evidence for a strong role of (i) phenotype/environment matching and (ii) functional convergence in shaping the composition of tropical forests and influencing their species distribution and trait composition. Our results also show that the directional shift in species composition is mirrored by a strong shift in ecological strategies reflected by key traits associated with carbon and water use strategies (as also similarly observed by Cornwell and Ackerly (2009) along a topographic gradient of water availability).

The moments analysis revealed the possible ecological processes occurring across the WT gradient, which promoted the shifting and filtering of the plant communities' trait composition, ranging from acquisitive (floodable) to conservative (dry) setup of traits. In addition, the skewness outcome suggests the observed increase in temperature and occurrence of drought is likely already influencing *restinga* plant communities as reflected in observed shift in trait distributions. Our results are consistent with the TDT hypothesis of an ongoing increase in temperature and the drought, matching the climate patterns observed along the last decades in the Southeast region of Brazil.

AUTHOR’S CONTRIBUTIONS

JLJ and CRD designed the project; LDT gave support to the taxonomy survey and plant species identification; JLJ analyzed the data; JLJ and BJE led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary Material

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Trait prediction

Appendix S2. PCA loadings and species shifting in two-dimensional centroid space

Appendix S3. Shapes of the traits distribution

Coding. Pred_trait.R function and text file script for the trait predictions

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SUPPLEMENTARY MATERIAL

Shaping the trait distribution of alpha and beta components of environmentally diverse tropical plant communities

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Contents

Appendix S1 – Traits prediction procedure

We tested the function we made for the trait prediction by providing six different simulated datasets containing five values of height and specific leaf area (SLA), simulating five individual trees. Each dataset variate in correlation strength (from 99% to 75%) by keeping the height constant and changing the SLA values. We iterate the prediction 1000 times using the same height values to see what SLA values could be found by the prediction.

The results of 1000 predictions (fig. 2) shows that the uncertainty of the predicted values increases as the correlation between the based (height) and the predicted trait (e.g. SLA) decreases. In higher correlation, the predicted trait values match closely the expected SLA values (190, 240, 280, 340 and 385 cm2/g). In any strength of correlation, the trait prediction is limited within the confidence interval informed by the linear regression of the prediction model (fig. 1), avoiding trait values variate indefinitely. The scheme in the figure 1 (A and B) depicts the prediction model, showing how it constraints the trait prediction within a known range of variation.

Appendix S2 – PCA loadings and species shifting in two-dimensional centroid space

We included the SLA, LDMC, SD, H and WD values of all individuals into the principal component analysis (PCA). The results (fig. 2 and Table 1) show the first PCA axis is mainly related to leaf dry matter content (LDMC), wood density (WD), and specific leaf area (SLA), whereas the second PCA axis is related to the variation in height (H) and stomatal density (SD). The first and second PCA components summed up an accumulative proportion of 68.4%. Thus, we collapsed the five traits information into the two PCA axis, following the score extraction, which were used to make the hypervolume analysis (BLONDER et al., 2014). This procedure is suggested to multidimensional dataset to avoid issues related to the low number of observations per species, and to optimize the time spent into the hypervolume calculations, as well. We made the hypervolume analysis for each ecotype, that is, for each species in a given site (floodable, intermediate, and dry), and then we extracted the hypervolume centroid, to find the species centroid position into the hypervolume space.

Wide-spread species shifted deeply in niche space (fig. 4), exhibiting high plasticity. It is interesting notice that the species adjust differently to fit the environmental conditions. Toward the dry end of the gradient, *Tapirira guianensis* shifts along the second centroid axis, decreasing the body size and increasing stomatal density. *Pseudobombax grandiflorum,* in turn, shift deeply along the first centroid axis, increasing the wood density and LDMC. It is interesting notice that *Micropholis venulosa* has an important reduction in number of individuals in the transition from intermediate to dry site, suggesting that this species has a ecological preference intermediary to wet and dry environmental condition, such as *Guapira opposita*, and *Rudgea reticulata*, while *Emmotum nitens* seems to have a preference for drier and well-drained environments.

Appendix S3 - Shapes of the traits distribution

In general, the analysis of the shape of the traits distribution reveals a directional shifting along the gradient, both at species and community level. For instance, toward the drier environment, there is a trend of increasing the LDMC values, thus, as the community level shape of LDMC values distributions shift to the right side along the water table gradient, wide-spread species, as *Protium icicariba*, follow the same trend, shifting the LDCM values distribution to adjust to the environmental conditions (fig. 4A).

The curves of traits distribution (fig. 4) depict the asymmetry discussed in the main text of the article. SLA, SD and H are positively skewed (right tail), while WD and LDMC are negatively skewed (left tail). We observe the bimodal shape of many traits distribution. The bimodality may reflect the coexistence of contrasting strategies within the plant communities, by multiple optimal trait values, or may reflect the community response to recent environmental change, which may result in the continued presence of individuals with optimal trait values for the historic environment (ENQUIST et al., 2015).

The bimodal curves of LDMC and height in floodable communities seems to depict the TDT hypothesis about the *coexistence of contrasting strategies*. The light gradient produced by the wider variation in plant height in floodable communities (fig. 2, main text of the article) seems to contribute to the coexistence of species with contrasting strategies to light requirements, resulting in the occurrence of abundant plant species in the understory (*Cyathea phalerata*) and canopy strata (*Calophyllum brasiliense*), which exerts a strong contribution for contrasting LDMC and height values within the floodable plant communities.

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Figure S1. Pred_trait function sets the upper and lower boundaries of the trait range to be predicted (a) and the prediction (b) is made within the 0.95 confidence interval provided by the linear regression (green line), for every single value. In this example, SLA was predicted by the values of height. We iterated 1000 trait predictions (c) by the pred_trait function using datasets with different correlation (75% to 99%) between the predicted trait (e.g. height) and the trait used for the predictions (e.g. SLA). The dashed lines represent the real values, which should be matched in an accurate prediction.

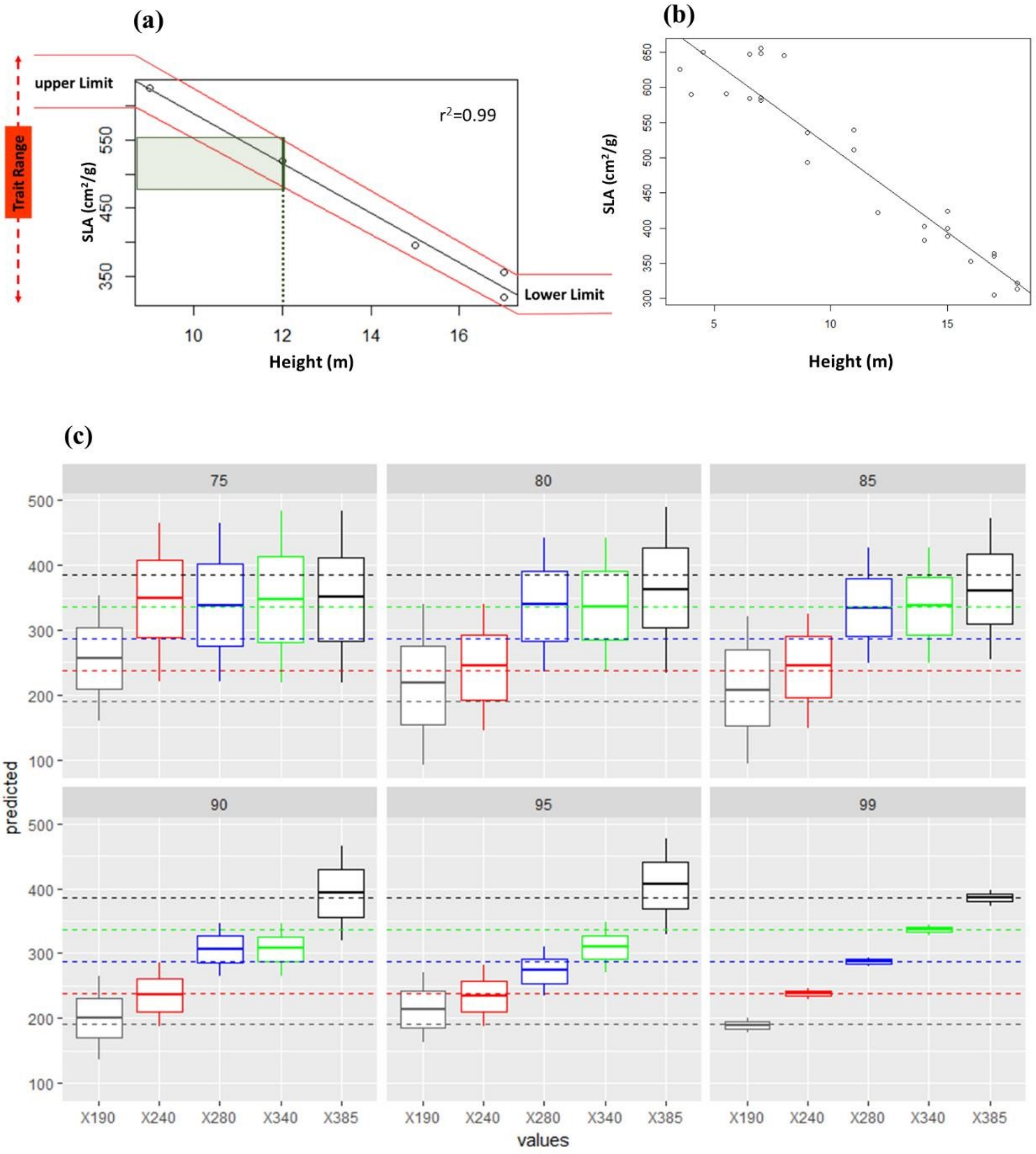

Figure S2. Principal components analysis (PCA) of specific leaf area (SLA); height (H); wood density (WD); leaf dry matter content (LDMC); and stomatal density (SD) from 626 individual trees within 38 species. The first PCA component is related to LDMC, WD and SLA, whereas the second component is related to H and SD (see table 1).

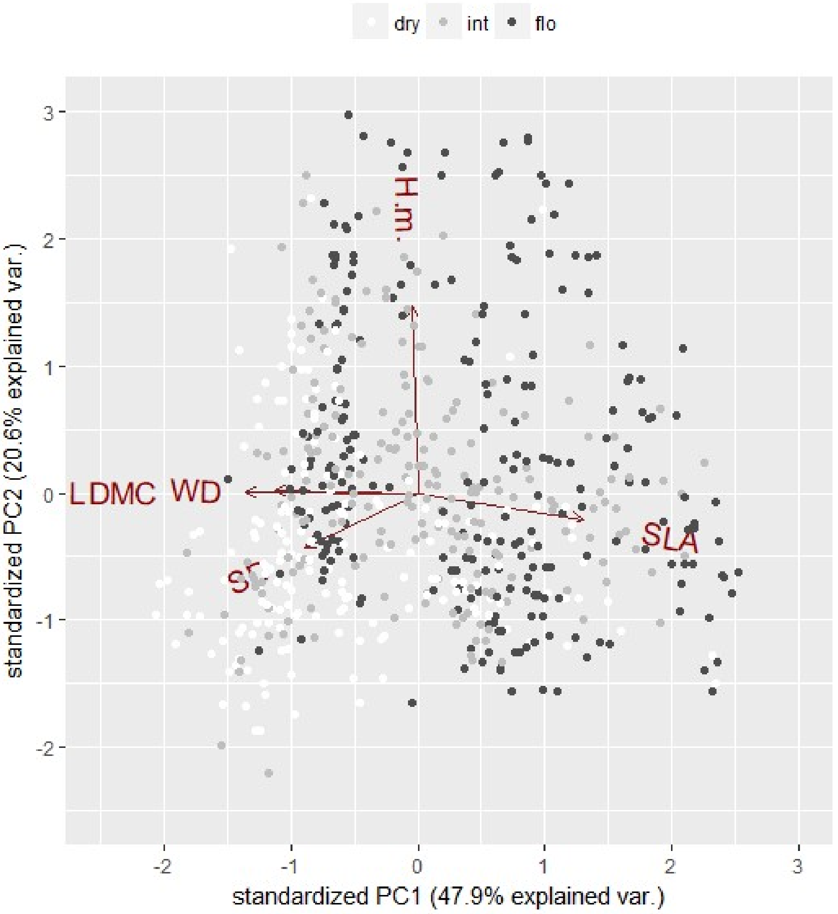

Table S1. Loadings of height (H), specific leaf area (SLA), leaf dry matter content (LDMC), stomatal density (SD) and wood density (WD) from 626 individuals across the gradient of water table depth and the importance of each principal component, showing standard deviation (StDev), Proportion of variance and cumulative proportion.

Loadings					
Traits	**Comp.1**	**Comp.2**	**Comp.3**	**Comp.4**	**Comp.5**
H.m.	–	**0.952**	-0.258	-0.163	–
SLA	**0.55**	-0.138	-0.151	-0.52	-0.62
LDMC	**-0.573**	–	–	0.31	-0.757
SD	-0.376	**-0.273**	-0.794	-0.336	0.202
WD	**-0.476**	–	0.527	-0.703	–
Importance of components					
StDev	1.547	1.014	0.909	0.678	0.540
Prop. of Variance	0.478	0.205	0.165	0.092	0.058
Cum.Proportion	0.478	0.684	0.849	0.941	1

Figure S3. Species hypervolume centroids for the scores of the PCA first and second principal components. Arrows indicate species ecotypes shifting along the two dimensions of the hypervolume, following the shifting in the site mean centroid (solid and dotted lines) across floodable (blue), intermediate (green) and dry (red) sites. The first PCA component is related to LDMC, WD and SLA, whereas the second component is related to H and SD (see fig. 2 and table 1). Species abundance is represented by the circle size and the species occurring in more than one site are identified by numbers in bold font. *1 - A. fraxinifolia; 2 - C. brasiliense; 3 - E. nitens; 4 - E. edulis; 5 - G. schotiana; 6 - G. opposita; 7 -I. laurina; 8 - P. heptaphyllum; 9 - P. icicariba; 10 - P. grandiflora; 11 - S. glandulatum; 12 - S. selloi; 13 - S. amara; 14 - S. globulifera; 15 - T. cassinoides; 16 - T. guianensis; 17 - C. arborescens; 18 - B. tetraphylla; 19 - C. guazumifolia; 20 - C. flexuosa; 21 - K. albopunctata; 22 - E. bahiensis; 23 - Eugenia.sp2; 24 - M. cestrifolia; 25 - M. venulosa; 26 - M.bergiana; 27 - M. floribunda; 28 - O. notata; 29 – Ocotea sp; 30 - P. coelomatica; 31 - R. grandiflora; 32 - R. reticulata; 33 - R. capixabensis; 34 - S. sycocapum; 35 - C. saligna; 36 - E. pentaphylla; 37 - Z. glabra; 38 - A. triplinervia*.

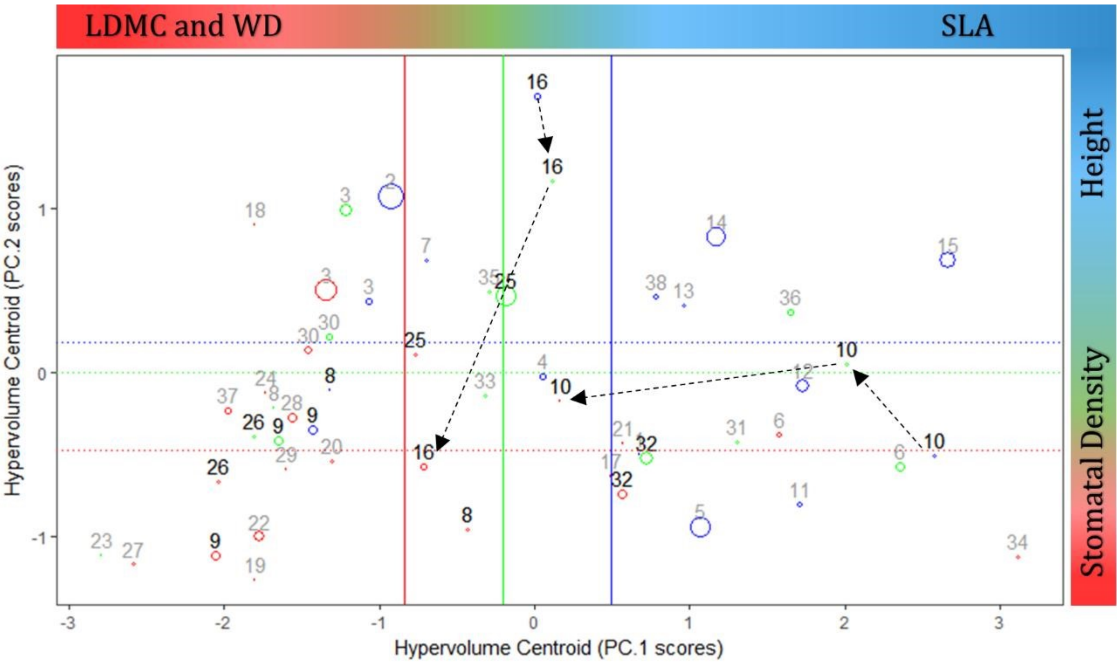

Figure S4. Shape of the traits distribution at species and community level, showing: a) specific leaf area (SLA), and leaf dry matter content (LDMC); b) height (H), and stomatal density (SD); c) wood density (WD), and leaf thickness (LT).

(a)

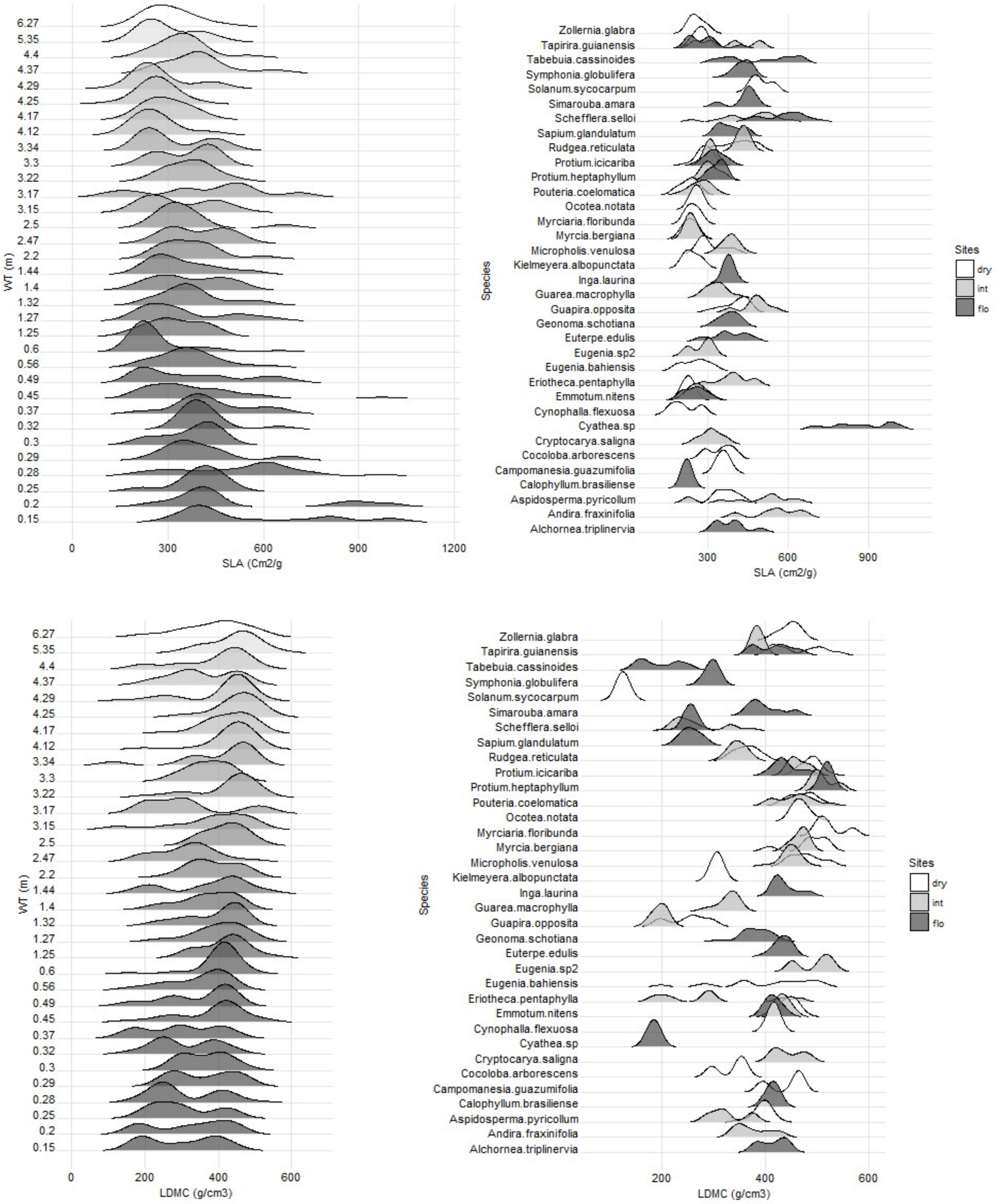

Figure S4. (Continued)

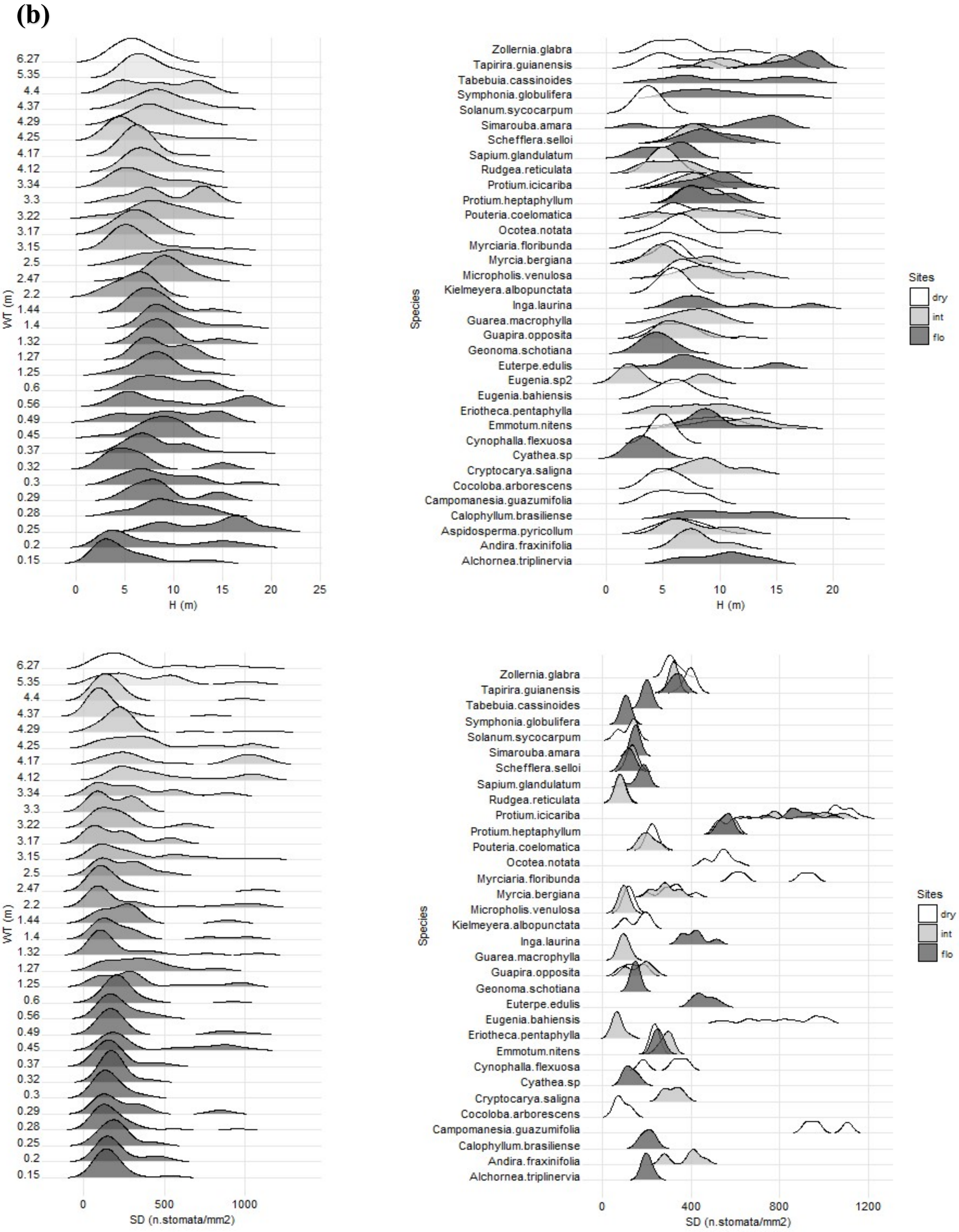

Figure S4. (Continued)

(c)

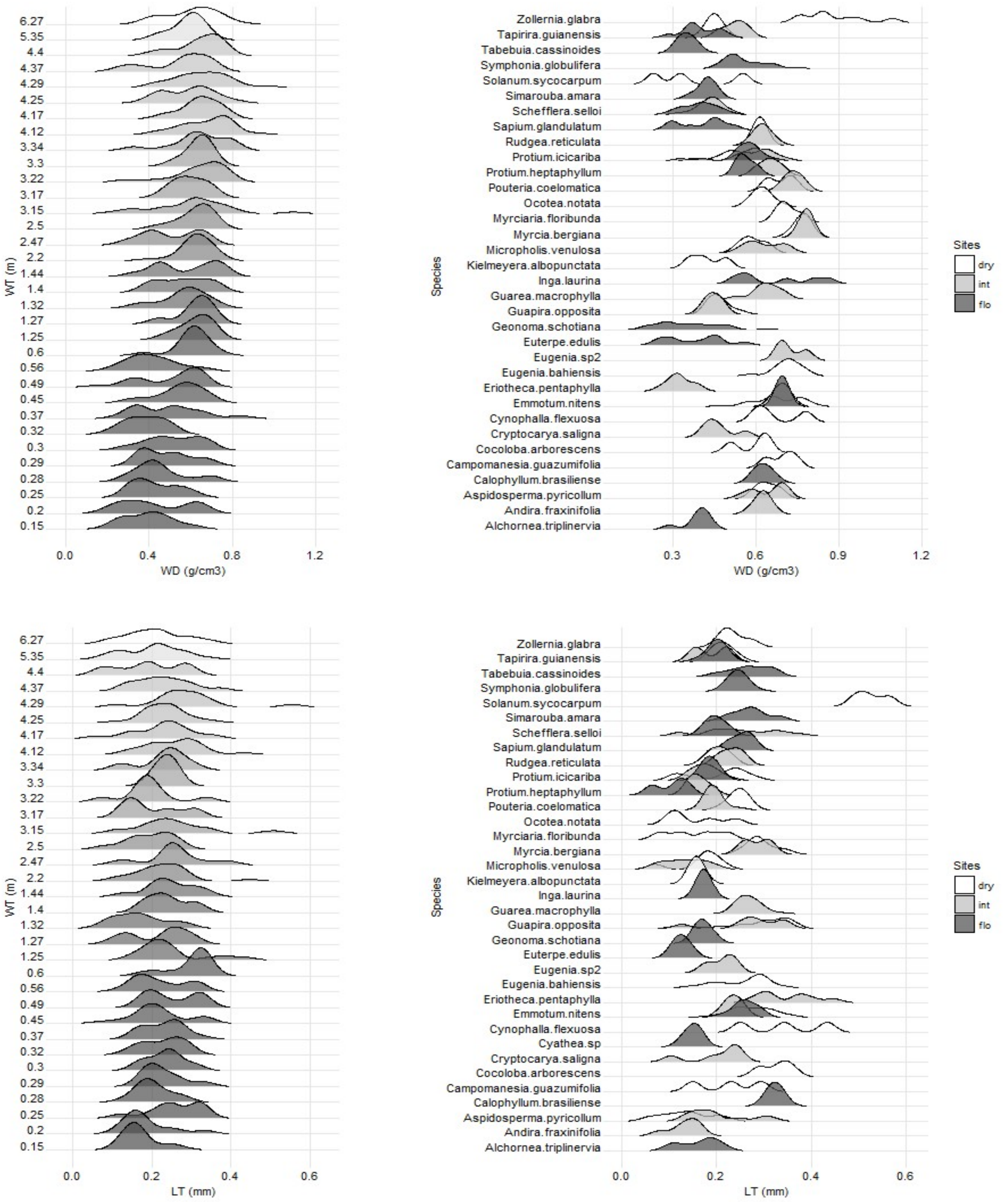

Figure S5. Scheme showing the shifting in trait distribution driven by the increasing of the drought, temperature, and the water table distance from the soil surface (WT). The deepening of the WT is owned to the increasing in the soil slope and is predicted to occur as the result of the drought, as well. In this example, TDT predicts a negatively skewed distribution of LDMC values.

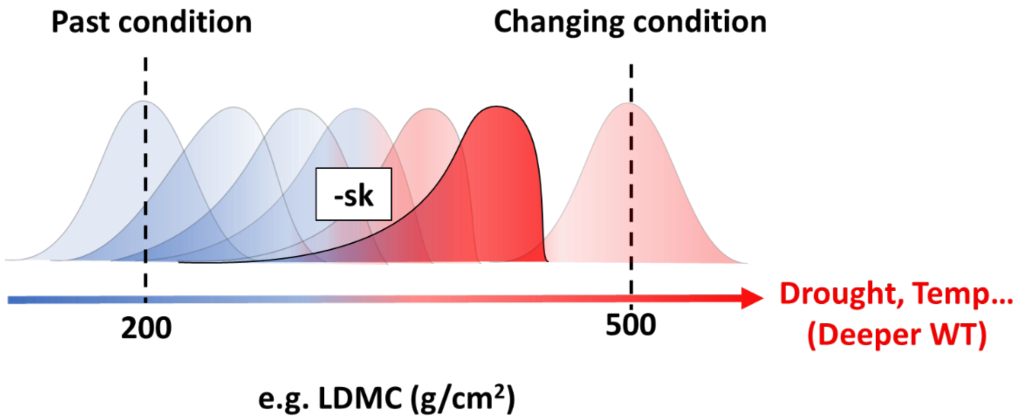

Table S2. Species list by site of occurrence (floodable, intermediate and dry). The water table depth variation is showed below to the site names, and species richness of each site and botany family is described within the parenthesis.

Taxon	**Sites**		
	Flo (36) 0.15-0.60m	**Int** (46) 1.25-3.34m	**Dry** (56) 4.12-6.28m
Anacardiaceae (1)			
Tapirira guianensis Aubl.	X	X	X
Annonaceae (3)			
Annona sp1		X	
Annona sp2			X
Xylopia sericea A.St.-Hil.	X		
Apocynaceae (3)			
Aspidosperma polyneuron Müll.Arg.			X
Aspidosperma pyricollum Müll.Arg.	X	X	X
Rauvolfia grandiflora Mart. ex A.DC.		X	X
Aquifoliaceae (1)			
Ilex sp.		X	X
Araliaceae (1)			
Schefflera selloi (Marchal) Frodin & Fiaschi	X	X	
Arecaceae (3)			
Astrocaryum aculeatissimum (Schott) Burret	X		
Euterpe edulis Mart.	X	X	
Geonoma schottiana Mart.	X		
Bignoniaceae (2)			
Jacaranda puberula Cham.		X	
Tabebuia cassinoides (Lam.) DC.	X		
Burseraceae (2)			
Protium heptaphyllum (Aubl.) Marchand		X	X
Protium icicariba (DC.) Marchand	X	X	X
Cactaceae (1)			
Brasiliopuntia brasiliensis (Willd.) A.Berger			X
Calophyllaceae (2)			
Calophyllum brasiliense Cambess.	X		
Kielmeyera albopunctata Saddi			X
Capparaceae (1)			
Cynophalla flexuosa (L.) J.Presl	X	X	X
Celastraceae (1)			
Maytenus cestrifolia Reissek			X
Chrysobalanaceae (1)			
Couepia ovatifolia Benth.		X	X
Clusiaceae (3)			
Clusia spiritu-sanctensis G.Mariz & B.Weinberg			X
Garcinia brasiliensis Mart.			X
Symphonia globulifera L.f.	X	X	
Combretaceae (2)			
Buchenavia tetraphylla (Aubl.) R.A.Howard			X

Terminalia catappa L.			X
Cyatheaceae (1)			
Cyathea phalerata Mart.	X		
Elaeocarpaceae (1)			
Sloanea guianensis (Aubl.) Benth.	X	X	
Erythroxylaceae (1)			
Erythroxylum sp.	X		X
Euphorbiaceae (2)			
Alchornea triplinervia (Spreng.) Müll. Arg.	X		
Sapium glandulatum (Vell.) Pax	X		
Fabaceae (7)			
Andira fraxinifolia Benth.	X	X	
Inga capitata Desv.			X
Inga fagifolia Willd. ex Benth.			X
Inga laurina (Sw.) Willd.	X		
Inga sp1			X
Leptolobium bijugum (Spreng.) Vogel			
Zollernia glabra (Spreng.) Yakovlev		X	X
Lamiaceae (1)			
Vitex polygama Cham.		X	X
Lauraceae (3)			
Cryptocarya saligna Mez	X	X	
Indet		X	
Ocotea notata (Nees & Mart.) Mez		X	X
Rhodostemonodaphne capixabensis J.B. Baitello & Coe-Teix.		X	
Leguminosae (1)			
Indet		X	
Malpighiaceae (2)			
Byrsonima bahiana W.R. Anderson			X
Byrsonima sericea DC.			X
Malvaceae (2)			
Eriotheca pentaphylla (Vell. & K.Schum.) A. Robyns	X	X	X
Pseudobombax grandiflorum (Cav.) A. Robyns	X	X	X
Melastomataceae (1)			
Miconia amoena Triana.	X		
Meliaceae (1)			
Guarea macrophylla Vahl		X	
Metteniusaceae (1)			
Emmotum nitens (Benth.) Miers	X	X	X
Moraceae (4)			
Ficus sp1		X	
Ficus sp2	X	X	
Ficus sp3	X	X	
Ficus sp4	X		
Myrtaceae (17)			
Blepharocalyx salicifolius (Kunth) O.Berg	X		
Campomanesia guazumifolia (Cambess.) O.Berg			X
Eugenia astringens cf Cambess.			X

Eugenia bahiensis DC.		X	X
Eugenia punicifolia (Kunth) DC.			X
Eugenia rostrata O.Berg			X
Eugenia sp1		X	X
Eugenia sp2			X
Indet			X
Marlierea obscura O.Berg		X	
Myrcia bergiana O.Berg			X
Myrcia neesiana DC.			X
Myrcia racemosa (O.Berg) Kiaersk.	X	X	
Myrciaria floribunda (H.West ex Willd.) O.Berg			X
Neomitranthes obtusa Sobral & Zambom			X
Plinia rivularis (Cambess.) Rotman			X
Nyctaginaceae (1)			
Guapira opposita (Vell.) Reitz		X	X
Olacaceae (1)			
Cathedra rubricaulis Miers			X
Peraceae (1)			
Pera glabrata (Schott) Poepp. ex Baill.	X		X
Polygonaceae (1)			
Coccoloba arborescens (Vell.) R.A.Howard			X
Primulaceae (1)			
Myrsine sp.		X	X
Rubiaceae (5)			
Amaioua guianensis Aubl.			X
Indet	X		
Psychotria sp.	X		
Rudgea reticulata Benth.		X	X
Simira sp.		X	X
Rutaceae (1)			
Rauia nodosa (Engl.) Kallunki		X	X
Salicaceae (1)			
Casearia sp.			X
Sapindaceae (1)			
Cupania emarginata Cambess.		X	
Sapotaceae (4)			
Micropholis venulosa (Mart. & Eichler) Pierre		X	X
Pouteria coelomatica Rizzini		X	X
Pouteria cuspidata (A.DC.) Baehni	X	X	
Sideroxylon obtusifolium (Roem. & Schult.) T.D.Penn.		X	
Simaroubaceae (2)			
Simaba cuneata A.St.-Hil. & Tul.		X	
Simarouba amara Aubl.	X		
Solanaceae (1)			
Solanum sycocarpum Mart. & Sendtn.		X	X
Theaceae (1)			
Laplacea fruticosa (Schrad.) Kobuski	X		
Thymelaeaceae (1)			
Daphnopsis sp.			X

Urticaceae (1)	
Cecropia pachystachya Trécul	X

CHAPTER 2 – Drivers of forest physiognomy and functional composition in environmentally diverse coastal plant communities from Atlantic forest

Running head: Drivers of *restinga* forest physiognomy and functioning across a flooding gradient

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Keywords: Above-ground biomass, soil gradients, soil-plant relationship, salinity, Aluminum, water availability, nutrients, tropical plant communities.

Journal of submission: Ecology

ABSTRACT

Background: The Atlantic forest ecosystem has suffered severe deforestation along the last decades and its fast decreasing highlights the importance of studying the remnants of its several habitats, as *restinga* plant communities. The assessment of the environmental drivers of forest physiognomy and functional composition can provide a better understanding about the *restinga* forest functioning, giving support for preservation and restoration of these fragile and diverse habitats, still insufficiently understood. We analyzed several soil parameters to identify the most important environmental drivers of *restinga* forests physiognomy and functional composition.

Methods: We performed a forest inventory in 42 patches of *restinga* plant communities, where we tagged and measured height and diameter at the breast height (dbh) of all plants with dbh ≥ 5cm. We also collected wood samples and leaves from around 85% most abundant plant species to assess the AGB and the community-weighted specific leaf area (SLA), respectively. We measured the water table depth and collected soil samples to quantify 24 physicochemical soils parameters. We analyzed the dataset by generalized additive models (GAM) and piecewise structural equation models (SEM) to determine the most important environmental drivers and its effects on forest physiognomy and trait composition.

Results: Water table depth, soil coarseness, potential acidity, sodium saturation index (SSI) and Aluminum were included into the best models explaining AGB, height, basal area, and SLA distribution. The increasing of water table depth, coarse sand, and Aluminum soil concentration along the gradient negatively impacted the forest trait composition, whereas Sodium had a positive effect on AGB and plant height.

Conclusion: Soil parameters related to water availability (WT and coarse sand) play a more significant role in the forest functional composition. The results also suggest that Na is an important component for the *restinga* forests functioning. Among the 25 environmental parameters analyzed, water availability related parameters (water table depth and coarse sand), followed by potential acidity (H+Al), Sodium saturation index (SSI) and Aluminum are the most important drivers of *restinga* physiognomy and functional composition.

INTRODUCTION

The Atlantic forest has supported the development of the Brazilian society, providing food, hydric resources for electricity production and drinking water to three-fourths of the Brazilian population (JOLY et al., 2014). Notwithstanding its importance, the Atlantic forest is one of the most impacted Brazilian ecosystems, whose deforestation resulted in a reduction which ranges from 84 to 88.6 % of its original cover (RIBEIRO et al., 2009). Such a large degradation is the result of a long history of disturbance, starting from the logging of the Pau-Brazil tree (*Caesalpinia echinata*) over than 500 years ago, followed by the expansion of the land use for agriculture (TABARELLI et al., 2005), cattle ranching, and Eucalyptus plantations for paper production (TORRES; ALVES; DE OLIVEIRA, 2007).

Moreover, all the state capitals from South, Southeast and Northeast regions of Brazil are within the Atlantic forest domain (TORRES; ALVES; DE OLIVEIRA, 2007; JOLY et al., 2014). The urban expansion has caused the fast reduction of the coastal Atlantic forest habitats, as *restinga* forests (SCARANO, 2009), which have been severely impacted by the vegetation removal for housing construction, change of its original substrate, and selective removal of species of economic importance (ROCHA et al., 2007). Given the fast degradation of the coastal plant communities, the most part of this habitat is expected to be lost, being confined to even smaller remnants along the Brazilian coast (ROCHA et al., 2007; DE CARVALHO; SOMNER; ALLEN, 2015).

The *restinga* forest comprises one of the most environmentally diverse habitats of Atlantic forest (SCARANO, 2009). Such environmental diversity is attributed to the conspicuous soils mosaicism and topographic variation in consequence of the recent transgressive and regressive events from the Quaternary (SUGUIO; MARTIN, 1990). The topography affects the water flow which tends to accumulate in lower sites with organic soils and shallow water table depth (floodable or permanently flooded forests), while upper sites (non-floodable areas) exhibit well-drained sandy soils, creating continuous soil gradients of water and nutrients availability.

In Espirito Santo State, the restinga of Paulo Cesar Vinha State park (PEPCV, fig. 1B) accounts with an impressive environmental variability, where several plant communities are discretely distributed side-by-side, increasing in structural complexity from the shore to the continent (PEREIRA, 1990; ASSIS; PEREIRA; THOMAZ, 2004), ranging from shrubs to tree communities over than 18 meters height. The causes of such remarkable change in *restinga* plant communities' physiognomy has been a long-standing question and is attributed to several environmental variables, as soil nutrients (LOURENÇO JUNIOR; CUZZUOL, 2009), salinity (LOURENÇO JUNIOR et al., 2013), organic matter and Aluminum saturation (RODRIGUES et al., 2013), soil texture and water table depth (COOPER et al., 2017). However, a more powerful test to assess the most important drivers of *restinga* forests physiognomy and trait composition remains to be made.

Have been suggested the importance of water table depth and microtopography in *restinga* forest structure, for driving deep changes in physicochemical soil properties, as nutrients content, base saturation, acidity, cation exchange capacity, organic matter and Aluminum saturation (DE ALMEIDA JR et al., 2011), which supposedly constraint *restinga* plants growth (MARQUES; SILVA; LIEBSCH, 2015). Such conditions may be exerting a strong environmental filtering over the forest taxonomic composition, selecting species adapted to grow in soils poor in nutrients, and with a recognized capacity of Aluminum tolerance (BRITEZ et al., 2002a, 2002b).

Furthermore, the porous *restinga* soils result in low soil fertility and low water retention capacity (COOPER et al., 2017), which may impose important constraints to plant biomass productivity (LAMBERS; CHAPIN; PONS, 2008). In such conditions, plants exhibit more conservative strategies, as small body size and low specific leaf area (CORNWELL; ACKERLY, 2009; KATABUCHI et al., 2012), producing thick and scleromorphic leaves with an increased longevity (WRIGHT et al., 2004; LAMBERS; CHAPIN; PONS, 2008), as typically found in *restinga* plant species (MANTUANO; BARROS; SCARANO, 2006; DE AGUIAR-DIAS; YAMAMOTO; DE MORAES CASTRO, 2012; MELO JÚNIOR; TORRES BOEGER, 2015; PINEDO et al., 2016).

The increasing of soil salt concentration toward the coast and the influence of the saline spray by the proximity to the ocean (GRIFFITHS, 2006) may be an important driver of species distribution of Atlantic forest ecosystem, as well. The increasing of the salt concentration in the soil could be filtering the functional and taxonomic composition of coastal plant communities, selecting species with salt tolerant traits, as high leaf mass per area, thicker leaves, and a remarkable capacity of salt exclusion (POORTER et al., 2009). Moreover, as salinity was recently reported as the explanatory predictor of *restinga* forest location in comparison to other Atlantic forest marginal habitats (NEVES et al., 2017), we believe that salt may be playing a role in the functioning of this system.

Given the high species richness, extremely high level of endemism and the small fraction of the original forest cover, have been increasing the understanding of prioritize the preservation of Atlantic forest habitats (SCARANO, 2009; JOLY et al., 2014), which are ranked among the top five hotspots of biodiversity in the world (Myers et al. 2000). The few studies addressing *restinga* plant-soil relationship highlights the necessity a more detailed study about the drivers of the forest physiognomy and functional composition, which may allow us to assess the functioning of these highly diverse and threatened plant communities.

Thus, we assessed 25 environmental variables in 42 *restinga* plant communities to address the following questions: 1) What the main environmental drivers of functional composition in *restinga* forests? 2) How the most important environmental drivers influence *restinga* forest physiognomy and functional composition?

MATERIAL AND METHODS

Taxonomic survey and above ground biomass analysis

Localized in Guarapari municipality of Espirito Santo State – Brazil, Paulo Cezar Vinha State Park (fig. 1A, coordinates 20°33'-20°38'S and 40°23'-40°26'W) is an environmentally diverse *restinga* protected area, where Pereira (1990) reported 11 well-defined plant communities, ranging from small shrubs to tree communities. The region is characterized by a conspicuous soil mosaicism and a slightly waved relief, which create microenvironmental conditions for the occurrence of steep flooding gradients, where plant species spread out from floodable sites with organic soils, to upper sites with well-drained sandy soils.

The study area (fig. 1B) comprises a short flooding gradient transition, where we set up 42 plots (5 x 25 meters) across three types of forests: floodable, intermediate, and drier forests (fig. 1C), and all trees with diameter at the breast high (dbh) ≥5cm were tagged and had height and dbh measured. The close spatial scale of communities allowed us to precisely track the strong continuous flooding gradient (207.4 ± 60.7 meters), increasing the sampling accuracy in detecting the effect of soil gradients in wood and leaf traits forest composition.

We estimated the above ground biomass by the Chave's pantropical models using the computeAGB function provided by the BIOMASS r package (CHAVE et al., 2014), which requires height, dbh and wood density data. We collected wood samples from five individuals per species and per site (floodable, intermediate and dry) to accurately determine the wood density of each species in each specific environmental condition. Around 85% of the most abundant plant species were sampled. The samples were previously hydrated for 12 hours, and the wood volume was calculated according to the water displacement method (CHAVE, 2005), with the support of a high precision balance. Afterwards, we dried the samples in an oven at 60ºC and weighted. The WD was determined by dividing the wood dry mass by its volume (g/cm^3).

We determined the WD of the 15% less abundant plant species by using a hierarchical approach, which comprised in taking as reference the mean WD value for the genus or family related species, whose occurrence matched the same site or environmental condition. If the species had no similar genus or family in the study area, the WD was taken by the getWoodDensity function from BIOMASS package (CHAVE et al., 2014), using "SouthAmericaTrop" as the “region” argument. For simplicity, each plot was defined as a plant community. The maps were drawn by using the maptools (BIVAND; LEWIN-KOH, 2017) and raster package (HIJMANS, 2017) in the R Statistical Environment.

Soil analysis and variables selection

We collected five soil samples per community at 15 cm deep, which were homogenized in the field to produce one compound soil sample for the quantification of nutritional and physicochemical soil composition from the 42 communities. Several parameters were determined by the soil analysis, including coarseness (proportion of fine and coarse sand, silt, and clay); nutrients (P, K, Na, Ca, Mg, Al, H-Al [soluble aluminum], Zn, Mn, Cu and B); organic matter (OM); pH; Sodium saturation index (SSI), Aluminum saturation index (m), base saturation (BS), base saturation index (V), effective cation exchange capacity (CEC) and cation exchange capacity in a pH of 7 (T), following the Brazilian Agricultural Research Corporation protocol (DONAGEMA et al., 2011).

In addition, we calculated the soil water retention capacity or field capacity (FC) for each plant community. First, we collected two soil samples (15 cm deep) per community using flexible pipes to remove the soil layer in a way to preserve the integrity of its structure. Then, we sliced the pipes and left one pipe tip opened to introduce the water and installed a fine net in the other tip to retain the soil and allow the water flow during the lab experiment, which consisted in carefully introducing water within the pipe until the soil exceed its maximum water retention capacity. When the water stopped to leak, the samples were weighted in a balance, dried in an oven at 60 ºC, and daily weighted until reach out a constant weight. The FC was determined following equation: FC = (Wet soil – Dry soil)/Wet soil, according to Donagema et al. (2011).

The water table depth (WT) was directly measured in the floodable areas by digging shallow holes in the soil, when necessary. For the intermediate and dry plots, we measured the slope variance, taking the WT of the nearby floodable area as a reference. This way, we estimated the WT of the intermediate and the dry plots according to the variance detected along the soil slope.

The selection of the most important environmental variables related to AGB, SLA, height and BA distribution was performed via generalized additive models (GAM) and generalized linear models (GLM) following the script provided by Neves et al. (2017), which comprised in 1) a forward selection method of environmental variable for redundancy analysis (RDA); 2) additional and progressive elimination of collinear variables based on their variance inflation factor (VIF) and ecological relevance, until maintaining only those with VIF<4 (QUINN; KEOUGH; PETRAITIS, 2002). The GAM, GLM, NMDS, and ordination analysis were conducted in the R statistical environment, using the packages vegan (OKSANEN et al., 2018) and recluster (DAPPORTO et al., 2015).

To assess the effect of the most important environmental drivers in forest trait composition, we performed piecewise structural equation models (SEM) and independent effects analysis (GRACE et al., 2012), taking the setup of environmental variables selected in GAM analysis for each response variable (AGB, SLA, BA and height). We followed the script provided by Michaletz et al. (2018). The Piecewise SEM analysis is performed with AIC model selection (SHIPLEY, 2016), and is more adequate than traditional SEM analysis for small datasets, containing non-independent observations of multivariate non-normally distributed variables (MICHALETZ; KERKHOFF; ENQUIST, 2018), as observed in our datasets for AGB, height and BA (S.M.: fig.S4). We used piecewiseSEM (LEFCHECK, 2016) and hier.part (WALSH; NALLY, 2013) packages for R software environment (R CORE TEAM, 2018).

RESULTS

Except for clay, all physical soil properties shifted deeply along the WT. There is an increasing of coarse sand, whereas fine sand and silt proportion decrease toward the drier end of the gradient, reducing the soil water retention capacity (S.M.: fig. S2). Soils become impoverished in nutrients (Zn, Al, Na, Ca, Cu, SOM, CEC, SB, H+Al and ASI) toward drier environments, as well. Exceptions are P, Mn, and B which increased slightly. Despite the trend of decreasing in soil acidity toward the drier communities (higher pH and lower H+Al), all communities presented strongly acid soils (Ph<5).

According to the GAM and SEM analysis (table 1, fig. 1 and fig. 2), the functional composition was primary affected by soil parameters related to water availability (wt and coarse sand), whereas soil nutrients (fig. 1d), and Al, Na, and H+Al (fig.3b) had a secondary role on the response variables. Interestingly,

Table 1. Environmental variables related to above ground biomass (AGB), height, specific leaf area (SLA), and basal area.

AGB ~	**adj. R^2 cum.**	**ΔAIC**	**F**	**Pr(>F)**
Coarse sand	0.182	216.99	10.13	0.006**
Sodium Saturation Index	0.238	214.92	3.96	0.050*
<All variables>	0.509			
Height ~				
Coarse Sand	0.268	34.476	16.008	0.002**
Saturation Sodium Index	0.410	26.332	10.665	0.004**
<All variables>	0.596			
SLA ~				
Water table depth (wt)	0.640	321.84	73.999	0.002**
<All variables>	0.681			
Basal Area ~				
Coarse Sand	0.249	679.83	14.644	0.002**
H + Al	0.364	673.80	8.225	0.002**
Al3+	0.431	670.03	5.589	0.028*
<All variables>	0.672			

We assessed goodness-of-fit of the predictor variables through adjusted coefficients of determination, Akaike information criterion (AIC), F-values and significance tests ($p < 0.01$ in all cases). VIF, variance inflation factor, obtained using the r-squared value of the regression of one variable against all other explanatory variables. adj. R^2 cum. = cumulative adjusted coefficient of correlation.

elements usually toxic (Aluminum) or stressful for plants (Sodium) were selected into the models (table 1). Basal area, Height, and AGB were negatively impacted by coarse sand soil content (fig.2a, c and b). However, Sodium saturation index (SSI) had a positive effect on AGB and height (fig.2a, 2b, and 3a). Potential acidity (H+Al) had a positive effect on the basal area (BA), despite its influence on the increasing of Al soil availability, which negatively impacted BA (fig.2c and 3a).

SLA was mainly affected by WT, which comprised a large amount of explained variation in SLA - 64% of 68.1%, considering the 25 environmental variables used in the global test of the forwarded selection step in the GAM analysis (table 1). In general, the models explained a high proportion of the variation in AGB (50.9%), BA (59.6%), and plant height (67.2%).

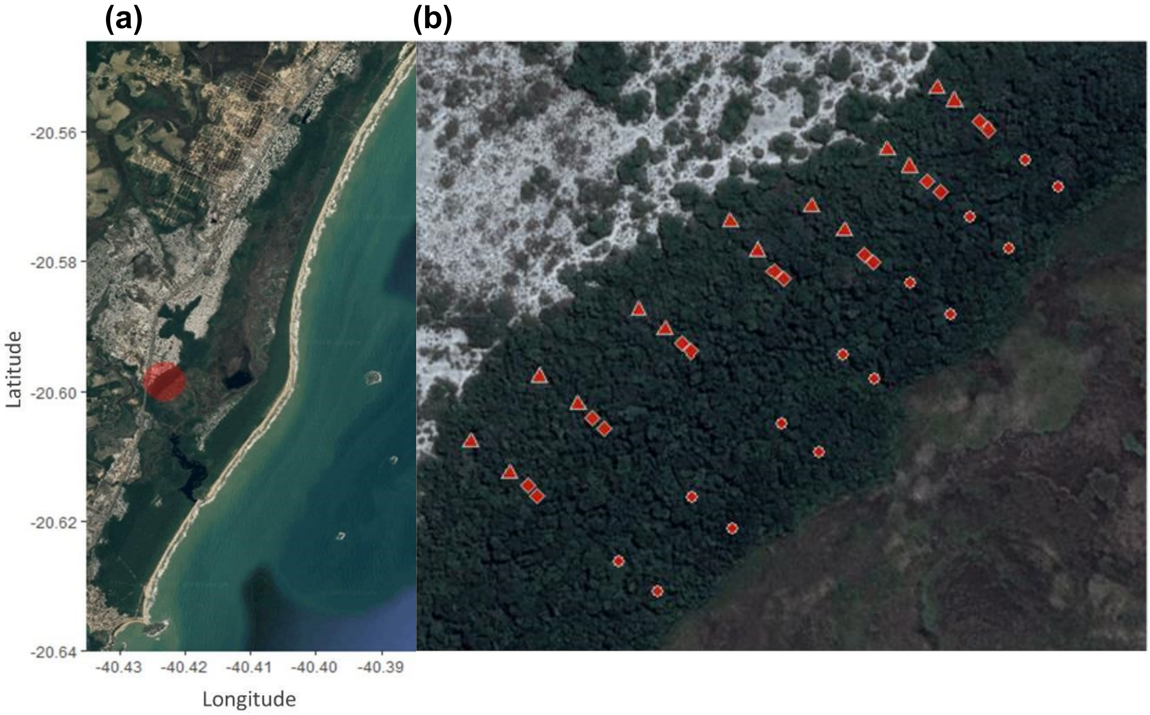

Figure 1. A – Paulo Cesar Vinha State Park and study area location (red dot). **B** – Detailed map of the study area, showing the 42 plots/communities distribution across floodable (○), intermediate (□), and dry (Δ) sites.of two assumptions of Trait Driver Theory. Skewness, and kurtosis of height, leaf dry matter content (LDMC), leaf thickness (LT), stomatal density (SD), specific leaf area (SLA), and wood density (WD) of 42 plant communities from floodable (○), intermediate (□), and dry (Δ) sites along the water table gradient. Statistically significant (*), p-value<0.05, or marginally significant (ms) [p-value=0.6].

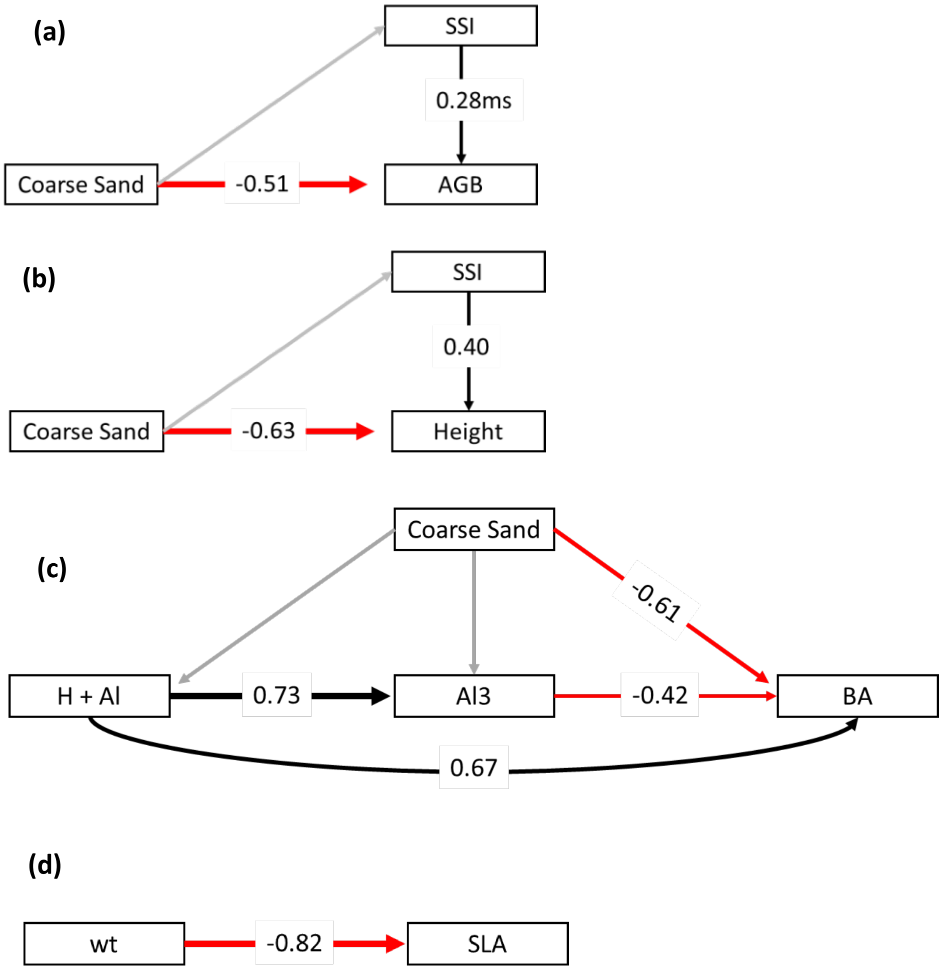

Figure 2. Simplified models obtained by piecewise structural equation models (SEM), using the environmental variable selected in the forwarded selection analysis (table 1). We explored the most important drivers of (a) AGB, (b) height, (c) BA and (d) SLA from 42 *restinga* plant communities. Sodium effect over AGB was marginally significant (ms), presenting a p-value = 0.0536. Notice that water table depth (wt) has a predominant effect on SLA, and the rest of all the other 24 environmental variables provided in GAM analysis (table 1) exhibited a lower fraction of explanation on AGB variation.

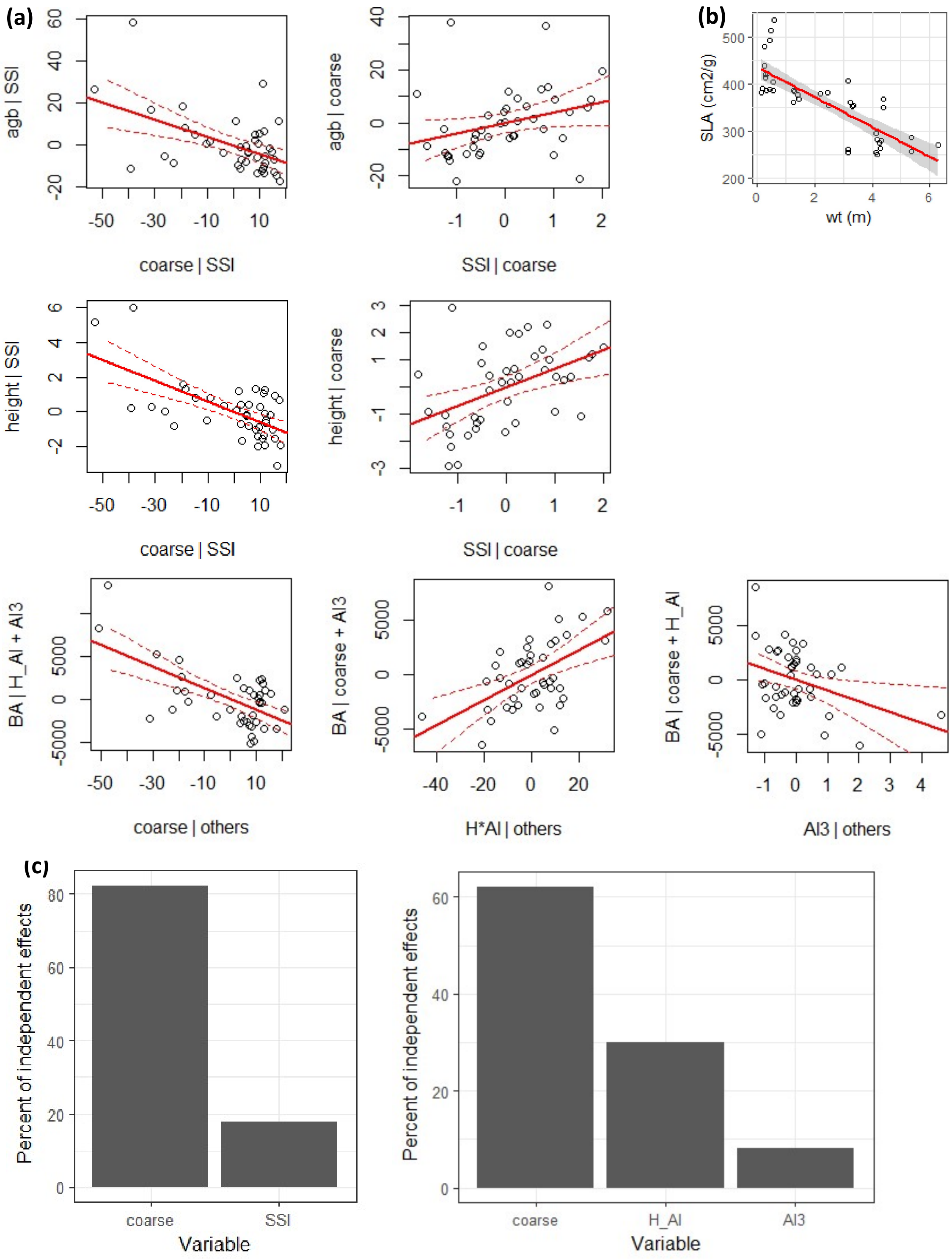

Figure 3. Drivers of above ground biomass (AGB), basal area (BA), specific leaf area (SLA), and height in 42 restinga plant communities. Piecewise structural equation model selection via d-separation and Akaike’s information criterion (LEFCHECK, 2016; SHIPLEY, 2016), which produced the models in figure 2, the partial correlations of direct effects (a), and the separate independent effects analysis (c). The water table has a direct and prevalent effect on SLA (b).

DISCUSSION

Toward the dry environments, the soils become more sandy, well-drained (S.M.: fig. S1) and poorer in SOM, Zn, Al, Na, Ca and Cu, whereas P, Mn and B soil content increases slightly (S.M.: fig. S2). Such nutritional impoverishment toward dry communities have been related to the higher leaching of nutrients in well-drained soils of *restinga* plant communities (LOURENÇO JUNIOR; CUZZUOL, 2009; MAGNAGO et al., 2010), as textural characteristics are highly associated to the retention of soil nutrient in *restinga* soils (COOPER et al., 2017). Furthermore, bases saturation (BS) lower than 10 (S.M.: fig.S2) indicate low nutritional reserve into the soil, which is even more impoverished in *restinga* soils by the high rainfall and the sandy soil texture, resulting in high Aluminum saturation (RODRIGUES et al., 2013).

Despite the slight changing in pH across the gradient, all soils are strongly acid (S.M.: fig. S2), exhibiting pH<5. Below this level of pH there is a severe depletion of the availability of essential nutrients as N, P, K, Ca and Mg, however, as a higher concentration of Hydrogen cations in the soil modestly increases the nutrients input by increasing weathering rates of soil organic matter (LAMBERS; CHAPIN; PONS, 2008), floodable communities may take advantage of higher acidity in organic soils, by the releasing of Nitrogen compounds from SOM, explaining the positive effect of potential acidity (H+Al) on forest basal area (fig. 2C and 3A). Moreover, flooding imposes anoxic conditions to the soil, which reduce the microbial decomposition activity, delaying the nutrients releasing from SOM (FRIDMAN, 2005). Such low rate of decomposition is appointed to favor a more efficient absorption of nutrients by plant root systems, which may enhance the nutrient use in *restinga* plant communities (BONILHA et al., 2012), and influence plant species distribution (DE ALMEIDA JR et al., 2011).

However, we did not find evidence to support a primary role of SOM in communities' trait composition (table 1). The most explicative soil variables for AGB, SLA, basal area, and height were those related to the water table depth and soil drainage (WT and coarse sand), whereas SOM was not selected into the models (table 1), and Sodium saturation index (SSI) and Aluminum had a lower explanatory power and independent effects on trait composition (table 1 and fig. 3b). Thus, water availability-related soil parameters (WT and coarseness) seem to have a key role in the trait composition of such systems, while SSI and Al seem to exert a coadjutant role.

In an interesting discussion about nutrient essentiality, Subbarao et al (2003) define functional nutrient as "*an element that is essential for maximal biomass production or can reduce the critical level of an essential element by partially replacing it in an essential metabolic process*" and present several arguments to consider Na as a functional nutrient. In conditions of soil nutrients shortage or even in high Na soil content, Na can replace K given their chemical similarity, by competition for the same absorption sites in the plant root system and assuming some K-related metabolic functions, as osmoticium for cell enlargement and accompanying cation for long-distance transport (SUBBARAO et al., 2003).

Thus, circumstances of high Na soil content as we found (SSI>4.5%, and Na>90 g/kg – S.M.: fig. S2), and its positive effect on AGB and height (figures 2a, 2b, and 3a) suggest that Na may be exerting a vital role in *restinga* plants nutrition, possibly supplementing the nutritional lack of K soil content, for instance (S.M.: fig. S2). Moreover, as salinity was recently reported to distinguish *restinga* forests from others environmentally marginal habitats of Atlantic Forest (NEVES et al., 2017), it seems reasonable to think that Na could play a role in the system functioning.

The aluminum soil content in the study area (fig. 3) exhibited even higher values (>6cmol/kg) than previously reported by earlier studies in *restinga* plant communities (MAGNAGO et al., 2013; RODRIGUES et al., 2013; MELO JÚNIOR; TORRES BOEGER, 2015). The increasing of soil Al solubility and availability is related to the increasing of soil acidity (LAMBERS; CHAPIN; PONS, 2008), which was the trend toward the wet end of the flooding gradient (S.M.: fig.S2), where soils are strongly acid, reaching out pH = 3.6. Moreover, the potential acidity (H+Al) exhibited a positive effect on Al soil content, which in turn produced a negative effect on BA (fig. 2b). This finding is supported by the scientific literature, as the combination of lower pH (<5.5) and higher Al soil concentration results in toxic condition for the most part of plants, constraining shoot and root growth, and the uptake of several essential nutrients, as well (US ENVIRONMENTAL PROTECTION AGENCY, 2014; BOJÓRQUEZ-QUINTAL et al., 2017).

In other hand, the application of Al can stimulate the uptake of N, P and K in native plants (OSAKI; WATANABE; TADANO, 1997), enhancing the growth in some native plant species that have adapted to acid soils by exclusion or accumulation of

Al (WATANABE; OSAKI, 2006), as reported for *Tapirira guianensis* (BRITEZ et al., 2002a) and *Faramea marginata* (BRITEZ et al., 2002b), species usually found in *restinga* forests. Other benefits of Al in adapted plants include increasing defense against pathogens, alleviation of abiotic stress, increased metabolism and antioxidant activity (BOJÓRQUEZ-QUINTAL et al., 2017).

The accumulation of Aluminum in leaves and seeds, and the positive effect of the soil exchangeable aluminum in plant survivance have been reported in some native plants from Cerrado ecosystem (HARIDASAN, 2008), which shares several plant species with *restinga* forests, as those we found in our study area – *Tapirira guianensis*, *Calophyllum brasiliense*, *Protium heptaphyllum*, *Myrcia rostrata*, *Xylopia sericeae*, *Alchornea triplinervia*, *Pseudobombax grandiflora*, *Emmotum nitens*, *Pera glabrata*, *Amaioua guianensis*, and *Cecropia pachystachya* (LOUNREÇO JUNIOR et al. 2018, *in prep*). The similarity of both systems regarding to the high Aluminum soil content and the co-occurrence of several plant species suggest the importance of the Aluminum affecting the composition of some Brazilian ecosystems, as Atlantic forest and Cerrado.

In general, the forests were differently affected by soil parameters which covariate with soil texture, such a way that coarse sand in covariation with Na, Al, and acidity influenced forest physiognomy and functional composition (AGB, height, basal area and SLA). The selection of SSI in the GAM analysis (table 1) and its positive relationship with AGB and height (figures 2 and 3) suggest that Sodium could be playing a secondary (fig. 3b) but not less vital role in the functioning of *restinga* plant communities.

It is worth it to mention that despite of the several environmental variables used in this study and the high level of retained explanation for all response variables (table 1, adj. R^2 cum. ranging from 51% to 68%), a considerable amount of variation remained unexplained (32% to 49%). These suggest the existence of other factors beyond the physicochemical soil properties influencing forest physiognomy and functional composition, highlighting the importance of the biotic interactions, as competition (GARBIN et al., 2016) and facilitation (GARBIN et al., 2014), whose investigation could be widening our comprehension about the functioning of this threatened Atlantic forest habitat.

Supplementary Material

Additional Supporting Information may be found in the online version of this article:

Figure S1. Soil coarseness, field capacity (FC) and soil organic matter (SOM) variation across water table gradient.

Figure S2. Soil physicochemical parameters variation across the water table gradient

Figure S3 Above ground biomass (AGB), community weighted specific leaf area (SLA), Height and basal area (BA) distributions along the water table depth gradient.

Figure S4 Chi-Square Q-Q plot of the variables from each equation model.

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US ENVIRONMENTAL PROTECTION AGENCY. **Appendix 5 : US EPA Ecological Soil Screening Level for Aluminium**. [s.l: s.n.].

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SUPPLEMENTARY MATERIAL

Drivers of forest physiognomy and functional composition in environmentally diverse coastal plant communities from Atlantic forest

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Contents

Figure S1. Soil coarseness, field capacity (FC) and soil organic matter (SOM) variation across water table gradient. Communities from floodable (○), intermediate (□), and dry (Δ) sites.

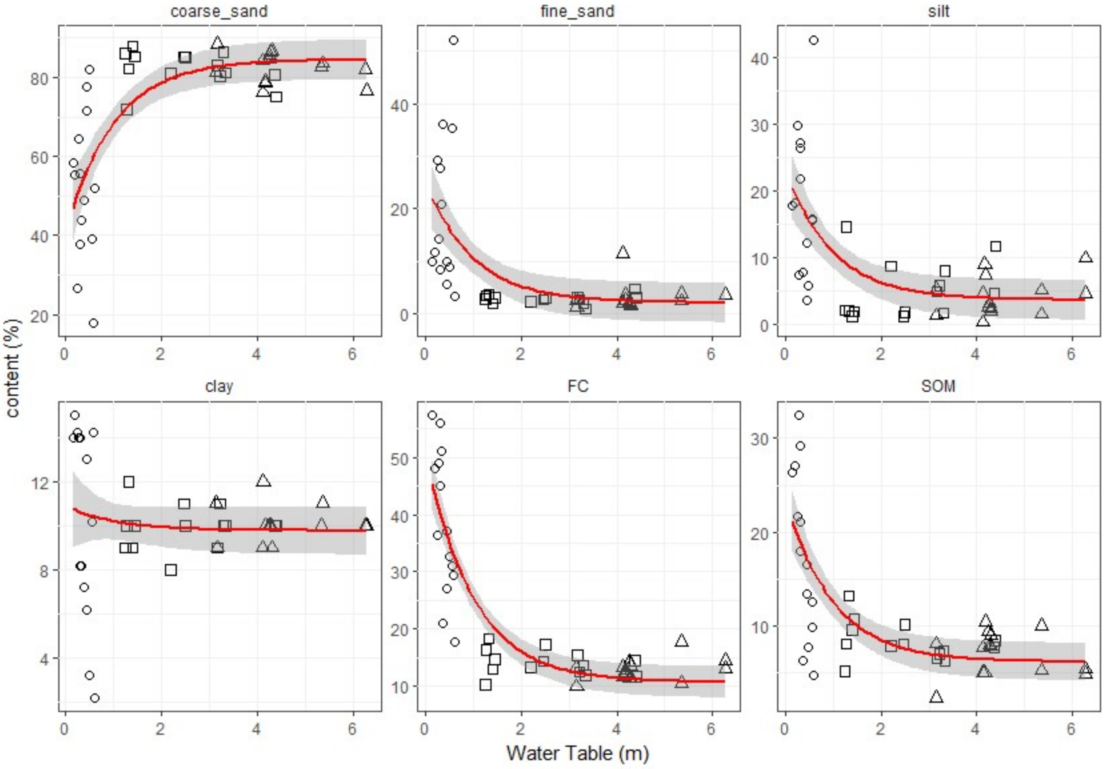


Figure S2. Soil chemical properties variation across the water table gradient, showing macronutrients; micronutrients; acidity (pH), cation exchange capacity (CEC), base saturation (BS), acidity related to Hydrogen and Aluminum (H+Al), Aluminum saturation index (m), Sodium saturation index (SSI). Plots from floodable (○), intermediate (□), and dry (∆) sites.

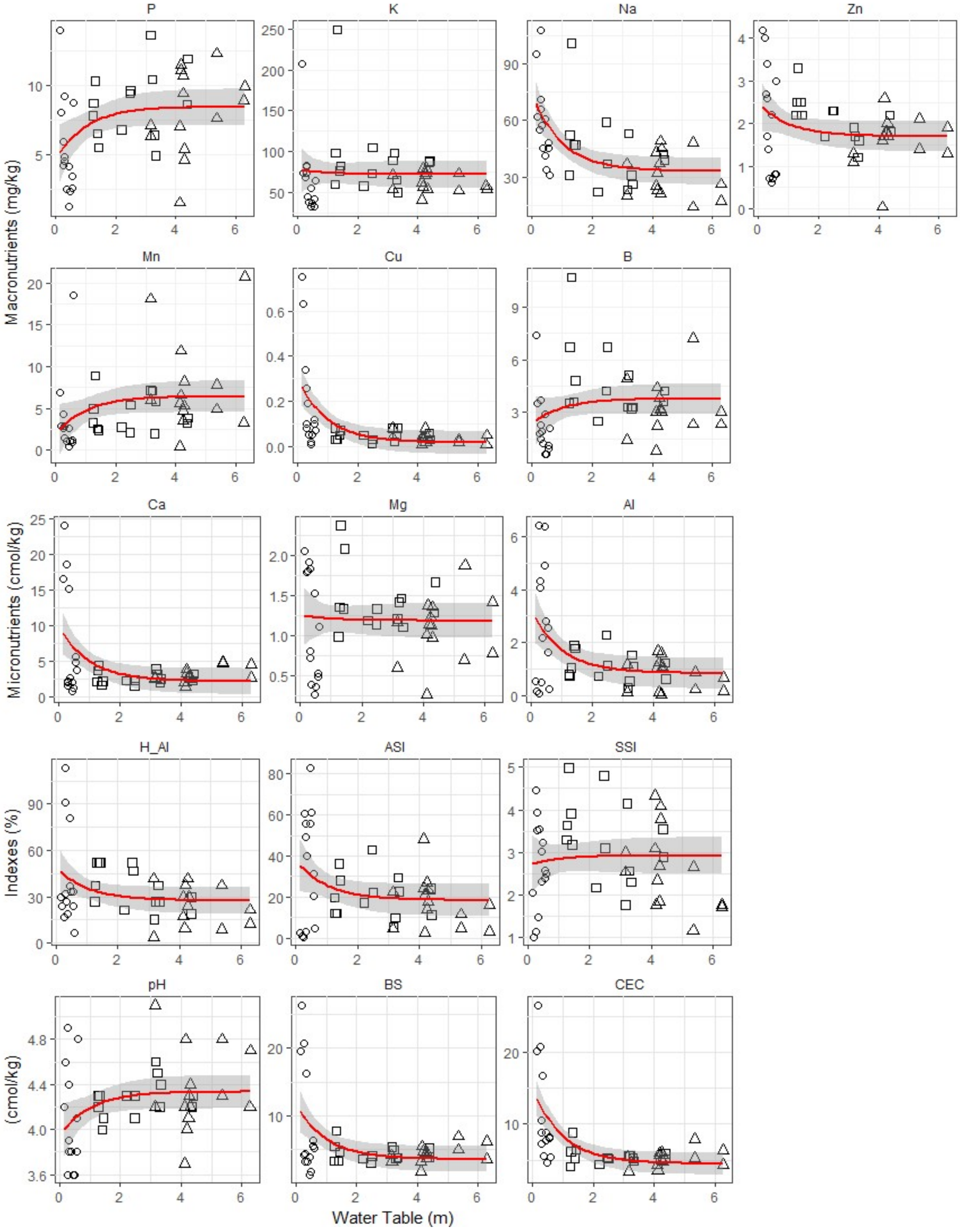


Figure S3. Above ground biomass (AGB), community weighted specific leaf area (SLA), Height and basal area (BA) distributions along the water table depth gradient. All correlations were statistically significant (p-value < 0.05). Each dot represents one of the 42 communities from Floodable (○), intermediate (□), and dry (Δ) sites.

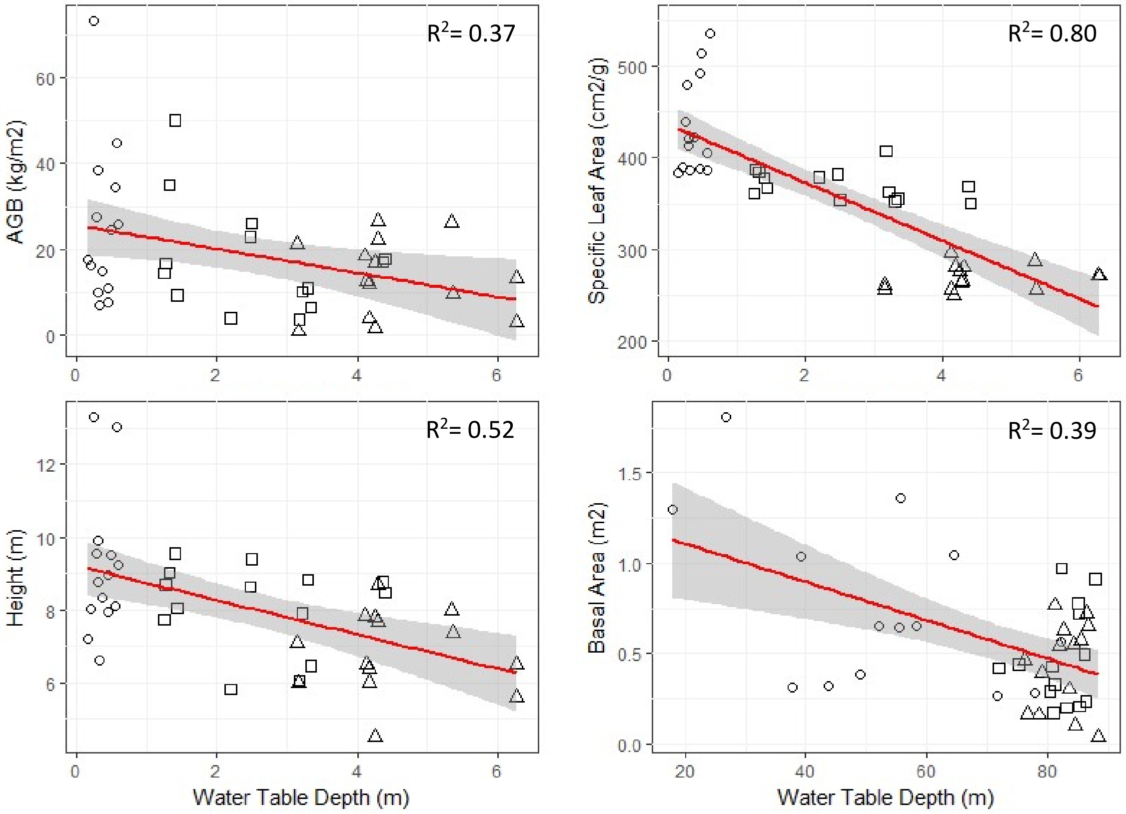
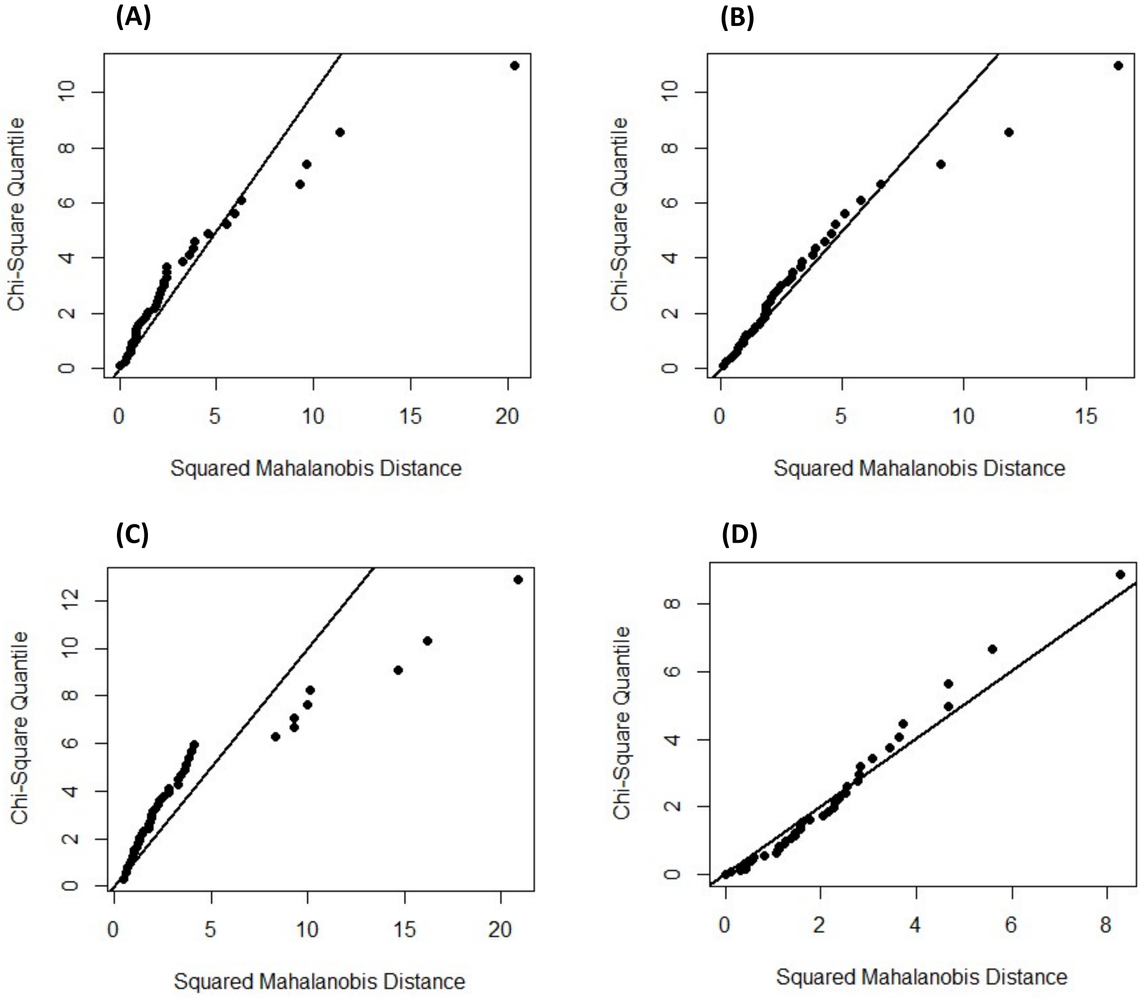


Figure S4. Chi-Square Q-Q plot of the variables from each equation model: AGB ~ coarse sand + SSI **(A)**; height ~ coarse sand + SSI **(B)**; BA ~ coarse sand + H_Al + Al3 **(C)**; and SLA ~ water table **(D)**. Except for the model D, all the models show a departure from multivariate normality.



Multivariate non-normality is showed by Mardia's test for variables from model A (P_{skew} = 0.004, $P_{kurtosis}$ = 0.86); model B (P_{skew} = 1.54, $P_{kurtosis}$ = 0.02); and model C (P_{skew} = 3.73 x 10^{-12}, $P_{kurtosis}$ = 3.21 x 10^{-9}). The variables from the model D presented multivariate normality (P_{skew} = 0.35, $P_{kurtosis}$ = 0.28). Statistical tests were conducted using the package MVN (KORKMAZ et al. 2014) in the statistical software R (R CORE TEAM, 2018).

CAPITULO 3 – The Trait-based Ecological Wood Anatomy: unveiling the shifting of anatomical wood traits and setups of wood space use along a steep gradient of water availability

Running head: Shifting in the wood trait composition of tropical plant communities along a steep water availability gradient

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Keywords: Environmental gradient. Safety-efficiency tradeoff. Trait-based ecology. Water availability. Wood anatomy.

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ABSTRACT

1. Background. Overcome the species-level paradigm is challenging to the ecological wood anatomy. The community-weighed metrics are well-established approaches in trait-based ecology to address several ecological questions in ongoing discussions about plant hydraulics, widening the perspectives in wood anatomy survey. We assessed the wood trait composition of 42 plant communities along a strong gradient of soil water availability to test several hypotheses from Bittencourt *et al.* (2016) about the setups of wood space-use and its correlation to the variability in the xylem-specific potential hydraulic conductivity (Ks). Moreover, we discuss the functionality of several wood anatomy traits.

2. Methods. We collected branches of the 80% most abundant tree species (36 species) from 42 environmentally diverse tropical plant communities. We sliced, stained (safranin), took microscopic photos (10 times of magnitude) and analyzed the wood anatomy of 156 wood samples. We calculated the community weighed metrics to assess the shifting of several wood anatomy traits along a steep soil water availability gradient.

3. Results. The strong water availability gradient allowed us to assess deep changes in wood anatomy traits, matching several expectations from the wood anatomy literature. Toward dyer conditions, there are reductions in Ks, vessel-size, vessel solitary fraction, and the space occupied by non-conductive tissues (e.g. parenchyma), whereas fiber area, bending resistance, cell-grouping, and cell-density increased. The variance in Ks was well-correlated to fiber-wall thickness and the variance in cell-size and conductive area.

4. Conclusion. We propose a new model based on Bittencourt *et al.* (2016) to explain the high variance in Ks presented previously by Gleasson *et al.* (2016). We did not find evidences that the variance in Ks is related to the variance in parenchyma proportion. We suggest that thin-walled fibers with water storage capacity (e.g. septate fibers) may be increasing the efficiency of water transport in species occurring in high water potential, ultimately producing “inefficient” low Ks species. The variability in cell-size, conductive area, and proportion of septate fibers may also produce different setups of wood space-use and explain the variance in Ks.

INTRODUCTION

Overcome the traditional species-level paradigm is challenging to the Ecological Wood Anatomy (EWA). Despite well succeed in developing methods to address ecological questions (BEECKMAN, 2016), the EWA may take advantage of using more quantitative and versatile approaches from trait-based ecology, widening the ecologic hierarchical levels of its application and increasing its predictive power to answer ecological questions.

The wood traits are considered important source of information about plant-environment relationship and can be potentially used in predictive models to assess timely ecological questions, as the impact of the climate change on plant communities world-wide (BEECKMAN, 2016). Despite being created to taxonomic identification purpose, several wood traits known in wood anatomy literature (COMMITTEE, 1989; BAAS et al., 2004a) and in recent reviews (CRIVELLARO; WIEDENHOEFT; RUFFINATTO, 2015) match functional traits definition of Violle *et al.* (2007), which consider "*Any trait which impacts fitness indirectly via its effects on growth, reproduction and survival"*.

However, the choice of the wood traits to access plant ecological strategies along gradients must be made with care (FOURNIER et al., 2013). Beeckman (2016) presents a detailed discussion about the multiplicity of wood anatomical traits and their potential funcionality, and suggest a wide diversity of traits and their potential application to address timely ecological questions, as those related to the impact of environmental changing on forest trait composition.

The use of community ecology approaches, as community weighed metrics, provide feasibly way to address several hypotheses in ecological wood anatomy. The weighed metrics are underpinned by the "mass-ratio" hypothesis, which states the prevalent influence of the most abundant species over the subordinate and transient in assessing ecosystem functions (GRIME, 1998).

Such assumption has a implicity concept of environmental optimality, where species relative abundance is mediated by their traits, that is, "community assembly by trait selection" (SHIPLEY; VILE; GARNIER, 2006). In a advanced standing point, the weighening procedure may be extend to the others statistical moments, as *variance, skewness* and *kurtosis* (ENQUIST et al., 2015; ASNER et al., 2017), increasing the potential of this analysis in describe ecological processes of interest in ecology and mediated by wood anatomy traits.

Following a recent discussion of Enquist *et al.* (2015), the “Holy Grail” of the trait-based ecology (and the trait-based ecological wood anatomy) has to be the use of functional traits, rather than species identities, which are better predictors of community and ecosystem dynamics in the context of the environmental change (DÍAZ; CABIDO, 2001; DIAZ et al., 2007; SUDING; GOLDSTEIN, 2008).

We argue that by community weighed metrics one can investigate the correlation between wood traits; test and propose hypotheses in wood anatomy about hydraulic traits; discuss the ecological functions of the wood traits; and scale up from organ to ecosystem functioning to access the ecological process (internal and external filters) structuring wood trait composition in plant communities.

Several interesting hypothesis tested in this paper come from a recent discussion (BITTENCOURT; PEREIRA; OLIVEIRA, 2016; GLEASON et al., 2016) about the existence of a tradeoff between protecting conduits against embolisms and the efficiency of water transport by plants (DIXON; WRIGHT, 1894; TYREE, 1997) – The safety-efficiency tradeoff (SET). This may be an important mechanism in plant ecology and evolution, as more efficient plants with high Ks and wider vessels are linked to fast-growing strategies (POORTER et al., 2010), ultimately influencing plant-plant interactions (e.g. light competition), whereas safety plants with low Ks and smaller vessels should be more resistant to the environmental stress imposed by the water deficit (BAAS et al., 2004b). Both strategies may be the consequence of divergent forces acting along the evolutionary history of plants (SPERRY, 2003).

Gleason et al. (2016) compared the relationship between these two conduit-level traits – hydraulic conductivity (Ks; kg m^{-2} MPa^{-1} s^{-1}) and safety – in a remarkable dataset, comprising 335 angiosperms and 89 gymnosperm species, and reported no species with high efficiency and high safety in hydraulic system, which support the idea of a SET. However, the finding of many species with low Ks (low efficiency) and safety, resulted in a weak correlation between such SET traits, challenging the understanding of the xylem evolution by the SET mechanism.

In interesting comments on Gleason et al. (2016) findings, Bittencourt et al. (2016) proposed to revisit the “efficiency” definition of Ks assumed by Gleason and colleagues, which is based on water transport – *“amount of water transported by a* *given xylem area"* - to a definition based on space-use efficiency – *"efficiency of wood space use allocated to the vessels"*. Thus, if Ks can be understood as a measurement of hydraulic space use efficiency, the Ks variability has to be understood based on the factors that limit or affect the wood space use (BITTENCOURT; PEREIRA; OLIVEIRA, 2016). These same authors explicitly affirm that the *"space allocation to conduits thus necessarily conflicts with space allocation to parenchyma, fibers and cell walls"*, and the Ks variability should be investigated under this perspective.

According to the wood space-use model proposed by Bittencourt et al. (2016), there is a limit and not a direct relationship between hydraulic safety and space-use efficiency, which could be explained by two ways: 1) the wood-space constrained by fibers and cell-wall thickness. Given that hydraulic safety plants under more negative water potentials must to deal with the trend of the vessel implosion, the resistance to the inner mechanical stress is provided by thicker conduit cell-walls (HACKE et al., 2001) and/or the increased transverse fiber area (JACOBSEN, 2005). 2) the parenchyma space-use. As parenchyma is considered a source of water more accessible to the leaves, which can buffer plants against the water stress – refilling hypothesis (ZWIENIECKI; HOLBROOK, 2009) – a plant with high parenchyma content and low Ks could also be associated with low hydraulic safety, as parenchyma could increase the water transport efficiency under non-steady state conditions.

Thus, Bittencourt *et al.* (2016) propose that such variability of Ks in high water potentials found by Gleason et al. (2016) could be explained by the variance in the wood space-use, where conduits (conductive area and frequency of vessels) and parenchyma could be variating freely, allowed by the smaller inner mechanical stress, given the smaller space occupied by the fibers and cell-walls.

Here, we tested the hypothesis made by the model of Bittencourt and colleagues, and assessed the shifting of several hydraulic traits by the community weighed mean and variance, to answer the following questions: 1) What hydraulic traits correlates with the variance in Ks? 2) How the water availability gradient affects the wood anatomy traits and the setups of wood space-use? 3) How the communities wood trait composition is affected by the ecological processes?

MATERIAL AND METHODS

Sampling procedure

We collected branches of 36 tree species or the 80% most abundant tree species at the Paulo Cesar Vinha state park (PEPCV) study area (fig. 1). We collected at least three individuals (branch) per species and per environment, to keep the sampling consistency and detect the fine scale influence of the environmental gradient over the wood trait composition. However, we collected a small number of individuals from *Solanum sycocarpum* (n=1), *Buchenavia tetraphylla*, and *Campomanesia guazumifolia* (n=2), given their rarity or low-frequency. We stablished a pattern in sampling strategy regarding to the branch position along the individual, the position along the branch, the branch diameter, and its maturity. Thus, we collected adult trees first basal branch with approximately similar diameter (1.10 ± 0.37 cm, without the bark).

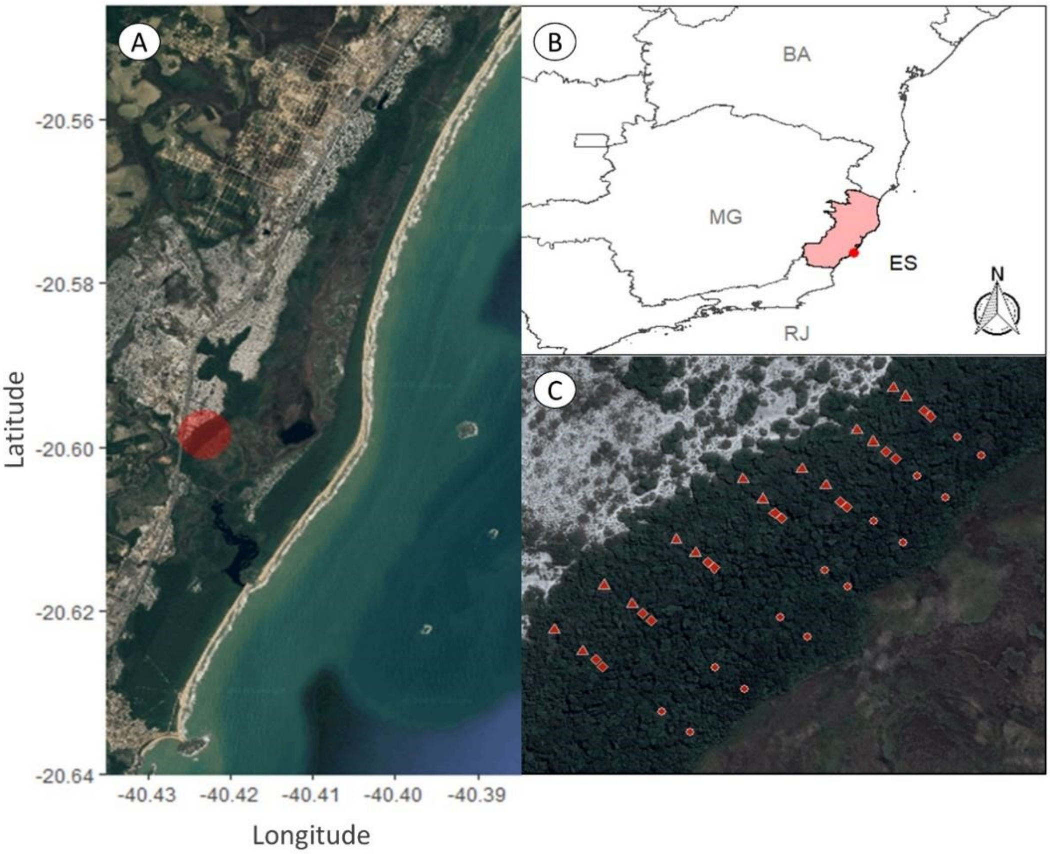


Figure 1. A) Paulo Cesar Vinha state park (PEPCV) and the study area (red dot) localized in the Espírito Santo State (B). Detailed map of the study area (C), showing the 42 plots/communities that spans a gradient ~ 207 ± 60 meters from Floodable (○), intermediate (□), and dry (∆) forest communities.

This dataset is part of an earlier study investigating wood density and leaf traits composition of *restinga* forests (LOURENÇO JUNIOR et al. 2018, *in prep*), which includes the determination of the water table depth gradient and the forest inventory where all individual plants with diameter at the breast height (dbh) ≥ 5cm were measured the height and dbh. We excluded a fern (*Cyathea phalerata*) and two palms species (*Euterpe edulis* and *Geonoma schottiana*) from this wood anatomy study.

Wood anatomy analysis

We previously boiled the samples to make easy the cutting procedure. We produced microsections of 10–20 µm thickness, as samples thicker than 20 µm can input variance in some anatomical features, making difficult to differentiate thin to thick-walled cells, for instance (VON ARX et al., 2016). The microsections were produced with the sledge microtome GSL1 microtome, which produces high quality thin-sections and it is easy to handle (GÄRTNER; LUCCHINETTI; SCHWEINGRUBER, 2014).

We did not set up semi-permanent or permanent histological slides, which speed up the wood anatomy analysis. Thus, the thin-section were dyed in Safranin (1%) and placed in histological slides for the image capture, using a microscope coupled with a high-resolution camera. We took several microscopic photos from the pith to the margin of the branch sample at 10 times of magnitude. The images were stitched with the PTGui software (New House Internet Services B.V., Rotterdam, NL), which is recommended for producing distortion-free composite images (VON ARX et al., 2016).

The final composite images were analyzed in the freely available software ROXAS 3.0 (VON ARX; CARRER, 2014), which is specialized for quantitative wood anatomy analysis and provides several parameters of ecological interest (VON ARX, 2016), as vessel density (no.mm^{-2}); vessel grouping index (no/group); vessel solitary fraction (%); vessel-wall thickness (µm); and mean vessel size (µm^{2}).

Others parameters included in this study are Xylem-specific potential hydraulic conductivity (Ks, [kg.m^{-2}.MPa^{-1}.s^{-1}]): Kh/XA, where “Kh” is the accumulated Potential hydraulic conductivity as approximated by Poiseuille's law and adjusted to elliptical tubes, and “XA” is the xylem area (mm^2); Overall mean bending resistance index (Bend): [t/b]^2, where “t” is the double cell wall thickness and “b” the length of the same cell wall (HACKE et al., 2001).

The proportion of fibers and parenchyma (%), and fiber-wall thickness (µm) were calculated in the Image-Pro Plus 7 (Media Cybernetics, Silver Spring, EUA). We call parenchyma proportion by “non-conductive area”, which include axial and radial parenchyma.

Community weighed metrics

We calculated the community weighted mean (CWM, eq. A) and variance (CWV, eq. B) for each of the 42 plots (or plant-communities, for simplicity) in this study. The calculations were made by the species trait values weighted by the species abundance, according to the equations:

$$\mathrm{CWM}_{\mathrm{j,y}} = \sum_{k=1}^{nj} A_{k,j} \,.\, z_k \qquad \text{(A)}$$

$$\mathrm{CWV}_{\mathrm{j,y}} = \sum_{k=1}^{n} A_{k,j} \,.\, (z_k - \mathrm{CWM}_{\mathrm{j,y}})^2 \qquad \text{(B)}$$

where n_j is the number of species sampled in plot j, $A_{k,j}$ is the relative abundance of species k in plot j, and z_k is the mean value of species k.

RESULTS

The wood trait composition was deeply affected by the water table gradient, and the setups of wood space-use exhibited consistent trends by changing fiber, non-conductive (parenchyma), and conductive (vessels) cross-sectional area proportions. Toward the drier end of the gradient, fiber proportion increases, while conductive and non-conductive area decrease (fig. 2a). The variance in Ks was

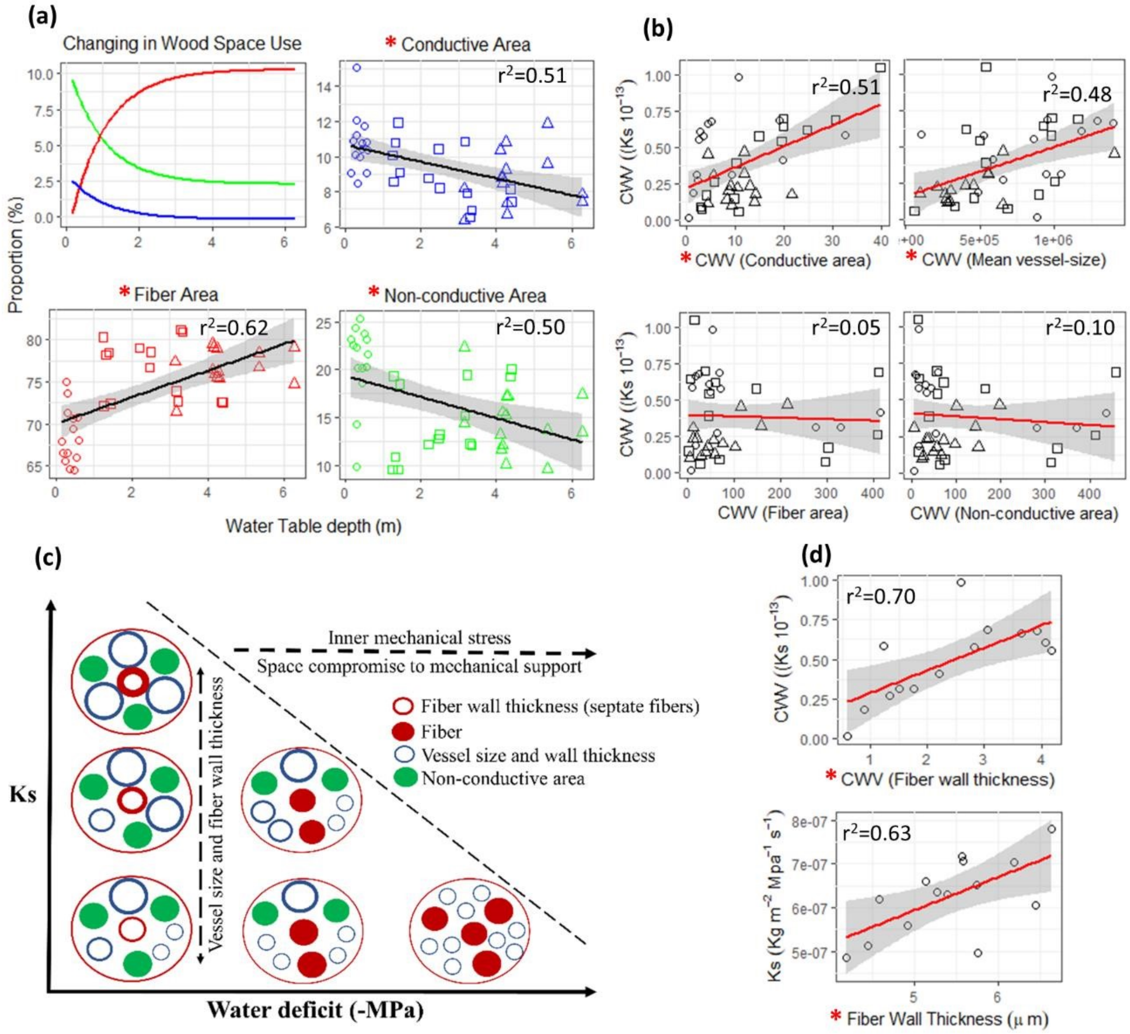


Figure 2. (a) Setups variation of wood space use along the water table gradient by the shifting of fibers, conductive (vessel lumen) and non-conductive area (parenchyma) in 42 plant communities. (**b)** community weighed variance (CWV) of potential hydraulic conductivity (Ks) in relation to the CWV of conductive area (xylem lumen), mean cell-size (vessels), non-conductive area, and fiber area. (**c)** Model modified from Bittencourt *et al.* (2016). **(d)** Positive correlation between fiber wall-thickness and Ks (both variance and mean) at higher water potential. In the floodable communities, we notice the presence of thin-walled fibers with larger lumem (fig. 4, septate-fibers), which could be surrogating the parenchyma functions of water storage, allowing Ks to variate. Statistically significant (*), p-value<0.05. Floodable (○), intermediate (□), and dry (Δ) forest communities.

correlated to vessel-size and conductive area variance, and uncorrelated to non-conductive area (parenchyma) and cell-wall thickness (fig. 2b). Thus, we propose a new model for Ks variance (fig. 2c) based on the model of Bittencourt et al. (2016).

From the wetter to the drier communities we notice strong trends of increasing vessel-density, bend and vessel grouping index, whereas Ks, mean vessel-size and vessel solitary fraction decreased (Fig. 2a). The changes in the anatomical features can be easily observed in the microscopic photos of the most

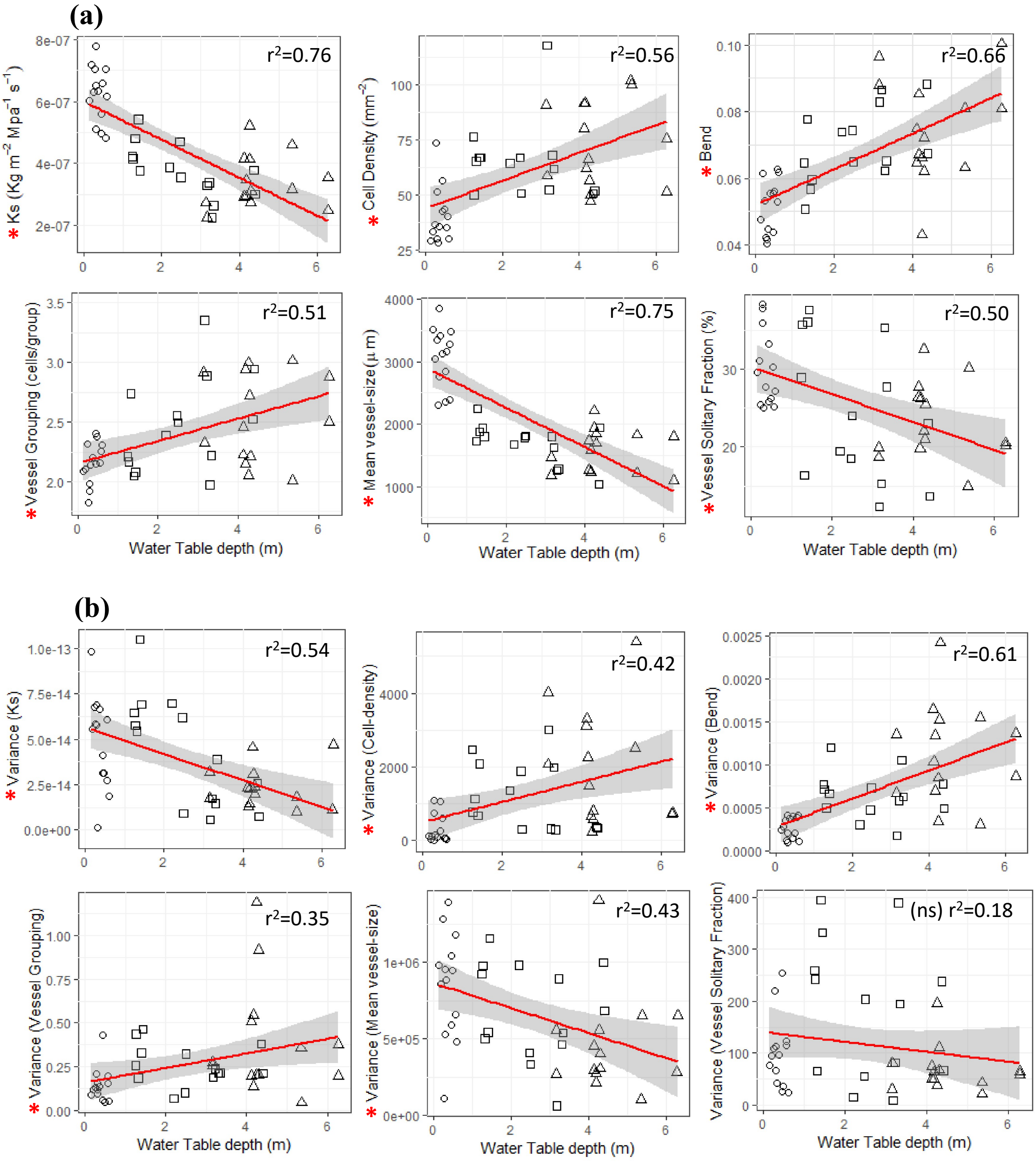


Figure 3. Community weighed mean (a) and variance (b) of Ks, cell density, resistance to the bending (Bend), vessel grouping index, mean vessel size (MCA), and vessel solitary fraction along the water table depth gradient. Statistically significant (*), p-value<0.05. Floodable (○), intermediate (□), and dry (Δ) forest communities.

abundant species for each forest (fig. 4, I-III, a-c). Moreover, *P. grandiflorum* has a conspicuous changing in fiber proportion, and *T. guianensis* exhibit changes in vessel-wall thickness and fiber lumen (fig. 4, d-e).

The community weighed variance of cell-density, bend, and vessel grouping index had a trend of increasing toward the dry end of the gradient, while Ks and mean cell area exhibited an opposite trend (fig. 3b). Vessel solitary fraction variance was weakly correlated to the water table gradient.

Species-level analysis and additional discussion can be assessed in the supplementary material of this article, available in the on-line version.

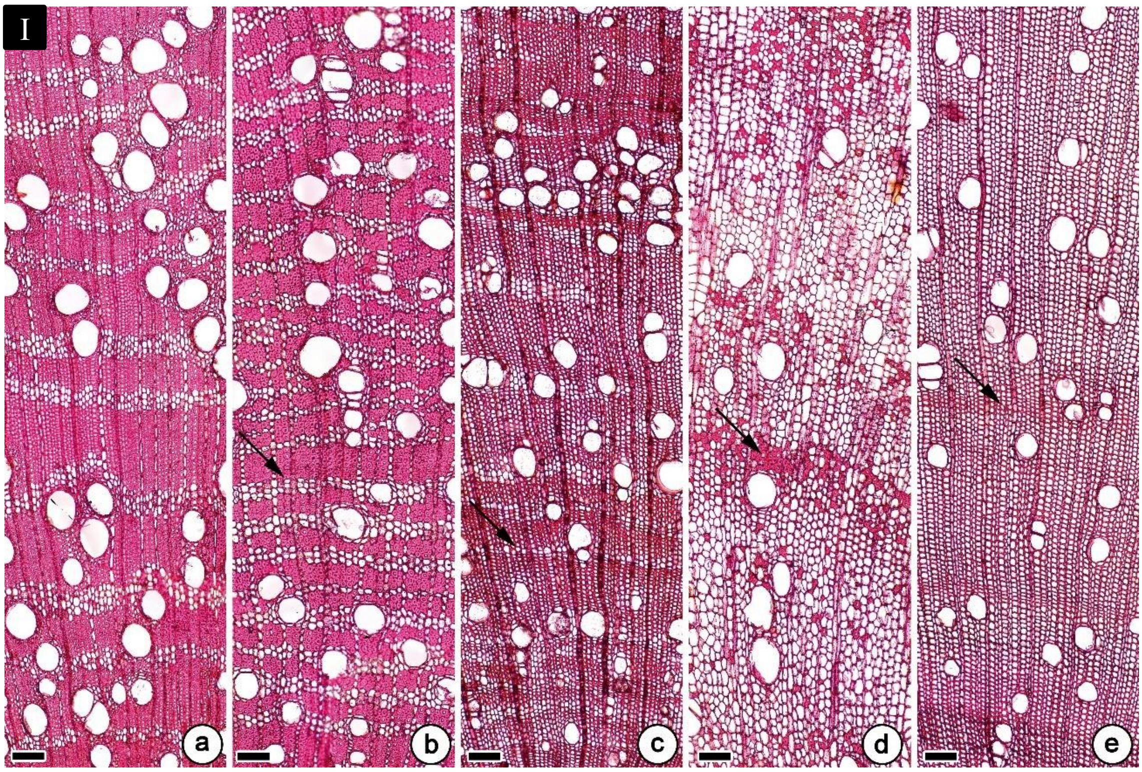
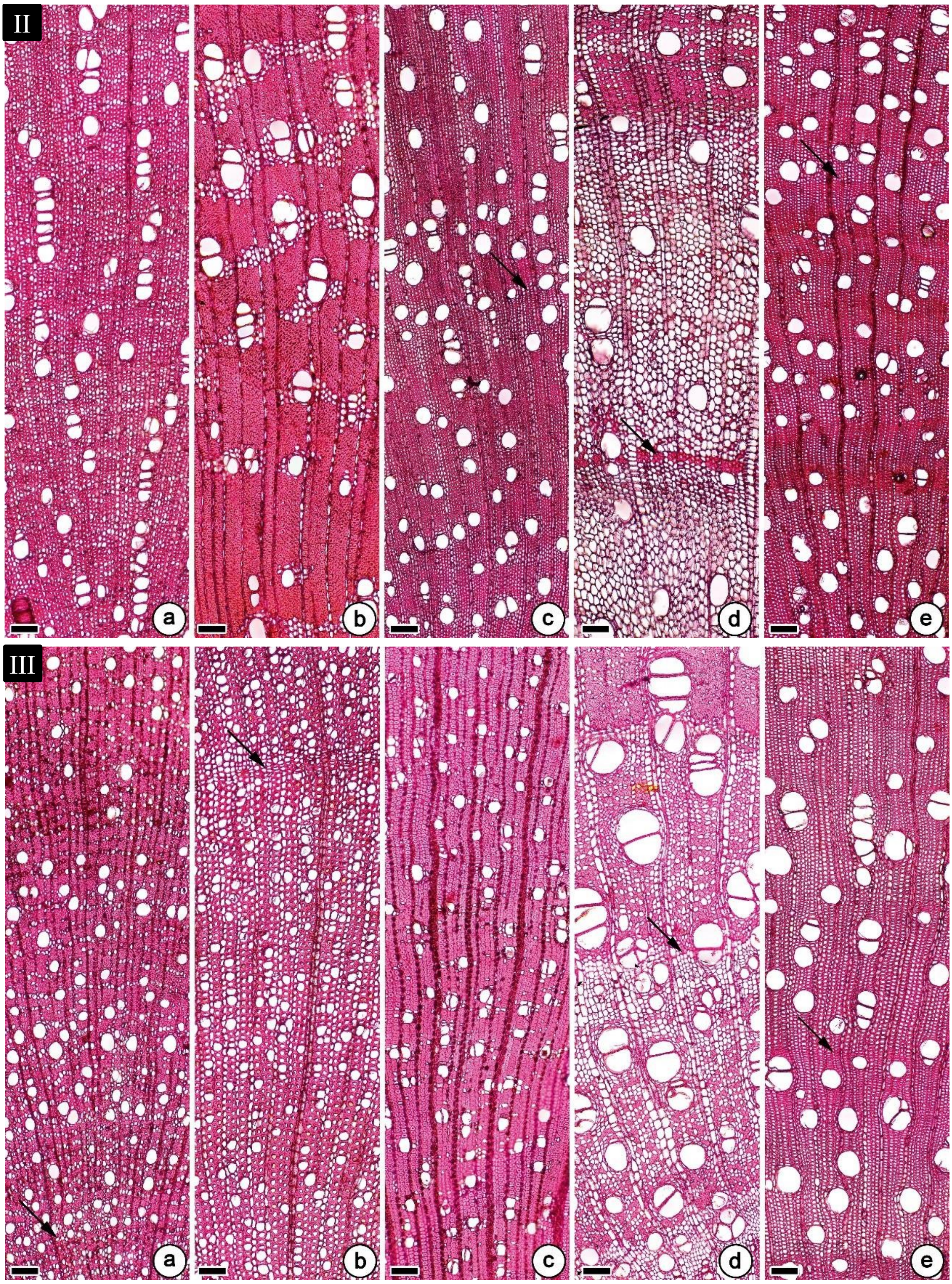


Figure 4. Floodable forest (I): a – *Calophyllum brasiliense*, b – *Symphonia globulifera*, c – *Tabebuia cassinoides*, d – *Pseudobombax grandiflorum*, e – *Tapirira guianensis*. Intermediate forest (II): a – *Micropholis venulosa*, b – *Guarea macrophylla*, c – *Protium icicariba*, d – *Pseudobombax grandiflorum*, e – *Tapirira guianensis*. Dryer forest (III): a – *Emmotum nitens*, b – *Rudgea reticulata*, c – *Pouteria coelomatica*, d – *Pseudobombax grandiflorum*, e – *Tapirira guianensis*. Notice the frequency and vessel diameter decreasing toward the drier site when comparing the species, and the intraspecific variation of *T. guianensis* and *P. grandiflorum*. Moreover, fiber-wall thickness of *T. guianensis* increases toward the dryer forest. The thick mark at the bottom of each figure represents 100 µm. Arrows indicate growth-rings.

Figure 4. (Continued).



DISCUSSION

The weighed metrics allowed us to assess the influence of the environmental gradient in the communities' wood trait composition, showing to be an interesting alternative to the traditional species-level approach in the ecological wood anatomy. The environmental gradient of water availability produced an interesting range of setups of wood space-use (fig. 2a). Toward the dry end of the gradient, there is an increasing of fiber content, which was balanced by the decreasing of conductive area (vessel lumen) and non-conductive area (parenchyma), suggesting a limiting relationship of the wood space use (BITTENCOURT; PEREIRA; OLIVEIRA, 2016).

In another understanding of the limiting relationship hypothesis, Bittencourt *et al.* (2016) propose an explanation for the variation of Ks in wood plants (GLEASON et al., 2016) based on the variance of the parenchyma space-use. According to this idea, in higher water potential, less space needs to be allocated to the tissues related to mechanical support (fiber and cell-wall), which reduce the inner mechanical stress in the wood space and create more space available to other tissues. Assuming the invariability of vessel-size and pit traits, if less space is allocated to conduits and more is allocated to parenchyma, the Ks value could be reduced. Thus, a range of space-use setups could produce different Ks values, explaining its high variance in high water potential.

However, we didn't find support for this hypothesis, given the uncorrelation between the variance in parenchyma and Ks, which was correlated to the fiber-wall thickness in floodable forest (fig. 2d) and the variance in Mean vessel-size and conductive area (fig. 2b). In fact, vessel-size seems to be a highly variable trait at the individual (fig. 4) and species-level (see fig. S2-4 in sup. material), which is corroborated by the literature (PRESTON et al., 2006).

Furthermore, the strong correlation between the Ks variance and the fiber-wall thickness suggests the presence of thin-walled septate fibers in floodable forest (fig. 4a), which has been reported as surrogate of the axial parenchyma functions (e.g. vessels water-refilling) in some tropical families (WHEELER; BAAS; RODGERS, 2007) and it is highly present in several Atlantic forest plant species (SOFFIATTI et al., 2016) from humid areas (ALVES; ANGYALOSSY-ALFONSO, 2002). A higher proportion of thin-walled fibers may increase the water capacitance and the efficiency of the hydraulic system, despite the low Ks of these supposedly “inefficient” plants (GLEASON et al., 2016). Our findings suggest that the variance in Ks observed by Gleason *et al.* (2016) may be also related to the presence of the thin-walled septate fibers and the variance in vessel-size and conductive area, whose variability could provide several setups of wood space use with different Ks values (fig. 2c).

The CWM analysis showed a spectrum of variation along the water table depth consistent to the hydraulic safety-efficiency tradeoff (SET), ranging from more efficient to more safety strategies of water transport by the xylem, as wetter communities exhibited higher Ks, larger vessel-size (fig. 3a), more conductive area, and lower fiber content (fig. 2a), whereas drier communities exhibited opposite trends. This shifting of wood trait composition is mirrored by the species replacement (fig. S4b in Sup. Material) along the environmental gradient (LOURENÇO JUNIOR *et al.*, 2018 – *in prep*), matching the results of a recent study along a flooding gradient in central Amazônia (COSME et al., 2017).

Furthermore, there is an increasing in tree height (and specific leaf area) toward the wet end of the gradient (LOURENÇO JUNIOR *et al.*, 2018 – *in prep*), suggesting that larger vessels in floodable communities may be minimizing the costs associated with the resistance in the hydraulic system, enabling plants to have larger body sizes (WEST; BROWN; ENQUIST, 1999) (fig.S7).

According to the SET, the efficiency in transport water should tradeoff with the safety in the water transport, as larger vessels increase both the amount of water transported to the leaves and the risk of cavitation, causing the failure of the hydraulic by the air seeding into the water column (TYREE, 1997; JACOBSEN, 2005). The SET may represent an important mechanism in the plant evolution and ecology (SPERRY, 2003; BAAS et al., 2004b), given that plants would tend to operate in the limit of the efficiency and safety.

Other interesting results were the increasing of the bending resistance of vessel-wall to implosion, cell-density, and vessel grouping toward the drier communities, whereas vessel solitary fraction decreased (fig 3.a). In general, larger vessels are associated with lower vessel densities, and this relationship is dictated by the limiting relationship in the use of the xylem space (SCHULDT et al., 2013). However, the increasing of vessel density have been related to the safety in water transport, as closely spaced vessels may enhance conductivity and the spread of embolism when cavitation occurs (HACKE et al., 2001; PRESTON et al., 2006; HACKE; JACOBSEN; PRATT, 2009).

Similarly, a higher vessel grouping and the consequent lower fraction of solitary vessels (fig. 3a) may increase the hydraulic integration, reducing the risk of cavitation by the redundancy of pathways in the sapwood (VON ARX; KUEFFER; FONTI, 2013). The vessel grouping metrics are potentially important hydraulic trait in angiosperms (VON ARX; KUEFFER; FONTI, 2013), particularly for the research in trait-based ecology assessing environmental gradients and the impact of the climate change.

The community-weighed variance is a standard approach in trait-based ecology (LAVOREL et al., 2011; RICOTTA; MORETTI, 2011; SIDES et al., 2014; ENQUIST et al., 2015), largely used to understand how community trait composition is affected by ecological processes, which can limit or increase the trait variance, as the result of the environmental filtering (KEDDY, 1992) and limiting similarity (MACARTHUR; LEVINS, 1967), for instance.

The decreasing of the variance in Ks along the water table depth gradient (fig. 3b) corroborates the discussions presented so far, which have shown a higher variance in wetter communities, while drier conditions imposed a filtering effect on Ks, suggesting a selection for strategies of increased safety in the water transport (low Ks). Likewise, the vessel-size of plants in drier communities tends exhibit less variance (fig. 3b and 4), as smaller vessels reduce the risk to the cavitation (PRESTON et al., 2006; HACKE; JACOBSEN; PRATT, 2009).

Interestingly, the variance in bending resistance of xylem-wall, vessel grouping, and density exhibit an increasing toward the dry end of the gradient (fig. 3b), that is, species in drier conditions seems to have different arrangements in the xylem architecture, which could represent different well-succeed ecological strategies to cope with the water deficit, instead of a dominant structural arrangement (COSME et al., 2017).

In contrast, the same traits in the floodable communities are virtually invariable (fig. 3a), which may represent that more efficient hydraulic strategy have been environmentally filtered, such a way that floodable communities have invariably low bending resistance, cell-density and vessel grouping (fig. 3b). Such an invariance in trait values may represent optimal phenotype for fitness and/or growth rate (NORBERG et al., 2001), and the external filters would promote the convergence of trait values around this optimum phenotype (VIOLLE et al., 2012).

The shifting of the trait mean values and the constraining of trait variance suggest the effect of the external filtering selecting for different phenotypes (SWENSON; ENQUIST, 2007; SPASOJEVIC; SUDING, 2012) along the environmental gradient and the SET axis. We suggest the limiting of the vessel size variance may provide a safety hydraulic system by strategically preventing the production of larger vessels, which reduce the risk of cavitation. In other hand, the limiting of the variance in vessel grouping and density in floodable communities suggests a strategy for a more efficient water transport, as xylem produces preferably larger and solitary vessels.

It is important to inform the relevant research communities of the rich treasure of functional traits that is wood, given the consolidation of approaches treating forest systems as specific assemblages of functional traits is gaining importance in the modern ecology and global change biology (BEECKMAN, 2016). We show several wood traits potentially relevant for hydraulic efficiency and safety, which are worthwhile to be included in analysis of ecosystem modeling assessing the climate change impact over plant diversity. Moreover, it is timely that such models include the role of local scale (e.g. microtopography) as a key factor selecting species with contrasting hydraulic traits (COSME et al., 2017).

Lastly, the results unveil an interesting variation of wood space-use setups along the local water table depth gradient, by the changing of parenchyma, fiber and conductive area proportion in the wood composition. However, we did not find support for parenchyma explaining the variance in Ks, as predicted by Bittencourt *et al* (2016), given the uncorrelation between both traits.

The variability in Ks was well-correlated to the fiber-wall thickness (likely by the presence of septate fibers), and the variance in cell-size and conductive area. In our new model based on Bittencourt et al. (2016), we propose that in wetter condition (e.g. floodable forests) the high water potential enables for less space occupied by dense fibers within the wood which may be filled by thin-walled fibers (e.g. septate fibers), increasing the water capacitance (WHEELER; BAAS; RODGERS, 2007). The variance of vessel-size, conductive area and the proportion of septate fibers may also explain the Ks variance in these tropical plant communities.

A deeper investigation of the effect of steep environmental gradients (e.g. water availability) on wood anatomy traits at community-level can unveil several hypotheses regarding to plant hydraulics. The functional ecology approach may deeply contribute to the advance of the ecological wood anatomy, allowing to scale up from trait to ecosystem level and overcome the species-level paradigm.

ACKNOWLEDGMENT

This work was conducted during a visiting scholar period at University of Arizona, sponsored by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES)- PDSE program grant n° 88881.131961/2016-01. We are grateful for the field work support of Oberdan Pereira, and the precious advices of Eduardo A. Mattos and Mayara Assis in the initials steps of this study. We are also grateful for the field work assistance of Douglas T. Wandekoken, Felipe Barreto, Fabiano Volponi, Jocimara S. P. Lourenço, Nilton E. Oliveira Filho, Rodrigo Theófilo, José M. L. Gomes, and Danielly hirata. The authors declare any conflict of interest.

SUPPLEMENTARY MATERIAL

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Setups of wood space-use at species level

Appendix S2. The intraspecific variation of the wide-spread species

Appendix S3. Vessel-wall thickness, fiber content, and maximum cell-size correlation

Coding. Text file script to the community weighed metrics calculations

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SUPPLEMENTARY MATERIAL

The Trait-based Ecological Wood Anatomy: unveiling the shifting of anatomical wood traits and setups of wood space use along a steep gradient of water availability

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Contents

Appendix S1 – Boxplot showing several setups of wood space use by species and environment

In general, floodable species have less fiber-content (fig. S1), thinner fiber-wall (fig. S3), more parenchyma/non-conductive area (fig. S1), more conductive-area and wider vessels (fig. S2), and thinner fiber-wall, whereas species from dryer communities exhibit opposite trends. Moreover, the mean site values represented by the colored lines was calculated by the mean value of the 14 community weighed means in each site, showing these shifting also occurs at site-level, which was tracked mainly by the most abundant species.

Appendix S2 – The intraspecific variation of the wide-spread species

We assessed the intraspecific variation of the four widely-spread species occurring throughout the flooding gradient and the correlation between the species replacement and the community-level shifting of the wood trait composition to address two major questions: Does intraspecific variation mirror the community-level changing along the environmental gradient? What explain the shifting of the trait composition along the environmental gradient?

Just *E. nitens* and *P. grandiflorum* exhibited statistically significant changes in trait composition along the gradient, tracking the changes at community-level in the most part of the cases. Toward the dry end of the gradient, *E. nitens* increase cell-density, vessel-wall thickness, and vessel grouping, while vessel solitary fraction and non-conductive area (parenchyma) decrease (fig. S4a and Table 1). Fiber area showed an unexpected and slight decreasing. The fiber area and vessel-wall thickness of *P. grandiflorum*, in turn, steeply increases toward dryer conditions, reducing the space available for parenchyma (fig. S4a), which can be easily observed in the microscopic photos (fig. 4, main text).

Plant species composition matrices were reduced to one dimension using non-metric multidimensional scaling (NMDS). Ordinations were based on species relative abundance, following the single site species exclusion and Hellinger transformation, according to the script provide by Neves and colleagues (NEVES et al., 2017). Communities were compared by the Simpson similarity index, thus, the shifting across the NMDS axes is given to the dissimilarity caused by the species replacement.

We used the first NMDS axis which is predominantly related to the species replacement along the water table depth gradient (LOURENÇO JUNIOR, et al. 2018 – *in prep*). The first NMDS axis was compared to the shifting of wood anatomy traits analyzed in this study. The analysis reached out 14.67% of stress and was performed in the R statistical environment (R CORE TEAM, 2018), using the packages vegan (OKSANEN et al., 2018), and recluster (DAPPORTO et al., 2015).

Except for vessel grouping, the shifting of the wood traits is driven by the species replacement gradient (fig. S4b).

Appendix S3 – Vessel-wall thickness, fiber content, and maximum cell-size correlation

Fibers are not the only possible way to increase the safety against implosion in conduits. Thicker vessel-wall in wider vessels can provide an important pathway to prevent vessel collapse (ALVES; ANGYALOSSY-ALFONSO, 2002) when fiber content is low (e.g. in high Ks plants), such a way is reasonable to think in a tradeoff between fiber proportion and vessel-wall thickness along the SET gradient.

Toward wetter communities, there is an increasing of vessel-wall thickness (fig. S7) and size, while fiber proportion decreases (fig. 3, main text). According to this idea, higher fiber content provides more protection against the vessel implosion, enabling plants to produce thinner vessel wall. However, the lower fiber content in woods with larger vessels makes needed a thicker cell-wall to protect larger vessel against implosion (fig. S7).

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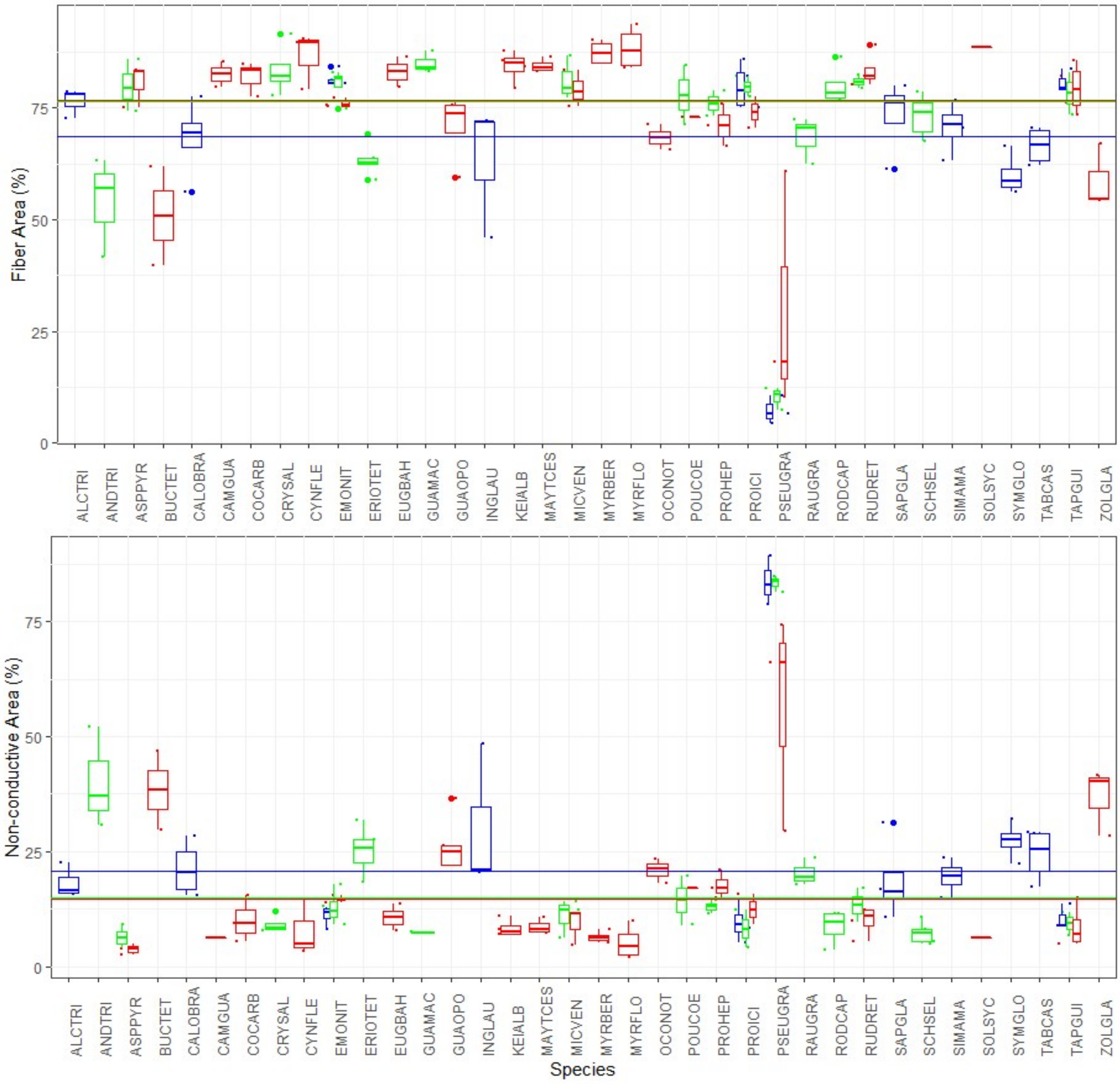
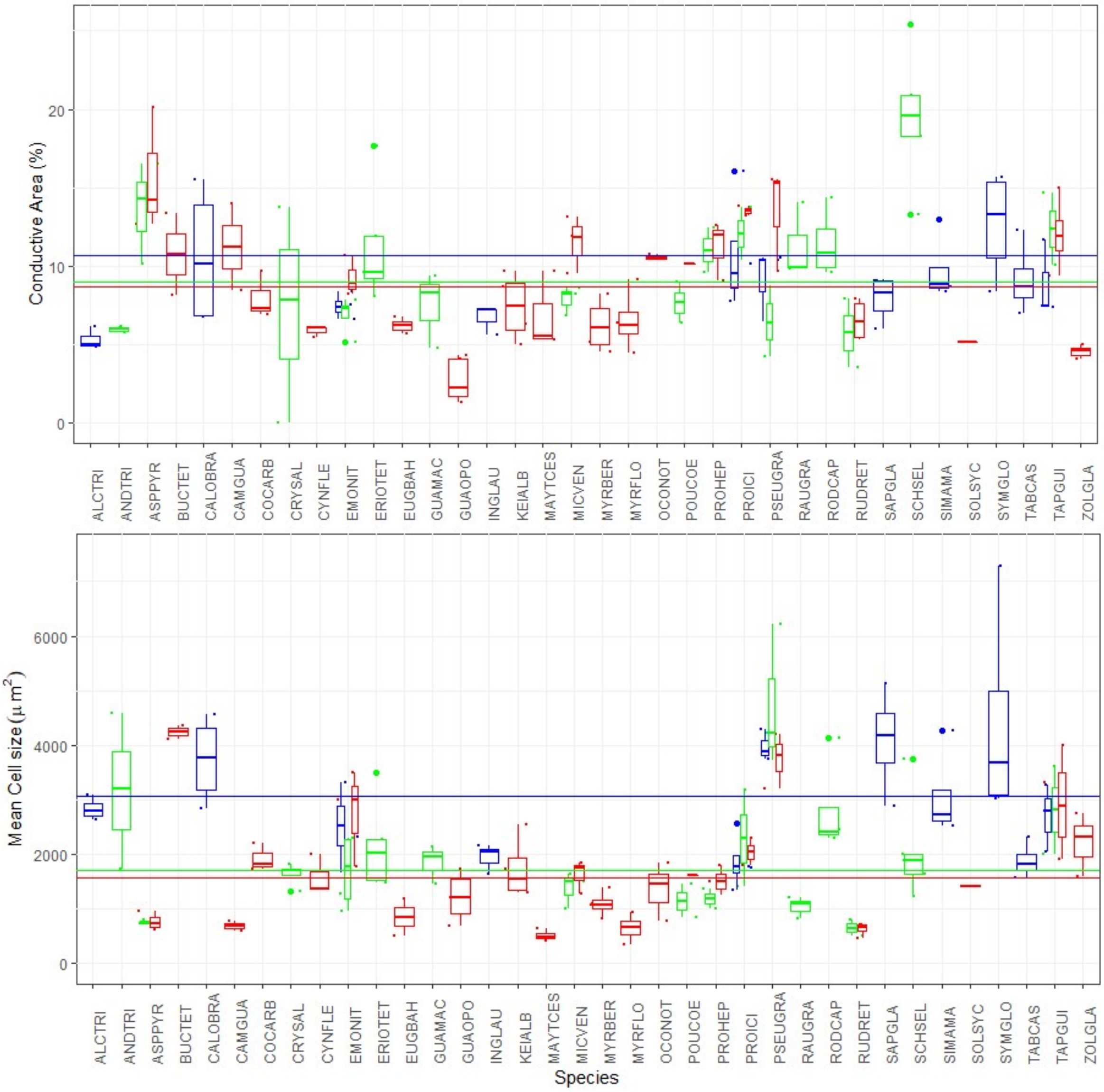
Figure S1. Species-level setups of wood space used by fiber and non-conductive area (parenchyma) in floodable (blue), intermediate (green), and dry (red) sites. Colored lines are the site means calculated by taking the 14 CWM or plant communities by in each site.



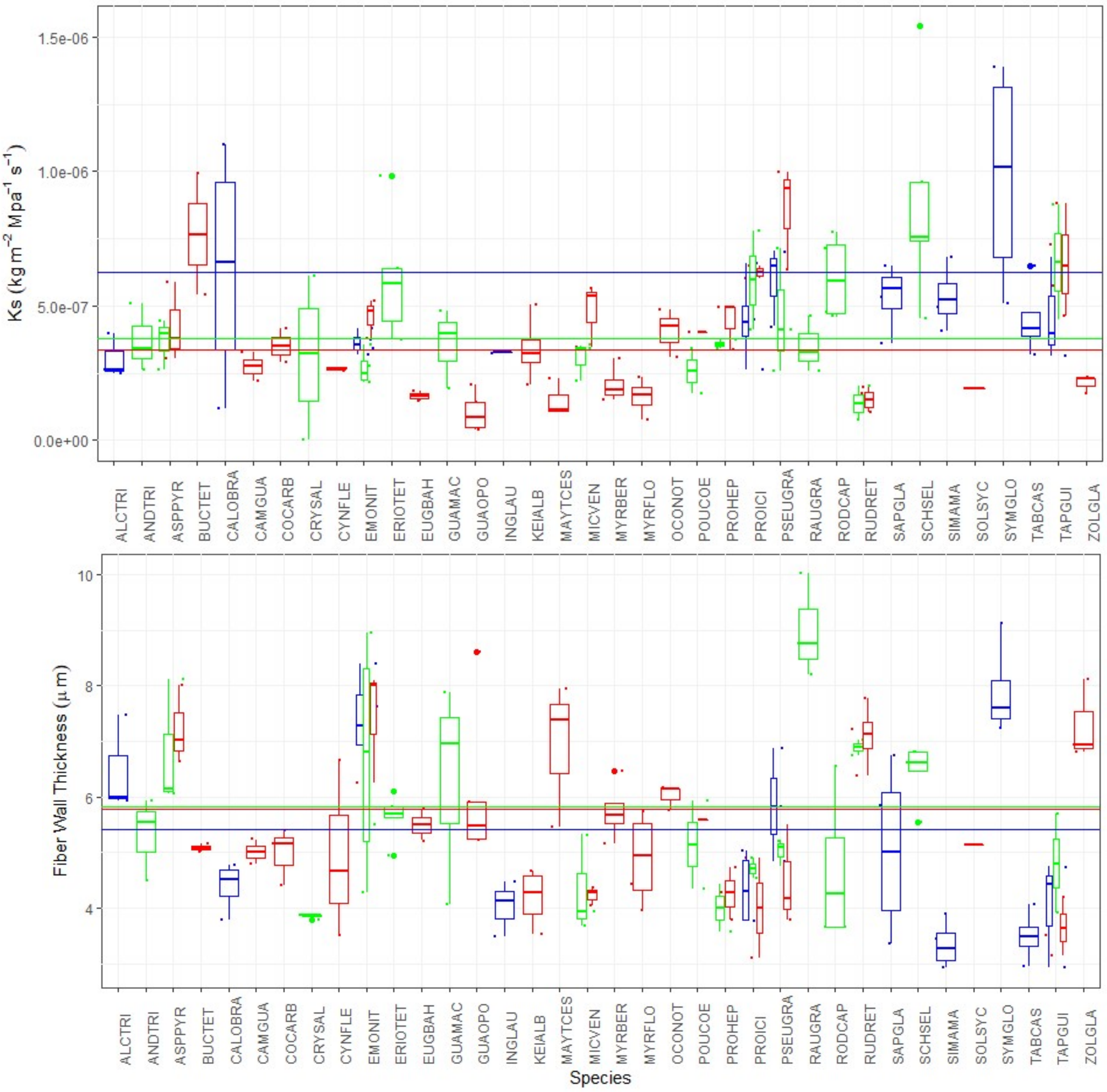
Species: *Alchornea triplinervia* (ALCTRI), *Andira fraxinifolia* (ANDTRI), *Aspidosperma pyricollum* (ASPPYR), *Buchenavia tetraphylla* (BUCTET), *Calophyllum brasiliense* (CALOBRA), *Campomanesia guazumifolia* (CAMGUA), *Coccoloba arborescens* (COCARB), *Cryptocaria saligna* (CRYSAL), *Cynophalla flexuosa* (CYNFLE), *Emmotum nitens* (EMONIT), *Erioteca pentaphylla* (ERIOTET), *Eugenia bahiensis* (EUGBAH), *Guarea macrophylla* (GUAMAC), *Guapira opposite* (GUAOPO), *Inga laurina* (INGLAU), *Keielmeyera albopunctata* (KEIALB), *Maytenus cestrifolia* (MAYTCES), *Micropholis venulosa* (MICVEN), *Myrcia bergiana* (MYRBER), *Myrciaria floribunda* (MYRFLO), *Ocotea notata* (OCONOT), *Pouteria coelomatica* (POUCOE), *Protium heptaphyllum* (PROHEP), *Protium icicariba* (PROICI), *Pseudobombax grandiflorum* (PSEUGRA), *Rauvolfia grandiflora* (RAUGRA), *Rhodostemonodaphne capixabensis* (RODCAP), *Rudgea reticulata* (RUDRET), *Sapium glandulatum* (SAPGLA), *Schefflera selloi* (SCHSEL), *Simarouba amara* (SIMAMA), *Solano sycocarpum* (SOLSYC), *Symphonia globulifera* (SYMGLO), *Tabebuia cassinoides* (TABCAS), *Tapirira guianensis* (TAPGUI), and *Zollernia glabra* (ZOLGLA).

Figure S2. Species-level setups of wood space used by conductive area and mean cell size in floodable (blue), intermediate (green), and dry (red) sites. Colored lines are the site means calculated by taking the 14 CWM or plant communities by in each site.



Species: *Alchornea triplinervia* (ALCTRI), *Andira fraxinifolia* (ANDTRI), *Aspidosperma pyricollum* (ASPPYR), *Buchenavia tetraphylla* (BUCTET), *Calophyllum brasiliense* (CALOBRA), *Campomanesia guazumifolia* (CAMGUA), *Coccoloba arborescens* (COCARB), *Cryptocaria saligna* (CRYSAL), *Cynophalla flexuosa* (CYNFLE), *Emmotum nitens* (EMONIT), *Erioteca pentaphylla* (ERIOTET), *Eugenia bahiensis* (EUGBAH), *Guarea macrophylla* (GUAMAC), *Guapira opposite* (GUAOPO), *Inga laurina* (INGLAU), *Keielmeyera albopunctata* (KEIALB), *Maytenus cestrifolia* (MAYTCES), *Micropholis venulosa* (MICVEN), *Myrcia bergiana* (MYRBER), *Myrciaria floribunda* (MYRFLO), *Ocotea notata* (OCONOT), *Pouteria coelomatica* (POUCOE), *Protium heptaphyllum* (PROHEP), *Protium icicariba* (PROICI), *Pseudobombax grandiflorum* (PSEUGRA), *Rauvolfia grandiflora* (RAUGRA), *Rhodostemonodaphne capixabensis* (RODCAP), *Rudgea reticulata* (RUDRET), *Sapium glandulatum* (SAPGLA), *Schefflera selloi* (SCHSEL), *Simarouba amara* (SIMAMA), *Solano sycocarpum* (SOLSYC), *Symphonia globulifera* (SYMGLO), *Tabebuia cassinoides* (TABCAS), *Tapirira guianensis* (TAPGUI), and *Zollernia glabra* (ZOLGLA).

Figure S3. Species-level potential conductivity (Ks) and fiber-wall thickness in floodable (blue), intermediate (green), and dry (red) sites. Colored lines are the site means calculated by taking the 14 CWM or plant communities by in each site.



Species: *Alchornea triplinervia* (ALCTRI), *Andira fraxinifolia* (ANDTRI), *Aspidosperma pyricollum* (ASPPYR), *Buchenavia tetraphylla* (BUCTET), *Calophyllum brasiliense* (CALOBRA), *Campomanesia guazumifolia* (CAMGUA), *Coccoloba arborescens* (COCARB), *Cryptocaria saligna* (CRYSAL), *Cynophalla flexuosa* (CYNFLE), *Emmotum nitens* (EMONIT), *Erioteca pentaphylla* (ERIOTET), *Eugenia bahiensis* (EUGBAH), *Guarea macrophylla* (GUAMAC), *Guapira opposite* (GUAOPO), *Inga laurina* (INGLAU), *Keielmeyera albopunctata* (KEIALB), *Maytenus cestrifolia* (MAYTCES), *Micropholis venulosa* (MICVEN), *Myrcia bergiana* (MYRBER), *Myrciaria floribunda* (MYRFLO), *Ocotea notata* (OCONOT), *Pouteria coelomatica* (POUCOE), *Protium heptaphyllum* (PROHEP), *Protium icicariba* (PROICI), *Pseudobombax grandiflorum* (PSEUGRA), *Rauvolfia grandiflora* (RAUGRA), *Rhodostemonodaphne capixabensis* (RODCAP), *Rudgea reticulata* (RUDRET), *Sapium glandulatum* (SAPGLA), *Schefflera selloi* (SCHSEL), *Simarouba amara* (SIMAMA), *Solano sycocarpum* (SOLSYC), *Symphonia globulifera* (SYMGLO), *Tabebuia cassinoides* (TABCAS), *Tapirira guianensis* (TAPGUI), and *Zollernia glabra* (ZOLGLA).

Figure S4. (a) Shifting of the hydraulic traits from the four species occurring along the water table gradient. *Emmotum nitens* (EMONIT), *Protium icicariba* (PROICI), *Pseudobombax grandiflorum* (PSEUGRA), and *Tapirira guianensis* (TAPGUI). The shift in wood traits along the water table gradient is due to the species replacement **(b)**, which was assessed by NMDS analysis. Statistically significant for P-value<0.01 (*).

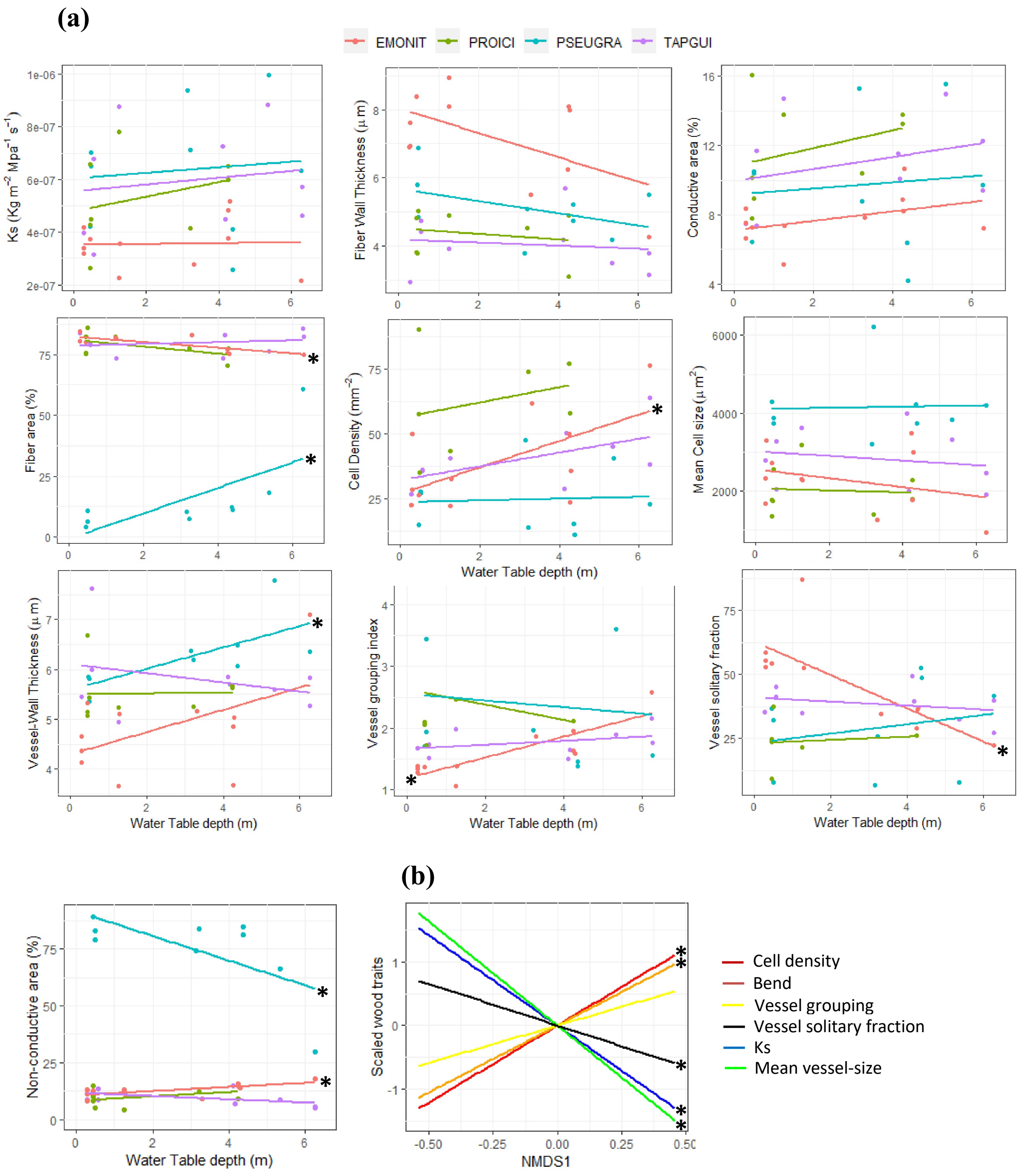


Table S1. Pearson correlation between traits and the water table gradient. Non-conductive area (NCoA), fiber area (FibA), fiber-wall thickness (FWT), cell-density (CD), conductive area (CoA), mean vessel-size (MCA), Vessel-wall thickness (CWT), vessel-grouping index (VGI), vessel solitary fraction (VSF), potential conductivity (Ks). Statistically significant (P-value<0.05) are denoted in bold.

Species	NCoA	FibA	FWT	CD	CoA	MCA	CWT	VGI	VSF	Ks
EMONIT	**0.70**	**-0.78**	-0.55	**0.61**	0.42	-0.30	0.50	**0.86**	**-0.78**	0.04
PSEUGRA	**-0.66**	**0.67**	-0.44	0.06	0.10	0.04	**0.70**	-0.11	0.23	0.10
TAPGUI	-0.52	0.21	-0.13	0.59	0.32	-0.20	-0.31	0.38	-0.30	0.16
PROICI	0.40	-0.53	-0.20	0.29	0.31	-0.07	0.02	0.38	0.30	0.29

Figure S5. Community weighed means and variance values along the water table depth gradient. Non-statistically significant (ns), marginally significant (m), for P-value=0.06, and statistically significant (*), for P-value<0.05.

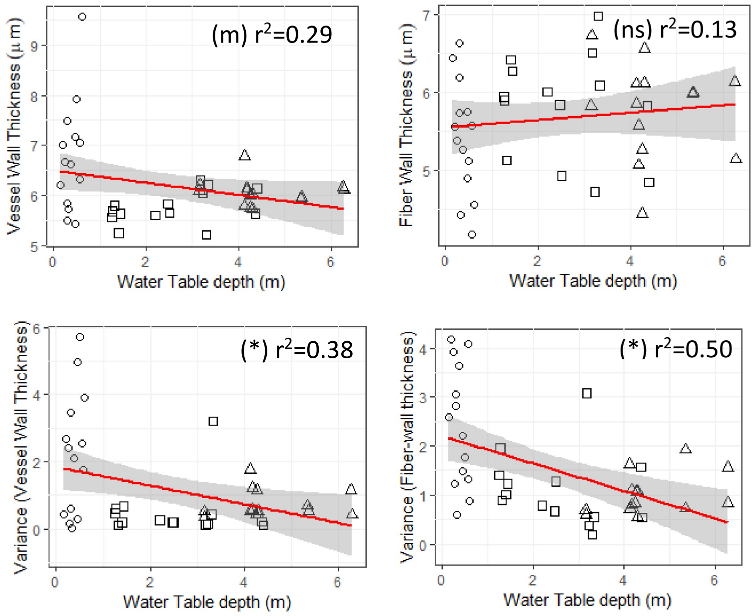


Figure S6. Vessel wall thickness (CWT), showing negative correlation with fiber proportion and positive correlation with maximum vessel-size (MaxCA). This finding suggests a tradeoff between these traits. The higher fiber content provides more protection against the vessel implosion, which, in turn, can produce thinner vessel wall. However, the lower fiber content in woods with larger vessels makes needed a thicker cell-wall to protect larger vessel against implosion. Statistically significant (*), for P-value<0.05.

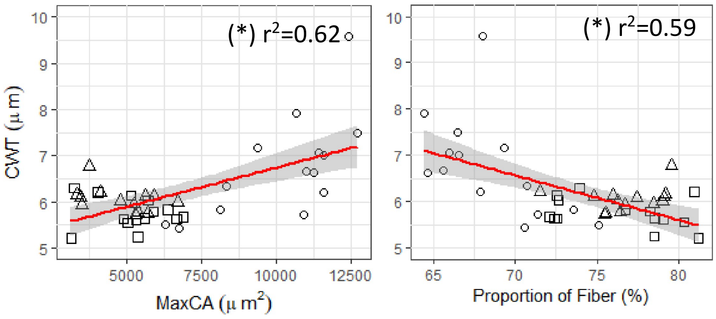
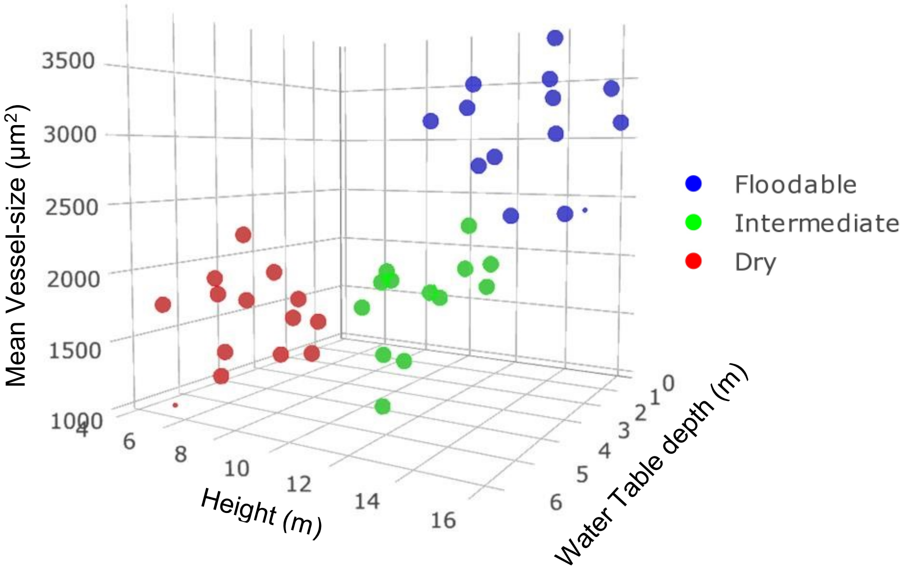


Figure S7. Height, vessel-size, and water table depth covariation, and the Pearson correlation coefficient. All correlations were statistically significant (P-value <0.01).



	Water table depth	**Height**	**Mean vessel-size**
Water table depth	1		
Height	-0.79	1	
Mean vessel-size	-0.77	0.61	1