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ANDRÉ LUIZ GILES DE OLIVEIRA

MUDANÇAS FUNCIONAIS EM ECOSSISTEMAS TROPICAIS EM RESPOSTA ÀS ALTERAÇÕES NOS FILTROS AMBIENTAIS

FUNCTIONAL SHIFTS IN TROPICAL ECOSYSTEMS IN RESPONSE TO CHANGING ENVIRONMENTAL FILTERS

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Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Doutor em ecologia.

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Orientador: RAFAEL SILVA OLIVEIRA

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

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RESUMO

Os ecossistemas tropicais estão experimentando modificações no clima, mudanças no uso da terra e aumento de invasões biológicas. Essas modificações afetam principalmente as condições e a disponibilidade de recursos, que são os principais componentes responsáveis por estruturar comunidades. A degradação de habitats e perda de espécies atingiram níveis alarmantes em florestas e em savanas neotropicais. Em consequência disso, novos filtros estão emergindo nos ecossistemas, modulando as comunidades e afetando as funções ecossistemas. O desafio do século está em compreender como plantas irão responder a essas mudanças e como mitigar os efeitos dessas mudanças utilizando técnicas de restauração ecológica para garantir a manutenção de serviços ecossistêmicos. Portanto, aqui avaliamos os efeitos de mudanças nas condições e disponibilidades de recursos no desempenho de plantas em ecossistemas florestais e savânicos. No primeiro capítulo apresentamos uma revisão teórica para demonstrar que as abordagens ecológicas de nicho baseadas em atributos funcionais permitem definir as maneiras como podemos prever as mudanças na comunidade em resposta à variação na disponibilidade de recursos. Propomos a hipótese de que mudanças na composição de espécies das comunidades em resposta à eventos extremos na maioria das vezes favorece a dominância de espécies aquisitivas do que espécies conservativas, e isso altera a estabilidade dos ecossistemas. Nos demais capítulos, utilizamos o experimento de seca na maior floresta tropical do mundo e único experimento de restauração de savanas neotropicais em larga escala para responder as questões relacionadas a esses desafios do século. Em termos de mudanças no padrão de precipitação, árvores pequenas do sobosque de florestas tropicais são capazes de tolerar a seca no solo e aclimatar seu sistema hidraúlico para maior ganho de carbono em resposta ao aumento de luz. Para ecossistemas de savanas neotropicais descobrimos que as novas condições ambientais de áreas degradadas aliadas à técnica de semeadura direta favorecem espécies com estratégias aquisitivas, com alta biomassa aérea e baixa biomassa subterrânea, o que confere baixa resiliencia ao sistema. Os sistemas restaurados são funcionalmente semelhantes às áreas degradadas pelo pastejo abandonado de gado. Entretanto, a alta biomassa em áreas restauradas favorece o processo de decomposição por invertebrados. Em constraste, a técnica de preparo do solo para semeadura direta não garante a manunteção das funções do solo pelos microorganismos, promovendo baixa biomassa e decomposição microbiana. Para o cerrado, a restauração do componente herbáceo continua sendo um desafio, principalmente em relação à necessidade de incorporar espécies conservativas e aumentar investimento em sistemas subterrâneos para garantir a resliência dos sistemas restaurados. Portanto, apesar do ganho em termos de decomposição por invertebrados a vegetação restaurada ainda é propensa a invasão e distúrbios podem se tornar catastróficos. Já os principais desafios para florestas tropicais está em elucidar se árvores pequenas serão capazes de manter a aclimatação ou se vulnerabilidade a seca pode aumentar com mudança ontogenética nas árvores pequenas. Finalmente, quanto aos desafios da década, muitas perguntas ainda continuam não elucidadas , e este trabalho representa primeiros passos sobre questões aplicadas para restauração, conservação e mudanças futuras em savanas e florestas tropicais

ABSTRACT

Tropical ecosystems are experiencing changes in the climate, changes in land use and increased biological invasion. These changes mainly affect the conditions and availability of resources, which are the main components responsible for structuring ecological communities. Habitat degradation and species loss have reached alarming levels in Neotropical forests and savannas. As a result, new filters are emerging in ecosystems modulating communities and affecting ecosystem functions. The challenge of the century is to understand how plants will respond to these changes and how to mitigate the effects of these changes using ecological restoration techniques to guarantee the maintenance of ecosystem services. Therefore, here we evaluate the effects of changes in conditions and availability of resources on the plant's performance in forest and savanna ecosystems. In the first chapter, we present a theoretical review to demonstrate that ecological niche approaches based on functional attributes allow us to define the ways in which we can predict changes in the community in response to the variation in the availability of resources. We propose the hypothesis that changes in the species composition of communities in response to extreme events most often favour the dominance of acquisitive species over conservative species, and this alters the stability of ecosystems. In the chapters, we use the drought experiment in the largest tropical forest in the world and the only large-scale experiment in the restoration of Neotropical savannas to answer the questions related to these challenges of the century. In terms of changes in the pattern of precipitation, small trees in the understory are able to tolerate drought in the soil and acclimate their hydraulic system for greater carbon gain in response to the increase in light. For neotropical savanna ecosystems, we found that the new environmental conditions of degraded areas combined with the technique of direct-seeding favours acquisitive species, with high aboveground biomass and low belowground biomass, which gives the system low resilience. The restored systems are functionally similar to areas degraded by abandoned cattle grazing. However, the high biomass in restored areas favours the decomposition process by invertebrates. In contrast, the technique of soil preparation for direct sowing does not guarantee the maintenance of soil functions by microorganisms, promoting low biomass and microbial decomposition. For the Cerrado, the restoration of the herbaceous component remains a challenge, mainly in relation to the need to incorporate conservative species and increase investment in underground systems to guarantee the resiliency of the restored systems. Therefore, despite the gain in terms of decomposition by invertebrates, the restored vegetation is still prone to invasion and disturbances can become catastrophic. The main challenges for tropical forests are to elucidate whether small trees will be able to maintain acclimatization or whether vulnerability to drought can increase with ontogenetic change in small trees. Finally, many questions are still unclear as to the challenges of the decade and this work represents first steps on applied issues for conservation and future changes in savannas and tropical forests.

SUMÁRIO

INTRODUÇÃO GERAL

Ecossistemas em todo o globo estão experimentando modificações no clima, mudanças no uso da terra e invasões biológicas (Foxcroft et al. 2017; Lewis & Maslin 2015; Rockström et al. 2009). Essas modificações afetam principalmente as condições e a disponibilidade de recursos que são os principais componentes responsáveis por estruturar comunidades biológicas pelo processo de filtragem ambiental (Cadotte & Tucker 2017; Cornwell et al. 2006; Steffen et al. 2015). Filtros ambientais determinam quais espécies são capazes de estabelecer na comunidade de acordo com seus nichos ecológicos (Thakur & Wright 2017). O nicho ecológico pode ser definido como um hipervolume n-dimensional contendo os conjuntos de fatores ambientais (dimensões) que filtram características evolutivas e ecológicas necessárias para uma reprodução bem-sucedida da população em determinado local (Blonder et al. 2018; He & Bertness 2014). Portanto, as comunidades biológicas no tempo atual estão sendo influenciadas pelas novas conformações dos filtros e modificação do nicho ecológico das espécies impostos pelas mudanças no antropoceno.

Para plantas o nicho ecológico é definido principalmente pelo espaço multidimensional composto pela disponibilidade de água, nutrientes, temperatura e luz (Chave et al. 2009; Ordoñez et al. 2009; Reich 2014). As variações em suas características (fisiológicas, anatômicas e morfológicas) ligadas à aquisição e processamento desses elementos determinam o nicho ocupado pela espécie, e em muitos casos, a sua capacidade de modificar esses filtros na escala microambiental (Ackerly & Cornwell 2007; Laughlin 2014; Laughlin et al. 2020; van der Putten et al. 2016). Esses filtros podem determinar quais características fenotípicas persistem nas comunidades, ampliando ou estreitando a largura dos nichos de cada espécie (Fernández-Pascual et al. 2017; Sommer et al. 2014). Sabemos que as combinações dessas características controlam muitos processos ecossistêmicos (Bardgett et al. 2014; Diaz & Cabido 1997; Lavorel & Garnier 2002). Por exemplo, a assimilação de carbono e os ciclos de nutrientes minerais são produtos de características foliares, como concentrações de nutrientes, capacidades fotossintéticas, defesas químicas e longevidade de vida (Cornwell et al. 2008; Freschet et al. 2012, 2013). Desse modo, as modificações nos filtros que definem as características da comunidade, consequentemente, afetará funções ecossistêmicas e a provisão de serviços ecossistêmicos.

De modo geral, florestas tropicais são especialmente determinadas pelos elevados níveis de precipitação (Hirota et al. 2011). Essa condição é responsável por filtrar um conjunto de atributos que permitem a coexistência de diferentes estratégias para condução de água e manutenção do ganho carbono (Eller et al. 2018; Brum et al. 2019). Contudo, mudanças preveem que esses filtros serão deslocados e espécies experimentarão novas condições na disponibilidade de água no solo (da Costa et al. 2014; Meir et al. 2018). Experimentos demonstram que a seca a longo prazo pode reduzir até 40% da biomassa de florestas tropicais ocasionado principalmente pela morte de arvores de grande porte (Rowland et al. 2015). Esse novo arranjo da comunidade gera novos filtros, especialmente o aumento da incidência luminosa e provavelmente diminuição na competição por água baixo do solo. Assim, espécies de pequeno porte estão experimentando novas disponibilidades de água, luz e interações com outras espécies. Isso poderá estreitar ou aumentar a amplitude do nicho realizado pelas espécies. Portanto, essas mudanças no padrão de precipitação poderá desencadear um novo processo de filtragem para florestas tropicais que irá afetar o funcionamento das espécies nesses ecossistemas.

Em savanas tropicais a invasão por gramíneas exóticas, somado ao uso e abandono do solo pela agricultura e pecuária geram novos processos de filtragem ambiental (Gorgone-Barbosa et al. 2014; Setterfield et al. 2018). Nesses ambientes que sofreram a conversão em pastagem, os novos filtros impostos pela dominância de exóticas torna a recolonização da vegetação nativa um desafio (Sampaio et al. 2019). Mesmo após 50 anos de abandono, as pastagens não conseguem recuperar espontaneamente a diversidade, estrutura e funcionamento do ecossistema (Cava et al. 2018). Desse modo, é necessário o processo de restauração ativa pelo método de plantio de mudas, transferência de estruturas subterrâneas ou semeadura direta (Sampaio et al. 2019; Silva & Vieira 2017). No entanto, para obter algum êxito contra as invasões biológicas esse processo exige o preparo do solo. Isso gera um solo com estrutura diferente da comumente encontrados em cerrado conservados (Coutinho et al. 2019). A modificação desses filtros pode favorecer um conjunto de características ligadas a um nicho ecológico diferente do desejado (Lohbeck et al. 2013; Sterck et al. 2006). Ainda, esses novos filtros podem continuar a favorecer processos de invasão ou manter a comunidade em estado instável, distinto de um cerrado nativo. Assim, entender e discutir como esses novos filtros podem auxiliar no processo de restauração e como o processo de restauração deve ser ecologicamente pensado por meio desses filtros demonstra alternativa para evitar problemas nesse processo.

Nesse contexto geral proponho avaliar como a emergência de novos filtros ambientais podem afetar as comunidades tropicais, focando principalmente nos efeitos da mudança na precipitação em florestas tropicais e mundaças nas condições edáficas em savanas tropicais. Usamos atributos funcionais em savanas e florestas para responder a questões aplicadas e fundamentais relacionadas ao funcionamento de plantas e ecossistemas. Dividimos a tese em quatro capítulos, sendo o primeiro um artigo de opinião baseado na revisão da literatura onde discutimos e propomos novas hipóteses sobre efeitos da modificação desses filtros nas comunidades biológicas, principalmente em savanas e florestas. No segundo, avaliamos experimentalmente como a modificação na disponibilidade de água do solo pode afetar o funcionamento de árvores pequenas que se localizam no sobosque em florestas tropicais. No terceiro e quarto capítulos avaliamos como a mudança do uso da terra afetam as comunidades vegetais recém-estabelecidas e como isso afeta funções de de ciclagem de nutrientes como a decomposição. Por fim, discutimos baseados nos nossos resultados como florestas e savanas vão responder as mudanças globais.

Objetivo geral

Avaliar como a emergência de novos filtros ambientais podem afetar as comunidades tropicais, focando principalmente nos efeitos da mudança na precipitação em floresta tropical e mudanças no uso da terra em savana neotropical.

CHAPTER 1 – Overview/Opinion

Global change, ecological niches and the future functional composition of tropical plant communities

Journal norms: *Annual Review of Ecology, Evolution, and Systematics*

Global change, ecological niches and the future functional composition of tropical plant communities

Abstract

The anthropocene is marked by extreme events, such as droughts, mega-fires, and over-use of soil fertilizers. These changes act as novel environmental filters, driving local species extinction and shifts in community composition. The traditional approach to forecast changes in biological communities is largely based on the assumption of niche conservatism. This approach invokes a correlative method where the ecological niche is defined by the climatic envelope occupied by the species. However, using the functional trait approach improve the to predict the n-dimensional hypervolume niche might forecast of the changes in environmental filtering because of its mechanistic nature. We propose the use of fast-slow continuum traits as a useful framework to predict plant community shifts in tropical ecosystems. We consider this approach useful to predict growth, survival and reproduction of species, elucidating trade-offs and species fitness. We discuss the use of ecophysiological traits and evolutive-life-history as performance and fitness predictors. Furthermore, the use of community weighted means based on these traits is useful to characterize and understand the viable ecological strategies with communities. These approaches allow us to define the ways we can forecast the shifts in community-based in response to changes in conditions and resource availability. Finally, we propose the hypothesis that shifts to acquisitive species are more likely in response to extreme events, and this has implications to ecosystem instability. To address these hypotheses, we suggest measuring individual-traits at the field and experimental approaches to better understand species potential niches and their role in community assembly. We need to understand the response of ecological strategies and the capacity of environmental filters to select certain strategies within communities to improve our capacity of predicting niche displacement and selecting adequate sets of species for restoration considering novel scenarios of water and nutrients in the next years.

Introduction

Anthropocene environmental changes are increasing in space and magnitude, imposing new conditions and resource availabilities levels that affect plant survival (Lewis & Maslin 2015). The high production and use of fertilizers, large-scale changes in land use, urban expansion and extreme climatic events impose novel conditions and resource availability in natural communities (Bardgett et al. 2013; Bradford et al. 2016; da Costa et al. 2014; Ripple et al. 2017). Following these changes new ecological filters emerge and determine new “assemblage rules” that shift the trajectories and functioning of ecosystems (Cadotte & Tucker 2017). These changes represent a major challenge to the conservation and management of natural ecosystems. In turn, understanding the potential ecological niche displacement in response to the new ecological filters might help to overcome these challenges.

The ecological niche is an emergent ecosystem property that determines the physiological range of tolerance to environmental factors and biological interactions threshold within the environment defined by a n-dimensional hypervolume. Originally proposed by Hutchinson (1957), this hypervolume can be separated into realized niche and potential niche (Soberón & Nakamura 2009). The realized niche can be defined by pack of interactions in response to biotic and abiotic properties. The fundamental niche results from evolved environmental tolerances (Soberon & Arroyo-Peña 2017). This means that the fundamental niche represents the capacity of species have given the range of environmental changes possibilities (Kearney & Porter 2004). For example, when looking to a set of traits on the multidimensional space that predict species performance, we can visualize the realized niche in current time. The Figure 1 shows a representation of the fundamental ecological niche represented by the box and environmental filter represented by an rectangle. Here we suggest that the overlap between box and rectangle is a hypothetical representation of the realized niche of a species (Fig 1a). We also proposed that because the environmental changes alter the conditions and new ecological filtering appears (represented by rectangle Tx displacement Fig 1 b), it would select the new optimal set of traits to species performance. Therefore, a new ecological niche space will emerge depending on how large is the fundamental niche for a given species (Lavergne et al. 2010). However, for a given species with a restricted fundamental niche, the species might be negatively selected and may become extinct. Thus, the potential to elucidate the fundamental niche to predict shift in communities in response to changes in environmental conditions rely in improving our capacity to accuretaly predicting the fundamental niche. However the central questions remains to be answered: How can we estimate the niche range using measurements of species-levelfunctional traits?

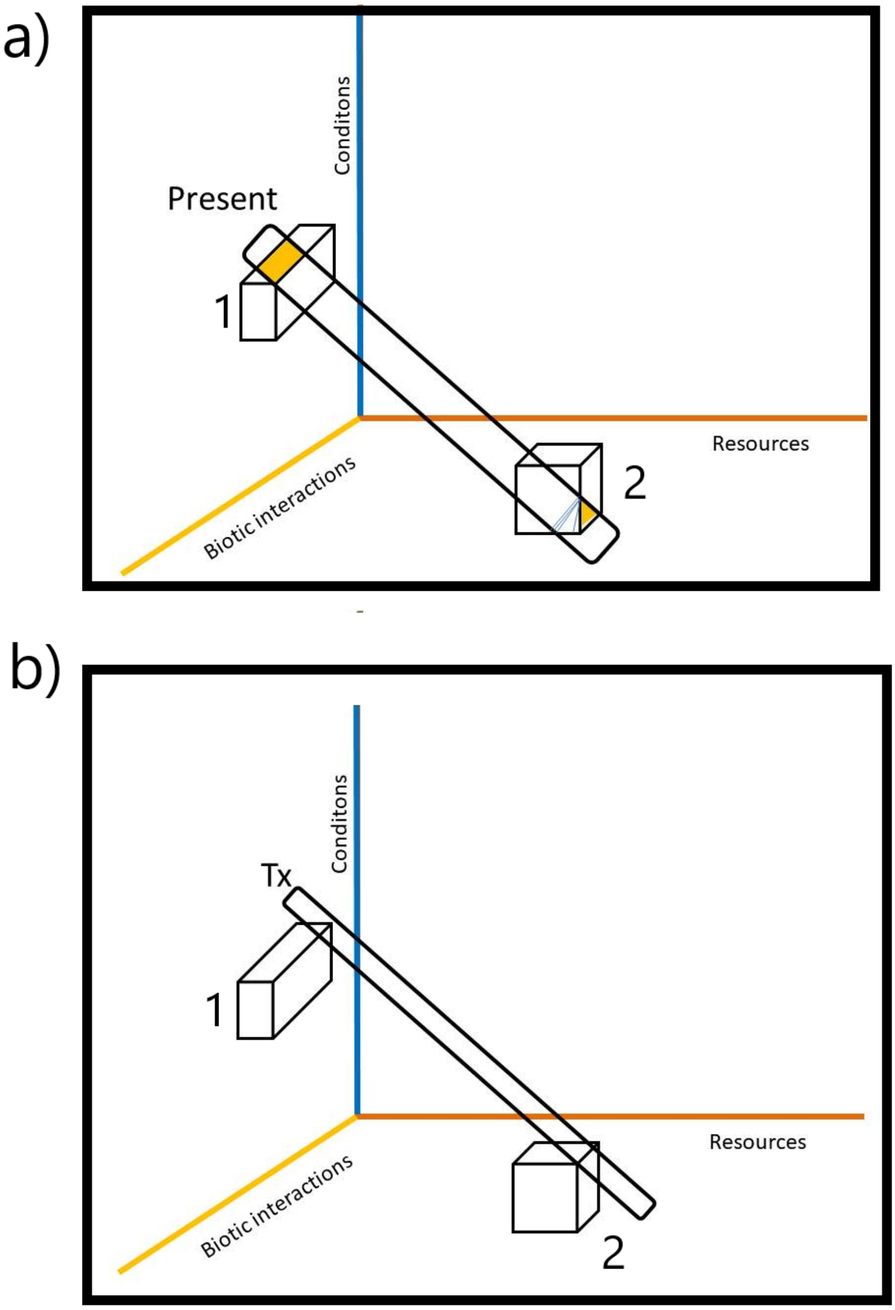

Fig.1. The summary of three dimensions of hypervolume niche considering a range of conditions and resources, and biotic interactions considering a) the ecological niche in current time, and b) ecological niche in hypothetical time (Tx) in the future. The rectangular and cube box represents the dimensions of niche for two species. The

rectangle represents an environmental filter. The overlay with rectangle and box represents (Yellow fill) . The rectangle presents a higher overlap in example A (higher niche amplitude), however, due the emergence of new environmental filters the niche amplitude decreases (B) showing how might affect the species differently, that sometimes could lead to species extinction.

The 'ecological niche' concept is an overarching theory to describe and predict the impact of environmental changes on plant performance and distribution (Hanspach et al. 2010). Traditionally correlative approach is used to take account the patterns of distribution and their relation with abiotic gradients predicted by climate variables (Species distribution models-SDM's) (Buckley et al. 2010; Elith et al. 2010; Ehrlén & Morris 2015). This approach is based on the niche conservatism premises disregarding niche displacement by acclimation, phenotypic plasticity (the ability of one genotype to express varying phenotypes when exposed to different environmental conditions), or via evolutionary adaptation (changes in allele frequency in populations) (Ackerly & Monson 2003; Hadly et al. 2009). Most models used to predict niche neglect information about plasticity and other variations within species, but it could be a conceptual problem. Correlative SDMs may predict large scale responses but the models not accurately predict local-level responses due to high environmental heterogeneity, especially in edaphic conditions, that rarely considered in these models (Diamond et al. 2012; Kearney 2006; Zuquim et al. 2020). Advances in functional ecology have paved the way for a more mechanistic approach to predict the species ecological niche (McGill et al. 2006; Silvertown 2004). The use of traits represent a mechanistic approach to define niches range because traits should describe/inform the organisms "sensitivity" to conditions and resources variability and consequently define the thresholds of performance by species (Adler et al. 2014; Ackerly & Cornwell 2007; Kraft et al. 2015)

The number of studies using the trait-based approach to predict ecological niche increased in the last two decades (McGill et al. 2006; Treurnicht et al. 2020). However, only recently we realized that most of the commonly used traits are unable to predict basic and key functions such as survival, growth and reproduction (REF). Also have low capacity to predict evolutionary and ecological history, limiting a mechanistic understanding necessary to predict the effect of local and global change (Adler et al. 2014; Sterck et al. 2011). To determine the multidimensional ecological niche, one needs to gather knowledge on different axes of trait variation for plants. For this, we need additional experimental approaches to develop hypotheses about the set of functional traits with most potential to aggregate and support the different axis of trait variation for plants.

The fast-slow continuum represents a comprehensive framework that reflects coordination of multiple plant attributes along one major axis (Wright et al. 2004). It is possible to create an integrated whole-plant economics spectrum (acquisitive/fast and conservative/slow spectrum) that influence performance and fitness consistent with a trait-based theory about underlying adaptive mechanisms. Specific sets of conditions and resource availability (water, light and nutrients) select the species traits/ strategies across the evolutionary time. Environmental conditions and resource availability also determine the prevalence of certain ecological strategies in plant communities. If climate/conditions change, one can expect changes in the relative abundance of certain ecological strategies. For example, increasing soil nutrient availability, the community can be shifted from a dominance of slow-growing to fast species (Lambers & Poorter 1992; Russo et al. 2005a). Changes in functional composition led to shifts in ecosystem process (Lavorel & Garnier 2002; Mori et al. 2013). Fast traits are associated with faster rates of ecosystem processes such as decomposition or primary productivity (Cornwell et al. 2009). Therefore, the Anthropocene environmental changes might change the functional composition and lead ecosystems into new fuctional states.

The discussion on ecosystems shifts based on shifts in the dominance of plant strategies offers a good starting point to answer whether there are contrasting trajectories among communities dominated by fast-slow species in response to multiple abiotic drivers. Under which conditions certain strategies are expected to become dominant? Specifically, in the tropics, savannas and forests represent the dominant ecosystems with high diversity and most important to productivity, carbon stocks, nutrients and the water cycle (Dexter et al. 2018). Precipitation is a first-order determinant of biome configuration according to mean annual precipitation (MAP) (Hirota et al. 2011), following disturbance, soil fertility (Lloyd et al. 2015) and the feedback mechanisms at play. To predict how environmental changes will affect all dimensions of biological diversity in tropical ecosystems, we need a well-grounded framework to offer subsidies to management, conservation and restoration studies. The focus of this viewpoint will be on discussing the mechanistic approach to define ecological niches and how it can be used to predict the responses of plants and communities in the tropics. We address the following questions: 1) Are commonly used traits really functional? 2)Which traits should be used to define the sensitivity of plants to abiotic drivers? 3) Which are the determinants of conservative or acquisitive strategies selection in ecological scale? 4) Does climate change have the potential to replace communities across the fast-slow axis? What are the implications?

Are commonly used traits really functional?

The current definition of functional trait is “any trait that is linked to species’ fitness and performance” (Díaz et al. 2016; Violle et al. 2007). Many traits measured at the organ and whole plant level have been proposed as “functional” before any a priori measurement of their functionality (Díaz et al. 2016; Reich 2014a). Most of these traits integrate multiple aspects of plant function making it difficult to interpret their values. For example, the exhaustively studied specific leaf area (SLA) is a trait related to foliar investment, leaf life span and photosynthetic capacity (Poorter & Bongers 2006; Poorter et al. 2009). However, SLA is also commonly used as a predictor of ecological strategies along the fast-slow continumm (Onoda et al. 2017; Wright et al. 2004). Several studies have shown that this trait is not a consistent indicator of plant performance (ie growth or survival). Plants can achieve a given growth rate through either low leaf biomass but high specific leaf area or high leaf biomass but low specific leaf area (Bonser 2006). In case of trees, where woody accounts for most of the plants biomass, the use of leaf foliar traits (SLA; Leaf Area- LA; Leaf Dry Mass Content-LDMC) to predic fast-slow strategies may not be adequate (Poorter et al. 2018, Rowland et al. 2019). Thus, to use a single trait to predict the growth performance maybe is not adequate (Worthy et al 2020).

Deciding which traits to measure is one of the most difficult aspects of predict ecological niches and their response to change in environmental conditions. It is often difficult to know, a priori, the mechanism responsible for driving a particular community- or ecosystem-level process, much less the organismal trait most closely linked to the mechanism (Funk et al. 2017). Overall, plants differentiate along the resources axis, most of the time driven by water, light and nutrients availability. The strategies of plants to deal with resources seem to range among traits correlated with reproduction, relative growth, and consequently determine survival and fitness (Martínez-Garza et al. 2013; Poorter 2009; Russo et al. 2005a; Sterck et al. 2016). Thus, there is an n-dimensional space called functional niche based on traits, phylogenetic relation and their relation with environmental resources (Blonder et al. 2018; Cornwell et al. 2006; McGill et al. 2006). Yet, it remains unknown which traits and environmental variables are most important to determining fundamental ecological niche, under which conditions and their potential of displacement. Even more difficult which traits can be incorporated into the alleles by epigenetic effects and guarantee the expansion of the species' niche (Burggren 2016; Latzel et al. 2013; Syngelaki et al. 2020). Ignoring variation in trait-fitness relationships across environments has hindered progress toward using traits to make general predictions about how species respond to environmental change.

The use of integrative traits that summarize the outcome of several plant processes (e.g. photosynthesis, respiration, nutrient allocation, life-history strategies) might be a good way forward. For example, based on fast-slow plant economics theory (Reich 2014), species with lower leaf mass per area (LMA) may be expected in high light environments (Lusk et al. 2008). Similarly, combinations of conservative traits (e.g., low root specific length investment and high LMA), may be expected to have superior growth in resource poor (e.g., low light) environments. However, multiple growth performance peaks occur at a given point on light or soil gradients and many of these peaks combine an acquisitive belowground strategy and a conservative aboveground or leaf level strategy (Worthy et al. 2020). Therefore, whole-plant coordination may be more common in systems where resource levels above- and belowground co-vary (Freschet et al. 2015). In Contrast, in a system that has the independence of the selective environments above- and belowground, it becomes more difficult to predict plant performance using only above- or bellow ground traits (Worthy et al. 2020).

Which traits should be used to define the ecological niche and the sensitivity of plants to abiotic drivers?

The use of species niche concept (i.e. adaptations to environmental conditions) has received much attention in functional diversity research (Blaum et al. 2011; Kattge et al. 2011; Reich 2014b; Salguero-Gómez et al. 2015; Wright et al. 2004). The functional diversity indexes have aims to capture the multidimensional space encompassing the trait variation within a plant community. Functional diversity (FD) is commonly measured as the diversity of trait combinations that reflect differences in species' adaptations to the environment and in their effects on other species, i.e. their functional roles (Petchey & Gaston 2002; Tilman et al. 2001). Although, the set of traits and the functional index does not adequately predict species ecological niche on the plant community (Dehling & Stouffer 2018). Indexes such as Community weighted mean (CWM) seems to be good descriptors of the dominance of certain ecological strategies within plant communities (Laliberte & Legendre 2010; Muscarella & Uriarte 2016). In practice, however, there is a recent tendency to measure FD as a general diversity of traits, i.e. without a definition of the ecological process of interest and without testing whether the selected traits are relevant for a specific process (Mlambo 2014; Rosado et al. 2013). The current practice of measuring FD indirectly via species traits restricts analyses to species with comparable sets of traits (Dehling & Stouffer 2018). However, species might represent different strategies to lead with environmental filtering and achieve the same fitness. For example, if a trait is negatively related to survival, then it may be positively related to individual growth or reproduction (Metcalf et al. 2006; Rüger et al. 2018). For example, wood density negatively affects individual growth rates but positively affects survival rates because faster tree diameter growth can be achieved by constructing low density wood, but this comes with a higher risk of damage and death from multiple causes (Visser et al. 2016; Wright et al. 2010).

Evolutionary history is another important variable to consider in trait-based ecology. Much of the trait variation between species is associated with different life forms and evolutionary history within a common environment (Ackerly & Monson 2003).The species lineage that experienced greater conditions and resources variabilities during their evolutionary history must have a high potential to express plasticity and, consequently, a higher breadth fundamental niche (Fox et al. 2019). This may underpins patterns of intraspecific variation among traits and across seasons (Donohue et al. 2007; McKown et al. 2013), acclimation responses to variable temperature (Cavender-Bares et al. 2005), and also trait shifts along ontogeny (Bartholomew et al. 2020; Lusk et al. 2008; Poorter 2007, 2009). However, traits with high plasticity may be a relatively poor indicator of life history strategy (Adler et al. 2014). For example, for trees, traits measured at the individual level were surprisingly poor predictors of individual growth, whereas traits measured at the species level were reasonable predictors of growth (Poorter et al. 2018). These demonstrate that the use of trait based on specie-level is a product of evolutionary history (life-history) (Snell-Rood et al. 2018), and might provides a evidence of ecological strategies (Fast-Slow). Overall the assumed links between functional traits and life history have been poorly tested. We encourage the field experimental approach to increase knowledge about the range of trait and species. Complementary the sampled of traits of species in long-term experimental, focusing in traits linked to survival and fitness and how it ranges in environmental gradients, seems a better way to predict the niche displacement for the future climate changes.

The good integrative traits are the onesrelated to organisms growth, survival, and reproduction (thus fitness).Therefore, there are few key plant traits that would be more integrative and efficient to predict responses to changes in abiotic filters than others. For example, the increasing concentration of carbon dioxide gas in the atmosphere might be causing changes species composition change (Phillips et al. 2002, 2004).In these case, plant performance in terms of carbon, nutrients and water exchange to be predicted based Leaf level gas exchange measurement, as Photosynthetic carbon assimilation rate (A), dark respiration rates (R_d), stomatal conductance to water vapor (g s) and Maximum carboxylation capacity values (Vc_{max}) (Bartholomew et al. 2020; Domingues et al. 2007). In terms of water relation, the hydraulic safety margin (HSM), given by the difference between minimum xylem water potential (Ψmin) and xylem vulnerability to embolism (P50 and P88 - the water potential when plants lose 50% and 88% of their maximum hydraulic conductivity) is an important trait affecting plant mortality rates during intense water stress (Anderegg et al. 2016; Barros et al. 2019). Embolism resistance is a property of the water-conducting tissues of plants, while the Ψmin is a function of both environments (including recent precipitation and soil type) and other hydraulic plant traits, such as rooting depth, stem capacitance and stomatal regulation (Bhaskar & Ackerly 2006; Brum et al. 2019; Choat et al. 2012). Also, plant hydraulic traits are correlated with plant's capacity to assimilate carbon, since stomatal regulation to prevent excessive embolism also reduces CO2 acquisition (Eller et al. 2018; Brodribb et al. 2010). In addition, these hydraulic traits are important to determine , have been identified as important in the species distribution of species across in environments with different water availability gradients. Consequently, plant hydraulics traits might play an important role in the growth-mortality tradeoffs, and determine fitness in future precipitation changes.

The traits related to seed reproduction and vegetative reproduction are important to determine reproductive axes from species performance. In general, the seed mass is a good predictor to seedling surviving (Baraloto et al. 2005), although the reproduction efficiency might also be influenced by vegetative reproduction (Pistón et al. 2019). Overall, belowground bud banks and traits of clonal growth are important type of reproduction principally in fire-prone savannas vegetation (Ott et al. 2019; Pausas et al. 2018). The amount of non-structural carbohydrate (NSC) in these organs might be a good predictor of surviving after disturbance (Miranda et al. 2020; Tolsma et al. 2007). However, only few studies addressed the mechanistic approach from belowground resprouts in the tropics. The advances in land use and the conversion of native vegetation to productive soil use in the last two decades (crops, livestock) draw attention to explore the belowground traits and how these organs determine plant surviving after disturbance.

The root structure and their functioning are responsible for soil resources acquisition (Lambers et al. 2008). Nutrient-impoverished soils frequently involves specialized root structures (i.e. cluster roots) or symbiotic structures (e.g. mycorrhizas, root nodules) (Lambers et al. 2006). Apparently, these specialized structures or symbioses are commonly associated with efficient nutrient uptake from the soil solution, conferring rapid growth, and roots proliferation in response to localized nutrient sources (Lambers et al. 2006). However, variation in soils P might generate P toxicity symptoms (P sensitivity). The P toxicity symptoms in plants are frequently observed in species from severely nutrient-impoverished soils in ancient landscapes when plants are exposed to slightly elevated P-supply (Lambers et al. 2003; Shane et al. 2004). Therefore, is expected the shifts in P availability potentially leads community species turn-over and consequently changes roots traits pattern (Zemunik et al. 2016). The eminent problem in P on the systems is that the might affect nutrients, growth and species surviving on the community (Penuelas et al. 2020). However, the root traits and symbiotic structure, are both low explored by mechanistic studies. Thus, studies analyzing the response of roots traits to changes in soils fertility are an important way to understand what is the limit from species ecological niche and how species communities will shift in response to soil environmental filter changes.

We reinforce the importance of examining traits in the context of the entire plant where plant responses to environmental variability require coordinated responses of whole-plant (Bonser 2006). We encourage the use of ecophysiological traits, such as embolism vulnerability, hydraulic safety margin, photosynthetic capacity, will exhibit the strongest mechanistic links to vital rates given their direct link to resource use (Lauglin et al 2020). Furthermore, use of below-ground traits, as roots, and their integration to aboveground traits and experimental and environmental conditions, is an important outstanding point to elucidate what are the determinants in whole-plant coordination and how they vary among environmental gradients to allow the survival, growth, and reproduction at the scale of individuals. Also, we emphasize the importance of quantifying relative growth rates in the different conditions, which is a necessary step for defining if the specie can tolerate a given environment and for quantifying the fundamental niche of a species.

Is the fast-slow framework useful to predict changes in community functional composition in response to climate change?

The most of the variance of economic functional traits at organ scales is not explained by broad-scale climatic influences (Cornwell et al. 2008; Freschet et al. 2012). However, community-weighted mean traits may be better explained by climate (Laughlin et al. 2012). Using the community-weighted mean traits we have ways to try to predict the shifts of dominant community and resilience from ecosystems. For example, soil nutrients are key drivers determining the dominance of slow x fast-growth strategies (Lambers & Poorter 1992; Russo et al. 2005b; Reich 2014), which have a great impact on ecosystem resilience, due to their influence on vegetation resistance and recovery. Plant communities at sites with high resource availability likely have higher recovery capacity, despite their lower resistance to disturbances. In this context, we propose nutrient availability as a strong driver on the shifts in ecosystems via changes in community (Fig. 2) .

Overall, soft traits can be used to predict global patterns of ecological niche and ecosystem functions in forest and savannas communities (Garland et al. 2020). However, the great variation among environmental filtering in these ecosystems provides different roles of traits to predict resources strategies and ecosystems functions (Hoffmann et al. 2012a; Ratnam et al. 2011; Silveira et al. 2020). For example, leaf traits seem important to grasses that have the whole aboveground biomass composed by leaves. In grasses, leaves have a higher effect on carbon gain to whole-plant higher than trees (Jackson et al. 1994; Pearcy et al. 1987). Therefore, using the only leaf traits to predict the fast-slow spectrum to forest seems the high level of generalization and shows the poor predictor individual performance related to ecological strategies (Poorter et al. 2018; Rowland et al. 2020b). In forests, stem traits are more integrative regarding water and C relations than leaf traits.

The most used stem trait is wood density. High wood density is usually associated with a low efficiency to water transport, but with stronger and more flexible mechanical properties and greater protection from drought stress. There is coupling of stem hydraulic, leaf hydraulic and leaf C flux dynamics ((Brodribb et al. 2007; Meinzer et al. 2009) leaf and canopy C and N dynamics (Ollinger et al. 2008; Wright et al. 2004); stem hydraulic conductivity with leaf area (McCulloh et al. 2010); and leaf area with C fluxes (Reich 2012; Stark et al. 2012). In adition, according with the growth-hydraulic safety margin trade-off (HSM), fast-growing plants have a high return in terms of carbon gain at the risk of xylem embolism propagation (lower HSM) compared to slow-growing plants and safe hydraulic system avoiding xylem embolism (higher HSM) (Oliveira et al. 2021, Eller et al. 2018).Together these strongly support the idea that stem traits associated with C, N and water dynamics represent a slow-safe x fast-risky' trade-off. Therefore for forests, this axis might represent a better framework to predict response to future changes.

Extreme drought events or N fertilization can be seasonal and increase the risk of mortality or favour the population increase of certain species in the community. If these events become recurrent or "chronic", a new set of environmental conditions may be established and favor a new assembly of the community. We propose here that in response to changes in nutrient availability or new climatic regimes, communities can shifts from acquisitive to conservative, and vice versa. For example, species with acquisitive strategies (high SRL) were less impacted by P and water stress than those with conservative ones (low SRL) (Fort et al. 2015). In addition, in savannas, the input of P in the soil favour invasive grasses that have fast resources use, leading the vegetation to an acquisitive station, dominated by invasive grasses (Lannes et al. 2016; Nardoto et al. 2006). In temperate ecosystems, simulations of potential future climate indicate that higher temperatures could favour acquisitive species and, thus, may contribute to changes in the relative abundance of conservative and acquisitive species (Wentz 2017;Griffin-Nolan et al. 2019). In conservative ecosystems (ecosystem having the dominance of the conservative strategy) the short-term warming can lead to the dominance of acquisitive fast-growing species over conservative species, thus reducing species richness leads low resilience of systems (Debouk et al. 2015). Despite the ecosystem having the dominance of the conservative or acquisitive strategy, the change in dominance strategy is only allowed to cause communities to maintain a range of diversity of strategies species still on the community, even in low dominance. However, there is a lack of experiments to investigate the shift of community dominance and vegetation stability of tropical ecosystems. One example, is a drought experiment in the Amazon rainforest that have last more than 17 years and offer interesting results for understanding the trajectories of the community and the real capacity of species response to change in the environmental filter (Bartholomew et al. 2020; Binks et al. 2016; Bittencourt et al. 2020; Rowland et al. 2020a). In fire-prone ecosystems, unburned communities usually have the lowest species diversity, the most acquisitive leaf traits, and the fewest phylogenetic lineages, but with higher phylogenetic distance (Wigley et al. 2016). Burned communities, on the other hand, usually have higher species richness, and more resource-conservative leaf traits, and higher number of phylogenetic lineages (Hoffmann et al. 2012b; Maracahipes et al. 2018; Wigley et al. 2016).

Can we predict the displacement of community strategies based on changes in resources avaibility?

Species with fast reproduction rates will present a better performance in newly founded and low-density populations, but as their population increases in size and reach the carrying capacity, the species with slower-reproduction rates will instead be favoured, instead, because of their ability to resist the low resources availability and a greater competition ability (Wright et al. 2019). Such shift is the main factor favouring the dominance of fast or slow species in the community. Thus, the fitness of fast or slow species depend more on resources that favour increases in population growth on the community (See fig 2).

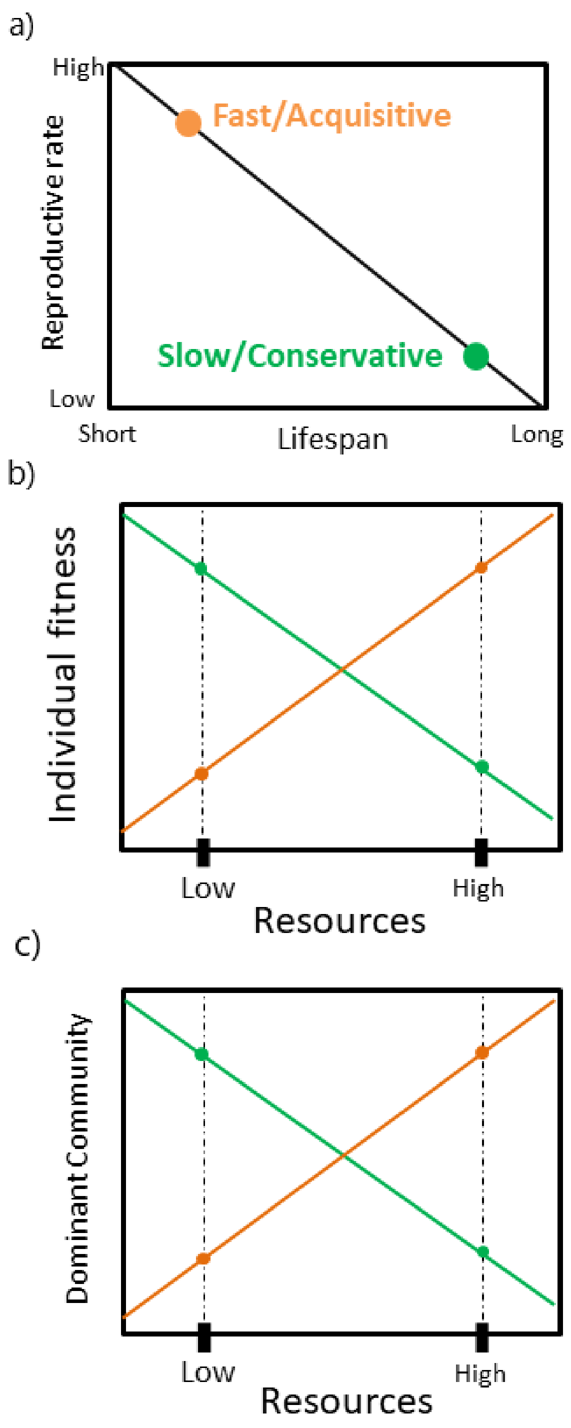

Figure 2. Fast-slow spectrum in environments with contrasting resource availability a)Relationship between lifespan and the reproductive rate. The Point represent species b) Individual Fitness in contrasting resources. Each point represents species. Arrows represent possibles trajectories to shifts increasing or decreasing fitness. c) Dominant strategies on the community in contrasting resources. The point represents the mean of community traits.

The segments represent the possible continuum trajectories to change the mean of community traits. The colour represents the fast-slow continuum.

The persistence of a specie and increase in population size under new environmental conditions can occur via the expression of phenotypic plasticity or evolution via selection for particular phenotypes (Fox et al 2019). Plasticity acts at the level of the individual is often hailed as a rapid-response mechanism that will enable organisms to adapt and survive in our rapidly changing world (Chevin et al. 2010; Fox et al. 2019; Snell-Rood et al. 2018). What seems possible is that if this environmental variation is sufficiently predictable, for example, using environmental cues or just before reproduction (e.g epigenetic), we can expect plasticity to evolve in individual reproductive effort throughout reproductive events. In this way, fast species may have greater plasticity because they are more responsive to changes (Rozendaal et al. 2006; Valladares et al. 2000), but lower heritability simply due to the fact that they have lesser reproductive events throughout life (short life). Reproduction events occur more often over the longer life of conservative species, which theoretically would be worth paying the costs of plasticity. However, at the population level, fast species can compensate for this by the greater number of generations in a short period of time (Fig. 4). This may provide greater chances of incorporating variation in attributes in the coming generations (epigenetic) in response to climatic fluctuations. In the long run, we could predict that this lack of heritability is more prevalent for slow species, which despite expressing plasticity at the individual level, is more difficult to incorporate into the population and become a dominant strategy in the community. Therefore, although the short-term dominance of fast-slow ecosystems is linked to the conditions mainly of nutrients and water, the question remains whether in the long term extreme events could favour acquisitive species (Fig 4).

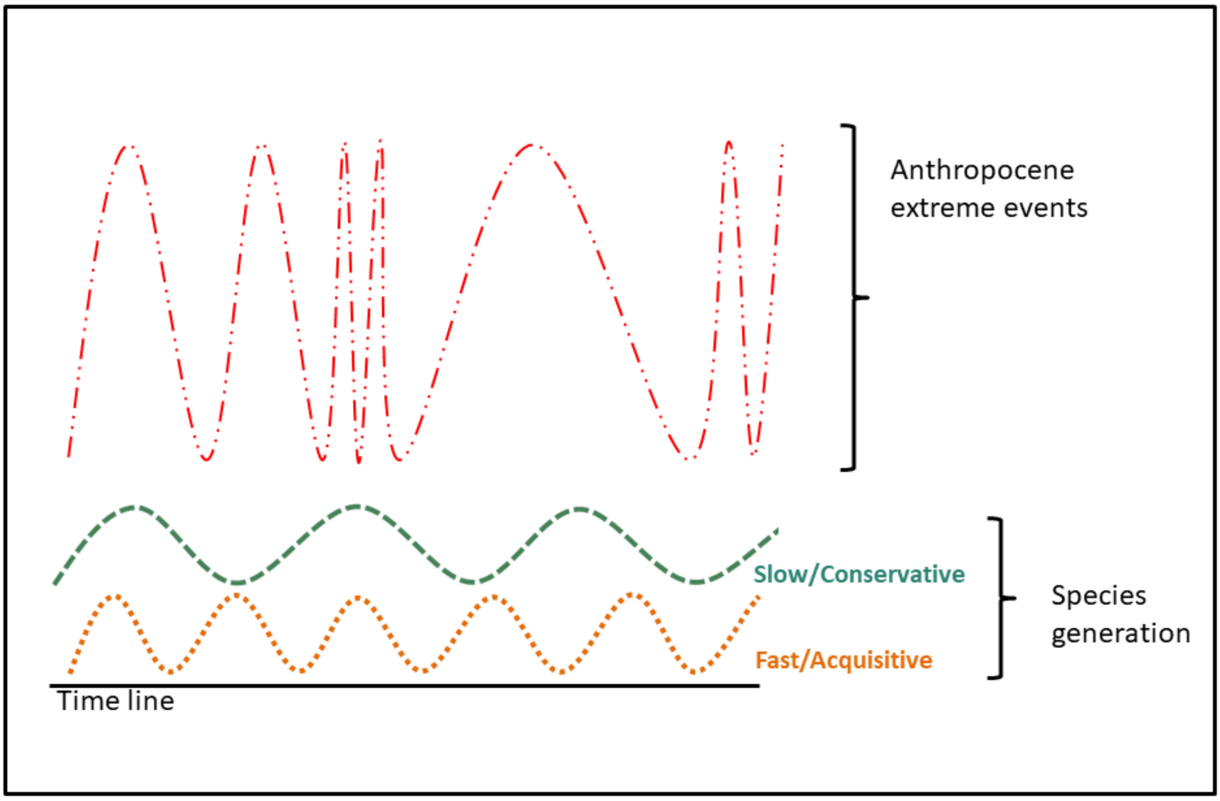

Figure 4. Fluctuations in extreme events and generation of fast-slow species over time. The red dashed lines represent the fluctuations of events over time, the green dashed lines represent generations of conservative species, in contrast, acquisitive species are represented by the orange

Whether this is true, one can expect from acquisitive communities more environmentally unstable populations that give rise to greater selection for a faster pace of life to be regulated more by the mortality of individuals with a faster pace of life, which will further contribute to the instability of the system. Thus, communities across the globe will become less resilient, more prone to biological invasion, and with high mortality rates from native species. Here we pose relevant questions regarding ecological strategies, phenotypic plasticity, resilience and environmental change:

1) *Are acquisitive species more plastic than conservative species in the tropics?*
2) *Is plasticity a heritable trait?*
3) *Do ecosystems with dominance of slow species have higher resilience?*
4) *What is the proportion optimum of acquisitive and conservative species on the plant community to increse ecosystem stability?*

Finally, experimental approaches are urgently needed to test with more accuracy which traits are important to define ecological strategies, niche breadth and to predict responses to environmental changes. At this stage, it seems that acquisitive plants show more trait variation and can deal better with ecosystems displacement mediate by change in land use, input of high nutrients concentration, and extreme events linkage of temperature and drought. Even an increase in disturbed, like as fire frequency, the conservative strategies will only be dominant if we can deal with changing soil phosphorus input after the fire. Therefore, with environmental filters shifts from land use and climate change the dominance of the community by acquisitive species might more recurrent than conservative species that seems favoured only when system shown nutrient-poor soil.

Conclusions

Acquisitive species have stronger positive responses to changing resources compared to resource-conservative species. We need to avoid the indiscriminate use of soft traits to make inferences about ecological strategies and test whether different traits are in fact functional. Most plant attributes are not good predictors of performance and attributes that are linked to high growth do not always mean superior performance. Performance metrics based on attributes are context-dependent and we suggest measuring traits in the field and at the individual level, instead of using indiscriminate database with species attributes measured in different environmental filters contexts. We need to understand the response of ecological strategies and the capacity of environmental filters to select theses strategies to be able to predict niche displacement. These allow the selection of species with an ecological function that to support extreme events, like as changes in nutrients and water availability in the next years. These ways seem important to restoration decision-makers to allow great outcomes in terms of guarantee ecosystems services and resilience.

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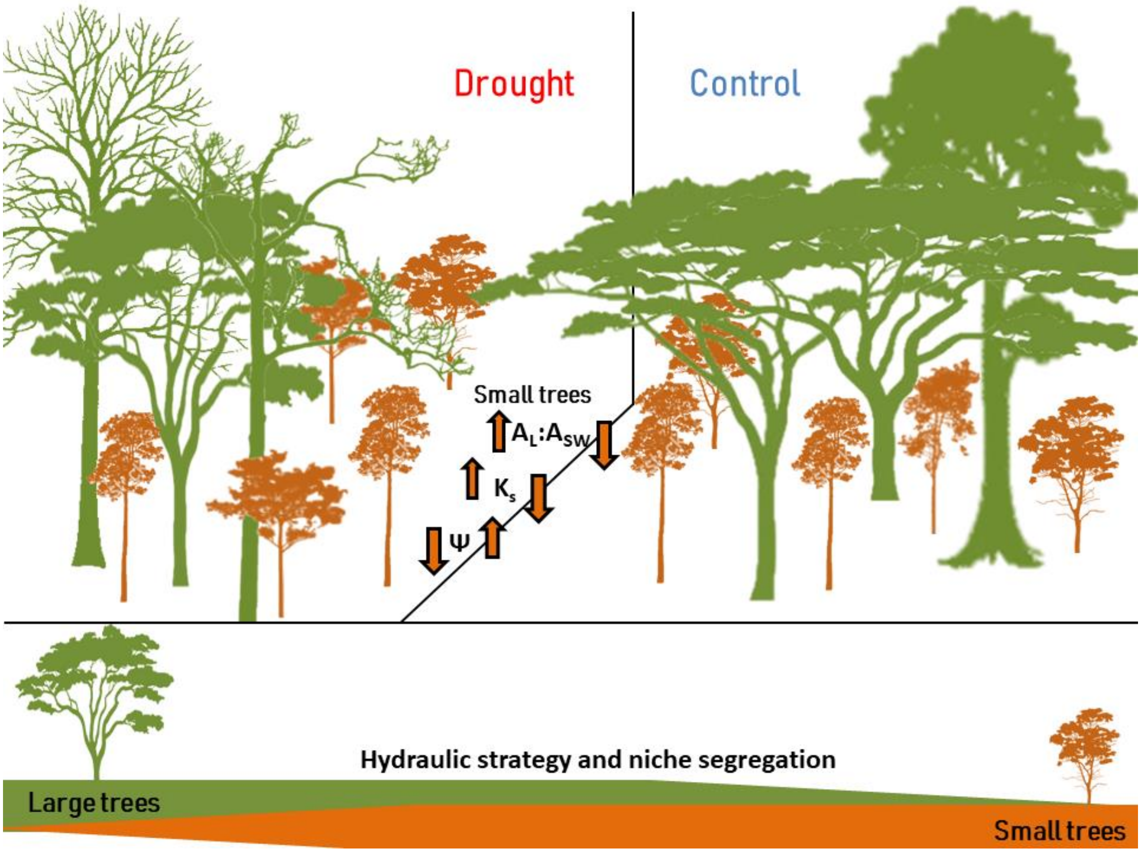
CHAPTER 2- Original Research

Small understorey trees have greater capacity than canopy trees to adjust hydraulic traits following prolonged drought in a tropical forest

Journal norms: Tree Physiology



Small understorey trees have greater capacity than canopy trees to adjust hydraulic traits following prolonged drought in a tropical forest

Running title: Tree size strongly controls plant hydraulic responses in a droughted tropical forest

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Abstract

Future climate change predictions for tropical forests highlight increased frequency and intensity of extreme drought events. However, it remains unclear whether the different niches occupied by large and small trees create distinct strategies that confer differential drought tolerance. The future of tropical forests is ultimately dependent on the capacity of small trees (<10 cm in diameter) to adjust their hydraulic system to tolerate drought. We evaluated multiple hydraulic traits indicative of drought tolerance of small trees across nine common neotropical genera at the world's longest-running tropical forest throughfall-exclusion experiment and compared their responses with surviving large canopy trees. Small understorey trees in both the control and the throughfall exclusion treatment had significantly lower minimum stomatal conductance and maximum hydraulic leaf-specific conductivity relative to large trees of the same genera, as well as significantly greater branch hydraulic safety margin (HSM), percentage loss of conductivity (PLC) and embolism resistance, demonstrating they occupy a distinct hydraulic niche. Surprisingly, in response to the drought treatment, small trees increased specific hydraulic conductivity by 56.3% and leaf:sapwood area ratio by 45.6%. The greater HSM of small understorey trees relative to large canopy trees likely enabled them to adjust other aspects of their hydraulic systems to increase hydraulic conductivity and take advantage of increases in light availability in the understorey, driven by the drought-induced mortality of canopy trees. Our results demonstrate that differences in hydraulic strategies between small understorey and large canopy trees drive hydraulic niche segregation. Small understorey trees can adjust their hydraulic systems in response to changes in water and light availability indicating natural regeneration of tropical forests following long-term drought may be possible.

Key-words: Long-term drought; Understorey trees; Hydraulic Safety margin; P50; Maximum conductivity; acclimation; Amazon forest.

Introduction

Future climate change predictions for tropical forests highlight increased frequency and intensity of extreme drought events (Aragão et al., 2018; Brodribb, Powers, Cochard, & Choat, 2020) and long-term reductions in soil moisture availability (Corlett 2016, Christensen et al. 2017). Most studies relating to drought focus on the impacts on large trees that comprise the highest proportion of forest biomass (Doughty et al., 2015; Rowland et al., 2015; Yuan et al., 2019), often finding the effect of drought stress on a plant's hydraulic system is a key driver of tree mortality (Bittencourt et al., 2020; Brodribb et al., 2020; Rowland et al., 2015). However, small understorey trees have a fundamental for recruitment and trees population maintenance and responsible for up to 20% of the forest carbon sink (Hubau et al. 2019). Thus, small trees may be critical in determining long-term drought responses if there is extensive loss of large canopy trees (Rowland, da Costa, et al. 2015, Esquivel-Muelbert et al. 2017).

Large trees occupy canopy positions (hereafter large trees) with high light levels and high vapor pressure deficit. In contrast, small trees occupying understory positions (hereafter small trees), grow slowly, generally in shaded conditions and have a lower atmosphere vapor pressure deficit (Sterck et al. 2011). The distinct resource partitioning between small and large trees, (Brum et al., 2019; Poorter, Bongers, Sterck, & Wöll, 2005) could cause strong differences in their water supply and demand relative to large trees. Reduced water supply from the roots, alongside lower capacitance, is likely to cause more negative water potentials in small trees relative to larger ones, during periods of low soil moisture (Salomón et al. 2017). Large trees are more likely to buffer periods of water deficit with greater water access by deep roots (Brum et al. 2019), higher capacitance (Mcculloh et al. 2014), and elevated carbohydrate storage that allows prolonged stomatal closure (McDowell et al., 2008). These potential size dependent variations in the structural and physiological traits suggest tree size potentially influences a tree's capacity to acclimate in response to severe drought stress.

Several key traits of a plants hydraulic system are essential to determining a trees capacity to survive prolonged drought stress. These traits are often related to controlling the risk of hydraulic failure, air bubble (emboli) formation in the xylem vessels(Sperry and Tyree 1988). This can lead to severe decreases leaf water supply, photosynthesis and other physiological functions (Martinez-Vilalta et al., 2019; McDowell et al., 2008; Sperry et al., 2002). These key traits include the water potentials at which tissues (i.e., stem xylem) lose

50% or 88% of their conductance (P50 or P88, respectively) and the hydraulic safety margin (HSM) (Meinzer, Johnson, Lachenbruch, McCulloh, & Woodruff, 2009), the difference between the minimum leaf water potential and P50, effectively a metric of the risk of a plant crossing a critical hydraulic threshold. Following sustained periods of drought stress a tree's capacity to survive is likely related to its capacity to acclimating certain key drought tolerance traits such as these or to limit their demand for water and thus stress on their hydraulic system (Sala et al. 2010, Meir et al. 2018). Existing studies on large trees show limited capacity for tropical trees to adjust plant hydraulic traits in response to drought stress (Binks et al., 2016; Bittencourt et al., 2020; Powell et al., 2017; Schuldt et al., 2011). Some studies have shown that the risk of embolism can be reduced by increasing HSM under drought conditions (Awad et al. 2010, Tomasella et al. 2018, Prendin et al. 2018). However, in tropical forest drought experiment, large trees were found to have limited plasticity in leaf level anatomy (Binks et al., 2016) and no capacity to acclimate their hydraulic systems, especially traits relating to embolism resistance (Bittencourt et al., 2020; Powell et al., 2017; Rowland et al., 2015). However, to our knowledge, no studies have evaluated whether small trees (<10 cm diameter at breast height, DBH), have the capacity to adjust their hydraulic system to prolonged drought stress. Following high mortality losses in large, more vulnerable trees in tropical forests, small trees can increase photosynthetic capacity (Bartholomew et al., 2020; Metcalfe et al., 2010) and lower canopy trees can elevates growth rates, even following drought (Brando et al., 2008; Rowland et al., 2015). This suggests that small trees can increase performance in response to elevated light, despite drier conditions. Increased light availability should require these small trees to adjust their hydraulic system to increase water supply and/or sustain lower xylem water potential driven by the increased atmospheric water demand. However, these adjustments in conditions of severe drought only seem to be possible as long as small trees have a greater drought tolerance, functioning with higher levels of embolism resistance and hydraulic safety margin (HSM). Consequently, consideration of ecosystem changes, such as canopy loss and shifting light availability, is likely to be as important as the consideration of the direct impact of soil moisture stress following long-term drought, as both factors may drive hydraulic acclimation within small trees.

Here we take advantage of a unique drought experiment located in northeast Amazonia to evaluate the response of small trees to combined changes in water and light availability. Previous research at this site has shown that large trees (>40 cm DBH) had significantly higher mortality rates, when compared to small trees and to trees on an equivalent control forest, leading to a 40% reduction in biomass as a consequence of drought (da Costa et al., 2010; Rowland et al., 2015). This biomass loss was almost entirely from the upper canopy, which led to increased level of light in the understory and increased growth rates of small understory trees in the wet season. (da Costa et al., 2014; Metcalfe, et al., 2010; Rowland et al., 2015). Furthermore elevated radiation loads are likely to have increased leaf vapour pressure deficit and temperature, increasing the atmospheric drought effect these small trees experience (Mulkey & Pearcy 1992a; Kamaluddin & Grace 1992; Krause, Virgo & Winter 1995). Using new data from this unique drought experiment (henceforth throughfall-exclusion experiment – TFE), we explore how small trees adjust hydraulic traits in response to increases in light availability coupled with increased drought stress. Specifically, if drought stress prevents small trees from adjusting traits to novel light conditions. Thus, we test how small trees (1-10 cm DBH) alter their plant hydraulic system in response to prolonged soil moisture stress and increased canopy openness, and determine how these responses vary relative to those of large trees (>20 cm DBH). We address the following hypotheses: Small trees have a different hydraulic strategy to large trees, specifically, relative to large trees, small trees have greater drought tolerance conferred by greater xylem embolism resistance and larger hydraulic safety margin. Small trees have a different hydraulic strategy to large trees, specifically, relative to large trees, small trees have greater drought tolerance conferred by greater xylem embolism resistance and larger hydraulic safety margin.

Methods

Site and plant material

Our study site is a lowland tropical rainforest located in the Caxiuanã National Forest, state of Pará, north-east Brazil (1°43′S, 51°27 W). It has an annual rainfall of 2000-2500mm, with a dry season (< 120 mm monthly rainfall) from July to December. A throughfall exclusion (TFE) experiment was established in 2002, where 50% of canopy throughfall is excluded by a plastic panel structure installed at 1-2m height over a 1 ha area. The TFE plot was studied alongside a 1 ha Control plot, where no throughfall exclusion took place. The plots have been monitored continuously since 2001 and further information on the experimental set-up can be found in earlier papers (da Costa *et al.*, 2010; Fisher *et al.*, 2007; Meir *et al.*, 2015 and Rowland *et al.*, 2015b).

From August-September 2017, during the peak of the dry season, we sampled 74 small trees with diameters ranging from 1 to 10 cm at breast height (1.3 m). 41 small trees were measured on the Control plot and 33 on the TFE, all taken from nine genera (20 species), replicated in each plot (two to five individuals per genera per plot). While we tried to maintain the same range of tree heights within each genus between plots, small trees had more variable height in the TFE, with light-exposed individuals reaching over 15 meters height, whilst no individuals in the Control reached 15 metres height (See Fig. S1). It was not possible to know the age of each sampled individual, because (destructive) sampling for age determination (tree-ring analyses; e.g., Brienen et al., 2016) was not possible. Consequently, we must assume that our sampled trees may have strongly varying ages (Groenendijk et al. 2014). We thus test the influence of tree stature and position within the forest strata (van der Sleen et al. 2015), while assuming that most of our sampled trees are likely to be young.

For each individual, we collected two branches from the top of the crown, representing the point maximally exposed to light. The branches were third to fourth order, counting from the leaves. We collected one set of branches before sunrise (0400 to 0600 hours) and used these to measure embolism resistance and predawn leaf water potential. We collected a second set of branches at midday (1130 to 1330 hours) and used these to measure midday leaf water potential, native embolism, leaf-to-sapwood area, xylem and leaf specific conductivity, minimum leaf conductance and wood density measurements. Immediately after collection, branches were bagged in thick black plastic sacks with moist paper to humidify internal air and minimise leaf transpiration. Branches were transported 100m from the plots to measure leaf water potential, and for the remaining measurements the branches were transported to a laboratory ~1km walk away.

We measured predawn leaf water potential (Ψpd), which represents when transpiration is at its minimum and the water potential of the plant is closest to equilibrium with that of the soil. Ψpd can be considered an integrated metric of maximum soil water availability across the rooting depth (Bartlett et al. 2016). We also determined midday water potential (Ψmd), to capture the minimum Ψ of the plant in the dry season. This measure is affected by any cuticular or stomatal transpiration and, thus, broadly captures the integrated effects of plant traits and the environment on the minimum water potential a plant reaches in natural conditions. All water potential measures are expressed in negative values. We also measured the native dry-season percentage loss of conductivity (PLC) and we used the difference between the minimum leaf water potential (Ψ_{md}) and P_{50}, to calculate the branch hydraulic safety margin (HSM). These two values (native PLC and HSM) were used as indicators of the cumulative damage from embolism.

Predawn and midday water potential

Predawn and midday leaf water potentials were measured in the field immediately after collection, using a pressure chamber (Model 1505, PMS), without being bagged. Branches collected for predawn water potential measures were sampled before sunrise, and for midday water potential, the sampling took place between 1130 to 1330 hours. For each tree we measured water potential of two leaves, or three leaves if the first two measures differed substantially (>0.5 MPa difference) from one another. Measurements from multiple leaves were averaged to create a single value per tree. All water potentials were all taken on the same day for small trees.

Wood density, leaf to sapwood area *ratio and minimum stomatal conductance*

We measured wood density (W_D) on woody sections 40 to 80 mm long and 4 to 7 mm diameter cut from the branch. We debarked samples, immersed them in water for 24 hours to rehydrate and measured saturated volume using the water displacement method (Pérez-Harguindeguy *et al.*, 2013). We then oven dried the samples at 60°C until they were a constant mass and measured their dry weight with a precision balance to 3 decimals.

We determined the leaf to sapwood area ratio (A_L:A_{SW}), on all branches by measuring leaf area and calculating sapwood area from two diameter measurements of the debarked basal part of the branch using precision callipers and standard distance from the tip . We measured leaf area by scanning all leaves on the branch and quantifying their area using Image J software (version 1.6.0_20; Schneider et al., 2012). We calculated the leaf area to sapwood area ratio as total branch leaf area divided by basal sapwood area. All branches had a similar size and were standardised by distance to the tip (~40-70 cm). The A_L:A_{SW} is a key indicator of the balance between transpiration and stem water supply (Mencuccini et al. 2019).

For minimum leaf conductance (G_{min}), we used the leaf conductance to water vapour measured in the abaxial surface of leaves kept 30 minutes in the dark, using an infrared gas analyser (Li-COR 6400, US). All leaves measured were fully formed, undamaged leaves. G_{min} is a key indicator of residual leaf water loss and likely a combination of stomatal conductance from leakage of partially closed stomata and cuticular conductance (see Rowland *et al.* 2020) and Bartholomew et al. (2020), for further leaf gas exchange measurement details.

Hydraulic efficiency and native embolism

We calculated maximum hydraulic specific conductivity (K_s) as a measure of xylem hydraulic efficiency and maximum leaf specific conductivity (K_{sl}) as a measure of leaf water supply capacity. We used the native percentage loss of conductivity of the collected branches (PLC) as a measure of native embolism. To estimate these variables, we measured branch xylem hydraulic conductivity before (K_{snat} – native conductivity) and after flushing to remove emboli and we quantified the leaf area distal to the sample to obtain K_{sl} from K_l (leaf conductance). Using samples from the branches collected at midday, we put the entire branch underwater and discarded a 10 cm long segment from the base. After this, we cut another 10-15 cm long segment from the base of each branch underwater, standard distance from the tip of the branch and let them rehydrate for 15 min to release tension and avoid artefacts (Venturas et al. 2015). Subsequently, to relax the tension in the branch we cut 1-1.5 cm of branch from base to leaves underwater, in steps of ~15 cm, and used the distal end of the branch for hydraulic measurements to ensure no artificially embolised vessels were present in the measured sample. All samples used for hydraulic measurements were second or third order branches, between 30-55 mm in length and 3-5 mm diameter and were recut underwater with a sharp razor blade before connecting to the apparatus, to ensure all vessels were open at both ends. We then measured flow using the pressure drop over a capillary method (Pereira and Mazzafera 2013), where a capillary of known conductance is connected in series with the sample to measure K_s and then the samples are flushed to remove emboli and estimate maximum conductance (Martin-StPaul et al., 2014). We note the samples remained under-water during the entire procedure. We calculated PLC as the ratio of K_{snat} to K_s multiplied by 100. We calculated K_{ls} as the sample hydraulic conductivity (i.e., sample conductance times sample length) after flushing divided by the leaf area distal to the measured sample.

Embolism resistance and hydraulic safety

As an index of xylem embolism resistance, we used P_{50} and P_{88}, the xylem water potentials where, respectively, 50% and 88% of hydraulic conductivity is lost. We also used P_{50} to calculate the hydraulic safety margin - the difference between P_{50} and Ψ_{md}, an index of tree hydraulic safety. Branches collected before sunrise were rehydrated for 24 hours and from each branch we cut two or three smaller branches of approximately 40-70 cm. We measured the xylem embolism resistance of each branch using the pneumatic method (Pereira et al. 2016, Zhang et al. 2018). With this method, the loss of hydraulic conductance is estimated from the increase in air volume inside the wood caused by embolism formation as the branch dehydrates. Air volume is estimated from the air discharge from the cut end of the branch into a vacuum reservoir (~50 kPa absolute pressure) of known volume during a given amount of time (2.5 minutes). We measured initial and final pressure inside the vacuum reservoir with a pressure transducer (163PC01D75, Honeywell) and calculated the volume of air discharged using the ideal gas law. A detailed protocol is presented in (Pereira et al. 2016, Bittencourt et al. 2018). Percentage loss of conductance for each branch is estimated from percentage air discharged (PAD) during its dehydration. PAD is calculated by standardising air discharge for each branch by its minimum (fully hydrated) and maximum (most dehydrated) air discharge state. We dehydrated branches using the bench dehydration method (Sperry et al. 1988). Before each air discharge measurement, branches were sealed in thick black plastic bags for one hour for leaf and wood xylem water potential to equilibrate. Directly after the air discharge was measured, we estimated wood xylem water potential by measuring the leaf water potential of one to two leaves. Drought embolism resistance is then given by the increase in PAD with decreasing xylem water potential for each tree. To calculate P_{50}, we pooled data from the two-to-three branch replicates from the same tree and fitted a sigmoid curve to the data, where P_{50} and slope (a) are the fitted parameters (Pammenter and Van der Willigen 1998) and P88 is predicted from the fit (Eqn 1):

$$\mathrm{PAD} = 100/\left(1 + \exp\left(a\left(\Psi - \mathrm{P50}\right)\right)\right)$$

Eqn1. Percentage air discharge equation (PAD). Ψ Water potential. P_{50} (xylem embolism resistance (MPa)

Data analysis

By comparing trees found on the Control and TFE experimental plots, we measure the effect of the experimental drought on our drought stress indicators (Ψ_{pd} - predawn water potential; Ψ_{md} - midday water potential; HSM – branch hydraulic safety margin to P_{50}; PLC – native dry season percentage loss of conductivity) and plant traits (W_D – wood density; A_L:A_{SW} - leaf to sapwood area; P_{50} - xylem embolism resistance; P_{88} - xylem embolism resistance; G_{min} – minimum stomatal conductance; K_s – maximum hydraulic specific conductivity; K_{sl} - maximum hydraulic leaf -specific conductivity) in small trees. We used linear mixed effects models in the package lme4 (Bates et al. 2015) to test for plot (TFE vs Control) and taxonomic effects (genus and species) on hydraulics traits in small trees (n = 66). We started with a full fixed and random effect model of the plot, genus and their interaction as fixed effects. We tested the significance of the random effect by removing it and evaluating if the model significantly worsened. We tested sequentially for the random effect of genus on: (a) the model intercept; (b) the fixed Plot effect (drought effect, difference between plots) on slope without intercept; and (c) both intercept and plot. When more than one model with a random effect was significant, we chose the most parsimonious random effect (i.e. intercept effect only), unless the Akaike information criterion (AIC) of the more complex model was at least 2 units lower than the simpler model (Burnham and Anderson 2004). After testing the random effects, we tested the fixed effects by first removing the interaction (plot with genus) and testing if this significantly worsened the model and after this using the same approach with the additive terms. If no random effect was significant (lmerTest), we changed to a fixed effect model (R base package ‘lm’ function) and analysed fixed effects in the same way. When the taxonomy was included as a random effect in our models, we tested for both genus-only and species-nested-within-genus effects. We tested the complete model (genus and species as a random effect) against a GLM containing only the fixed effects. When genus was not significant, linear models were used to test the significance of the fixed effects. To quantify model goodness of fit, we considered the marginal and conditional R^2 (Mulkey and Pearcy 1992b). The marginal R^2 indicates how much of the model variance is explained by the fixed effects only, whereas the conditional R^2 indicates how much of the model variance is explained by the complete model, fixed and random effects. All the analyses were done in R (version 3.3.0; R Core Team, 2016)

Small and large tree comparisons

We tested for differences in individual tree-level responses to the TFE treatment for large (n = 72) and small trees (n = 39). We use the large trees data from Bittencourt et al. (2020) conducted in the same experimental plots and collected during 2017 with the same methodological procedures. For this comparison we restrict the samples to those trees whose genera are replicated on both plots and replicated between the large and small trees, with a minimum sample size of 2 individuals per size group per plot and genus. Consequently, the number of genera and individuals employed in this comparison is lower than the available number of individual small trees and the full dataset published in Bittencourt et al., (2020). In total we use five genera (*Eschweilera, Inga, Licania, Protium, Swartzia*), with 15 small trees on the Control and 24 small trees on the TFE, and 35 large trees on the Control and 37 large trees on the TFE. We used linear mixed-effect models to test the effects of the TFE treatment, tree size with two classes (Large and Small), and the interactions between treatment and tree size on drought stress indicators and hydraulic traits. Taxonomic effects were included by using genus as random effects, following the same protocol used for the small tree analyses, above. We selected the most parsimonious model from a full model set according to the Akaike information criterion with a correction for small sample sizes (AICc scores) (Barton, 2018). Within this paper, all data presented represent the mean and standard errors of the mean. A summary of available trait data by genus is presented in Table 1.

To test for an overall difference in the hydraulic strategy between small and large trees, we used the multivariate approach conducting non-metric multidimensional scaling (NMDS) using an individual-traits matrix (McCune et al 2002). We construct a matrix of data consisting of rows of individuals of each species and columns of traits values. We standardized the individual trait values for each genus and built the similarity matrix using Gower distance. NMDS searches for the best position of individuals variables on k dimensions (axes) to minimize the “stress” of the resulting k-dimensional configuration.

We use k axes = 2 from that ordination as the initial configuration. The "stress" is obtained by comparison among the pair-wise distances (differences) of each individual's variables in reduced ordination space (expressed in terms of axes) and the original distance matrix (Gower). The regression is fitted using least-squares regressions and the goodness of fit is measured as the sum of squared differences between ordination-based distances and the distances predicted by the regression. A goodness of fit, or stress value, between 0.1 to 0.2 represent a good fit within the specified number of dimensions analysed to enable points to be interpreted relative to the NMDS axes (Dexter et al 2020). Therefore, the axis represents the data in a way that best represents their dissimilarity, points on the graph that are closer together are more similar. In addition, we use MANOVA to test the difference in multidimensional space filled by tree size (Small and Large groups) and by plot effect (Drought and Control groups) separately (Anderson 2001). We use a MANOVA to compare Gower distance among observations in the same group versus those in different groups. We conducted a MANOVA first using Small and Large tree groups and them using Drought and Control groups using both tree sizes together. The size and plot effects were tested separately. Finally, we use permutations of the observations to obtain a probability associated with the null hypothesis of no differences between groups.

Results

The reduced soil moisture availability and increased canopy openness caused by 15 years of the TFE (Fig. S2) caused significant changes in the hydraulic traits of the small trees (Fig. 1). Maximum specific conductivity (K_s) increased by 56.3±41.5% in the TFE small trees relative to the Control (Fig. 1, $p<0.01$), similarly there was a 45.6±38.2% increase in the leaf: sapwood area ratio (Fig.; $p<0.001$). The TFE also had significant effects on key physiological indicators of drought stress (Fig. 1). Ψ_{pd} was 0.56 MPa lower on the TFE relative to the Control ($p <0.001$) and Ψ_{md} was 0.61 MPa ($p < 0.001$) lower. In contrast, other key hydraulic traits including xylem embolism resistance (P_{50} and P_{88}), leaf specific conductivity (K_{sl}), minimum stomatal conductance (G_{min}) and wood density (W_D) showed no significant change between the TFE and the Control plots (Fig. 1; Table 2; Table S1).

Taxonomic effects on hydraulic traits and their interactions with drought

Using mixed-effect modelling analysis we found that variance explained by taxonomy had only a limited role in affecting the overall drought responses. Of the four variables which demonstrated significant changes in trait values in response to the TFE, only Ψ_{md} had a genus or species nested within genus effect that significantly influenced the intercept of the relationship with drought treatment (Table 2). When genus by genus responses to the drought effect were examined separately, it was clear that there were highly variable responses to the treatment between genera and sometimes these were inconsistent in terms of direction, as well as magnitude. We cannot separate the taxonomic effect from the residual variance because genus-specific influences on the plot effect were highly variable (Fig. 2). Given the low replication (between 2 and 5 for each genus on each plot treatment) and high variation within each genus, it was not always statistically viable to test the plot effect within each genus (Fig. 2), however where this was possible, clear statistical differences were seen for some genera (Kruskal-Wallis test) but not for others (Fig. 2). For example, *Inga* showed consistent response in leaf:sapwood ratio and K_s while *Ocotea* did not show differences between plots (Fig. 2). K_s showed the most consistent drought treatment response across all genera, as, except for *Ocotea* and *Tetragatris*, all other genera showed a clear, significant, increase in K_s on the TFE (Fig. 2). The patterns described here were also maintained when we analysed the data at a species level (data not shown).

Large versus small trees

We compared the responses of hydraulic traits between large (>20 cm DBH) and small trees (1-10 cm DBH). Except for Ψ_{pd}, the results we obtain considering only the five genera, which were also sampled within the large tree study, were similar to when considering all nine genera of trees present in Control plot and TFE experiment (see Fig. S4 supplementary material and Table S3 for n values for the small to large tree comparisons). Using these five genera we find replicated in the small and large trees, we used NMDS ordination using all of the trait data to demonstrate the niche space occupied by the small trees was significantly different from the trait space of large trees. The traits space separated on to a clear 2-dimensional axis with a stress score of 0.18, indicating a good fit between the data and an analysis consisting of two axes (Fig. 3 a). Different associations amongst the nine hydraulic traits separated the individuals in the small and large tree groups. This result was driven predominantly by the first axis, which was positively related to PLC, P_{50} and P_{88} that influencied Small tree agroupment (Fig. 3b). While the first axis was negatively related to K_s, K_{sl}, G_{min} influencing Large Trees agroupment (Fig 3b, Table S4). Using the complete set of hydraulic traits, we show that the hydraulic niche of small trees was significantly different from that of large trees ($MANOVA_{(1,66)}$; F=7.96; p<0.001; Table 1). However, there was no difference in hydraulic niche space occupied by the Control and TFE groups ($MANOVA_{(1,64)}$; F=1.22; =0.30), except that K_s that showed plot and size effect ($MANOVA_{(1,64)}$; F=3.5; p=0.05).

In contrast to the large increase in K_s observed in the small trees from the Control to the TFE trees (Figs 1 & S3), the plot level average values of K_s were similar among large trees (4.82±3.93 TFE and 4.86±2.79 Control plot). Similar to Ψmd, large plot level differences were present in small trees, but these were absent in the large trees (-1.72 ± 0.48 TFE and -1.70±0.48 Control treatment). However, small trees had values of Ψ_{md} which were 17.12±0.03% higher (values closer to 0) than the large trees. Furthermore, for the variables which had no treatment effect amongst the small trees, we find on average, across both the TFE and Control plots, the small trees had a 38.2±32.1% (p<0.01) more negative P_{50} and a 68.4±58.8% and 20.7±30.4% lower G_{min} and K_{sl}, respectively, than the large trees (Fig. 4b, 3d, 3f; p<0.001). HSM and PLC were 72.97±36.34% and 44.41±14.62% greater, respectively, in the small trees relative to large trees (Fig. 4g, 4i, 4j; p<0.01).

We analysed the influence of genus on the combined effect of treatment and tree size effect (i.e., large and small trees on the Control and TFE plot) for the five genera we could replicate across plots and tree size classes. We found that the effects of tree size varied substantially among genera (Fig. 5). For example, the difference in P_{50} between large trees and the small trees was 61.48±52.51% for *Licania* and 38.96±3.7% for *Inga* (Fig. 5). In contrast, G_{min} was significantly lower in the small trees relative to large trees across almost all genera (Fig. 5b). The drought-response pattern also changes when doing within-genus comparisons between large and small trees, for example the mean P_{50} response for *Inga* was different between small and large trees (Fig. 5). A difference in trait values between the Control and TFE plots that was present either for small tree or large trees, but not for both size classes simultaneously, occurred multiple times (Fig. 5), especially for the genus *Inga*. We note the relatively low replication per genus, per plot, per size group (n values from 2-8 individuals) and high intra-genus variation (Fig. 5), makes the interpretation of genus level differences in traits complex. Mixed effect modelling results did however, identify a strong influence of genus on trait variation between our two size classes (Table 2), yet there are limited cases where we find significant models demonstrating trait differences between the Control and the TFE plot had a significant tree size and genus effect (Table 2).

To test for size (small and large) and genus effects in each treatment (Control and TFE), we created a model with both size and genus as fixed effects. In the Control plot the full model (trait ~ genus*size) was a better predictor of variation across almost all traits, except for K_s, where there was a genus only effect and Gmin, P50 and P88 where there was a size only effect. An interaction between size and genus was only significant for PLC (Table S5). The full model was also the best predictor of trait variation in the TFE plot. Although HSM, W_D and G_{min} only showed significance size effect. Significant interactions between genus and size were found for P_{50} and P_{88} (Table S6).

Discussion

Our results provide strong evidence that small trees can adjust their functioning in response to drought, allowing them to maximize carbon gain in high-light levels following mortality of large trees. We find that small trees (1-10 cm DBH) have the capacity to increase maximum specific hydraulic conductivity and leaf to sapwood area ratio in response to prolonged (15 year) soil moisture stress. Despite having significantly lower pre-dawn and midday leaf water potentials, small trees had the capacity to adjust key hydraulic traits to allow a positive response to a higher light environment. This suggests that despite soil drought stress, small trees can still increase water transport efficiency and crown water demand in response to increases in light availability, following drought-induced mortality of large trees, potentially allowing them to maximise productivity in periods of the year when water is available. We also show the different hydraulic strategy that provides niche segregation between small and large trees, with small trees being more drought tolerant than large canopy trees.

The impact of drought on the hydraulic system of small trees

The substantial loss in biomass from large trees (da Costa et al., 2010; Rowland et al., 2015) led to an increase in the light availability in the lower canopy of the TFE, driving increases in the maximum photosynthetic capacity (71.1% and 29.2% increase in J_{max} and Vc_{max} respectively) and a 15.1% increase in the LMA of the same small trees we study here (Bartholomew et al. 2020). These differences in response to the prevailing light environment have also been observed elsewhere in tropical tree canopies (Ruggiero et al. 2002, Domingues et al. 2010, Cavaleri et al. 2010) and are indicative of plants changing their allocation strategy in response to increased light availability (Poorter et al., 2009; Wright et al., 2004). Critically, these allocation shifts are likely to result in a net increase in photosynthesis and growth (Metcalfe et al., 2010; Rowland et al., 2015), which require higher water supply to the canopy of each individual. The elevated soil moisture stress in the TFE relative to the Control trees, manifested itself as significantly more negative pre-dawn and midday leaf water potential values (Figs 1h-1i), key indicators of plant water stress (Bhaskar & Ackerly, 2006; Kramer, 1988; Martínez-vilalta & Garcia-Forner, 2017). Interestingly however, these more negative water potentials did not translate into a significant change in HSM between plots, possibly because of a trend, albeit statistically insignificant, towards more negative P_{50} values on the TFE plot trees relative to those on the Control (Fig. 1). When examined at the genus level, five of the nine genera have consistently more negative P_{50} values on the TFE relative to the Control, with two remaining roughly equal and two less negative on the TFE (Fig. 2). These data suggest that, despite operating at more negative water potentials, it is still possible for small trees to adjust their hydraulic system to support the increased growth in response to greater light availability.

Consistent with increases in photosynthetic capacity (Bartholomew et al., 2020), we observe an increase in leaf area to sapwood area ratio in the small trees on the TFE, relative to the Control. Combined with greater hydraulic specific conductance, small trees are able to supply more photosynthetic tissue without increasing the volume of sapwood. A global study, including multiple sites from the tropics showed plant hydraulic systems are highly sensitive to changes in this ratio and may be one of the main factors controlling trade-offs in other plant hydraulic traits (Mencuccini et al. 2019). Increasing leaf area increases the total water demand of the tree. However, the observed increases in photosynthetic capacity (high values of Vcmax and Jmax, Bartholomew et al. 2020), may allow slightly lower stomatal conductance for any given CO_2 concentration (Bartholomew et al., 2020; Sperry et al., 2017). This may, in part, compensate for the increase in demand for water that elevated leaf areas may cause. However, even with the observed increases in photosynthetic capacity, these small trees probably still experience increased total water demand due to increased exposure to higher temperatures and VPD, suggesting that small trees must increase maximum hydraulic conductivity and/or tolerate reductions in water potential and therefore greater embolism risk (Sperry et al., 2017). In our study, sampled small trees in the TFE were sligthly taller than the small trees in the Control plot (Fig. S1). This difference may be in part-contributing to the slightly elevated conductance in the branches, as taller trees can have larger vessels at the base and greater vessel tapering from the trunk to branch tip (Olson and Rosell 2013, Olson et al. 2020). It is, however, unlikely that these differences had a large influence on our K_s results. Overall, the difference in height were small and the genera with the greatest height differences between the TFE and Control (*Protium, Octea, Voucoupoa*, Fig. S1) showed no changes in Ks (Fig. 2).

Differential hydraulic strategy between small and large trees

The comparison between small trees and large trees using NMDS showed that they occupied distinctly separate hydraulic niche space, considering a multidimensional space, which included all our measured traits. This revealed that smaller trees do indeed have a different water use strategy to larger canopy trees (Fig 3). The differences in the traits we observed were far greater, and in most cases significantly so, between the large and the small trees than for trees of the same size class between treatments (Fig 4). In addition, we show that smaller trees across both the Control and the TFE plot have significantly more negative P_{50} values and lower G_{min} values and significantly greater hydraulic safety margins (HSM), midday leaf water potentials and PLC (Fig. 4). Consistent with the results from large scale studies (e.g. Choat et al., 2012), where taxonomic matching between size classes was not conducted and small trees (<10cm) were not considered, in our study we find the HSM is 1.94 MPa more positive in the small trees relative to large trees, where they are close to zero on both plots. Our results are consistent with the hypothesis that the smaller trees are shallow rooted and compensate for the lack of access to deep water through developing greater xylem embolism resistance and greater stomatal control (Brum et al., 2019; Tardieu, 1996, Sperry et al. 2017). It is possible that the greater hydraulic safety margin in small trees enables them to adjust more effectively to increased light availability, despite the lower water availability in the TFE, as it enables these trees to tolerate greater drought stress without passing critical thresholds.

The carbon gain associated with allowing greater photosynthesis when higher light is available is more likely to be translated into new xylem growth in smaller trees. This growth implies that rapid replacement of damaged tissues is likely to be a more viable strategy for smaller trees, relative to large trees (Damián et al. 2018, Trugman et al. 2018), which would reduce the risk associated with higher PLC levels. Furthermore, maintaining significantly lower G_{min} and higher midday leaf water potential (Fig. 4d, g), relative to the large trees, despite having similar pre-dawn leaf water potentials, suggests that small trees are able to more tightly regulate water loss, during both the day and night. This greater degree of control further reduces the risk of runaway embolism when photosynthesising during periods with low water potential, particularly if these trees can repair cavitated vessels (Nardini et al., 2011; Salleo et al., 2004; Salleo et al., 1995) or grow new vessels between consecutive dry seasons (Eller *et al.* 2018). Combined, these factors are likely to allow small trees to have greater flexibility in terms of the strategy they use to adjust to combined changes in water and light availability. However, as we highlight in our results, there is lots of variability both within and between taxonomic groups concerning how small trees may alter their traits to alter their drought tolerance strategy.

This study highlights the importance of forest structural changes in controlling the traits of what are likely to be the next generation of trees growing up during prolonged drought stress. We show that small trees have higher capacity to acclimate their hydraulic systems to increases in light availability following drought-induced mortality of canopy trees relative to large trees. Our results suggest that small trees are able to acclimate despite experiencing prolonged soil moisture stress, which resulted in lower leaf water potentials and greater PLC. Our results demonstrate that there is a consistent and larger shift in the plant hydraulic strategy of saplings relative to large trees across most of Amazonia's hyper-abundant taxonomic groups. A key uncertainty which remains to be answered, however, relates to the long-term development of these trees. Assuming these small trees continue to develop under the experimental drought stressed conditions, it would be of interest to know if the trajectory of change in hydraulic traits we observe can be sufficient to increase the hydraulic resistance of these trees as they approach full size. Ultimately, continued acclimation of hydraulic systems throughout a tree's lifespan may allow a more drought-resilient ecosystem to develop.

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Conflict of interest

The authors have no conflict of interest to declare.

Authorship

ALG collected and compiled the data alongside LR, PRLB, IC, PBC, PG, LVF, DDV, JASJ, DCB and ACLdC. LR designed the study with MM, ACLdC, PM and RO. ALG, PRLB and LR performed the statistical analysis and ALG, LR and RO wrote the paper, all other authors substantially contributed to revisions.

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Figures and tables

Table 1 – Mean and standard deviation of P_{50} - xylem embolism resistance (MPa); P_{88} - xylem embolism resistance (MPa); G_{min} – minimum stomatal conductance (mol m^{-2} s^{-1}); K_s – maximum hydraulic specific conductivity (kg m^{-2} s^{-1} MPa^{-1}); K_{sl} - maximum hydraulic leaf-specific conductivity (kg m^{-2} s^{-1} MPa^{-1}); A_L:A_{SW} – leaf to sapwood area ratio (m^2 m^{-2}); W_D- Woody density; Ψ_{pd} - predawn water potential (MPa); Ψ_{md} - midday water potential (MPa); HSM – branch hydraulic safety margin to P_{50} (MPa); PLC – native dry season percentage loss of conductivity (%), separated by genus and treatment.

Hydraulic traits												
Genus	**Treatment**	**P_{50}**	**P_{88}**	**G_{min}**	**K_s**	**K_{sl}**	**A_L:A_{SW}**	**W_D**	**Ψ_{pd}**	**Ψ_{md}**	**HSM**	**PLC**
Eschweilera	**Control**	-2.91±0.07	-5.08±0.31	0.028±0.023	1.12±0.19	0.57±0.30	112.07±32.41	0.73±0.12	-0.32±0.23	-1.68±0.24	1.13±0.32	49.14±4.9
Eschweilera	**TFE**	-3.66±2.01	-6.32±3.80	0.026±0.019	2.80±2.62	4.71±6.12	92.12±65.44	0.59±0.09	-0.52±0.24	-1.87±0.26	1.89±2.28	9.22±4.36
Inga	**Control**	-4.60±1.63	-7.84±3.10	0.02±0.014	2.3±1.43	1.56±0.85	84.59±47.51	0.64±0.18	-0.37±0.26	-1.51±0.57	3.09±2.07	11.33±10.19
Inga	**TFE**	-3.48±0.58	-6.22±1.62	0.02±0.006	4.56±1.68	1.93±0.73	160.81±58.68	0.63±0.08	-0.39±0.22	-1.35±0.78	2.13±0.4	19.28±13.21
Licania	**Control**	-5.28±1.98	-9.62±4.40	0.025±0.014	0.15±0.04	0.12±0.07	66.15±24.41	0.76±0.062	-0.25±0.07	-1.65±0.85	3.62±2.75	38.29±28.52
Licania	**TFE**	-6.18±1.59	-9.07±1.77	0.024±0.02	2.17±2.19	0.37±0.40	104.90±45.99	0.761±0.014	-0.85±0.81	-1.388±0.78	5.183±1.70	68.667±28.13
Mouriri	**Control**	-4.77±0.54	-7.69±1.31	0.025±0.017	0.62±0.05	0.22±0.20	154.33±59.51	0.867±0.003	-0.24±0.09	-0.943±0.08	3.829±0.48	58.031±27.65
Mouriri	**TFE**	-5.55±0.74	-7.35±2.18	0.077±0.022	3.63±3.03	1.32±0.88	143.30±92.13	0.751±0.17	-1.07±1.32	-2.583±0.95	2.972±0.77	60.769±15.83
Ocotea	**Control**	-3.59±1.49	-8.72±2.63	0.007±0.003	1.63±0.81	0.84±0.17	125.38±54.22	0.638±0.05	-0.36±0.4	-0.6±0.364	2.994±1.26	36.718±18.42
Ocotea	**TFE**	-5.04±2.08	-8.61±4.88	0.03±0.024	1.58±0.66	0.60±0.46	84.83±32.64	0.68±0.13	-1.44±1.17	-2.41±0.81	2.62±2.45	65.27±24.19
Protium	**Control**	-2.30±0.71	-4.16±2.40	0.017±0.01	1.68±0.94	0.75±0.41	78.60±6.37	0.74±0.07	-0.332±0.3	-1.23±0.31	1.07±0.78	54.73±17.02
Protium	**TFE**	-3.64±1.47	-5.65±0.73	0.013±0.01	1.10±0.07	0.44±0.07	90.57±17.71	0.72±0.049	-0.48±0.16	-1.00±0.24	2.55±1.73	49.74±11.94
Swartzia	**Control**	-3.17±1.28	-5.98±1.89	0.06±0.04	1.67±0.26	0.78±0.55	72.45±18.20	0.73±0.02	-0.23±0.12	-1.57±0.16	1.60±1.36	59.73±9.94
Swartzia	**TFE**	-4.34±0.57	-6.94±0.06	0.06±0.02	2.78±0.51	0.89±0.54	210.45±67.51	0.72±0.005	-0.79±0.48	-2.36±0.09	1.98±0.66	49.13±8.57
Tetragastris	**Control**	-2.31±1.48	-4.34±1.81	0.03±0.01	2.22±1.66	2.29±3.12	83.86±59.38	0.64±0.05	-0.28±0.13	-1.06±0.58	1.25±1.31	22.12±15.60
Tetragastris	**TFE**	-4.36±1.19	-6.52±2.90	0.016±0.01	1.33±0.62	1.04±0.45	88.10±34.28	0.58±0.04	-1.43±0.40	-2.44±0.13	1.92±1.06	43.24±6.33
Vouacapoa	**Control**	-3.57±0.13	-5.37±1.45	0.015±0.003	1.00±0.16	0.95±0.64	56.71±22.69	0.69±0.13	-0.39±0.18	-1.59±0.19	1.97±0.31	43.78±11.37
Vouacapoa	**TFE**	-2.22±0.79	-3.54±1.63	0.012±0.004	0.83±0.51	0.67±0.78	229.76±101.26	0.70±0.01	-0.77±0.35	-2.07±0.24	0.15±0.72	33.24±19.67

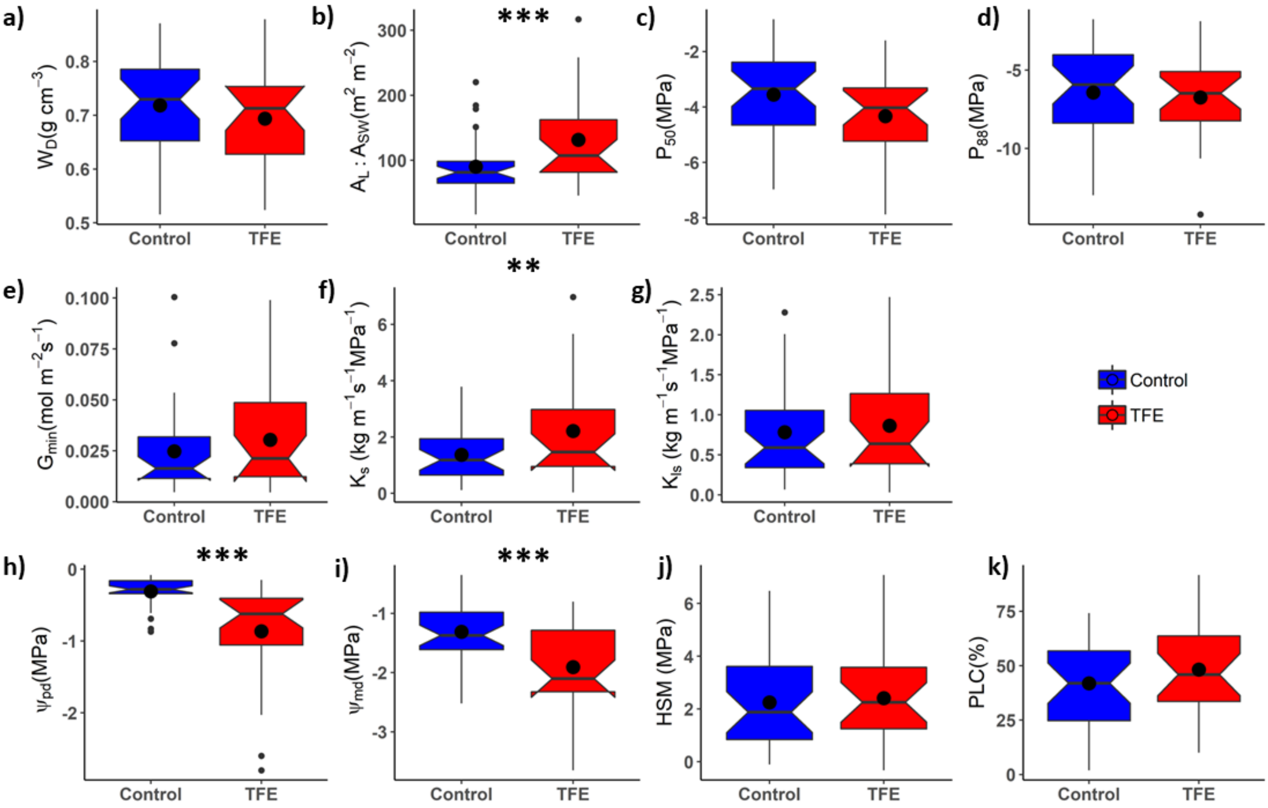


Figure 1 Stress indicators and hydraulic traits on the Control plot (blue) and through-fall exclusion (TFE, red). a) W_D – wood density b) A_L:A_{SW} - leaf to sapwood area ratio c) P_{50} - xylem embolism resistance; d) P_{88} - xylem embolism resistance; e) G_{min} – minimum stomatal conductance; f) K_s – maximum hydraulic specific conductivity; g) K_{sl} - maximum hydraulic leaf -specific conductivity; h) Ψ_{pd} - predawn water potential; i) Ψ_{md} - midday water potential. j) HSM – branch hydraulic safety margin to P_{50}; l) PLC – native dry season percentage loss of conductivity. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above quartile 3, if outliers are present and notches represents a confidence interval around the median represented by central line. Traits for which plot had a significant effect are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). P-values are from mixed effects analysis (see Table 2 for models and analysis section in Methods

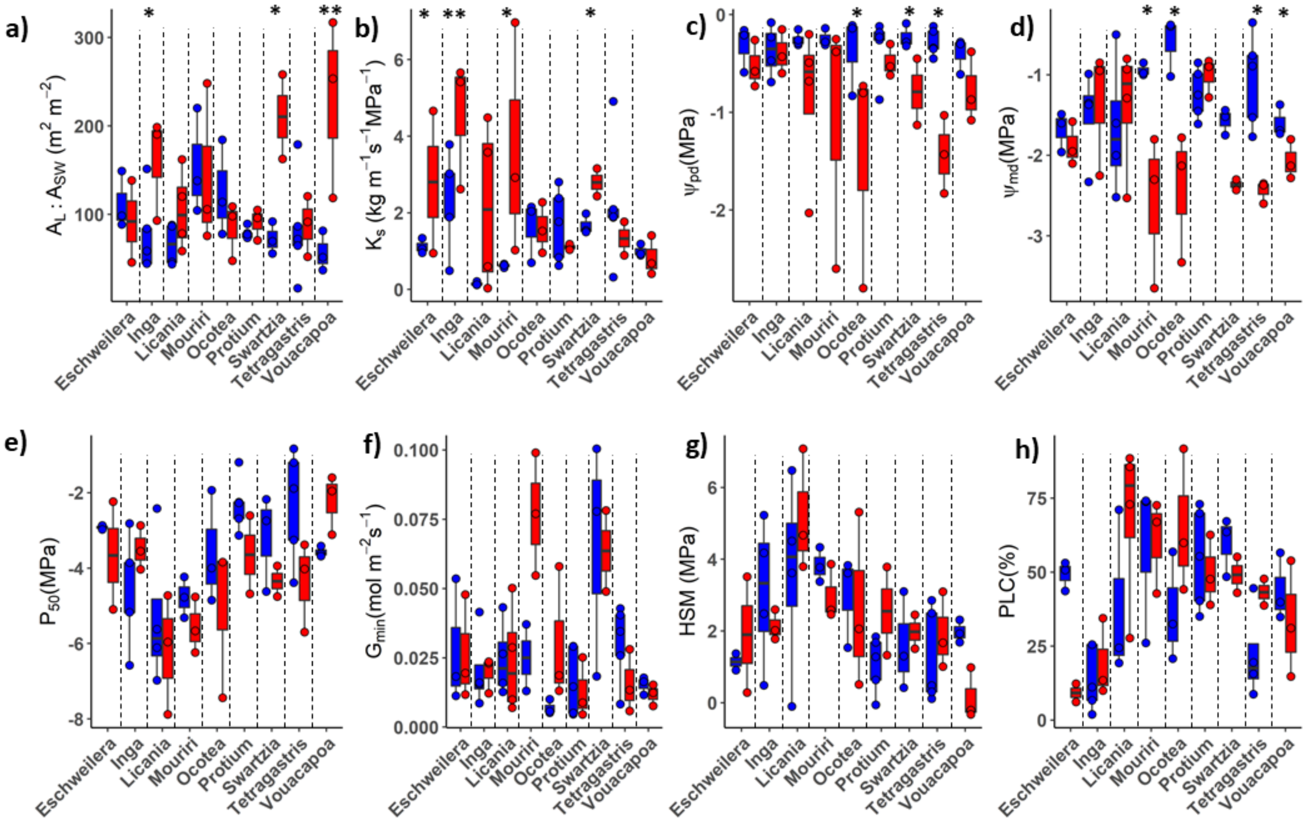


Figure 2 Drought stress indicators and hydraulic traits considered by genus on trees surviving after 15 years of throughfall exclusion (TFE – red) and the Control plot (blue). a) A_L:A_{SW} - leaf to sapwood area ratio (m^2 m^{-2}); b) K_s – maximum hydraulic specific conductivity; c) Ψ_{pd} - predawn water potential; d) Ψ_{md} - midday water potential; e) P_{50} - xylem embolism resistance; f) G_{min} – minimum stomatal conductance g) HSM– hydraulic safety margin to P_{50}; h) PLC – native dry season percentage loss of conductivity. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. The points represent individuals by genus in each treatment. Traits for which plot had a significant effect are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). P-values are from mixed effects analysis (see Table 2 for models and analysis section in Methods.

Table 2. Results of linear mixed effect models of plot (Control versus TFE) on the four key hydraulic variables (A_L: A_{SW}- Leaf to sapwood area ratio; K_s – maximum hydraulic specific conductivity; Ψ_{pd} - predawn water potential; Ψ_{md} - midday water potential, all of which showed significant changes between the TFE and Control plots. Intercept is the Control and the Plot effect is the difference of the TFE from the Control and random genus effects (on intercept is Control effect and Plot effect) are shown (see analysis section in Methods for details). The Numbers under Random Effects is standard deviation and under Fixed effects is coefficient values ± standard error. Significant intercept and fixed effects parameters are shown from F test with a standard error. Marginal (fixed effects only) and conditional R^2 (random and fixed effects) are shown (Mulkey & Pearcy 1992). The effect of species nested within genus was tested and, except for Ks, it did not yield a model with lower AIC (difference of more than 2) than genus alone (Table S3).

	Fixed Effects		**Random Effects**				
Variable	**Intercept**	**Plot**	**Intercept**	**Plot**	**Residual**	**R^2 M**	**R^2 C**
K_s	1.37±0.28***	1.02±0.43*	0.59	-	1.37	0.11	0.25
A_L:A_{SW}	87.65 ± 11.18**	54.68±17.09**	-	-	-	0.17	0.17
Ψ_{pd}	-0.31 ± 0.09**	-0.43± 0.14***	-	-	-	0.16	0.16
Ψ_{md}	-1.78 ± 0.13***	-0.60 ± 0.27 *	0.26	0.71	0.52	0.12	0.44

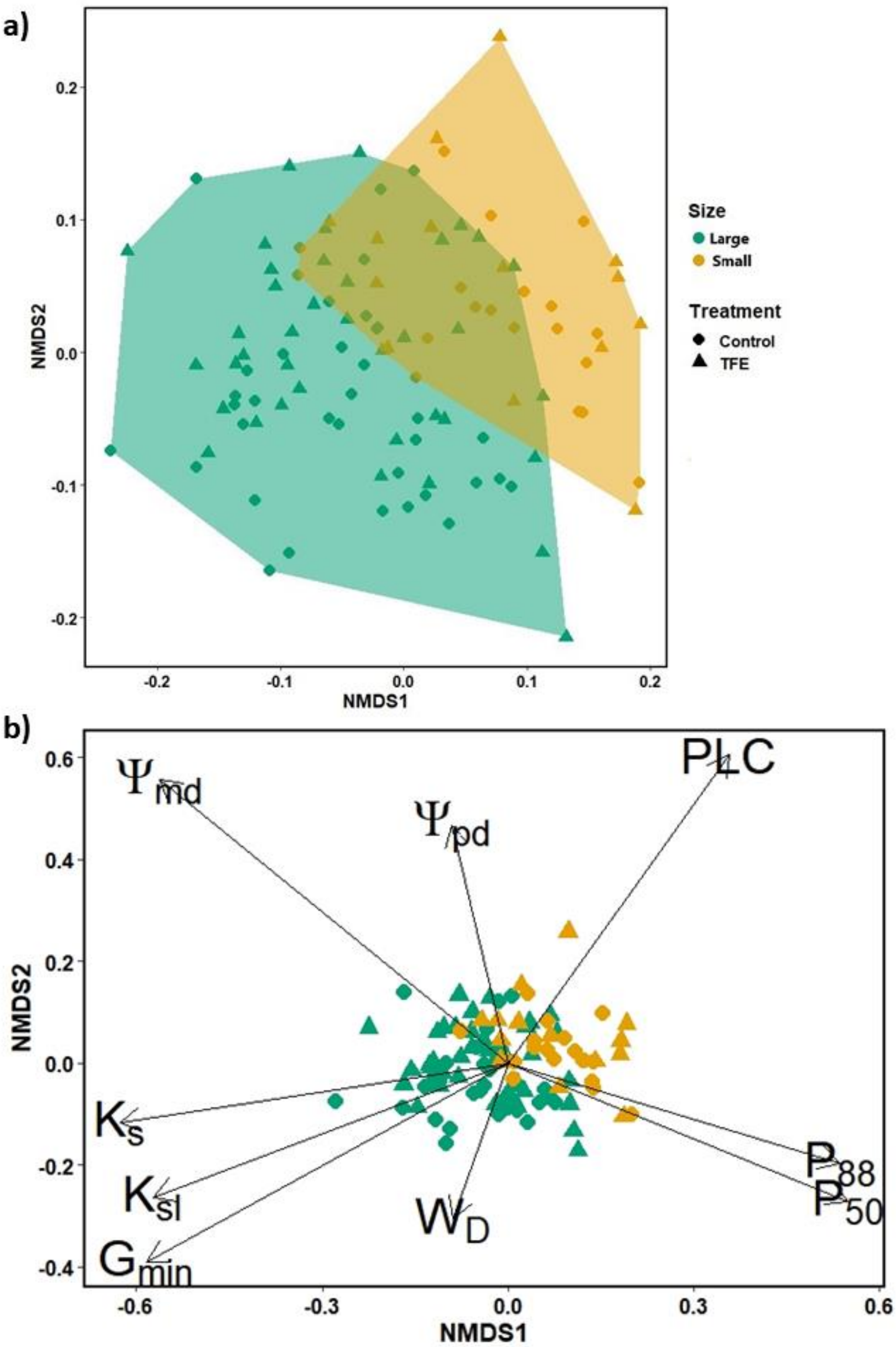


Figure 3 Non-metric multidimensional scaling (NMDS), a) ordination showing difference multidimensional filled between small and large trees indicating distinct hydraulic ecological strategies (MANOVA; $P < 0.05$) among TFE and Control. b) NNDS with 9 hydraulic traits represented by arrows (Arrow length represent predictor “strength”). Dots represent individuals in Control and triangles individuals in TFE treatment. The green colour represents Large trees and Yellow represents Small trees.

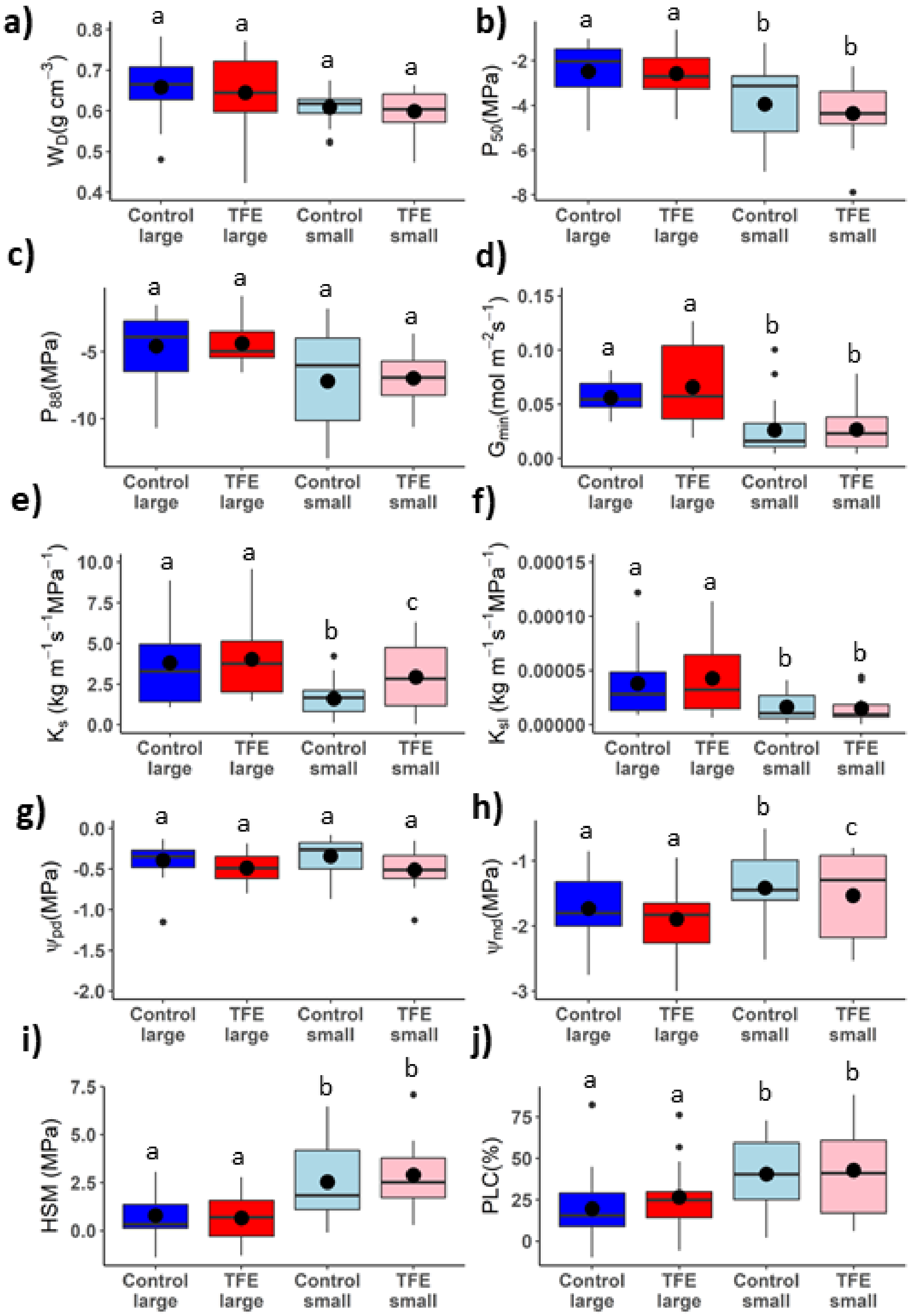


Figure 4 Comparison between small trees and large trees from the throughfall exclusion (TFE) and Control plots. a) W_D – wood density; b) P_{50} - xylem embolism resistance; c) P_{88} - xylem embolism resistance; d) G_{min} – minimum stomatal conductance; e) K_s – maximum hydraulic specific conductivity; f) K_{sl} - maximum hydraulic leaf -specific conductivity; g) Ψ_{pd} - predawn water potential; h) Ψ_{md} midday water potential; i) PLC – native dry season percentage loss of conductivity; j) HSM – branch hydraulic safety margin to P_{50}. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. Different letter indicate significant differences, $p<0.001$.

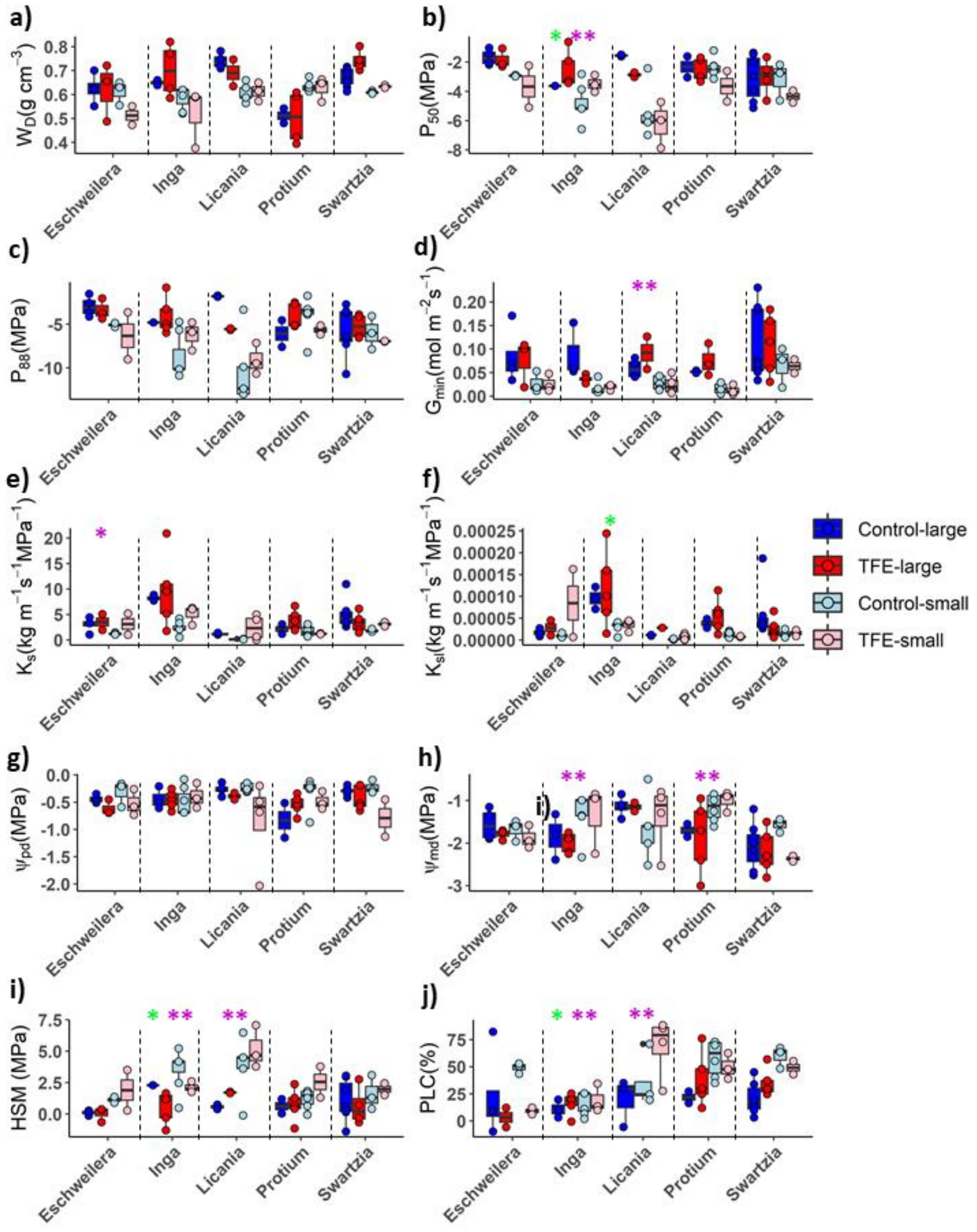


Figure 5 Comparison between small trees and large trees from throughfall exclusion (TFE) and Control plot. a) W_D – wood density; b) P_{50} - xylem embolism resistance; c) P_{88} - xylem embolism resistance; d) G_{min} – minimum stomatal conductance; e) K_s – maximum hydraulic specific conductivity; f) K_{sl} - maximum hydraulic leaf-specific conductivity; g) Ψ_{pd} - predawn water potential; h) Ψ_{md} midday water potential; i) HSM – hydraulic safety margin to P_{50}; j) PLC – native dry season percentage loss of conductivity. The box represents quartiles 1 and 3, with the central line indicating the median. The dots represents individuals values in each genera. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present.

Traits for which plot had a significant effect are marked with a green asterisk and traits for which size had a significant effect are marked with pink asterisk. P-values are from Wilcox test * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

Table 3. Results of linear mixed-effect models of size (large trees versus small trees) and plot effect (TFE and Control) on drought stress indicators and hydraulic traits. We tested the random genus effects on intercept and/or on plot and size (see Methods). Values for fixed effects are fitted parameter ± standard error. Values for Intercept column indicate the mean of the variable of large trees in Control plot, and the size column indicates of values of small trees on average in relation to large trees. Values for plot column indicate the values on the TFE plot in relation to Control plot. Values of size:plot column indicates the mean values of the interaction of small trees on TFE plot in relation Control and Large trees. Values for random effects are the standard deviation of the normal distribution from which coefficients were fitted. Marginal (R^2m - fixed effects only) and conditional R^2 (R^2c -random and fixed effects) are shown (Nakagawa & Schielzeth, 2013). Blank cells indicate that the effect is non-significant. P_{50} - xylem embolism resistance (MPa); G_{min} – minimum stomatal conductance (mol m^{-2} s^{-1}); K_s – maximum hydraulic specific conductivity (kg m^{-1} s^{-1} MPa^{-1}); Ψ_{md} - midday water potential (MPa); HSM – branch hydraulic safety margin to P_{50} (MPa); PLC – native dry season percentage loss of conductivity (%); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Variables	Fixed effect				Random effect			R^2m	R^2c
	Intercept	Size (small)	Plot (TFE)	Size:plot	Intercept	Plot	Resid.		
P_{50}	-2.64 ± 0.16***	-1.33±0.51**			0.98		1.12	0.20	0.39
G_{min}	-0.08±0.007*	-0.05±0.008			0.013		0.04	0.23	0.30
K_s	4.05±1.03**	-2.58±0.90*	0.45±0.6**	1.08±1.00	2.05	1.36	2.29	0.14	0.44
K_{ls}	6.13±0.45	-1.13 ±0.42			0.85		2.01	0.11	0.33
Ψ_{md}	-1.75 ± 0.12***	-0.60 ± 0.27 *			0.24		0.48	0.04	0.24
HSM	0.89± 0.30**	1.70± 0.31***			0.49		1.37	0.25	0.33
PLC	19.80± 4.74***	22.20±4.07			8.92		19.13	0.20	0.34

SUPPORTING INFORMATION

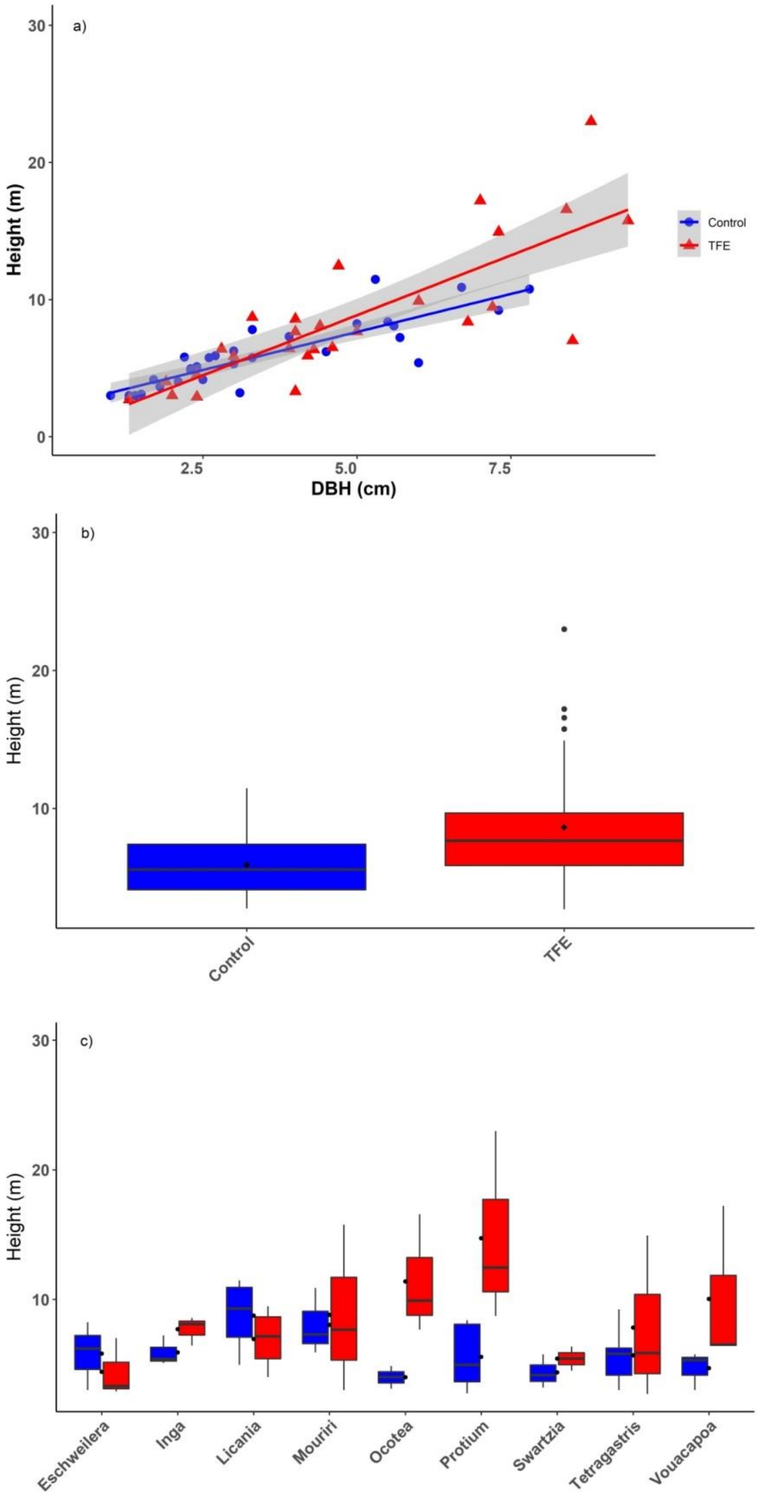


Figure S1. Height and diameter in each treatment (TFE vs. Control) and by genus for the most common small tree genera in this study (9 genera). a) Relationship between diameter (DBH) and Height by treatment, b) Height of Small trees by treatment c) Height of Small trees by genus. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present.

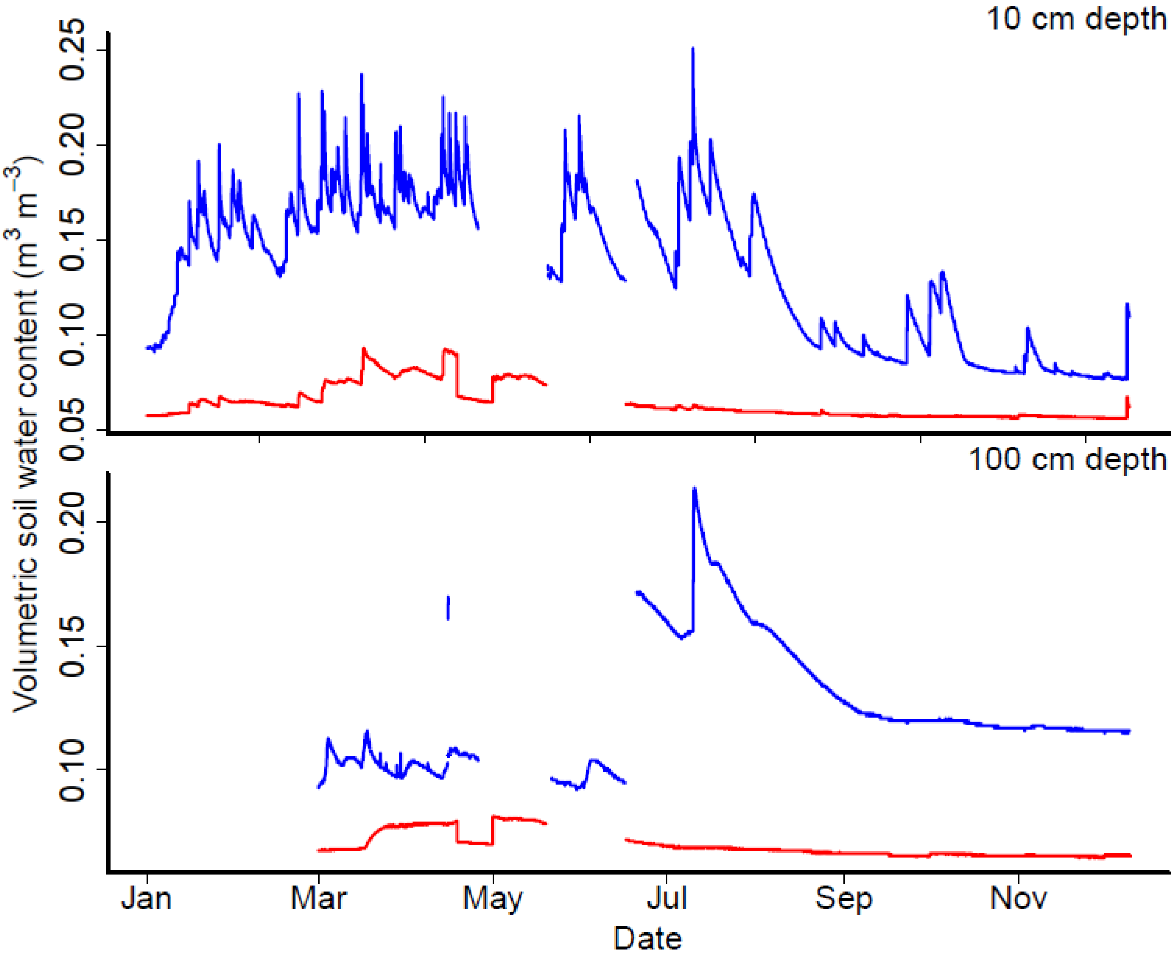


Figure S2. Soil water content during 2016 in the Throughfall Exclusion Experiment plot (red) and in the control plot (blue) at 10 cm and at 100 cm depth adapted from Bittencourt et al. 2020. The TFE had a mean reduction, in relation to control, of 48% and 56% in soil water content at 10 cm and 100 cm depth, respectively. Data are missing for periods when sensors failed.

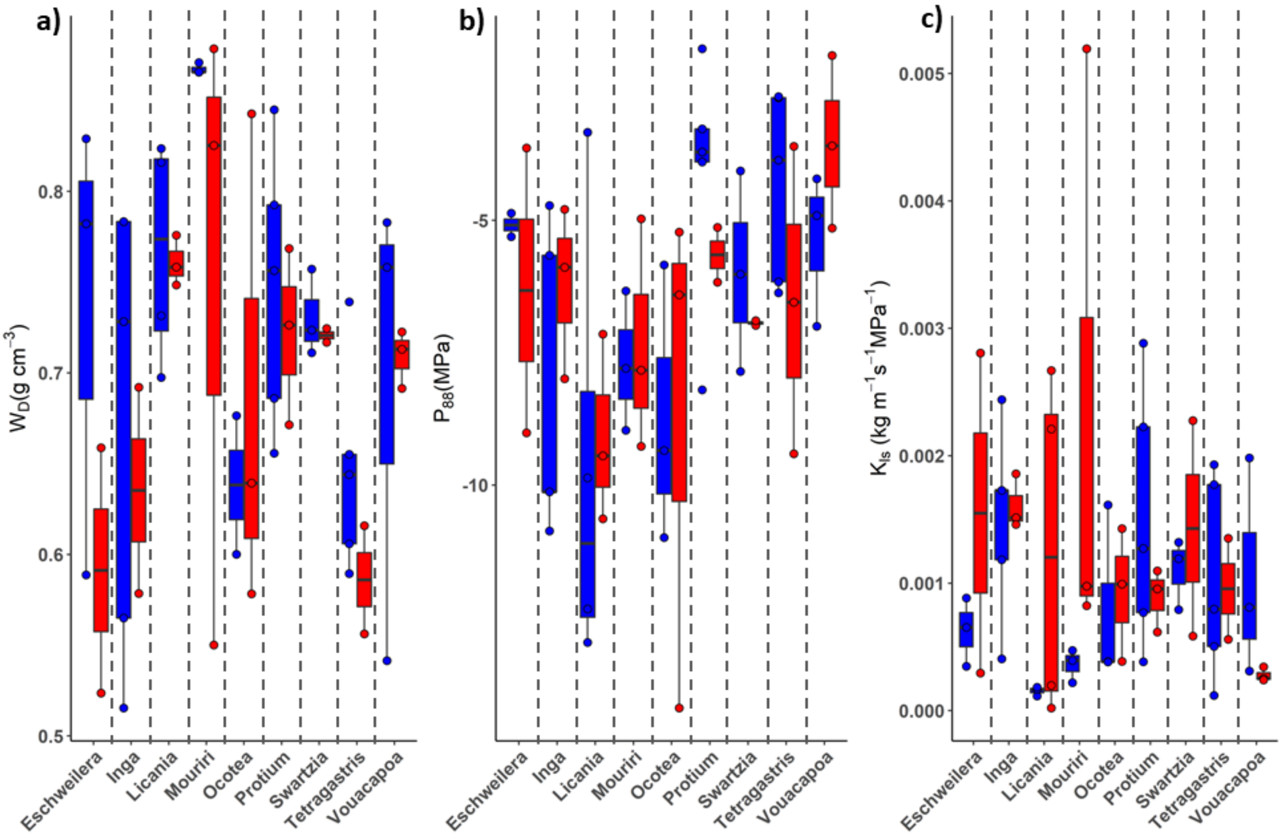


Figure S3. Hydraulic traits by genus for small trees from the throughfall exclusion (TFE) and Control plot. a) W_D – wood density b) P_{88} - xylem embolism resistance; c) K_{ls} - maximum hydraulic leaf -specific conductivity. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. P-values are from mixed effects analysis (see Table 2 for models and analysis section in Methods).

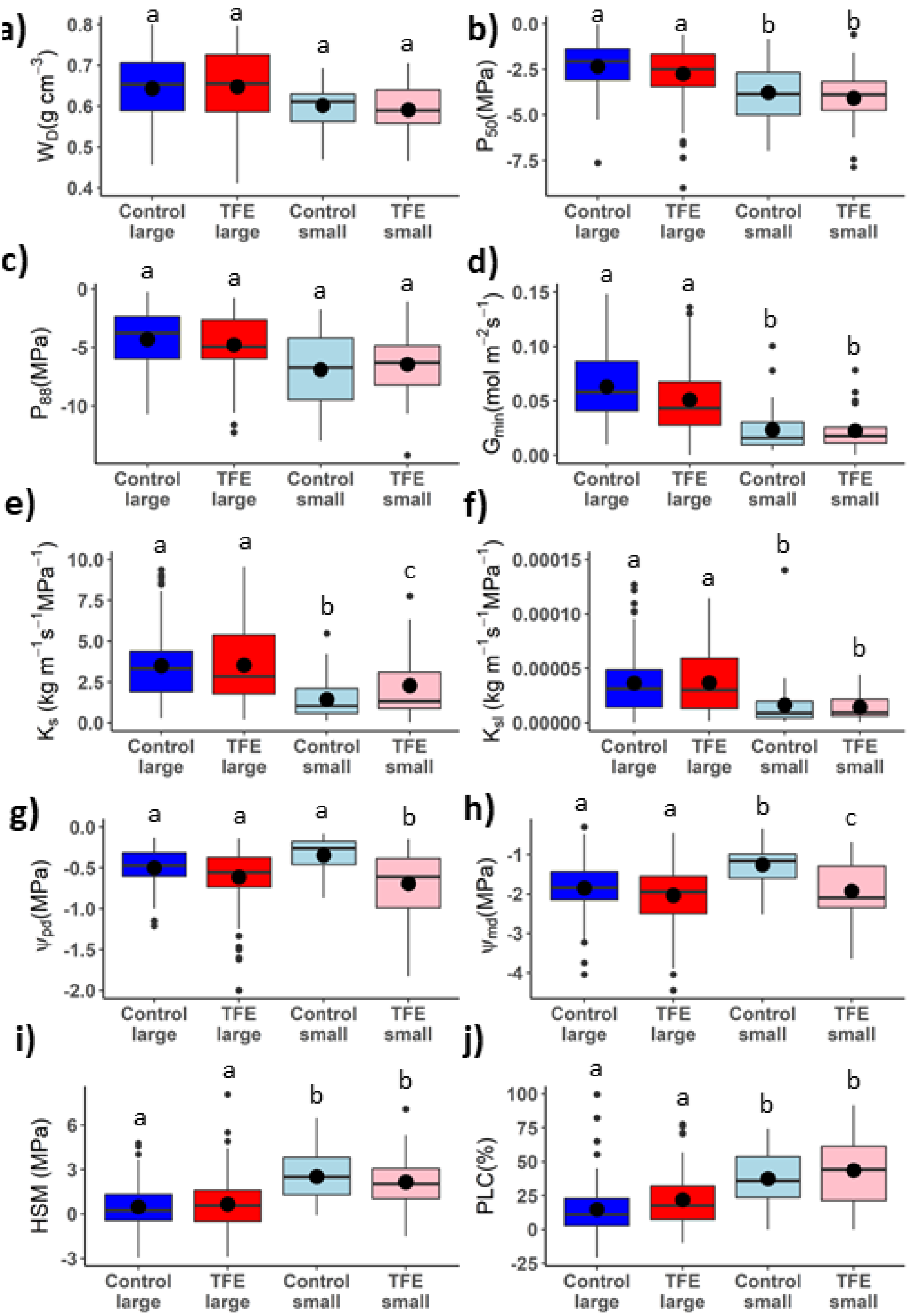


Figure S4 Comparison between the small trees and large trees from the throughfall exclusion (TFE) and Control plots from grouping all 9 genera available within the large and small tree groupings. Note that four of these genera were not present in both of the large and small tree groupings, and were thus excluded from Figure 3. a) W_D – wood density; b) P50 - xylem embolism resistance (MPa); c) P88 - xylem embolism resistance; d) G_{min} – minimum stomatal conductance; e) K_s – maximum hydraulic specific conductivity; f) K_{sl} - maximum hydraulic leaf -specific conductivity; g) Ψ_{pd} - predawn water potential; h) Ψ_{md} midday water potential; i) PLC – native dry season percentage loss of conductivity; j) HSM – hydraulic safety margin to P50. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. Different letter indicant significant differences, $p<0.001$. P-values are from Wilcoxon test.

Table S1. Linear mixed effect model analysis of stress indicator variables and hydraulic traits. Random genus effects on intercept and plot are tested against a null linear model without random effects. Values in bold highlight the significant (or more parsimonious for random effects) terms of the model. The final, significant model notation is: fixed effects outside brackets and random effects inside brackets; “1” indicates a fixed intercept; 1|genus is a random genus effect on intercept; or plot|genus indicate a random genus effect on plot effect; 1+ plot|genus indicates a random genus effect on intercept plus a random genus effect on height or plot effect (i.e. an interaction term of genus modelled as a random variable with plot). The numbers in random effects represent the standard deviation and in Fixed effects the coefficient values. Significant intercept and fixed effects parameters are shown with a standard error. R^2m is the marginal R² squared and R^2c is a conditional R squared. Blank cells indicate that the effect is non-significant

	P_{50}				**P_{88}**				**W_D**			
Random genus effect	Null	Intercept	Plot		Null	Intercept	Plot		Null	Intercept	Plot	
p-value		0.0012	0.01			0.005	0.04			0.14	0.26	
AIC	188.82	**182.25**	185.6		246.34	**242.22**	246.22		**-66.26**	**-66.38**	-62.58	
Fixed effects	Plot				Plot				Plot			
p-value	0.28				0.7				0.32			
Final model	P50 ~ plot + (1|genus)				P88 ~ 1 + (1|genus)				Wood density ~ 1			
	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation
	Intercept	-3.82	0.41		Intercept	-6.93	0.72		Intercept	0.71	0.014	
	Plot	-0.41	0.37							0		
	Genus			0.92	Genus			1.54		0		
	R2m	0.01			R2m	0			R2m			
	R2c	0.34			R2c	0.27			R2c			
	Kls				K_s				Ψpd			
Random genus effect	Null	Intercept	Plot		Null	Intercept	Plot	specie	Null	Intercept	Plot	
p-value		0.3	0.18			0.12	0.216	0.27		0.96	0.68	
AIC	**171.26**	172.19	172.39		178.87	178.46	**177.75**	179.7	**77.3**	79.3	77.3	
Fixed effects	Plot				Plot				Plot			
p-value	0.26	0.002			0.002**	<0.0001			**< 0.0001**			
Final model	Kleaf ~ 1				Ks max ~ plot				Ψ_{pd} ~ plot			

					(1|genus)							
	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation
	Intercept	1.01	0.29		Intercept	1.37	0.28		Intercept	0.31	0.09	
		0			plot	1.02	0.43		Plot	0.43	0.14	
		0			Genus				Genus			
	R2m				R2m	0.1			R2m	0.16		
	R2c				R2c	0.1			R2c	0.16		
	G_{min}				**A_L:A_{SW}**				**Ψmd**			
Random genus effect	Null	Intercept	Plot		Null	Intercept	Plot		Null	Intercept	Plot	
p-value		<0.001	0.006			0.97	0.19			0.96	0.03	
AIC	**-192.59**	-201.39	199.04		**521.56**	523.564	522.83		103.52	105.53	**100.93**	
Fixed effects	Plot				Plot				Plot	Genus		
p-value	0.2				<0.001				**0.001**	See tab 3		
Final model	G.min ~ plot+(1|genus)				LS ~ plot				Ψmd ~ plot +1 (plot|genus)			
	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation
	Intercept	0.027	0.006		Intercept	87.65	11.183		Intercept	1.32	0.13	
	Plot	0.007	0.0054		Plot	54.68	17.09		Plot	0.6	0.27	0.71
	Genus			0.017	Genus				Genus			0.26
	R2m	0.02			R2m	0.17			R2m	0.12		
	R2c	0.45			R2c	0.17			R2c	0.44		

	HSM				**PLC**			
Random genus effect	Null	Intercept	Plot	Height	Null	Intercept	Plot	
p-value		0.004	0.03	0.03		**<0.01**	0.03	
AIC	184.43	**208.06**	211.69	211.45	433.36	**427.66**	430.326	
Fixed effects	Plot				plot			
p-value	0.49				0.43			
Final model	HSM ~ 1 + (1|genus)				PLC ~ 1 + (1 + |genus)			
	Value	Standard error	Standard deviation		Parameter	Value	Standard error	Standard deviation
	2.4	0.39			Intercept	43.52	4.82	
			0.95					
					Genus			19.38
	0				R2m	0		

	0.3				R2c	0.31		

P_{50} - xylem embolism resistance (MPa); P_{88} - xylem embolism resistance (MPa); Ψ_{pd} - predawn water potential (MPa); Ψ_{md} midday water potential (MPa); HSM – hydraulic safety margin to P_{50} (MPa); PLC – native dry season percentage loss of conductivity (%); K_s – maximum hydraulic specific conductivity (kg m^{-1} s^{-1} MPa^{-1}); K_{sl} - maximum hydraulic leaf specific conductivity (kg m^{-1} s^{-1} MPa^{-1}); A_L:A_{SW} - Leaf to sapwood area ratio (m^2 m^{-2}); G_{min} – minimum stomatal conductance (mol m^{-2} s^{-1}); W_D – wood density (g cm^{-3}).

Table S2. – Comparison between large and small trees using linear mixed effect model analysis of hydraulic status variables and hydraulic traits. Random genus effects on intercept, plot and size are tested against a null linear model without random effects and against each other. Coefficients for the final fixed (mean and standard error) and random effects (standard deviation) are presented. Values in bold highlight the significant (or more parsimonious for random effects) terms of the model. The final, significant model notation is: fixed effects outside brackets and random effects inside brackets; "1" indicates a fixed intercept; 1|genus is a random genus effect on intercept; plot|genus indicate a random genus effect on the plot effect; 1+ plot|genus indicates a random genus effect on intercept plus a random genus effect on height or plot effect (i.e. an interaction term of genus modelled as a random variable with plot). Values for fixed effects are fitted parameter ± standard error; values for random effects are standard deviation of the normal distribution from which coefficients were fitted. The numbers in random effects are standard deviation and in Fixed effects are coefficient values ± standard deviation. Significant intercept and fixed effects parameters are shown with a standard error. Marginal (fixed effects only) and conditional R2 (random and fixed effects) are shown (Nakagawa & Schielzeth, 2013). R2m is the marginal R^2 squared and R2c is a conditional R squared. Blank cells indicate that the effect is non-significant

	Hydraulic traits											
	P_{50}				**P_{88}**				**G_{min}**			
Random genus effect	Null	Intercept	size	plot	Null	Intercept	plot	size	Null	**Intercept**	Size	plot
p-value		289.44	**<0.001**	0.14		0.07	0.31	**0.31**		**0.02**	0.15	0.17
AIC	291.66	0.04	**281.18**	292.18	395.01	393.85	397.49	**397.49**	-339.47	**-342.46**	-338.7	-338.46
Fixed effects	null	**Size**	plot	Plot:Size	Null	**Size**	plot	Plot:Size	Null	Size	Plot	Plot:size
p-value		**0.03**	0.32	0.13		0.06	0.78	0.3		**<0.001**	<0.001	0.88
AICC	289.12	**279.85**	283.16	282.64	**389.08**	387.66	391	291.42	-329.23	**-358.05**	-354.8	-327.25
Final model	P50~ size (1+size|genus)				P88 ~ 1+ (1+size|genus)				g.min ~size+(1|genus)			
	Parameter	Standard deviation	Value	Standard error	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation
	Intercept		-2.64	0.16	Intercept	-5.07	0.28		Intercept	0.08	0.007	
	Plot		-1.22	0.51	Plot				plot	-0.05	0.008	
	Genus	1.12			Genus			0.26	Genus			0.013
	Size	0.98			Size			2.76	Size			
	Plot:size				Plot:size				Plot:size			
	R2m		0.2		R2m	0			R2m	0.23		
	R2c		0.39		R2c	0.36			R2c	0.3		
	K_{sl}				**K_s**				**Ψ_{pd}**			
	Null	Intercept	Size	Plot	Null	Intercept	Size	plot	Null	Intercept	Size	plot
Random genus effect		0.001	**0.001**	0.008		<0.01	**<0.001**	<0.001		0.97	0.21	
p-value	465.24	456.7	**456.05**	459.49	489.37	472.47	**468.02**	473.44	**24.76**	26.76	26.31	
AIC	Null	Size	Plot	Size:plot	Null	Size	Plot	Plot:size	null	**Size**	plot	Plot:Size
Fixed effects		0.17	0.91	0.55		0.03	0.07	**0.03**		0.97	**0.001**	<0.001
p-value	**457.36**	457.53	459.35	461.27	472.9	469.9	471.16	**469.45**	24.76	22.76	**15.59**	16.23
AICC	Kleaf ~ 1 +(1+size|genus)				Ks max ~ plot				Ψ_{pd} ~ plot			
Final model	Parameter	Value	Standard error	Stardard deviation	Parameter	Value	Standard error		Parameter	Value	Standard error	Standard deviation
	Intercept	5.76	0.44		Intercept	4.05	1.033		Intercept	-0.37	0.03	
	plot				plot	0.45	0.6		Plot	-0.14	0.05	
	Genus			1.6	Genus			2.05				
	Size			1.92	Size	-2.58	0.9	1.36				
	Plot:size				Plot:size	1.08	1					
	R2m	0			R2m	0.14			R2m	0.07		
	R2c	0.26			R2c	0.44			R2c	0.07		
	Ψ_{md}				**HSM**				**PLC**			
Random genus effect	Null	Intercept	Plot	size	Null	Intercept	Plot	size	Null	Intercept	Plot	size
p-value		<0.001	<0.001	<0.001		0.002	0.08	0.006		<0.001	<0.001	<0.001

AIC	195.31	174.94	175.71	**171.79**	309.76	**306.41**	309.24	207.76	875.22	863.31	865.77	**863.28**
Fixed effects	Null	Size	plot	Size:plot	Null	size	plot	Size:plot	Null	Size	Plot	Size:plot
p-value		**0.01**	0.01	0.03		0.01	0.01	0.03		<0.001	0.41	<0.001
AICC	174.77	**169.77**	173.62	171.89	174.27	169.77	169.9	171.89	897.12	**872.87**	898.46	873.22
Final model	Ψ_{md} ~ +size (1|genus)				HSM ~ size+ (1|genus)				PLC ~ size+ (1 + |genus)			
	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation	Parameter	Value	Standard error	Standard deviation
	Intercept	-1.6	0.11		Intercept	0.89	0.3		Intercept	19.8	4.7	
	Plot				Size	1.7	0.31		Size	22.2	4.07	
	Genus	0.34		0.26	Genus			0.48	Genus			8.92
	Size	0.39		0.71								
	Plot:size											
	R2m	0			R2m	0.25			R2m	0.2		
	R2c	0.32			R2c	0.33			R2c	0.34		

P_{50} - xylem embolism resistance (MPa); P_{88} - xylem embolism resistance (MPa); Ψ_{pd} - predawn water potential (MPa); Ψ_{md} - midday water potential (MPa); HSM – hydraulic safety margin to P_{50} (MPa); PLC – native dry season percentage loss of conductivity (%); K_s – maximum hydraulic specific conductivity (kg m m^{-2} s^{-1} MPa^{-1}); K_{sl} - maximum hydraulic leaf specific conductivity (kg m^{-1} s^{-1} MPa^{-1}); Leaf: sapwood ratio - leaf to sapwood area (m^2 m^{-2}); G_{min} – minimum stomatal conductance (mol m^{-2} s^{-1}); WD – wood density (g cm^{-3}).

Table S3 - Numbers of individuals for small and large tree in each treatment (TFE an controls) and mean and standard deviation of P_{50} - xylem embolism resistance (MPa); Ψ_{pd} - predawn water potential (MPa); Ψ_{md} - midday water potential (MPa); HSM– hydraulic safety margin to P_{50} (MPa); PLC – native dry season percentage loss of conductivity (%); K_s – maximum hydraulic specific conductivity (kg m m^{-1} s^{-1} MPa^{-1}); W_D- Woody density; K_{sl} - maximum hydraulic leaf-specific conductivity (kg m m^{-1} s^{-1} MPa^{-1}); A_L:A_{SW} – leaf to sapwood area ratio (m^2 m^{-2}); G_{min} – minimum stomatal conductance (mol m^{-2} s^{-1}); WD – wood density (g cm^{-3}), separated by genus, size and treatment.

			Small				**Large**		
Genus				**Mean±sd**				**Mean±sd**	
	Traits	N TFE	N Control	TFE	Control	N TFE	N Control	TFE	Control
Eschweilera	Ψ_{pd}	3	3	-0.52±0.24	-0.32±0.24	7	7	-0.61±0.14	-0.44±0.07
Inga	Ψ_{pd}	7	3	-0.39±0.23	-0.51±0.23	7	7	-0.45±0.16	-0.44±0.21
Licania	Ψ_{pd}	5	4	-0.85±0.81	-0.23±0.81	7	7	-0.39±0.08	-0.27±0.11
Protium	Ψ_{pd}	6	3	-0.48±0.17	-0.31±0.17	6	8	-0.55±0.17	-0.83±0.45
Swartzia	Ψ_{pd}	3	2	-0.79±0.48	-0.23±0.48	8	8	-0.43±0.22	-0.3±0.07
Eschweilera	Ψ_{md}	3	3	-1.87±0.27	-1.68±0.27	7	7	-1.79±0.13	-1.56±0.37
Inga	Ψ_{md}	7	3	-1.35±0.78	-1.29±0.78	7	7	-1.98±0.23	-1.84±0.54
Licania	Ψ_{md}	5	4	-1.39±0.79	-1644±0.79	7	7	-1.15±0.14	-1.13±0.24
Protium	Ψ_{md}	6	3	-1±0.24	-1.17±0.24	6	8	-1.86±0.84	-1.7±0.21
Swartzia	Ψ_{md}	3	2	-2.4±0.09	-1.57±0.09	8	8	-2±0.52	-2.08±0.52
Eschweilera	P_{50}	2	2	-3.7±2.02	-2.92±2.02	6	5	-1.82±0.67	-1.67±0.55
Inga	P_{50}	7	3	-3.48±0.58	-4.85±0.58	3	6	-2.52±1.25	-3.62±NA
Licania	P_{50}	5	3	-6.19±1.59	-5.45±1.59	4	4	-2.86±0.22	-1.55±0.08
Protium	P_{50}	6	2	-3.64±1.47	-2.37±1.47	3	6	-2.59±0.76	-2.32±1.02
Swartzia	P_{50}	3	2	-4.34±0.58	-3.18±0.58	7	5	-3±1.26	-3.08±1.55
Eschweilera	P_{88}	2	2	-6.3±3.8	-5.09±3.8	6	5	-3.38±1.19	-2.96±1.16
Inga	P_{88}	7	3	-6.22±1.63	-8.82±1.63	3	6	-4.08±2.08	-4.81±NA
Licania	P_{88}	5	3	-9.08±1.77	-10.3±1.77	4	4	-5.56±0.13	-1.81±0.09
Protium	P_{88}	6	2	-5.65±0.73	-4.02±0.73	3	6	-4.13±1.39	-6.07±2.15
Swartzia	P_{88}	3	2	-6.94±0.06	-5.98±0.06	7	5	-5.23±1.41	-5.84±2.79
Eschweilera	HSM	2	2	1.9±2.28	1.14±2.28	6	5	0.03±0.59	0.1±0.18
Inga	HSM	7	3	2.13±0.42	3.56±0.42	3	6	0.54±1.29	2.3±NA
Licania	HSM	5	3	5.18±1.7	3.8±1.7	4	4	1.71±0.08	0.57±0.26
Protium	HSM	6	2	2.55±1.74	1.2±1.74	3	6	0.73±1.29	0.62±0.8
Swartzia	HSM	3	2	1.98±0.67	1.61±0.67	7	5	0.63±1.54	1.09±1.64
Eschweilera	PLC	3	2	9.22±4.36	49.15±4.36	7	6	3.23±12.66	22.71±40.73
Inga	PLC	7	3	19.29±13.	17.43±13.	7	7	16.14±10.	10.95±8.2

				22	22			15	6
Licania	PLC	4	4	68.67±28.13	34.84±28.13	5	3	NA±NA	19.82±22.08
Protium	PLC	6	3	49.75±11.95	57.77±11.95	5	8	38.24±24.86	21.86±7.6
Swartzia	PLC	3	2	49.13±8.58	59.73±8.58	6	7	35±13.09	20.98±15.62
Eschweilera	G_{min}	3	3	0.03±0.02	0.03±0.02	7	7	0.08±0.05	0.08±0.06
Inga	G_{min}	7	3	0.02±0.01	0.02±0.01	7	6	0.04±0.01	0.09±0.06
Licania	G_{min}	5	4	0.02±0.02	0.03±0.02	7	6	0.09±0.05	0.06±0.02
Protium	G_{min}	6	3	0.01±0.01	0.02±0.01	6	6	0.07±0.03	0.05±0
Swartzia	G_{min}	3	2	0.06±0.02	0.07±0.02	8	8	0.11±0.07	0.11±0.08
Eschweilera	K_s	3	2	3.12±2.93	1.25±2.93	7	6	3.54±2.23	3.03±1.41
Inga	K_s	7	3	5.09±1.88	2.36±1.88	7	7	9.68±7.24	8.23±0.57
Licania	K_s	4	4	2.42±2.44	0.16±2.44	5	3	NA±NA	1.22±0.17
Protium	K_s	6	3	1.23±0.08	1.72±0.08	5	8	3.91±1.95	2.16±1.31
Swartzia	K_s	3	2	3.1±0.57	1.87±0.57	6	7	3.44±1.85	5.15±3.07
Eschweilera	K_{sl}	3	2	3.7±0.58	5.67±0.58	7	6	5±1	3.25±0.5
Inga	K_{sl}	7	3	5±1	4.67±1	7	7	5.4±1.14	3.33±0.58
Licania	K_{sl}	4	4	4.25±0.96	4.2±0.96	5	3	7.5±2.12	7.5±0.71
Protium	K_{sl}	6	3	4.3±0.58	4.5±0.58	5	8	7.4±2.41	8±1.41
Swartzia	K_{sl}	3	2	8±1.41	8±1.41	6	7	7.2±2.39	7.75±3.2
Eschweilera	W_D	3	2	0.51±0.06	0.61±0.06	7	7	0.62±0.12	0.63±0.06
Inga	W_D	7	3	0.52±0.12	0.59±0.12	6	6	0.7±0.11	0.65±0.01
Licania	W_D	5	3	0.61±0.04	0.61±0.04	6	7	0.69±0.08	0.74±0.03
Protium	W_D	6	3	0.63±0.05	0.63±0.05	4	7	0.5±0.11	0.51±0.04
Swartzia	W_D	3	2	0.63±0.01	0.61±0.01	8	7	0.74±0.04	0.67±0.04

Table S4. Statistics from the NMDS modelling shown in Figure 3. The R2 and the significance' of fitted vectors (traits) in the analysis, assessed using permutation of environmental variables and the loading of the traits on to the two axes.

	NMDS1	**NMDS2**	**R2**	**Pr(>r)**
G_{min}	-0.83186	-0.55499	0.493	0.001
W_D	-0.27786	-0.96062	0.1015	0.044
Ψ_{pd}	-0.19057	0.98167	0.2255	0.001
Ψ_{md}	-0.71157	0.70262	0.6243	0.001
P_{50}	0.89701	-0.442	0.3749	0.001
P_{88}	0.9392	-0.34337	0.3264	0.001
K_s	-0.98323	-0.18239	0.4069	0.001
K_{sl}	-0.90904	-0.41671	0.3983	0.001
PLC	0.50789	0.86142	0.4935	0.001

Table S5–Results of linear effect models of size (Size vs TFE) and genus on the hydraulic variables on Control plot. Significant intercept and fixed effects parameters are shown with a standard error. Values in bold highlight the significant (or more parsimonious for random effects) terms of the. R^2 is a R square, and blank cells indicate that the effect is non-significant.

		Fixed Effects					
Variable	Model			genus	P value	AIc	R^2
Ψ_{pd}	null					23.15	
	Size				0.16	23.15	
	Genus				0.78	29.26	
	Size*Genus	size	genus	Size:genus	0.15	27.09	0.20
	F-value						
	Df						
	P						
Ψ_{md}	Model			genus	P value	AIc	R^2 C
	null				<0.01	99.71	
	Size				0.12	99.22	
	Genus				<0.01	83.53	
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**81.81**	
	F-value	38.532	2.42	1.16			
	Df	1	4	4			
	P	0.05	**<0.001**	0.09			
HSM	**Model**			genus	**P value**	**AIc**	R2 C
	null					150.36	
	Genus				<0.01	144.95	
	Size				<0.01	137.95	
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**134.14**	0.43
	F-value	**21.74**	**4.16**	0.62			
	Df	1	4	4			
	P	**<0.01**	**<0.01**	0.64			
P_{50}	**Model**			genus	**P value**	**AIc**	R2 C
	null					135.67	
	Genus				0.08	134.67	
	Size				<0.01	124.50	
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**124.84**	0.38
	F-value	**17.73**	2.57	0.99			
	Df	1	4	4			
	P	**<0.01**	0.05	0.42			
P_{88}	**Model**			**genus**	**P value**	**AIc**	R2 C
	null				<0.01	23974	
	Genus				0.20	239.23	

	Size				<0.01	234.27	
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**22.58**	0.37
	F-value	**10.85**	**2.48**	**3.9**			
	Df	1	4	4			
	P	**<0.01**	0.06	**<0.01**			
PLC	**Model**			**genus**	**P value**	**AIc**	R2 C
	null				**<0.01**	**414.64**	
	Genus				0.15	402.96	
	Size				**<0.01**	**410.37**	
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**390.66**	0.39
	F-value	**11.58**	**7.22**	**3.37**			
	Df	1	4	4			
	P	**<0.01**	**<0.01**	**0.01**			
K_S	**Model**			**genus**	**P value**	**AIc**	R2 C
	null					244.94	
	Genus		**genus**		<0.01	235.12	
	Size				0.09	249.95	
	Size*Genus				**<0.01**	**237.34**	0.54
	F-value		**4.85**				
	Df		4				
	P		**<0.01**				
K_{sl}	**Model**			**genus**	**P value**	**AIc**	R2 C
	null					216.35	
	Genus				0.08	215.61	
	Size				**0.01**	212.32	
	Size*Genus	**size**	**genus**	**Size:genus**	**0.01**	**211.55**	
	F-value	**2.39**	**4.44**	**2.54**			0.22
	Df	1	4	4			
	P	**<0.01**	**<0.01**	0.05			
G_{min}	**Model**			**genus**	**P value**	**AIc**	R2 C
	null					**-147.52**	
	Genus				0.12	-147.07	
	Size	**size**			**0.04**	**-157.94**	
	Size*Genus				**<0.01**	**-149.94**	0.20
	F-value	**12.79**					
	Df	1					
	P	**<0.01**					
W_D	**Model**			**genus**	**P value**	**AIc**	R2 C
	null					-74.17	
	Genus				**0.02**	-78.79	
	Size	**size**			**0.02**	-77.48	
	Size*Genus				**<0.01**	**-87.79**	0.20
	F-value	**7.75**	**4.10**	**2.83**			
	Df	1	4	4			
	P	**<0.01**	**<0.01**	**0.03**			

Table S6 Results of linear effect models of size (Size vs TFE) and genus on the hydraulic variables on TFE plot. Significant intercept and fixed effects parameters are shown with a standard error. Values in bold highlight the significant (or more parsimonious for random effects) terms of the. R² is a R squared. Blank cells indicate that the effect is non-significant.

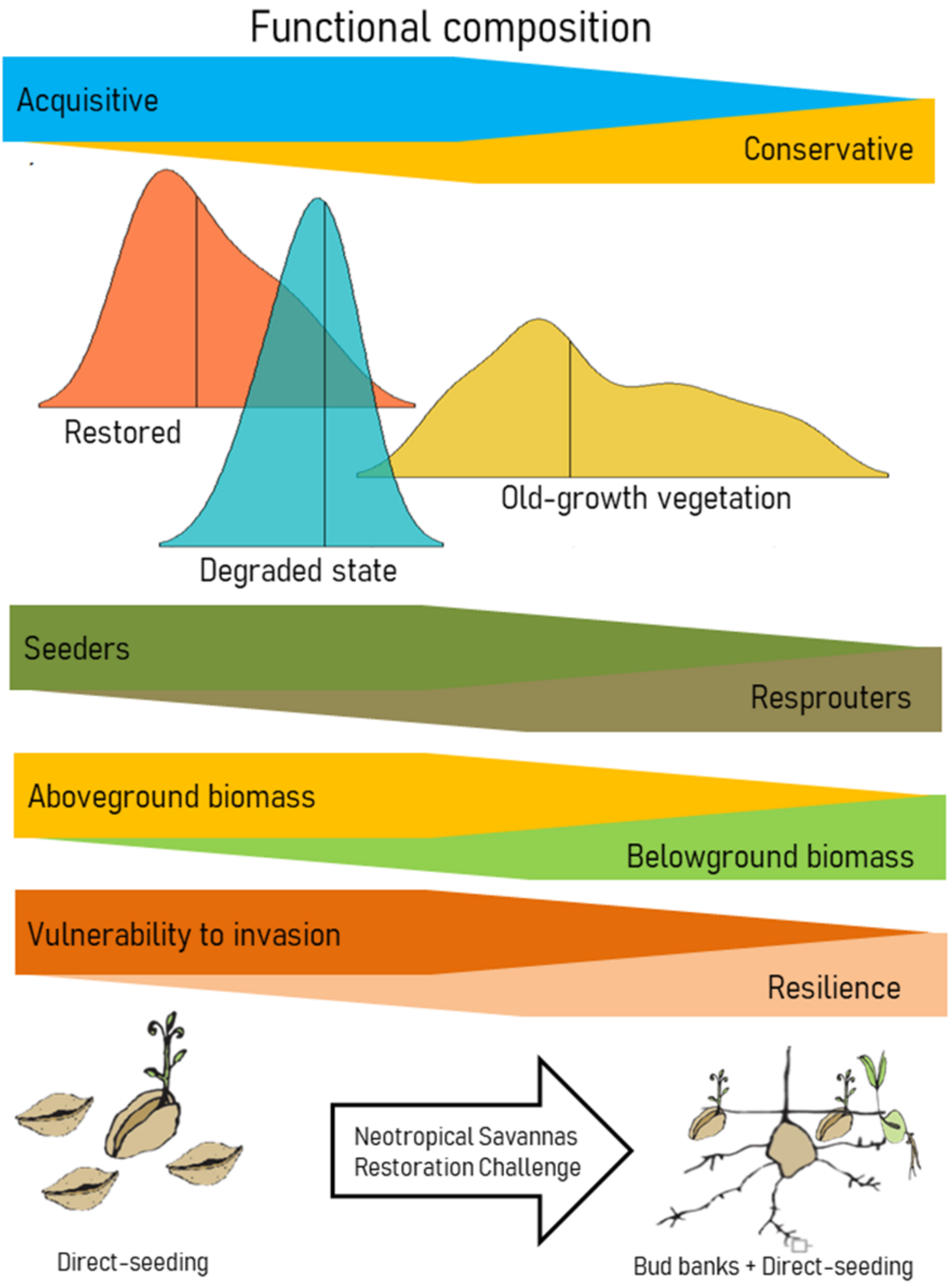
		Fixed Effects					Random Effects			
Variable	Model			genus	P value	AIc	Genus	size	R^2 M	R^2 C
Ψ_{pd}	null					-10.8	-	-		
	Size				<0.01	-9.85				
	Genus				<0.01	-13.23				
	Size*Genus	size	genus	Size:genus	**<0.01**	**-16.24**			0.20	0.20
	F-value	21.95	**3.54**	2.68						
	Df	1	**4**	4						
	P	0.27	**<0.01**	0.06						
Ψ_{md}	Model			genus	P value	AIc	Genus	size	R^2 M	R^2 C
	null				<0.01	88.57	-	-		
	Size				<0.01	85.7				
	Genus				<0.01	85.55				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**80.81**			0.23	
	F-value	38.532	2.42	1.16						
	Df	1	4	4						
	P	**0.01**	**0.04**	0.05						
HSM	**Model**			genus	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null					185.31	-	-		
	Genus				<0.01	174.64				
	Size				<0.01	182.55				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**173.04**				0.35
	F-value	**16.7**	**2.5**	3.31						
	Df	1	4	4						
	P	**<0.01**	0.05	0.18						
P_{50}	**Model**			genus	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null				<0.01	176.15	-	-	0.35	
	Genus				<0.01	172.70				
	Size				0.03	167.15				
	Size*Genus	**size**	genus	Size:genus	**<0.01**	**157.50**				0.43
	F-value	**16.25**	3.63	3.26						
	Df	1	4	4						
	P	**<0.01**	**0.013**	**<0.02**						
P_{88}	**Model**			**genus**	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null				<0.01	23974	-	-		
	Genus				0.20	239.23				
	Size				<0.01	234.27				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**22.58**				0.37
	F-value	**10.85**	**2.48**	**3.9**						
	Df	1	4	4						

	P	**<0.01**	0.06	**<0.01**						
PLC	**Model**			**genus**	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null				**<0.01**	**491.58**	-	-		
	Genus				0.15	492.29				
	Size				**<0.01**	**474.98**				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**468.99**				0.39
	F-value	**27.38**	**3.32**	**2.20**						
	Df	1	4	4						
	P	**<0.01**	**0.01**	0.08						
K_S	**Model**			**genus**	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null				**<0.01**	253.90	-	-		
	Genus				**<0.01**	239.17				
	Size				**<0.01**	243.69				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**220.07**				0.54
	F-value	**30.94**	**8.27**	**1.82**						
	Df	1	4	4						
	P	**<0.01**	**<0.01**	0.14						
K_{sl}	**Model**			**genus**	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null				**<0.01**	252.65	-	-		
	Genus				**<0.01**	246.02				
	Size				0.19	252.89				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**242.34**				
	F-value	**2.39**	**4.44**	**2.54**						0.29
	Df	1	4	4						
	P	**<0.01**	**<0.01**	0.05						
G_{min}	**Model**			**genus**	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null				**<0.01**	**-174.4**	-	-		
	Genus				**<0.01**	**-176.76**				
	Size				**<0.01**	**-193.44**				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**-191.86**				0.35
	F-value	**26.69**	**2.36**	**1.02**						
	Df	1	4	4						
	P	**<0.01**	0.06	0.40						
W_D	**Model**			**genus**	**P value**	**AIc**	Genus	size	R2 M	R2 C
	null				**<0.01**	**-122.23**	-	-		
	Genus				**0.11**	**-122.32**				
	Size				**<0.01**	**-127.86**				
	Size*Genus	**size**	**genus**	**Size:genus**	**<0.01**	**-148.67**				0.39
	F-value	**13.07**	**2.89**	**7.82**						
	Df	1	4	4						
	P	**<0.01**	0.03	**<0.01**						

CHAPTER 3- Original research

How effective is direct seeding to restore the functional composition of neotropical savannas?

Journal norms: Restoration ecology



How effective is direct seeding to restore the functional composition of neotropical savannas?

Running head:

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Author contributions

IBS, ABS, conceived the restoration practice for this research. ALG, PBC, RSO and AA conceived the experimental design. ALG, GW, PBC, AB, LL, LV, MC, AP performed the data sampling in the field and laboratory; ALG; AMD analysed the data; ALG, LR, RSO, AA and MCS wrote and edited the manuscript. All Authors had substantial contributions do manuscript writing.

Abstract

Species loss leads to changes in ecosystem function and services, impacting human well-being. Although biodiversity restoration is pivotal to circumvent this situation, the techniques for restoring old-growth tropical grassy biomes (TOGGBs) are still limited and the restoration outcomes remain unpredictable. Here, we use a trait-based approach to understand the functional outcomes of ecological restoration via direct seeding in a Brazilian savanna (*Cerrado*). We compared the functional composition and total biomass and biomass allocation of a restored relative to a degraded savanna (abandoned pasture) dominated by exotic grasses and a well-preserved old-growth native savanna. We found that the functional composition of restored communities was very similar to those dominated by exotic grasses, both characterized by a greater abundance of species with acquisitive traits, higher above-ground biomass, and lower investment in root biomass. In contrast, the native vegetation exhibited a dominance of conservative traits and higher investment in belowground rather than aboveground biomass. Even though the acquisitive traits in the restored savanna allow a fast aboveground biomass accumulation and soil cover, the lower belowground biomass investment in the restored savanna may limit its resistance and resilience to droughts and fires. Our findings suggest that restoration efforts in *Cerrado* should focus on fostering the establishment of slow-growing species to recover the ecosystem services provided by *Cerrado* biodiversity.

Key words: *Cerrado*; Seeders; Resprouters; Acquisitive; Conservative; Restoration practice.

Implications for Practice

- Current large-scale techniques for restoring Brazilian savannas (*Cerrado*) did not recover the slow-growth component of old-growth savannas
- Aboveground biomass and soil cover can be quickly recovered after direct-seeding restoration but not belowground biomass
- The lack of belowground biomass pools in restored savanna compared to the old-growth reference may threaten long-term restoration success that implies in low resilience and invasion resistance

Introduction

The process of human occupation in the tropics led to the conversion of hyper-diverse native vegetation into degraded ecosystems (Barlow et al., 2018, IBPES 2019). Especially in this decade, the United Nations will support a "Decade on Ecosystem Restoration" from 2021 to 2030 in an attempt to restore lost ecosystem services and stem the rapid decline of biodiversity (UNEP and FAO 2020, Strassburg et al., 2020). Most restoration knowledge is focused on forest successional trajectories, involving mainly woody plants (Silveira et al. 2020; Veldman, Buisson, et al. 2015). However, *the old-growth tropical grassy biomes* (TOGGBs) occupy >40% of the land surface (Veldman, Buisson, et al., 2015). TOGGBs include neotropical savannas with open-canopy where >50% of the ground layer is covered by grasses (Veldman, Buisson, et al., 2015). Although most of the vegetation is composed of grass and herbs, these components of vegetation have been widely neglected for conservation (Veldman, Overbeck, et al. 2015; Parr et al. 2014; Bond 2016). Thus, studies seeking to understand how to effectively restore TOGGBs are imperative. Achieving restoration success in TOGGBs depends on adequate context-specific knowledge to restore functional diversity, ecosystem functions and resilience (Buisson et al. 2020).

In recent decades, the Brazilian savanna (TOGGBs, hereafter neotropical savannas) lost 88 Mha (46%) of its original cover, and only 20% remains well-preserved (Strassburg et al., 2017). The invasion of non-native plant species is among the main causes of species loss and ecosystem degradation (Veldman et al., 2014). These invasions occur by conversion of native vegetation to livestock pasture or agricultural fields that deplete the seed and especially the underground bud bank, trapping the system in a degraded state. Therefore, these areas have slow natural regeneration and requires active restoration practices (Ferreira et al. 2017). Meanwhile, neotropical savanna restoration is still in its infancy relative to forest restoration and the success of most existing techniques remain uncertain at a large scale. The restoration of these ecosystems requires very different approaches than major forest restoration based on tree plantation and successional trajectories (Veldman et al. 2019; Andrade et al. 2015). In fire-prone ecosystems, natural plant populations persist mostly through clonal reproduction (Pausas et al. 2018a), and the investment in belowground structures (e.g., roots and bud banks) is the main mechanism by which plants re-sprout after disturbances, boosting then savanna resilience over disturbances (Ott, Klimešová, &

Hartnett, 2019). Even though there is an increasing number of neotropical savanna restoration studies, just a small number of successful models applies to the broad scale (Bustamante et al. 2019; Schmidt et al. 2019). Furthermore, the main challenge in restoration practices of neotropical savannas is the recovery of the belowground component of these communities as a way to ensure higher resilience.

Restoration practices that aim to convert abandoned pastures into biodiverse savannas need to deal with the potential invasion from persistent seed- and/or bud-bank of exotic grasses (Gorgone-barbosa et al. 2016; Dantas-Junior et al. 2018). After land clearing, alien grasses are often introduced as forage to feed the livestock or disperse from former pastures in the vicinity. Alien grasses frequently outcompete the native species, altering the ecosystem to a degraded state which also limits savannas' restoration success (D’Antonio & Meyerson 2002; Damasceno et al. 2018; Zenni et al. 2019). The potential restoration activity to reduce non-native invasion is to strategically increase the abundance of native species that are functionally similar to the non-native species (D’antonio & Chambers, 2011; Funk, Cleland, Suding, & Zavaleta, 2008). Invasive grasses usually have a fast-growth strategy and high aboveground productivity (Milbau & Nijs 2004). Generating fast soil cover by native grasses is a restoration practice which could potentially hamper the invasive potential of exotic grasses (Sampaio et al. 2019). However, invasive plants often generate strong priority effects, self-induced soil modifications that can persist after their removal or death, commonly termed “soil legacies” (Hess et al. 2019), and prevent the establishment of native species. Evaluating the functional composition of restored communities may offer insights about restoration success and help to predict vegetation trajectories.

Trait-based approaches have contributed to our understanding of the mechanisms driving community assembly (HilleRisLambers et al. 2012) and have been extended to explain ecosystem functions and processes (Funk et al. 2008). This approach considers the ecological niche as a result of the knowledge of different axes of plant-trait variation. One of the most comprehensive trait variation axes is the fast-slow continuum (Wright et al. 2004). Environments with high resources are characterized by the dominance of plants with traits associated with fast resource acquisition and the maximum allocation of resources to growth (Wright et al. 2004). Conversely, in environments where soil nutrients or climate limits plant growth, we expect dominant species to exhibit traits that reflect the conservation of long-lived tissues (Wright et al. 2004; Jardine et al. 2020). Several studies have used a trait-based approach for improving ecological restoration outcomes (Funk et al. 2008; Laughlin 2014) and a growing number of empirical studies have linked specific traits to plant establishment, survival, and persistence in restored systems (Larson & Funk 2016; Zirbel & Brudvig 2020). However, trait-based approaches of neotropical savannas restoration practices remain unknown, especially field-based studies with a focus on individual traits (Laughlin 2014). Several studies use traits from literature databases, not taking into account the trait-dependent context (Passaretti et al. 2020; Coutinho et al. 2019; Cava et al. 2018).

Savanna species tend to grow slowly and have high tissue construction costs, which allow them to persist under strong environmental stressors such as nutrient-poor soils, seasonal droughts, and fire (Hoffmann et al., 2012; Maracahipes et al., 2018; Viani, Rodrigues, Dawson, & Oliveira, 2011). Early stages of forest restoration are known for their trees with acquisitive traits and even old-growth forests have more acquisitive traits than the woody-component of neotropical savannas (Maracahipes et al. 2018). However, savannas and grasslands most likely do not follow successional trajectories because these ecosystems are dominated by resprouters (Pilon et al. 2020; Pausas et al. 2018b; Bond & Midgley 2001). Principally in these fire-prone ecosystems, the fast-slow strategy also is linked to specific reproductive strategies (Enright, Fontaine, Lamont, Miller, & Westcott, 2014;). Overall, acquisitive species are also species that tend to spread through seeds, that are usually small and with high germination rates, while rapidly growing aboveground (Power, Cramer, Verboom, & Chimphango, 2011; Verdú, 2000). In contrast, conservative species tend to resprout as a main reproductive strategy, they grow more slowly and allocate a greater proportion of their resources belowground (Eller & Oliveira 2018; Power et al., 2011; Verdú, 2000. In the *Cerrado* vegetation, the main technique known for large-scale restoration is direct seeding (Schmidt et al. 2019; Pellizzaro et al. 2017; Sampaio et al. 2019). By using an expressive number of seeds to cover the soil fastly, this technique is likely to favor mostly seeders/acquisitive species, which may be a problem if the goal is to restore an ecosystem where resprouters/conservative species are prevalent in the community (Pilon et al. 2020).

In this study, we used a trait-based approach to understand the functional composition of savanna communities and offer insights into the functional outcome of current restoration practices in the Brazilian *Cerrado*. We evaluated the functional composition of a 3-years-old restored savanna by direct-seeding when compared to areas dominated by exotic grasses (abandoned pastures) and well-preserved old-growth vegetation. We evaluated multiple leaf traits (see Table 1) of 61 native species, and species from restored and invaded savanna communities. Additionally, we measured exotic cover, bare soil cover and below- and aboveground biomass as biomass allocation has major implications for ecosystems resilience. We addressed the following questions and hypotheses:

- What is the functional composition of restored savanna vegetation via direct seeding compared to pastures and native savannas? Because direct seeding uses mostly seeders/fast growing plants, we expect the restored vegetation through this technique to be dominated by acquisitive traits, thus occupying the same functional space and functioning as abandoned pastures (H1).
- Does the restored *Cerrado* recover the functional diversity and biomass allocation of an old-growth state? We expect restored savanna to have higher Functional dispersion (FDis) and Functional richness (FR) than abandoned pasture, and exhibit higher soil cover and higher aboveground investment that might avoid invasive grasses. Besides, the higher aboveground biomass allocation in restored communities in comparison to higher belowground allocation in native communities may have implications to resilience to disturbance (H2).

Methods

Study site

The study was conducted in the Chapada dos Veadeiros National Park (14°07’03” S, 47°38’31” W), located in Central-Western Brazil in the municipality of Alto Paraíso de Goiás – Goiás; mean altitude: 1240 m (Pellizzaro et al. 2017). Mean annual precipitation is 1453 mm, 60% of which occurs between October and May and the mean annual temperature is 21°C (Pellizzaro et al. 2017). We selected three areas with different vegetation states, old-abandoned pasture (hereafter abandoned pasture), native old-growth vegetation (hereafter native), and restored vegetation.

The areas that are being restored in the park have generally been used for extensive livestock grazing before the park establishment in 1961 (ICMBIO 2009). In these areas, the soil was ploughed and limed, exotic grasses were sown, and the areas were constantly burned to stimulate grass growth (ICMBIO 2009). These areas were traditionally used for extensive livestock production, but have since been abandoned in two main periods: 1985 and 1995 (ICMBIO 2009). Our sites of abandoned pasture are dominated by the invasive exotic species: *Urochloa eminii* (Stapf) R.D.Webster and *Melinis minutiflora* P. Beauv. (Pellizzaro and others 2017).

In restored areas, the vegetation was restored using direct-seeding of native species applied in 2016. This area was previously dominated by invasive grasses. To reduce the biomass of invasive grasses, these sites were burnt, and then ploughed five times (250 mm deep) followed by inversion tillage (400 mm deep) to reduce the seed bank and regeneration of exotic grasses (Coutinho et al. 2019; Sampaio et al. 2019). Seeds of 75 species of native herbs, shrubs, and trees were collected at undisturbed sites in the surroundings of the restoration plots (Pellizzaro et al 2017; Coutinho et al. 2019; Schmidt et al. 2019). The restored area in 2016 corresponded to a total of 40 ha. Sixty-two out of the 75 seeded species were established, and the areas presented 60% cover by native species two years after seeding in a previous experiment at the same site (Pellizzaro et al. 2017).

Finally, we also selected an undisturbed native area adjacent to the restoration and an abandoned pasture, yet to undergo restoration. We consider this native area as a vegetation target because it is the native area closest to the restored area (<100m) and it is characterized by the dominance of non-woody species with <20% woody species cover (similar proportion achieved by direct-seeding; 78 ± 6% grass cover; Sampaio et al., 2019). These systems are ancient ecosystems characterized by slow-growth species, high herbaceous species richness, high endemism, and unique species compositions, commonly called old-growth savannas (Veldman, Buisson, et al. 2015). Therefore, the reference ecosystem selected is well preserved and consequently have a representative functional composition to *Cerrado*. While the abandoned pasture is characterized by a degraded state of vegetation with dominance of fast-growth species, which has remained in a similar state for the last 30 years, with livestock production abandoned in1985 (hereafter abandoned pasture).

Vegetation survey

To determine vegetation composition, we installed 10 plots of 4m² in each vegetation type, 40 m apart from each other. In each plot, we measured all individuals over 10cm in height and determined the vegetation cover by species based on the vertical projection of the aerial part of the plant (Mueller-Dombois; Ellenberg 1974). We surveyed the vegetation at the peak of the wet season during the year 2019 (Jan/2019).

We sampled soils to measure the soil fertility to investigate the driver to different functional composition. We took soil samples with 20cm deep soil core around each plot per vegetation type. We measured the concentration of total soil N, available P, and soil organic matter (SOM), Aluminum concentration, and soil pH, as a proxy of the nutrient pool. The soil N concentration was determined by sulfuric acid digestion followed by Kjeldahl distillation (Bremner 2016); soil P and potassium (K) concentrations were determined following a Mehlich-1 extraction (Raij et al. 2001); and SOM was determined by the Walkley-Black reaction (Nelson 1996) (Fig S3 a-f). Separately, we assessed soil water holding capacity at the 3 soil samples with 3 depth (surface, 50 cm, 100cm) at plots level in each area to estimate the slope of water-saturation by the Van Genuchten method (Genuchten V M 1980).

We measured biomass during the peak of wet season, and exotic cover and bare soil cover during the peak of the dry season (Jul 2018) and the peak of the wet season (Jul 2018 for the dry and Jan 2019 for the wet season). To measure aboveground biomass production, we clipped all plant material in a 50 x 50 cm plot around each vegetation plot at the peak of the dry season and the peak of the wet season in around each plot. To measure belowground biomass, we took 3 cm^2 by 10 cm deep soil cores from the corner of each plot at the end of the growing season. We sorted root samples to remove litter and oven-dried them at 65 °C for at least 48h before weighing. We took 10 cm² by 10 cm deep soil cores until 100 cm. All samples were taken from the corner of each plot in the same area where the aboveground biomass sample was collected. We sieved all soil samples (< 2mm) to collect all roots which were then washed and dried at 65°C for 48 h before weighing. We calculated the root:shoot ratio (Dry weight for roots/dry weight for aboveground biomass for each plot and for each treatment. To measure exotic cover and bare soil cover we use the grid of 4m² separated into 64 subplots of 6.25 cm² and visually estimated the number of subplots covered by exotic species, native species, and bare soil cover.

Community trait measurement

To determine functional composition we collected trait data for species that were present during the vegetation survey using standard methods (Pérez-Harguindeguy et al. 2013). We sampled traits of 61 species, 13 in restored, 15 in abandoned pasture, 44 in native savannas, covering 80% of species dominance. We focused on leaf traits – leaf area (cm²) (LA), Specific leaf area (g.cm^{-2}) (SLA), leaf thickness (mm) (LT), Leaf dry matter content (g.g^{-1}) (LDMC), leaf nitrogen (LNC), and leaf phosphorus concentration (LPC), and plant maximum height (H_{max}) (See Table 1 for details and ecological significance of traits). Vegetative height is related to a species' competitive ability with taller species better adapted to compete for light (Weiher et al. 1999). We chose these traits because in the grassland community most of the aboveground biomass consists of leaves. Added to that, leaf traits are a good predictor of acquisitive and conservative strategies using the leaf economic spectrum (Poorter, Niinemets, Poorter, Wright, & Villar, 2009; Wright et al., 2004). Acquisitive foliar traits include high SLA, leaf area, small LT and LDMC (Lohbeck et al., 2015; see Table 1). We obtained trait data from 3 individuals at each vegetation site. While we recognize the important role that intraspecific trait variation can play in community assembly (Jung et al. 2010; Laughlin et al. 2012), we could not measure it. These traits represent important facets of the leaf economic spectrum, a known gradient between fast and slow-growing species that can be associated with ecosystem processes, successional trajectories and reproductive strategies after disturbance (Reich 2014; Van Der Sande et al. 2016). Mean values of each trait for each species are available in Table S2 in supplementary information.

We measured the Community Weighted Means (CWM) for all functional traits by species dominance through the species cover in each plot per treatment (Laliberté & Legendre 2010). The CWM is suggested as a predictor of functional composition in the plant community, taking into account the dominant species in a community. We used a functional diversity index, functional richness (FR), that indicated the breadth of the niche based on the variation in a set of traits within a community. This is calculated as the minimum convex hull (minimal intersections of the convex sets containing the trait data) which includes all the species considered; the convex hull volume is then the volume inside this hull (Villéger et al. 2008). In addition, we calculated functional dispersion (FDis) that is the mean distance of individual species to the centroid of all species in the community. This index takes into account species relative abundances weighting distances of individual species by their relative abundances. In this study, we use FDis as an indicator of the niche complementary and as estimate the community functioning in each vegetation type (See (Laliberté & Legendre, 2010) for more details). Vegetations with similar index values probably have similar functioning.

Statistical analyses

To evaluate how leaf traits of species were associated, a principal component analysis (PCA) was done with leaf traits, using CWM values in each plot per treatment and then using species (n= 61) as data points. A correlation analysis was carried out, to test how leaf-traits and vegetation were correlated with soil nutrients. A one-way ANOVA with Tukey HSD post-hoc tests was used to evaluate whether leaf traits varied between species belonging to different vegetation types. Data were tested for homoscedasticity and leaf area was $\log_{10}$-transformed before analysis. To evaluate differences in above and below-ground between the vegetation types, we developed generalized linear models (GLMM), considering all vegetation types (native, restored, abandoned pasture) as fixed factors and plot as random factors . The GLMM were fitted separately for each response variable, using Poisson and Gaussian distribution.

To investigate the relation among soils properties and functional diversity index and functional diversity index and ecosystem functions, we use linear models. Firstly, we considering soil PCA axis as the predictor variable and FDis and FR as the response variable. All statistical analyses were carried out using R 3.3.

Results

Functional composition

The CWM SLA in the restored area was on average 44.28% higher than in the native area and similar to the abandoned pasture. The leaf thickness (LT) in restored area was in average 30.4% greater than the abandoned pasture and 25.8% lower than in the native vegetation. In relation to the leaf dry mass content (LDMC), was 28.8% greater in the restored area than in the abandoned pasture and 26.2% higher than in the native vegetation that has similar values when compared to the abandoned pasture. Leaf P content (LPC) in the restored area was in average 8.4% greater than in the native area and similar to the abandoned pasture. Leaf N content (LNC) in the restored area 16.9% lower than in the native area and similar to the abandoned pasture. The higher CWM SLA, lower LT in the restored area compared to native are, indicate that the functional outcome of direct seeding is a community with acquisitive strategies, contrasting with the conservative native (All average statistical values are showed in Table 2 and Fig. 2 a-f). All native species that were evaluated were used in the no-tillage method. No plant was a ruderal plant or would originate from regrowth, with the exception of invasive exotic grasses.

Associations amongst the six leaf traits and maximum height were analyzed with a PCA (Fig. 3). The first axis explained 46.5% of the variation, and was positively related to SLA, LDMC, and P concentration in leaves and negatively related to leaf area. The second axis explained 25% of the variation and was positively related to N concentration and negatively related to leaf thickness. The three vegetation types (native, restored, pasture) were grouped in this multivariate trait space according to their trait community weighted means in each plot per community. Acquisitive strategies were related to the abandoned pasture and restored area, with high SLA, LPC,and H_{max}. Conservative strategies were related to the native area, with high LA and LT.

In the restored area 78.3% was covered by grasses and 21% by shrubs. In contrast, the abandoned pasture had 90% cover of grass with 70% dominated by *U. eminii* and 11% by *M. minutiflora*; while, native areas had 46.2% dominated by grasses, 27.7% by shrubs, 8.7% by herbs and 3.3% of cover by trees (Table S3). In the restored area, the shrub *Lepidaploa aurea* was the most dominant species (15.2%), followed by the grasses S*chizachyrium*, *Axonopus pressus* (8.9%) and *Axonopus chrysoblepharis*

(6.13%), but also by the exotic *Melinis minutiflora* (6.08%) and *Urochloa eminii* (5.96%).

In restored areas, the high values of LA and SLA were strongly influenced by two invasive grasses, *U. decumbens* and *M. minutiflora* and the native species *Axonopus pressus*. The high values of SLA were mostly associated with species of the genus *Schizachyrium*. The shrubs *L. aurea* and *Mimosa clausennii*, and grasses from the genus *Axonopus* showed higher LDMC values (Table S3). The restored area showed overlap of dominant species with the abandoned pasture (*U. decumbens* and *M. minutiflora*). Both exotic species have acquisitive traits, with high SLA values and lower LT, and together covered more than 12% of restored community. In contrast, the species overlapping with natives was the grass *Axonopus pressus* and the shrub *Mimosa clausenii*, both exhibiting conservative foliar traits. The grass species *Trachypogon spicatus* was the only species that had conservative traits among grasses in the restored area, covering less than 4% of the plots. Overall, dominant species in the native area have lower values of SLA, and high values of LDMC and LT. In contrast, the species in the abandoned pasture have with higher SLA and lower LT (Table S3).

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Functional diversity, biomass allocation

The Functional Richness index was higher in the native area than in the abandoned pasture, and the abandoned pasture was similar the restored area ($F_{(2,27)}$=7.76, p=0.001, Fig 4 a). When the species abundances are taken into account, the Functional Dispersion index in the restored area was similar to that of the native area (Tukey test, p=0.084) and higher in relation to the abandoned pasture ($F_{(2,27)}$=5.16, p<0.001, Fig 4 b). That means, the multivariate space filled by traits are more similar between native and restored areas than to abandoned pasture.

Restoration practices resulted in higher aboveground biomass (6254.56 ± 2368.63 kg ha^{-1}) than in native areas (4710.64 ± 3068.31 kg ha $^{-1}$) and abandoned pasture (3774.2 ± 1585.13 kg ha^{-1}) ($F_{(2,27)}$= 2.684, p<0.005, Fig 4 c). However, the restored area (25.6 ± 6.4 kg ha $^{-1}$) showed lower belowground biomass when compared to the native area (50 ± 35.2 kg ha $^{-1}$) and abandoned pasture (11.6 ± 5.6 kg ha $^{-1}$), where the last was lowest than native areas ($F_{(2,27)}$=5.93, p=0.01; Fig 4 d). The root: shoot ratio in the restored area was lower when compared to native and abandoned pasture ($F_{(2,27)}$=4.63, p=0.02;Fig S2).The mean bare soil cover in the wet season was

21.5% in the restored area, 24.2% in the abandoned pasture and 13.2% in the native area. In the dry season, the bare soil cover was 33.75% in the restored area, 21.51% in the abandoned pasture and 29.37% in the native area. When compared among seasons, the restoration practice provided a decrease of 65% in bare soil cover from dry to wet season. These values were similar to native areas that decreased 55.3% the bare soil cover from the dry to the wet season (GLMM see table S1). In contrast, the abandoned pasture remained unaltered among seasons (GLMM see table S1). However, when we compare the exotic cover among seasons, we find an increase in 56.7% of exotic cover in the restored area from dry to the wet season, while abandoned pasture and native areas and remained unaltered in (GLMM see table S1).

Associations amongst the soil nutrients were analyzed with a PCA (Fig. S4). The first axis explained 41.5% of the variation and was used to correlate with traits and functional diversity. The first axis of soil PCA was positively correlated with N, organic matter, K, and silt, separating native area to abandoned pasture and restored. These areas were in another group positively correlated with %Sand, P, and Al soil content (negative values of PCA). The correlation between the PCA axes from soil nutrients with vegetation showed the decoupling of restored vegetation with the soil proprieties. None of the leaf traits were correlated with the soil PCA axis (Table S3). Yet, when we fit the values only for native and abandoned pasture areas the PCA1 axis showed a good predictor of functional richness and functional dispersion to both communities (Table S3; Fig S2). This relationship disappears when restored areas are included.

Discussion

To our knowledge, this is the first study evaluating the recovery of functional composition following direct-seeding restoration in Neotropical savanna communities. We evaluated how a *Cerrado* restored area varied in its community-weighted mean (CWM) trait composition, and whether total biomass and biomass allocation changed when compared to the degraded state (abandoned pasture) and well preserved old-growth savannas (native). Multivariate CWM trait composition differed strongly between native, restored and abandoned pasture areas. The restored communities and abandoned pasture showed a greater abundance of species with more acquisitive traits while the native vegetation presented conservative traits. Hence, the functional composition of a restored *Cerrado* via direct seeding is more similar to an abandoned pasture than old-growth reference areas. Our results showed that the species pool used in direct-seeding is an effective technique to establish a large number of acquisitive native species with high aboveground biomass investment and provides a fast gain in soil cover. Nevertheless, this fast aboveground recover did not guarantee resistance to invasion. In contrast, the restored *Cerrado* maintained lower belowground investment compared to the reference system, which implies in a lower vegetation resilience against common local disturbance such as fires (Ott et al. 2019; Pausas et al. 2018b).

Functional composition

For the majority of the analyzed traits, the restored area was functionally similar to the abandoned pasture, both harboring a great dominance of acquisitive species whereas the native vegetation harbored conservative species. The exception was LDMC which had greater values in the restored area, relative to the pasture area, due to presence of a particular woody species (See table S3). *Cerrado* woody species usually have slow growth and higher leaf carbon investments compared to grass species (Maracahipes et al., 2018; Rossatto, Kolb, & Franco, 2015). *Mimosa clausennii*, a slow-growing shrub, frequent in the restored site, has a LDMC of 4.44 $g.g^{-1}$ which can explain the higher LDMC following restoration (See table S3). Thus, restoration shows a bimodal distribution of LDMC (Fig. 1 D), the first was attributed to grasses with values similar to those of abandoned pasture and the second was an attribute to woody species. In fact, in our study LDMC had a lower contribution to discriminate vegetation along the PCA axis thus may not reflect the filtering processes among the studied plant communities (Fig 3b). In addition, the SLA often discriminates between communities better than LDMC, because SLA is influenced by both light and soil fertility, whereas LDMC largely reflects soil fertility (Hodgson et al., 2011). In fact, the restored area showed higher SLA values. Dominant grasses in the restored area have inexpensive short-lived leaves, which leads to the high CWM values of LPC and high SLA. These traits facilitate greater photosynthetic capacity, faster resources use, and high above-ground biomass investment to dominant species (Osnas, Lichstein, Reich, & Pacala, 2013). These patterns also were also present in the abandoned pasture area, suggesting similar ecosystem functioning and consequently low gain in terms of functionality when compared to the degraded state.

These fast-growing species in the restored area are associated with a high density of individuals, which can cover the soil and supposedly compete with invasive grasses (Hulvey & Zavaleta 2012). In fact, the fast soil cover and high aboveground biomass suppresses the exotic-grass spread in the first year of restoration. The use of a fast-growing shrub (*Lepidaploa aurea* – Asteraceae) also assisted in avoiding exotic-grass invasion in the first year (Sampaio et al. 2019; Lopes et al. 2018). However, the effect of this fast soil cover restricting the invasion of exotic grasses is short-lived as we observed an increase in exotic cover from the dry to the following wet season (Fig 4B). This suggests that only the high aboveground investment and soil cover in the area does not prevent the invasion. Furthermore, the use of species that have allelopathic potential demonstrates success only in the first years, because *L. aurea* is an opportunistic species and has a short life cycle (2- years) and its death offers a window of opportunity for exotic invasive grasses to spread, especially in the site that is closer to sources of propagules.

The high SLA, associated with a fast-growth strategy are characteristic that define seeders which tend to produce high numbers of smaller seeds (Power et al. 2011; Verdú 2000). Seeders are a dominant strategy in highly productive habitats with some form of disturbance, e.g. fire (Fynn et al. 2011). However, the *Cerrado* is an ecosystem with low nutrient availability and, in general, species associated with low-fertility soils have comparatively low SLA and slow growth (Jager, Richardson, Bellingham, Clearwater, & Laughlin, 2015; Maracahipes et al., 2018; Pinho, Tabarelli, Engelbrecht, Sfair, & Melo, 2019). Slow-growth strategies are following to resprouters reproductive strategy, that tend have allocate a greater proportion of their resources belowground (Eller & Oliveira 2018; Power et al., 2011; Verdú, 2000). In fact, *Cerrado* native plant communities have a higher dominance of resprouter species (Pilon et al. 2020) differently from what was observed in restored community where the high dominance of seeders/acquisitive species implied in a low investment in belowground biomass and high accumulation of aboveground biomass strongly associated to ecosystem resilience after disturbance.

Functional composition, Biomass investment and implications for resilience

Overall, the conservative traits of resprouter species observed in the native area underpins slow aboveground growth (Pellizzaro et al., 2017; Silva, Oliveira, da Rocha, & Vieira, 2015) and high investment in belowground tissues (De Castro & Kauffman, 1998; Hoffmann & Franco, 2003). In contrast, most acquisitive seeders grassland species often have a high specific leaf area, a low root-tissue density, and thin roots (Lachaise, Bergmann, Rillig, & van Kleunen, 2020), which explain the low belowground biomass investment and low root:shoot biomass in the restored area. However, in grasslands, more than 40% of the total biomass and carbon stocks are located belowground, mostly in roots (Fidelis et al. 2013).These are composed of roots and rhizomes, important components to resprouting after disturbances, especially fires (Fidelis et al. 2014; Ott et al. 2019). Several studies show that the most capacity of resilience after fires are in re-sprouting from underground organs (Bond & Midgley 2001; Bardgett et al. 2014; Pausas et al. 2018a; Ott et al. 2019). Rhizomes, lignotubers, and belowground stems can allow plants to resprout and survive disturbances, such as fire, and promote regrowth after unfavorable seasons (Ottaviani et al. 2020). Given that the recruitment of resprouter species occur mostly from belowground organs, we need to consider including vegetative reproductive tissues (e.g. bud banks) and not only seeds to achieve a greater resilience in *Cerrado* under restoration.

Another possible explanation for low belowground and high aboveground biomass allocation is the early-stage of the vegetation establishment (4 years after seeding) (Wilson 2011). In forest ecosystems, early successional stages have a high abundance of acquisitive species, followed by conservative species in the later stages (Lohbeck et al., 2015, 2013; Poorter et al., 2019). However, succession characterized by a high turn-over of species through time is most likely improbable in the *Cerrado* given that the species in surrounding native patches are resprouters (Silveira et al. 2020). The pioneer, secondary, or late-successional species are far from adequate to categorize plant species colonizing, covering the ground, and persisting in *Cerrado* vegetation (Dayrell et al. 2018). Fire-prone vegetation are characterized by the coexistence of conservative and acquisitive species, with low recruitment by seeds banks (Pilon et al. 2020). The source of seeds for direct seeding restoration in this ecosystem is most likely biased towards seeder species because only seeds are easily collected in the field (Schmidt et al. 2019). The seeder species are most used, also most favoured by the technique of direct seeding that provides seeds with high germination rates (Pellizzaro et al. 2017; Sampaio et al. 2019), leading to an acquisitive community in the restored area. The main problem of investing in restoration mainly with acquisitive species relies on the possibility of trapping the restored area in an ecosystem state composed of acquisitive species, which is likely more vulnerable to invasion and to have a lower fire-resilience. Because conservative species tend to reproduce vegetatively, the probability of arrival and establishment of resprouter/conservative species is very low because they have low investment in seed production. In addition, the legacy effect mediated by the presence of invasive grasses in the restored area will potentially increase the exotic dominance in the restored area (Hess et al. 2019). Therefore, to provide a more stable system to exotic invasion, we need to incorporate vegetative reproduction organs such as underground buds in restoration efforts (Pilon et al. 2019). Whether we only keep the direct seeding with a high abundance of seeders species, we run the risk of creating unstable ecosystems, with a low resistance to invasion and low resilience to fires.

Overall, the restoration strategy based on fast-growth by acquisitive species and high accumulation of aboveground biomass can also increase the risk of severe fires (as occurred in 2020 in the restored site, see fig 6 D). The imminent risk of fires mediated by the increase in the length of dry period and favored by the accumulation of dry aboveground biomass (combustible material in the dry season) in the restoration means that any disturbance can be catastrophic and supposedly with low chances of recovery of native species by sprouting (fig 6 D). Therefore, the low investment in belowground biomass and high accumulation of aboveground biomass provided by acquisitive species have implications for the risk of fires. Therefore, futures studies need to evaluate the resilience of restored vegetation to fire in neotropical savannas.

Restoration and soil uncoupling

The coupling among soil and vegetation provides high resistance and resilience to disturbance (Mack, Eppinga, & Bever, 2019). The results of uncoupling among functional index's and soil proprieties from the restored area (Fig S2) is another evidence that the first years of grassland restoration have low resilience and are sensitive to any disturbance. In addition, this uncoupling in restored areas provides evidence that the use of acquisitive species by the direct-seeding practice does not match *Cerrado* soil properties. Direct seeding eliminates dispersion filters responsible for community assembly rules, and the time since sowing might influence these results. However, it is clear that the species pool favoured by direct-seeding is not the species pool found in old-growth savanna communities that were built with different environmental filters than those proposed by direct seeding. Therefore, to achieve restoration success in the neotropical savannas we need to foster the fast coupling between soil and vegetation feedbacks. To target this, future restoration research should focus on the use of more slow-growing species and adjustments of soil conditions to support the development of conservative species.

Conclusions

Using the first large-scale restoration experiment by direct seeding in a Neotropical savanna, we evaluated the plant functional composition, and showed that the community under restoration is dominated by acquisitive species, with high aboveground and low belowground investment. Our results show that despite the high biomass in the restored community, the dominance of exotic grass was not avoided. The use of a restoration practice that favours seeders and acquisitive species have implications for the resilience of the restored system. Studies and efforts are needed to understand how to increase the abundance of species with conservative and resprouter strategies in large-scale restoration efforts. Restoration practices that restore both bud banks and seeds are still a major challenge to neotropical savannas restoration.

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Figures and tables

Table 1. Traits included in the study, the trait category the functional strategy that high values of this trait refer to (A = acquisitive, C = conservative) description of the ecological significance, units and abbreviations List of measured traits, abbreviations, and units used (Adapted from Lohbeck et al., 2013).

Code	**Trait**	**Ecological significance**	**Strategy**
SLA	Specific leaf area ($cm^2\ g^{-1}$)	Resource use, maximum photosynthetic rate, and the related environment disturbance	A
LA	Leaf area (cm^2)	Light intercepting area, dry matter production, respiration, leaf cooling, gas exchange	A
LT	Leaf thickness (mm)	Resistance to lower water availability, nutrient-poor soil	C
H_{max}	Maximum height (cm)	Access to light and competitive ability	A
LNC	Leaf nitrogen (N) content (g/kg)	Response to soil nutrient availability, photosynthetic capacity	A
LPC	Leaf phosphorous (P) content (g/kg)	Photosynthetic capacity , plant development and growth	A
LDMC	Leaf dry-matter content ($g\ g^{-1}$)	Relative growth;, leaf lifespan , construction costs, nutrient retention	C

Table 2. List of measured traits showing mean± standard deviation, in each vegetation type. .

	Vegetation type			**test**
Trait	**Abandoned pasture**	**Native**	**Restored**	
LA (cm^2)	15.2±3.4	24.36 ±8.81	8.05±31.67	F(2,27)= 20.44, p<0.001
SLA ($cm^2.g^{-1}$)	89.00±8.99	65.82 ±16.72	94.97±14.57	F(2,27)= 12.98, p<0.001
LT (mm)	0.17±0.03	0.31±0.08	0.23^{+}-0.05	F(2,27)= 20.97, p<0.001
LDMC ($g.g^{-1}$)	2.74±0.16	2.84±0.19	3.85±0.42	F(2,27)= 79.59, p<0.001
LNC ($mg.g^{-1}$)	1.10±0.04	1.18±0.14	0.98±0.17	kruskal-test, 10.415, p<0.001
LPC ($mg.g^{-1}$)	0.062±0.004	0.054±0004	0.059±0.006	F(2,27)= 16.15 p<0.001

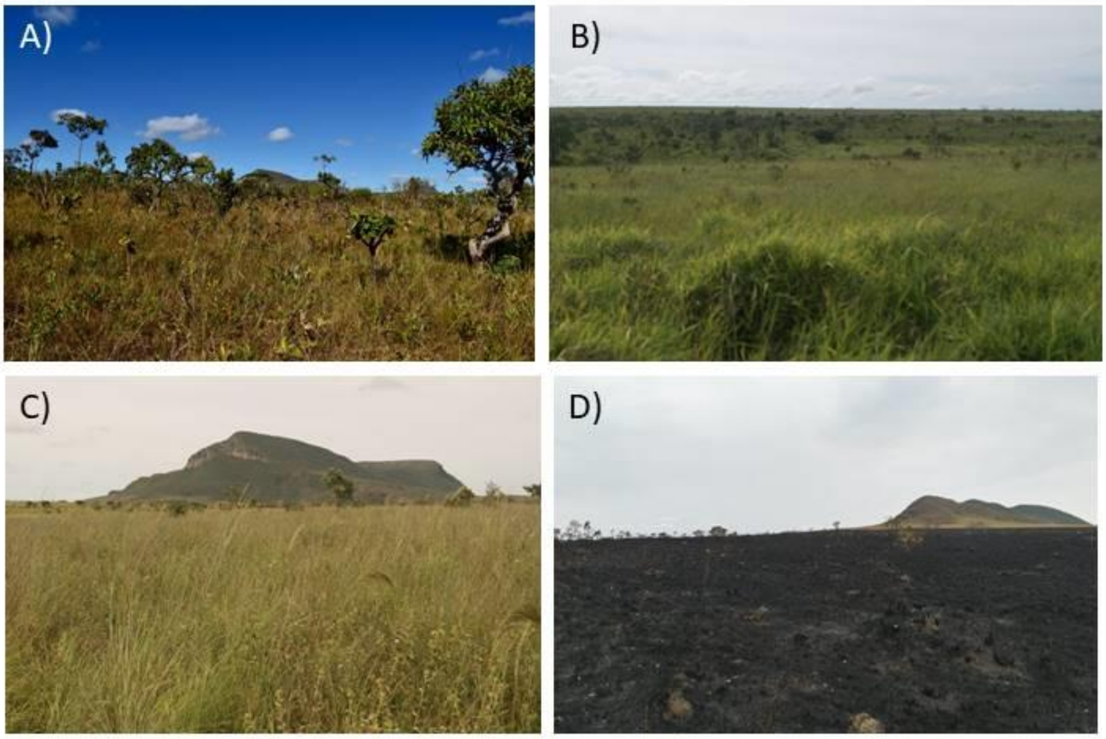

Figure 1. Vegetation types from Chapada dos veadeiros National Park, Brazil. A) well-preserved old-growth vegetation (Native area); B) degraded vegetation dominated by exotic grasses (abandoned pasture); C) Restoring area by direct seeding of native species; D) After light-fire in Restoring area.

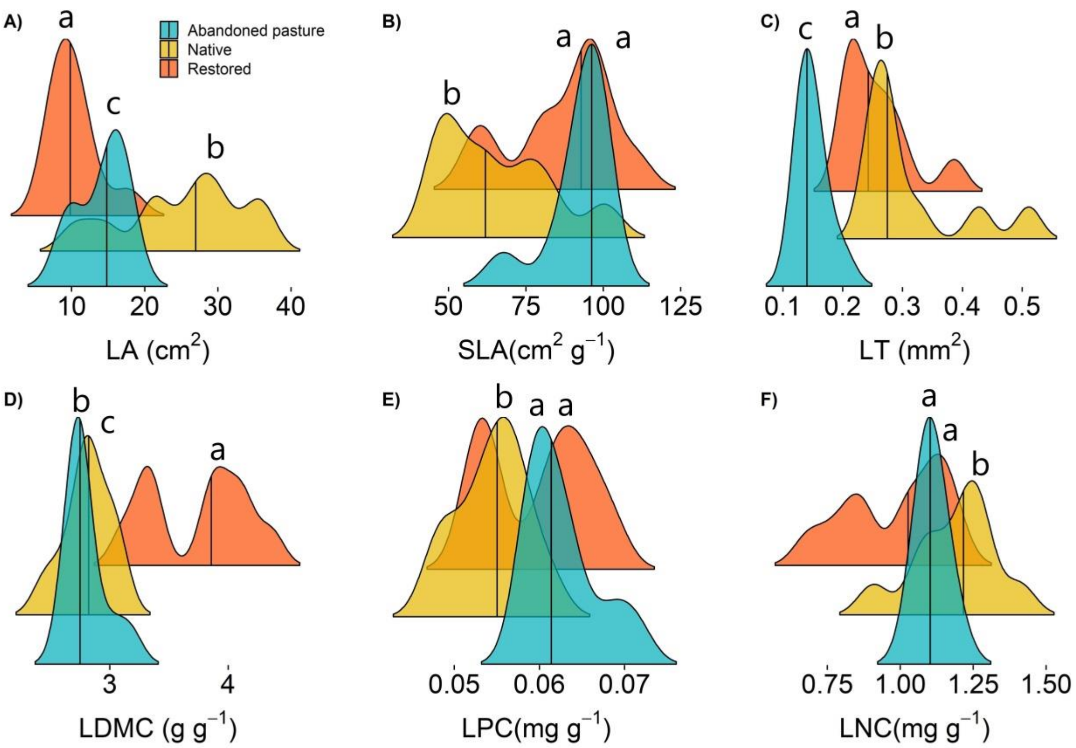

Figure 2. Leaf traits in the abandoned pasture, native vegetation, and restored vegetation in wet season a) Leaf area;) (b) Specific leaf area (SLA); (c) Leaf thickness, (d) Leaf dry mass content (LDMC). (e) Leaf P concentration (LPC); (f) Leaf N concentration (LNC).Different letters represents statistical significance (P<0.01).

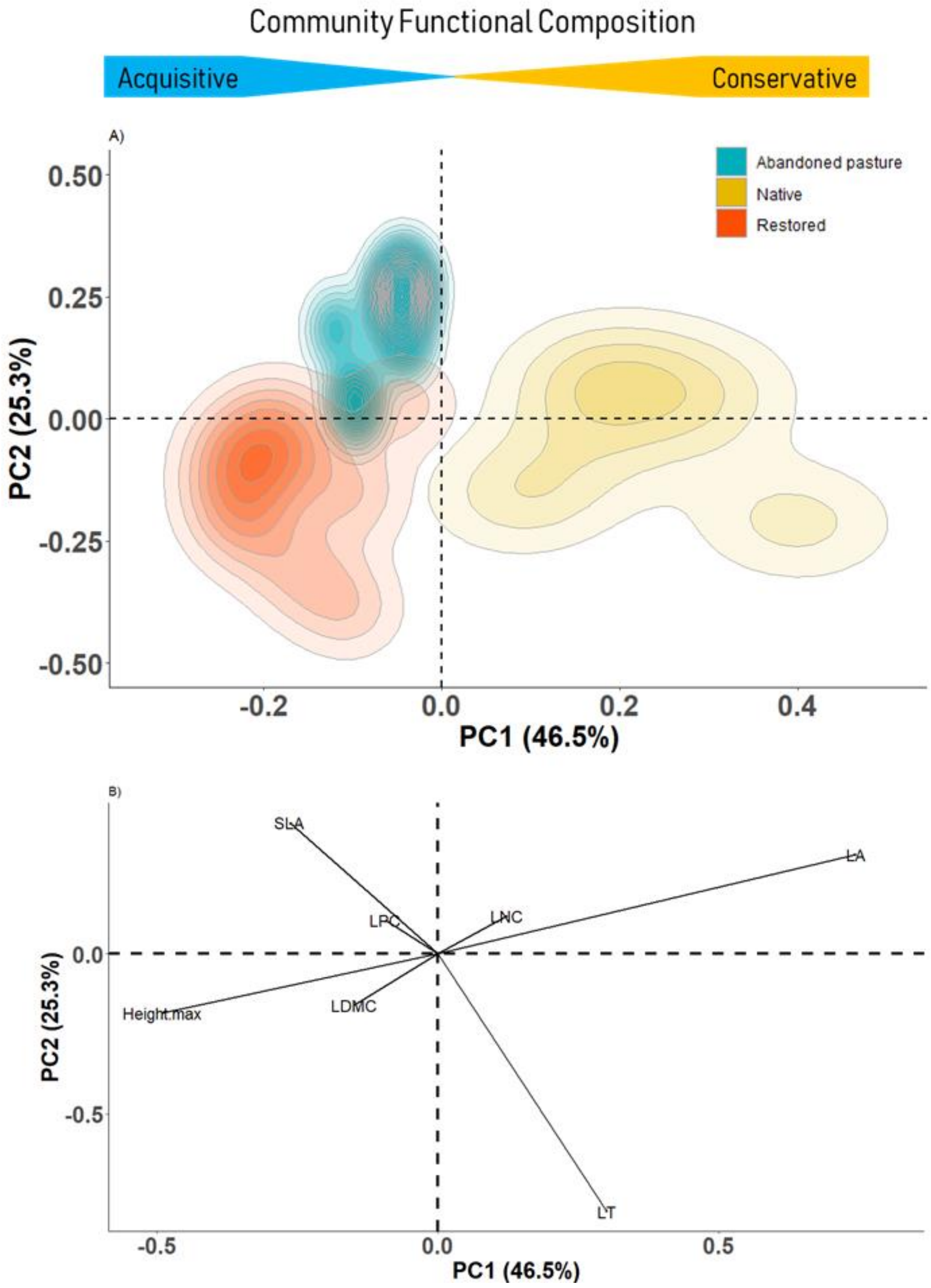

Figure 3. Principal component analysis of 6 leaf traits and maximum height max of the restored, native, and abandoned pasture community. Traits included are LA, leaf area; LT, leaf thickness; SLA, specific leaf area; LDMC, leaf dry matter content; LD, leaf density; LNC, leaf nitrogen concentration; LPC, leaf phosphorus concentration. The small circles represent the plots in each community, the large circles represent centroid mean, the arrows size represents the contribution of PCA axis.

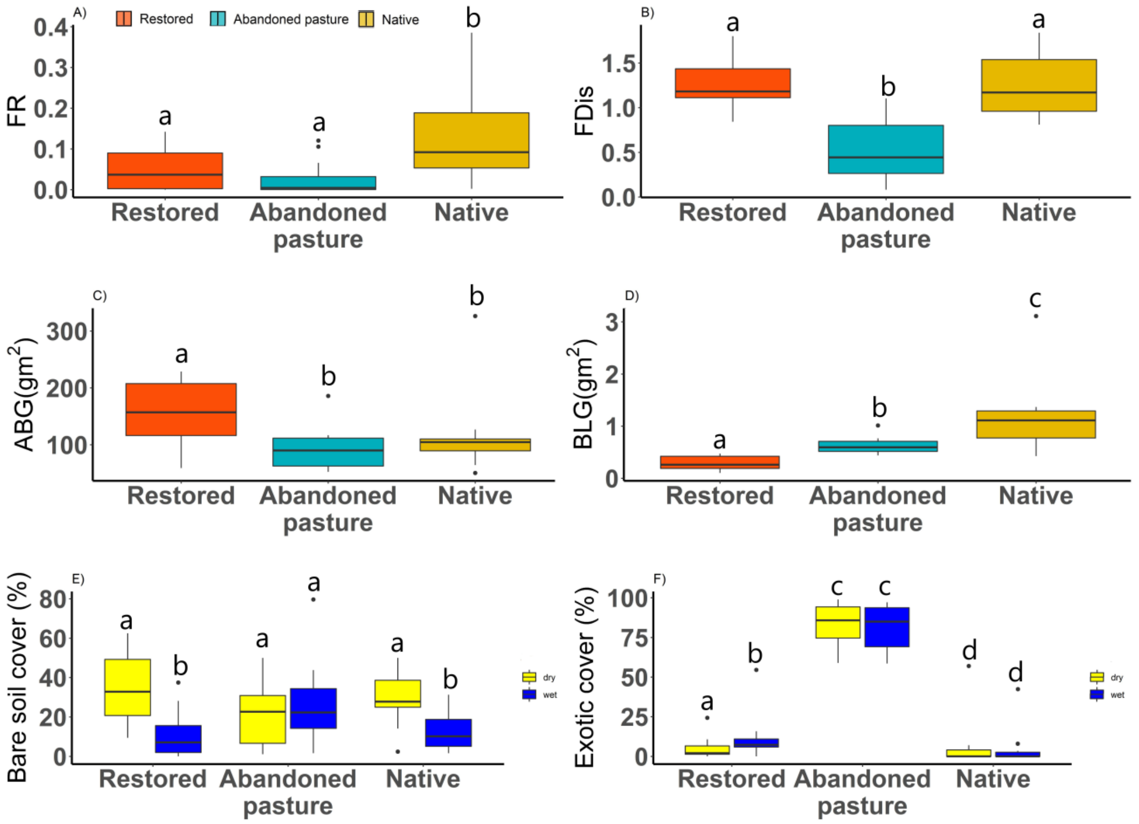

Figure 4. Functional diversity index, biomass investment and soil cover by vegetation areas. A) Functional Richness (FR); B) Functional Dispersion (FDis); C) Bare soil cover; D) exotic cover; E) Above-ground biomass; F) Belowground biomass. The blue represents the wet season, yellow represents the dry season. The central lines represent the medians . The colours from the box from figure (A) to D) represent each vegetation type analyzed and The colours from the box from figure E) and F represent season (Wet and Dry season). Different letters represents significance difference from pos-hoc test ($P<0.01$, see details Table S2).

Suplemmentary material

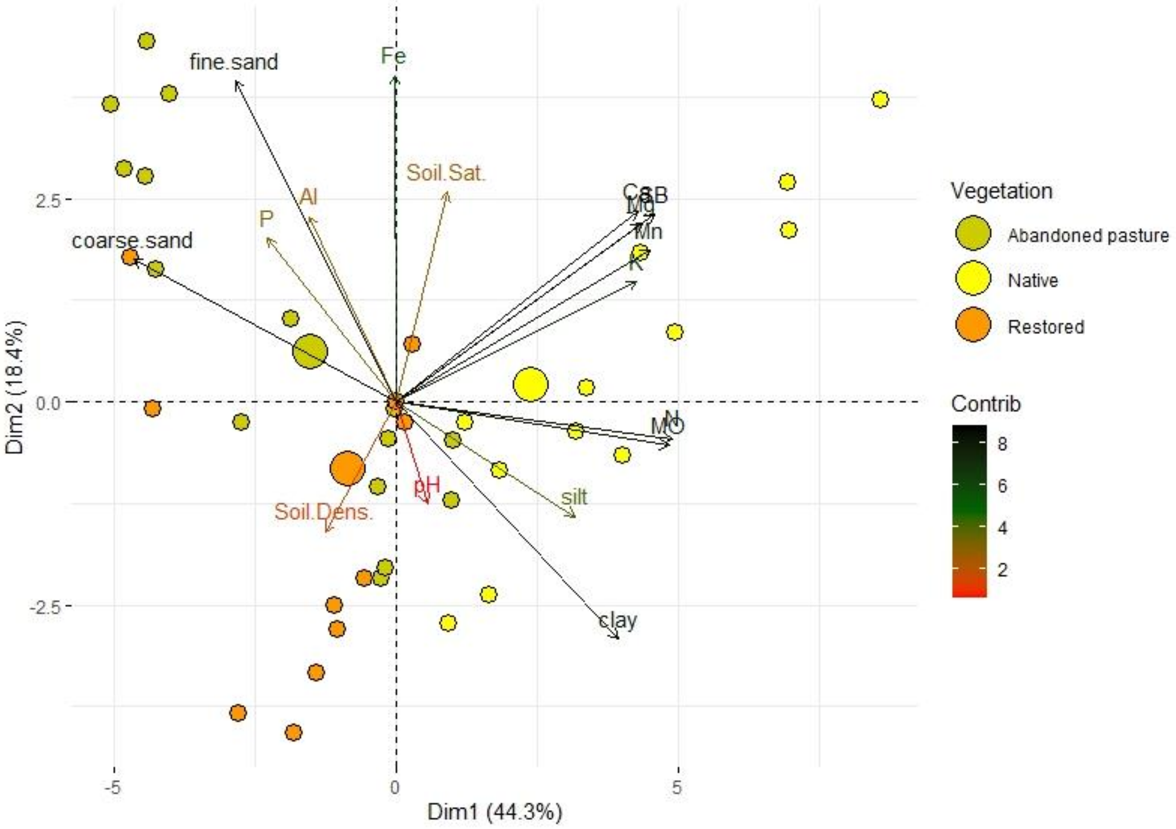

Figure S1. Principal component analysis of soil proprieties restored, native, and abandoned pasture community. The small circles represent the plots in each community, the large circles represent centroid mean, the colors of arrows represents the contribution of PCA axis.

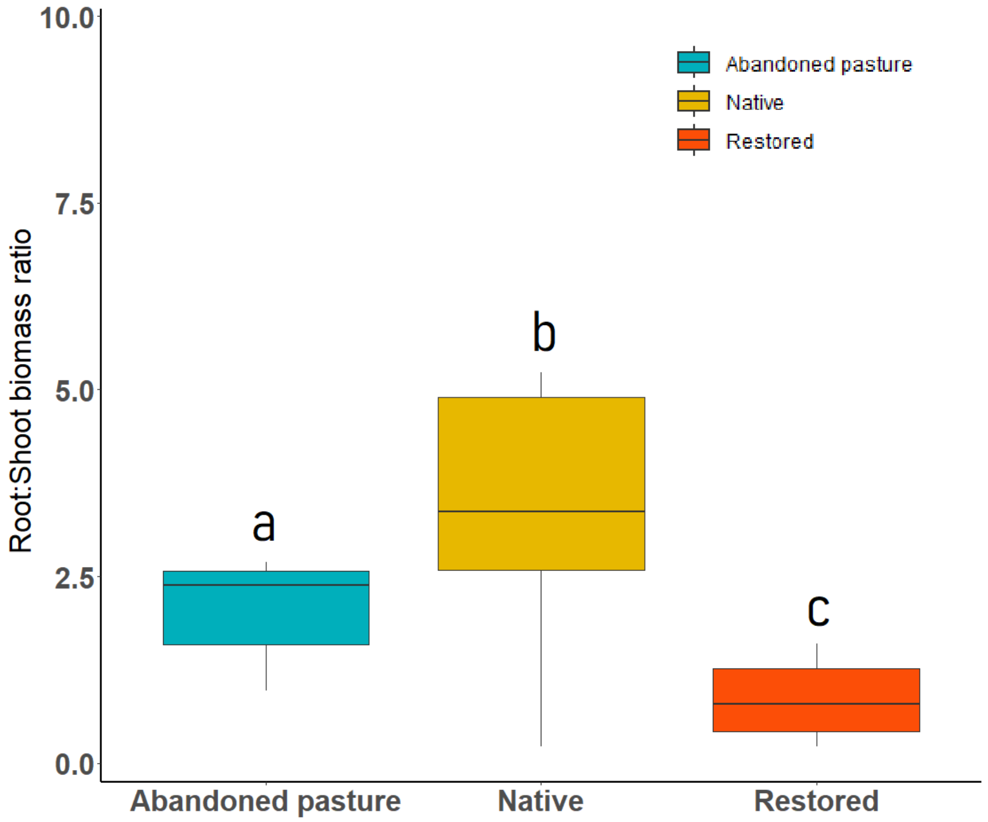

Figure S2. Root:Shoot biomass ratio in restored, native, and abandoned pasture community. Different letters represents statistically different significance ($P<0.05$).

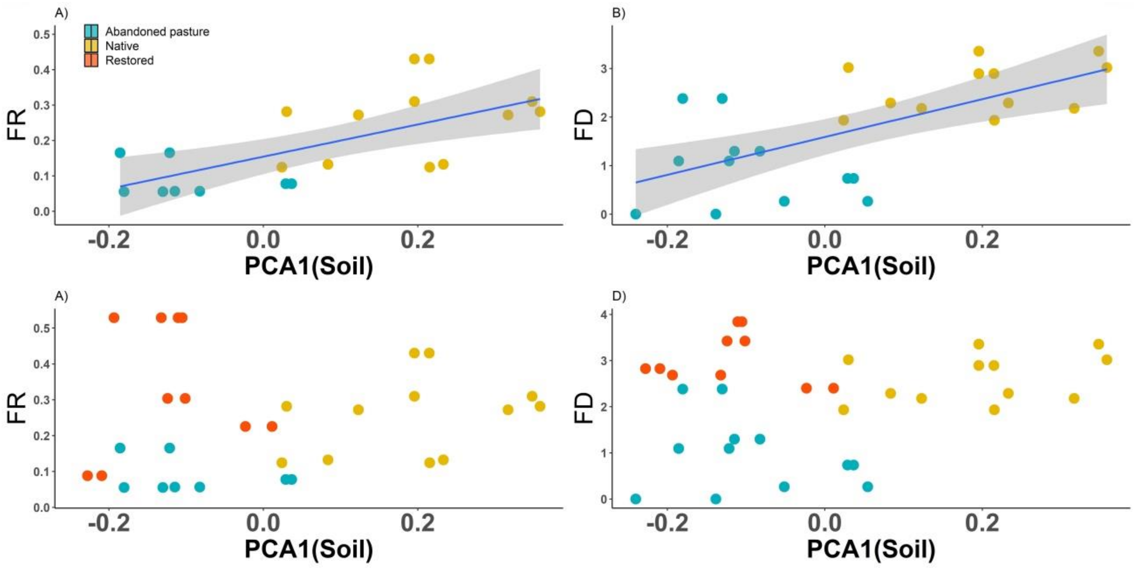

Figure S3. Relation with PCA soil axis with functional diversity index. A) relation PCA1 with functional richness (FR) considering all vegetation; B) relation PCA1 with Functional Richness (FR) considering only abandoned pasture and native vegetation; X) relation PCA1 with functional dispersion (FD) considering all vegetation; D) relation

PCA1 with functional dispersion (FD) considering only restored and native vegetation. The points represent the plot and colors represent each vegetation.

Table S1: Results of the Generalize linear mixed effect model evaluating the relations of the vegetation type (abandoned pasture , restored and native), and season (wet and dry) on Bare soil cover and Exotic cover

	Degrees of Freedom	**F-value**	**p-value**
Bare soil cover			
Vegetation type	2, 57	1.034	0.84
Season	1, 56	4.7	**<0.01**
Vegetation type : Season	2, 54	2.5	**0.01**
Exotic cover			
Vegetation type	2, 57	3.84	< **0.01**
Season	1, 56	0.30	0.75
Vegetation type : Season	2, 54	1.08	**0.01**

Table S2. Mean of functional traits by species and life forms from restored, native, and abandoned pasture community. Traits included are LA, leaf area; LTh, leaf thickness; SLA, specific leaf area; LDMC, leaf dry matter content; LD, leaf density; LNC, leaf nitrogen concentration; LPC, leaf phosphorus concentration. The small circles represent species in each community a) Abandoned pasture; b) Restored; c) Native, the large circles represent centroid mean, the colors of arrows represents the contribution of PCA axis.

Vegetation	**Specie**	**Life form**	**Relative Dominance (%)**	**H_{max}**	**LNC**	**LPC**	**LA**	**LT**	**SLA**	**LDMC**
Abandoned pasture	*Brachiaria decumbens*	grass	70.47	71.00	1.21	0.07	0.04	0.20	4.33	2.56
Abandoned pasture	*Melinis minutiflora*	grass	10.99	42.50	1.81	0.10	0.02	0.51	26.53	5.10
Abandoned pasture	*Echinolaena inflexa*	grass	4.21	76.10	1.10	0.06	1.74	0.13	99.58	2.77
Abandoned pasture	*Andropogon leucostachis*	grass	2.97	19.00	1.55	0.07	3.42	0.43	65.47	2.07
Abandoned pasture	*Baccharis dracunculifolia*	shrub	0.64	52.00	0.86	0.06	0.99	0.16	61.05	1.76
Abandoned pasture	*Kielmeyra abdita*	shrub	0.59	30.00	0.93	0.05	1.28	0.26	84.46	2.24
Abandoned pasture	*Pisidum mircinitis*	shrub	0.35	46.25	0.96	0.07	0.33	0.21	53.55	2.18
Abandoned pasture	*Porophyllum angustissimum*	herb	0.35	20.00	1.16	0.06	1.68	0.23	32.66	1.92
Abandoned pasture	*Casearia sysvestris*	shrub	0.20	82.00	1.16	0.06	1.68	0.23	32.66	1.92
Abandoned pasture	*Campomanesia pubescens*	shrub	0.10	40.88	1.00	0.07	0.47	0.15	95.25	2.74
Abandoned pasture	*Senna rugosa*	shrub	0.10	83.00	1.78	0.08	0.35	0.46	50.01	2.77
Abandoned pasture	*Spigelia pulchella*	herb	0.10	31.00	1.45	0.06	1.17	0.34	95.67	2.86
Abandoned pasture	*Chresta excuca*	herb	0.05	42.00	1.92	0.10	3.13	0.49	49.99	2.11
Abandoned pasture	*Erythroxylum suberosum*	shrub	0.05	34.00	1.51	0.07	0.16	0.21	80.94	3.84
Abandoned pasture	*Sthylosantes capitata*	herb	0.05	26.00	1.51	0.10	0.06	0.12	67.98	2.49
Native	*Aristida torta*	grass	9.19	57.80	0.75	0.04	4.60	0.41	49.11	2.59
Native	*Bauhinia dumosa*	shrub	8.10	37.25	1.14	0.08	1.01	0.28	48.23	2.20
Native	*Echinolaena inflexa*	grass	7.70	22.00	0.69	0.04	0.22	0.15	42.02	2.20
Native	*Poaceae*	grass	6.82	47.33	0.83	0.04	0.18	0.21	21.28	2.18

Native	*Mimosa clausenii*	shrub	5.87	51.00	1.43	0.08	9.11	0.29	62.53	2.90
Native	*Brachiaria decumbens*	grass	5.67	55.00	1.18	0.06	0.31	0.22	56.44	3.29
Native	*Aspidosperma tomentosa*	tree	3.33	48.67	1.18	0.06	0.31	0.22	56.44	3.29
Native	*Hypenia paradisi*	herb	2.93	92.00	1.14	0.06	1.05	0.25	55.34	2.76
Native	*Axonopus pressus*	grass	2.69	33.00	1.67	0.06	0.04	0.28	44.20	3.10
Native	*Poaceae 1*	grass	2.62	40.56	1.51	0.08	1.70	0.21	104.18	2.67
Native	*Axonopus chrysoblepharis*	grass	2.18	75.67	1.15	0.06	3.07	0.14	126.55	3.02
Native	*Aldama robusta*	herb	2.18	27.00	1.42	0.06	1.80	0.48	40.81	2.57
Native	*Casearia altiplanensis*	shrub	2.01	61.00	1.53	0.05	22.10	0.69	56.13	2.64
Native	*Campomanesia pubescens*	shrub	1.82	33.33	1.42	0.06	1.80	0.48	40.81	2.57
Native	*Allagoptera cf campestris*	palm	1.59	35.50	1.28	0.06	1.10	0.45	86.55	5.14
Native	*Andropogon leucostachis*	grass	1.43	29.71	1.33	0.05	1.13	0.23	81.25	2.79
Native	*Jacaranda ulei*	shrub	1.43	50.75	1.66	0.07	1.52	0.18	75.85	2.42
Native	*Byrsonima verbascifolia*	shrub	1.35	38.50	1.30	0.06	2.97	0.65	77.20	5.57
Native	*Mimosa cyclophila*	shrub	1.35	22.00	1.20	0.05	0.98	0.25	83.40	2.94
Native	*Erythroxylum suberosum*	shrub	1.13	49.33	1.21	0.05	0.41	0.21	101.03	3.20
Native	*Senna rugosa*	shrub	1.03	41.25	1.24	0.05	2.05	0.29	53.44	2.05
Native	*Casearia sysvestris*	shrub	1.03	32.67	1.69	0.06	1.88	1.43	56.96	2.26
Native	*Poaceae 2*	grass	0.95	38.20	1.26	0.04	0.42	0.19	79.71	4.22
Native	*Poaceae 6*	grass	0.95	19.00	1.28	0.06	1.10	0.45	86.55	5.14
Native	*Palicourea rigida*	shrub	0.87	55.00	1.30	0.06	2.97	0.65	77.20	5.57
Native	*Poaceae 10*	grass	0.87	39.71	1.41	0.05	3.21	0.91	42.84	3.26
Native	*Poaceae 9*	grass	0.87	38.00	1.59	0.05	3.61	0.59	33.39	2.86
Native	*Panicum campestre*	grass	0.82	13.50	1.59	0.05	3.61	0.59	33.39	2.86
Native	*Cissanpelo ovalifolia*	herb	0.76	30.00	0.99	0.06	1.54	0.41	42.63	2.08
Native	*Hyptis villosa*	herb	0.69	69.67	1.55	0.06	14.50	0.74	38.75	3.24
Native	*Cantiona violacea*	herb	0.67	38.00	1.00	0.05	3.29	0.19	91.36	3.11
Native	*Erythroxylum campestre*	shrub	0.57	51.33	0.94	0.04	0.94	0.17	70.02	2.50

Native	*Byrsonima*	shrub	0.48	59.00	0.74	0.06	3.84	0.29	67.01	2.63
Native	*Poaceae 12*	grass	0.48	30.00	0.86	0.04	0.27	0.12	38.21	2.40
Native	*Poaceae 8*	grass	0.44	69.00	0.97	0.04	0.52	0.24	50.51	2.59
Native	*Axonopus aureus*	grass	0.36	20.00	1.86	0.07	1.38	0.20	85.42	3.41
Native	*Ichthyothere connata*	herb	0.32	59.00	0.56	0.04	0.12	0.15	44.96	2.71
Native	*Poaceae 11*	grass	0.32	40.00	0.77	0.04	0.14	0.20	30.65	2.90
Native	*Croton goyazensi*	shrub	0.23	54.00	0.74	0.06	3.84	0.29	67.01	2.63
Native	*Baccharis cf varians*	shrub	0.20	39.00	0.76	0.06	0.55	0.17	148.68	4.61
Native	*Myrcia tomentosa*	shrub	0.16	12.00	1.86	0.07	1.38	0.20	85.42	3.41
Native	*Poaceae 5*	grass	0.16	33.00	0.99	0.06	1.54	0.41	42.63	2.08
Native	*Psidium laruotteanum*	shrub	0.16	50.50	1.72	0.07	1.96	0.23	63.81	2.56
Restored	*Lepidaploa aurea*	shrub	15.13	90.67	0.66	0.04	0.36	0.27	35.31	2.22
Restored	*Schizachyrium tenerium*	grass	10.28	45.67	0.64	0.04	0.48	0.28	88.15	4.02
Restored	*Axonopus pressus*	grass	8.91	92.60	0.65	0.06	0.27	0.19	78.54	3.52
Restored	*Axonopus chrysoblepharis*	grass	6.14	153.00	0.64	0.05	1.21	0.42	52.81	3.22
Restored	*Melinis minutiflora*	grass	6.08	79.75	0.75	0.05	2.41	0.14	142.98	3.24
Restored	*Brachiaria decumbens*	grass	5.97	120.00	0.94	0.06	5.02	0.27	75.67	3.02
Restored	*Loudetiopsis chrysothrix*	grass	4.11	99.80	1.64	0.09	0.76	0.24	113.26	5.56
Restored	*Trachypogon spicatus*	grass	3.48	86.67	0.75	0.05	0.47	0.22	46.54	2.35
Restored	*Aristida flaccida*	grass	2.83	65.00	0.71	0.04	1.26	0.12	171.68	3.65
Restored	*Schizachyrium sanguineum*	grass	2.80	28.33	2.24	0.09	4.71	0.49	53.66	4.45
Restored	*Mimosa clausenii*	shrub	1.54	120.75	0.59	0.04	0.77	0.23	75.78	2.86
Restored	*Axonopus barbigerus*	grass	1.08	125.33	0.83	0.05	0.64	0.22	80.54	3.04
Restored	*Canastra aristella*	grass	1.03	81.25	0.77	0.05	0.19	0.46	12.59	2.73

Table S3: Results of the linear mixed model evaluating the relations of soil PCA axis 1 on Heigh max, Functional diversity, and the vegetation type (abandoned pasture, restored and native)

	Df	**F-value**	**p-value**
Height max			
PCA1		32.54	**< 0.01**
vegetation		5.32	**0.04**
Vegetation:PCA1		3.58	0.09
Residuals			
Final model (H.max~PCA1*vegetation)			
R²= 0.76			
Functional richness			
PCA1		6.53	**0.05**
vegetation		0.69	**0.43**
Vegetation:PCA1		0.06	**0.81**
Residuals			
Final model (FR~PCA1*vegetation)			
R²= 0.39			
Functional diversity			
PCA1		14.95	**0.04**
vegetation		2.97	**0.12**
Vegetation:PCA1		3.24	**0.10**
Residuals			
Final model (FD~PCA1*vegetation)			
R²= 0.57			

Chapter 4 – Original Research

Does vegetation restoration restore soil function? Exploring invertebrates and microbial decomposition post restoration in Brazilian savannas

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Does vegetation restoration restore soil function? Exploring invertebrates and microbial decomposition post restoration in Brazilian savannas.

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Abstract

Changes in vegetation structure impacts biogeochemical cycling mediate by on plant-organism interactions, as decomposition. The main agents to decomposition include the microbial and invertebrates community. Both decomposition agents are influenced in different ways by substrate quality, vegetation structure and composition. In Brazilian savannas, the conversion of native grasslands to pastures for livestock production affects the nutrient cycling through impacts on soil properties (substrate quality) and on vegetation structure and composition. Hence, to increase the efficiency of restoration practices, we should aim to recover soil processes and plant-macro- and microorganism interactions. Using an experiment with woody-block decomposition in 50 year old abandoned pasture, old-growth savanna vegetation and in 3-year old restored restoration process, we evaluate the role vegetation plays on macro and micro decomposition. We found similar decomposition rates by invertebrates among vegetation types, which was related to aboveground biomass. In contrast, the wood decay rates by microorganisms activity was substantially lower in restored relative to native areas. Wood decay rates by microorganisms, was however related to soil moisture content, with sites with higher moisture having greater wood decay rates by microorganisms. The restored areas actually had higher biomass than the native area, due to the use of fast-growing native plant species. This promoted the high decomposition rates by invertebrates, but not by microorganisms, which was probably lost with the soil-management undertaken prior to restoration. We conclude that restoration practices must therefore be further developed in terms of soil treatment prior to restoration and the species composition used to promote better microbial decomposition, to facilitate soil restoration.

Highlights

The early evaluation of restoration practice reveal that it does not restore the wood decay rates by microorganisms activity to a state similar of native habitats. However, wood decay rates by invertebrates were similar among vegetation types and increased with biomass gain over independently of vegetation types.

The potential to recover plant biomass through recruiting fast-growing plant species promoted elevated wood decay rates by invertebrates.

The low wood decay rates by microorganisms in restored sites is related with low soil microbial biomass, that is likely related to soil-management practices prior to restoration, such as burning and plowing which reduced microbial biomass and potential microbial decomposition. These effects might attenuate with changing physical substrate conditions, as high soil moisture combined with high soil cover provides fast microbial colonization.

Introduction

The ecosystems functioning dependent on the link between aboveground and belowground processes (Wolters et al. 2000). Overall, above and belowground organisms are a critical component of biogeochemical cycles (Crowther et al. 2019; Fry et al. 2019). The interactions between soil microbial communities and plants are a well-documented drivers of nutrient cycling (Chapman et al. 2006; Knops et al. 2002; Reynolds et al. 2003). High trophic levels in invertebrates food webs i also important to regule the decomposition rates and soil food web dynamics (bacterial and fungal decomposers and their predators), strongly affecting the terrestrial carbon pool (Ulyshen 2016). The close relationship between vegetation change and soil carbon (C) dynamics (Jobbágy & Jackson 2000) suggests that any disruption of the coupling between plants and soil organisms, as a result of land use change or native vegetation loss, may have consequences for ecosystem function. Therefore, changes in vegetation structure are highly likely to impact biogeochemical cycling within ecosystems.

Decomposition in terrestrial ecosystems is essential to maintain nutrient and C cycling (Canadell et al. 2007). During decomposition, plant tissues undergo a loss of easily soluble compounds, including starch, amino acids and soluble sugars, due to leaching, microbial activity and soil invertebrate attack (Bani et al. 2018). Invertebrates activities occur through enzymatic digestion, substrate alteration or microbial symbiosis (Ulyshen 2016). The termites (epifamily Termitoidae) and wood-borring beetles (various families of coleopteran) are the invertebrates responsible for the most decomposition rates within an ecosystem. These invertebrates have the potential to both promote and retard microbial fungi activity (Ulyshen, 2016; Ulyshen, WAgner, & Mulrooney, 2014). Positive influences of invertebrates on fungal activity include facilitating the fungi establishment by creating tunnels into the heartwood and promoting wood fragmentation. Despite the importance of invertebrates, the microbial community remains the main agent of soil and wood decomposition, with fungi and, to a lesser extent, by bacteria being the key actors (Allmér et al. 2009; Purahong et al. 2015; Tláskal et al. 2016).

Decomposition is intrinsically influenced by temperature and precipitation regimes. However, it is also effected by other environment variables, at both regional and micro-site scales, for example the quality of the litter substrate, and composition of the decomposer community (Cornwell et al. 2008; Parton et al. 2007). At the local scale, microbial communities are influenced by substrate characteristics that influence the ability of different taxa to access and metabolize specific structural compounds (Purahong et al., 2016a, Tlaskal et al., 2016). Physical substrate composition includes soil temperature, moisture and pH, while chemical substrate properties include the amount of C, N lignin and their ratio (C:N). Carbon (C), nitrogen (N) and phosphorus (P) play especially important roles in regulating microbial growth (Elser, Dobberfuhl, Mackay, & Schampel, 1996, Zechmeister-Boltenstern et al., 2015). Consequently, substrate composition plays a key role in controlling the structure and function of microbial communities, through selecting the type of predominant life strategies.

A substrate with a high concentration of labile C and nutrients stimulates mineralization rates and favours r-strategist microorganisms. This strategy is characterized by fast-growing bacteria with low retention of elements and accumulation of biomass. With low litter mineralization rates, K strategists microorganisms are favoured, represented by slow-growing fungi with high nutrient retention and biomass accumulation (Boeddinghaus et al. 2019; De Vries & Bardgett 2012; Leff et al. 2015). Thus, any alteration in soil substrate composition induced by vegetation change, is likely to affect microbial community. For example, soil disturbance affect biomass and diversity of microbial communities through altering soil temperature, moisture, C and N content and pH values (Mabuhay et al. 2006) and this lead to changes in decomposition rates mediate by soil substrate due to change in vegetation (Blumenthala et al. 2009; Ehrenfeld 2010; Freschet et al. 2013; van der Putten et al. 2016).

Plant-soil feedbacks are related to changes plant exert on biotic and abiotic soil characteristics, which are determined by functional characteristics. These functional characteristics can affect the decomposition process in the ecosystem (Bradford et al. 2017; Cornwell et al. 2008). For example, plants with conservative strategies are likely to confer tissues with recalcitrant compounds that are slowly decomposed, while plants with more acquisitive strategies are associated with tissues with high labile compounds that decompose faster (Cotrufo et al. 2013). Acquisitive plant communities tend to have a high investment in above-ground biomass (Giles et al in prep. Oliver, & Roberts, 1987; Song et al., 2013). Several studies show that the same system, for example, grassland, the higher aboveground biomass is linked to high plant diversity (Grace 2001; Hector et al. 1999; Tilman et al. 2001). Overall, the higher plant diversity also provides higher abundance and diversity of arthropods decomposers and as a consequence of this higher species richness fast decomposition (Ebeling et al. 2014, 2018). Thus, the increase in biomass provides increased decomposition by invertebrates decomposers, also high vegetation biomass might maintain the optimal humidity and temperature conditions for fungal microorganisms to further accelerate the decomposition process (Thakur et al. 2015).

In Brazilian savannas, the conversion of native grasslands to pastures for livestock production affects the ecosystem nutrient cycling dynamics through impacts on soil properties and decomposition rates (D'Angioli et al. in prep; Da Silva et al., 2014; Ehrenfeld, 2003). The establishment of exotic grasses with acquisitive functional characteristics, which will decompose faster facilitates a more rapid nutrient turnover that leads to a positive feed-back for exotic plant establishment. These degraded areas need the active restoration practices to restore the plant community (Suding et al. 2004), however, restoration techniques rarely take into account of restoring soil functional processes, such as decomposition through restructuring decomposer community. This is problematic as soil management processes during agricultural practices often increases pH and disadvantaging native vegetation and altering the microbial composition (D'Angioli et al. in prep, Silveira, de Mello, Silva, Krüger, & Bustamante, 2020). Thus, ecosystems restoration practices should aim to recover soil function and plant-macro and microorganism interactions to increase the efficiency of restoration practices of Cerrado tropical grassland. Restoration in other ecosystems has shown great success in terms of restoring biodiversity (Funk et al. 2008; Zirbel & Brudvig 2020), whilst the recovery of soil functions remains underlying restoration practices.

Thus, in the decade of restoration (UNEP/FAO 2021), studies that focus on understanding ecosystems functions will be essential to evaluate the long-term efficiency of restoration practice. Here our goal was answer: ii) Does early stages of restoration process in neotropical savannas restore the soil decomposition rates; ii) What are the relationships between vegetation structure, soil substrate conditions and micro an decomposition by invertebrates (hereafter wood decay rates by invertebrates and microorganisms). To address these questions we evaluated the potential of invertebrates and microbial decomposers in different vegetation states. We used 50 year-old abandoned pasture, an old-growth native savanna and a three year old restoration savanna site. We expected that: i) Abandoned pasture will have faster decomposition rates than native areas, which have more similar decomposition rates to restored site, indicating the decomposition process was restored; ii) Greater biomass

(Vegetation structure) and higher soil moisture results in higher wood decay rates in tropical savannas ii) Aboveground biomass drives higher decomposition rates, whilist soil substrate driver wood decay rates by microorganisms.

Material and Method

Study site

The present study was conducted at the Chapada dos Veadeiros National Park (14°07’03”S, 47°38’31”W), located in Central-Western Brazil at the municipality of Alto Paraíso de Goiás – Goiás; mean altitude: 1240 m (Pellizzaro et al. 2017). Mean annual precipitation is 1453 mm, 60% between October and May, and mean annual temperature is 21°C (Sampaio et al. 2019). We selected three areas with different vegetation types, abandoned pasture, native old-growth savanna vegetation and restored savanna vegetation.

Traditionally the areas that have been restored inside the national park have been used for extensive livestock grazing pre-1970 (ICMBIO 2009). In these areas, exotic grasses were sown, and the areas were constantly burned in order to stimulate grass growth (ICMBIO 2009). These areas were abandoned in two main events: 1985 and 1995 (ICMBIO 2009). Our abandoned pasture sites are dominated by the invasive exotic species: *Urochloa uminii U. humidicola* (Rendle) Morrone & Zuloaga, *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster, *Andropogon gayanus* Kunth, *Melinis minutiflora* P. Beauv. *and Hyparhenia rufa* (Nees) Stapf (Pellizzaro et al. 2017).

In the restored area, native savanna species was sown by direct seeding of in 2016. Prior to the direct seeding, the restoration area was dominated by invasive grasses. To reduce the biomass of invasive grasses these sites was burnt, and then it was ploughed five times (250 mm deep) followed by an inversion tillage (400 mm deep) in order to reduce the invasive grass seed bank and regeneration rate of exotic grasses (Coutinho et al. 2019; Sampaio et al. 2019). Seeds of 75 species of native herbs, shrubs and trees were collected from surrounding native areas (Coutinho et al. 2019; Pellizzaro et al. 2017; Schmidt et al. 2019). A total of 58 ha was restored using this technique in 2016. Sixty-two out of the 75 seeded species established, and the areas had 87% cover of native species and 12% of exotic grasses on two years after seeding (Pellizzaro et al. 2017).

Finally, we also selected undisturbed native areas adjacent to the restoration site and abandoned pasture. This area is characterized by the dominance of non-woody species, containing <20% woody species. The native area is characterized by high herbaceous species richness, high endemism, and unique species compositions, commonly called old-growth savannas, locally called *Campo cerrado* (Veldman et al. 2015a).

Experimental Design

For each vegetation type (restored, native and abandoned pasture), we selected two areas (A and B). The areas differed in conditions and land-use history. Specifically, area A had a higher water content in the top-soil than area B, with events of waterlogging during summer. Furthermore, the areas A and B had distinct land-use histories.

Two years after seeding, in 2018, in each block (A and B), for each vegetation type we installed one transect of 400 m. At 10 points, 20 m apart along the transects, (6 transects × 10 points per transect = 60 samples) we measured four ecosystem functions: aboveground biomass stock, belowground biomass stock, decomposition rate and carbon stock. A fire in abandoned pasture in area A (block A) caused the loss of this transect therefore here we report 5 transects × 10 points per transect × 2 seasons = 50 samples.

We took soil samples with 20cm deep soil core around each plot per vegetation type. We measured the concentration of total soil N, available P, soil organic matter (SOM), Aluminium concentration and soil pH, as a proxy of nutrient pool. The soil N concentration was determined by sulfuric acid digestion followed by Kjeldahl distillation (Bremner 1996); soil P and potassium (K) concentrations were determined following a Mehlich-1 extraction (Raij et al. 2001) ; and SOM was determined by the Walkley-Black reaction (Nelson 1996). Separately, in the same places, we assessed soil water holding capacity at 3 soil samples with 3 depths (0-10 cm, 50 cm and 100cm) at plots level in each area to estimates the slope of water saturated by Van Genuchten method (*van genuchten, 1980)*. Complementary to that we installed soil moisture sensor in 3 points for each vegetation type at the same three depths as stated above. The sensors were programmed to take measurements with at 15 min intervals over 1 year, starting in July of 2018 and finishing in July 2019.

We measured aboveground biomass production by clipping all plant material in 50 x 50 cm plot around each vegetation plot at the peak of dry season and the peak of the wet season. To measure belowground biomass, we took 10 cm^2 by 10 cm deep soil cores from the corner of each plot at the end of the growing season. We sorted samples to remove litter and oven dried at 65 °C for at least 48 h before weighing. We took 10 samples of 3 cm^2 soil cores from 0-100cm at 10cm intervals. All sampled were taken from the corner of each plot during the same periods as aboveground biomass was collected. We passed soil cores through a 2 mm sieve to collect all roots, which were then washed and dried at 65 °C for 48 h before weighing. To measure exotic cover and bare soil cover we use the grid of $4m^2$ separated into 64 subplots of 6.25 cm^2 and estimate visually the number of subplots covered by exotic species, native species, and bare soil cover.

To assess the decomposition potential of microbial (hereafter wood decay rates by microorganisms) and invertebrates (hereafter wood decay rates by invertebrates) we measured the decomposition rates (in situ) (percentage of mass loss per day), using 40 woody-blocks placed in each transect, 4 blocks per transect point. Untreated *Pinus radiata* plank pieces 1.9 cm thick × 7 cm wide were cut to 15 cm lengths, with one bait-block consisting of two stacked-pieces (399 cm^3). All blocks were then dried at 120°C for 48 h. Drying provides a means of standardizing wood pre-treatment, given bait wood may have been dried and stored under different conditions. Two blocks were enclosed in 300 μm nylon mesh (Plastok, Merseyside, UK) bags to only allow for microbial decomposition, and the other two was enclosed in 5 mm nylon mesh to include all invertebrate decomposers. All blocks bags were sealed by rolling and securing mesh ends with stainless-steel staples. We followed a generalizable protocol for examining the influence of microbes and invertebrates on wood decay (Chesmaan et al. 2017). Using the traces on the wood was possible to detected termites, beetle and fungi presence in wood blocks. We removed intact leaf litter from the surface soil layer, this was homogenized via scraping and replaced. The experiment lasted for 24 months and was installed in july 2018. Half of the blocks (1 invertebrate-exposed and 1 microbial-exposed at each point along our 5 transects) was harvested at the peak of the dry season (July 2019) in the first year and the other half was harvested in the peak of dry season in the next year (July 2020). At harvest, blocks were sealed in plastic bags and stored in an air-conditioned lab until processing. Within a day of collection, mesh bags were cut open and external mud was removed from the block with a brush. Blocks were then placed in paper bags and dried at 120°C for 48 hours. Final decomposition rate was measured with proportional mass loss calculated for each block as in Equation 1.

Eq.(1):

Decay rates (mg g^{-1} d^{-1}) = (1000 * (MI - MF) / MI) / DAYS

MI = initial mass (g); MF = final mass (g); DAYS = days since fielding

We measured microbial activity using soil respiration (SR) during peak wet season (March 2019) in each plot in all sites. SR was measured with an EGM-4 (IRGA; EGM4, PP Systems, Hitchin, UK). Circular gas sampling collars (25 cm diameter) were inserted into the soil surface during 48 hours. At each measurement time, clear flux chambers were sealed over the collars. Microbial respiration can then be calculated by subtracting root respiration from total soil respiration (Kelting et al. 1998).We measured SR as soil CO2 efflux in each plot separating roots to soil trough a 2 mm sieve and them back the soil sample into the soil on the floor 24 hours before the first soil respiration measure. We measured soil respiration rate (mg C m^{-2} h^{-1}), R_{ref} is base soil respiration (mg C m^{-2} h^{-1}), normalized to 25°C, Q_{10} is temperature sensitivity of SR and T_S is the measured soil temperature (°C) at 5 cm depth. We use the equation below (*eq.2* R_{uc}) and apply the chamber volume correction to measure soil CO2 efflux (*eq.3* R_c).

Eq. (2):

$$R_{uc} = \frac{C_{10} - C_1}{t_{10} - t_1} \frac{P}{(T_a + 273.15)} \frac{V_d}{A} * \frac{44.01 * 0.36}{R_u} \text{ g CO}_2\text{ m}^{-2}\text{ h}^{-1}$$

Eq. (3):

$$R_c = R_{uc} * \frac{V_d + V_{added}}{V_d}$$

Finally, we measured C in the microbial biomass (C_{mic}) using the chloroform fumigation-extraction method (CFE; Brookes et al. 1985).

Statistical analyses

To assess if vegetation type – abandoned pasture, restored and native – had different soil decomposition we performed a linear mixed effect model (LME). In these models wood decay rates by microorganisms and invertebrates were response variables and we considered the block – A or B -, the area – abandoned pasture, restored and native - as predictive fixed effects variables, and years of decomposition as random variables nested in the plot, since the decomposition was repeated at the same plot in two years. We also considered aboveground biomass, exposed soil, exotic cover, soil moisture and soil fertility parameters (pH, concentration of N, P, K and SOM) as response variables. For the LMEs in which we found significant effects of the predictive variables, we performed a post-hoc Tukey's honestly significant test (Tukey HSD) in order to identify which communities differed from each other. All analyses and plots were made in R software v4.0.0 (R Core Team 2020). We used the 'nlme' package (Pinheiro et al. 2020) for the LME, and the 'emmeans' (Lenth 2020) package for the post-hoc Tukey HSD test. For our figures we used the 'ggplot2' package (Wickham 2016).

Results

Soil microbial respiration and biomass

Soil Microbial CO^2 efflux in the restored area was 45.6% lower than abandoned pasture, and 20% lower than native vegetation (Table 2, Fig. 1a). To Soil C_{mic} was related to vegetation type (Table 2, Fig. 1b). Soil C_{mic} of restored sites was lower than that of native sites, while abandoned pasture sites did not differ from the other vegetation types.

Soil Relative Water Content (RWC) was different between blocks and among vegetation types. The soil in block A presented higher RWC when compared to block B, being RWC 27.6% higher than block B in all vegetation types (Fig 1c). Natives areas soil showed the RWC 40.4% higher than restored and 30.3% higher than the abandoned pasture areas. The soil at block A in restored area had a 39.4% RWC higher than the block B at restored area (Table S3).

Soil pH was higher in restored sites than abandoned pasture sites (Figure S1A; Table S4). Soil total N concentration was lower in the restored sites relative to both the native and abandoned pasture sites (Fig S1B). Soil available P concentration was higher in abandoned pasture sites than in native and restored sites (Fig S1C). Soil K concentration was higher in the native sites than in abandoned pasture and restored sites

(Fig S1D). Soil organic matter concentration was lower in restored sites than in abandoned pasture and native (Table S4E).

Aboveground biomass and vegetation

Aboveground biomass (AGB) was different among vegetation types and had an interaction with block (Table S3). The restored area has high aboveground biomass with a mean of 37% more biomass than abandoned pasture and similar to native area. Native area block A had 40% less AGB than native block B. Restored area in block A shown more biomass and lower variation than block B (Fig 1d).

The exotic invasive grass cover was different among vegetations types. Pasture areas showed higher exotic cover than native and restored area (Fig 1e and table S3). The restored area in block A shown in average 58.2% higher values of exotic cover than restored in block B. The similar results were found for bare soil cover. The restored area in block B showed an average 52.3% higher bare soil cover than restored in block A, the same patterns were found in native areas (Fig 1f and table S3).

Wood decay rates

The decomposition rate was affected by block (A and B), and interaction between vegetation, block and year. The restored site block A was 36% higher than the native block A (More humid soil), while restored areas was similar to the native area in block B (less humid soil; Fig. 1 a and Fig. 2 a) Wood decay rates by microorganisms increased between year 1 to year 2 to all vegetation types. The wood decay rates from year 1 to year 2 in restored areas increased 53.3%, the native 47% and the abandoned pasture 63% (Table 1, Fig. 2a). Specifically in the restored area at block B, wood decay rates by microorganisms increased from 21.2% from year 1 to 2, which was lower than the mean 31% annual increase from the native block B and the 41% increase in the abandoned pasture (Table 1, Fig 2a).

The wood decay rates by invertebrates were similar among all vegetation types and block A and B (Table 1, Fig. 2b). The wood decay rates by termites was 58.3% in native areas; 22% in abandoned pasture; 37% in restored area, while other insect taxa, principally beetles, were responsible by 17.7% in restored area; 13.8% in native areas and 12.5% in the abandoned pasture. The wood decay rates by invertebrates increased between year 1 and 2 to all vegetation types (Table 1, Fig. 2b). Native areas showed a greater variance of invertebrates decomposition and the high values of decomposition rates were made by termites . Also, the wood decay rates by invertebrates was similar among block A and block B during both years.

Drivers of decomposition rates

The wood decay rates by microorganisms did not have a relationship with aboveground biomass or any other parameters related to the vegetation, shown only year effect (Fig. 3 a; table 4). Furthermore, wood decay rates by microorganisms was not related to C_{mic} and soil fertility parameters. Wood decay rates by invertebrates was correlated with aboveground biomass ($R^2=0.12$; $p<0.01$, Fig. 3 b).

Discussion

Here we evaluated how restoration using soil plough and direct-seeding techniques to restore open savanna vegetation influenced soil decomposition. In general wood decay rates by microorganisms were lower in restored block B than native and abandoned pasture. The low microbial decomposition followed the low C_{mic} and low microbial respiration in restored area. The soil-management practices at restoration sites most likely caused the low abundance of microorganisms due to a direct effect on the mortality of microorganisms that leaded low decomposition rates (Fierer et al. 2009; Serna-Chavez et al. 2013; Zuber & Villamil 2016). However, the soil management might be attenuate by soil moisture, that provided high fungi colonization and high wood decay rates in sites with high soil moisture (Fig 2 A). Finally, the wood decay rates by invertebrates were related to aboveground biomass, independently of acquisitive or conservative vegetation types. Thus, the wood decay rates by microorganisms seem to be more influenced by physical soil parameters linked to soil management. In contrast, the role of invertebrates in decomposition is more influenced by vegetation structure.

Restored areas were previously used for livestock grazing, when conventional techniques of soil management were applied such as tillage and liming, and also burning to stimulate grass growth (ICMBIO 2009; Pellizzaro et al., 2017). Recently, in 2015, the restored sites were plowed and burned before native species were sown (Pellizzaro et al. 2017; Sampaio et al. 2019). These soil-management practices most likely caused mortality of soil microorganisms and the SOM concentration reduction, which would limit microbial growth (Fierer et al. 2009; Serna-Chavez et al. 2013; Zuber & Villamil 2016). These practice, also provided the low CO^2 efflux (Fig. 2C) mediate by low microbial C in restored area. Consequently, it is likely that the soil treatment prior to restoration reduced the wood decay rates by microorganisms in the restoration site, relative to the native and pasture site. Therefore, the differences in microbial decomposition among the sites might be related to land use history and soil preparation before restoration practice (D'Angioli et al. in prep).

Despite similarities in the decay rates between vegetation types, the differences in soil moisture and vegetation structure might still indicate differences in patterns of Wood decay rates by microorganisms when comparing blocks A and B. The great variation in soil moisture among blocks (A and B) in the restored area (Fig 2 A) drives distinctly wood decay rates by microorganisms (Fig. 1 A). Overall, wood decay rates by microorganisms is more likely to be carried out by fungi and, to a lesser extent, by bacteria (Allmér et al. 2009; Bässler et al. 2010; Purahong et al. 2016; Tláskal et al. 2016). Several studies have shown that the ability of fungi to colonize dead wood and access C compounds are enhanced by the wood-soil contact that increases the moisture content of wood (Nicholas & Militz 2008; Van Der Wal et al. 2007). In sites with low levels of moisture, the colonization by fungi is slow (A'Bear et al. 2014; Progar et al. 2000). Thus, the low RWC at areas in block B (Fig. 2 A) explains the slower decomposition rates than block A.

Beyond moisture, soil surface temperature are likely to affect the decomposition process and decay rates (Cornwell et al. 2008; Gholz et al. 2000; Purahong et al. 2016; Santonja et al. 2015; Trofymow et al. 2002). Studies in forest systems demonstrate that the removal of the overstory layer can increase the temperature fluctuations on soil surface, leading to a shift in the decomposer communities (Lado-Monserrat et al. 2016; Purahong et al. 2015; Waldrop et al. 2004). Also, has been observed that different warming treatments alter the fungal community structure and wood respiration rates by microbial activities (Austin & Zanne 2015). Therefore, the higher aboveground biomass and soil cover in restored Block A probably provides a low variance of temperature that drives low soil surface evaporation. These minor temperature variations maintain the surface soil moisture that provides fast wood decay rates by microorganisms. We concluded that the restoring practice might damage soil microbial functions in early stages vegetation leading to low wood decay rates by microorganisms. Although, these impact in restored area might be softened in sites with higher soil moisture and low bare soil cover.

The invertebrates decomposition is accomplished by distinct invertebrates guilds (Kampichler & Bruckner 2009; Ulyshen et al. 2014). Apparent competition and other forms of interference among invertebrate taxa that compose the guilds also have the potential to alter decomposition rates, especially when involving major wood-boring taxa (Ulyshen et al. 2014). In general, termites are a major drivers of wood decomposition, consuming 15–20% of wood volume, after 2 years (Ulyshen et al. 2014). In our study, although there were similar decomposition rates mediated by invertebrates across all vegetation types (Fig 1B). Native areas are characterized as old-growth neotropical savannas where there is a high abundance of termites (Jones & Eggleton 2010). This results in a high number of nest, which increases the probability to find the wood blocks in native area (Ulyshen et al. 2014). In fact, termites were most present in woody block in native areas than abandoned pasture and restored areas. The restored area had a higher percentage of presence of other insects taxa, principally beetles. These different invertebrates are possible attracted by the early vegetation stage (De Deyn et al. 2003; Scheu & Schulz 1996).

The conversion from native vegetation to pasture is accompanied by changes in plant biodiversity and in the soil community (Maharning et al. 2009) and over time these include changes in abundance, diversity and activity of the soil organisms. There is evidence of an increase in invertebrates diversity in the first few years of old pasture succession (Loranger et al., 2014, Curry 1994). These increase of faunal diversity associated to an increase in plant diversity that drives higher grasses biomass, and consequently a higher abundance and diversity of decomposing arthropods (Ebeling et al. 2014, 2018). Therefore, the increase in wood decay rates by invertebrates can be attributed to the increase in biomass that likely increased decomposer species richness (Ebeling et al. 2014, 2018; Milcu et al. 2008). In fact, the high biomass provides resources to aboveground herbivores (Loranger et al. 2014), that consequently attract consumers, structuring the food web, that includes ‘saproxylic’ insects responsible for decomposing. Thus, the high biomass in the restored area is probably a great way to structure trophic relations and provide invertebrate decomposition process. Although, we have similar increases in decomposition rate between the years in the all vegetation type. This means that biomass is not the only driver determining the decay rates.

In our treatment of decomposition by invertebrates, all wood blocks showed fungi decomposition evidence. Therefore, the potential decomposition in all vegetation types is an interaction between wood decay rates by microorganisms and invertebrates. The abandoned pasture and native area despite lower aboveground biomass than restored show similar wood decay rates by to the restored, due to the higher fungi decomposition. Additionally, the restored area kept similar wood decay rates by invertebrates to other vegetation types due to the high aboveground biomass that provides trophic effect mediated by vegetation, even with low microbial biomass. Therefore, the interaction between vegetation structure (biomass) might compensate for the effect of low microbial biomass in the restored area.

Conclusion

Using the direct-seeding restoration experiment site to restore open-savanna areas in a Neotropical savanna, we find the restored vegetation potentially maintains similar wood decay rates by invertebrates mediated by trophic effects supported by the fast-growing plant community. On the other hand, the soil-management practices prior to restoration reduced microbial biomass, soil organic matter content, and consequently the potential of microbial decomposition. These effects might attenuate with changing physical substrate conditions, as high soil moisture combined with high soil cover provides fast microbial colonization. Thus, the conditions of the substrate and the vegetation structure needs take into account in restoration whose objective is recovery ecosystem functions relate to nutrient cycling.

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Figures and Tables

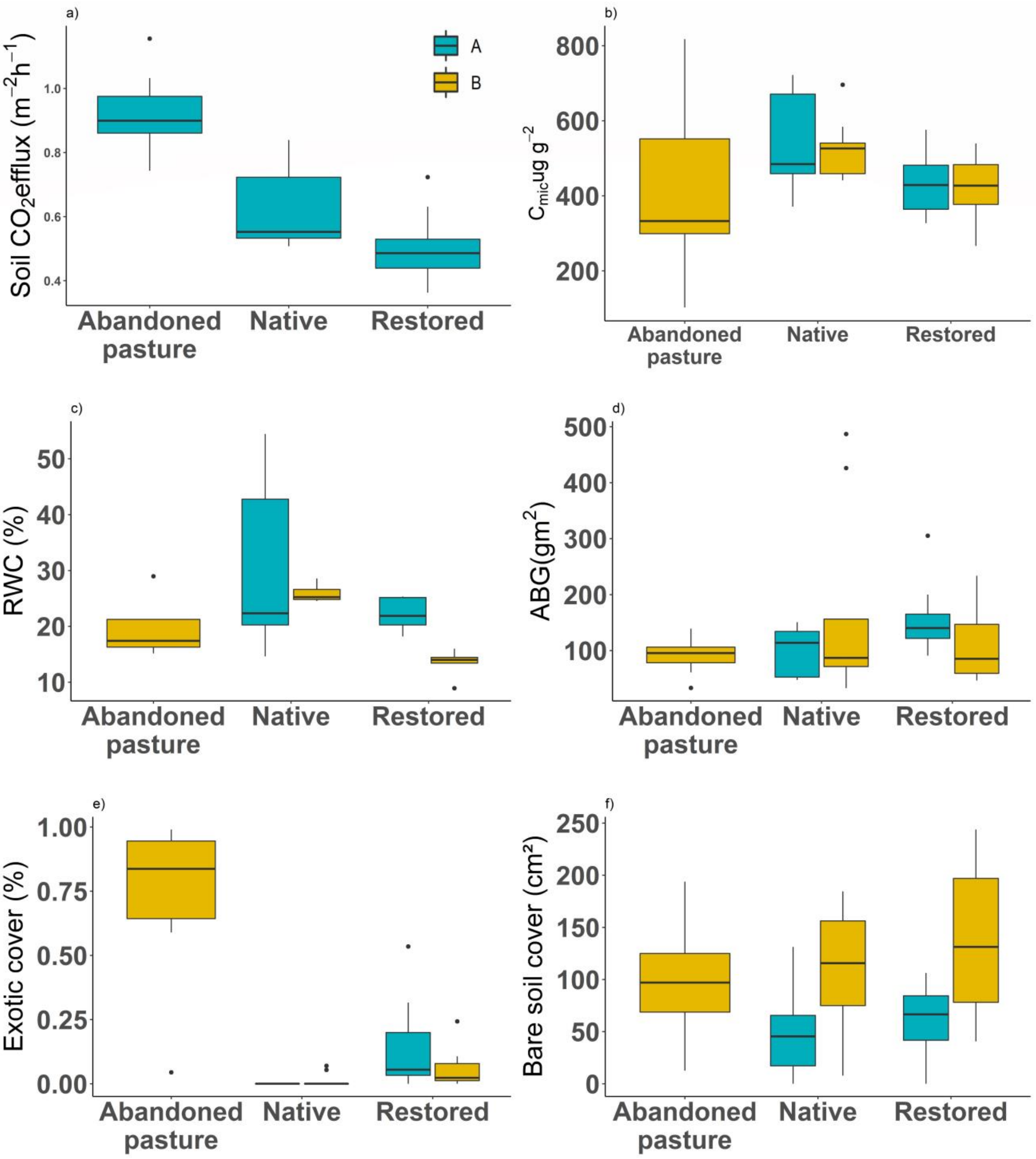
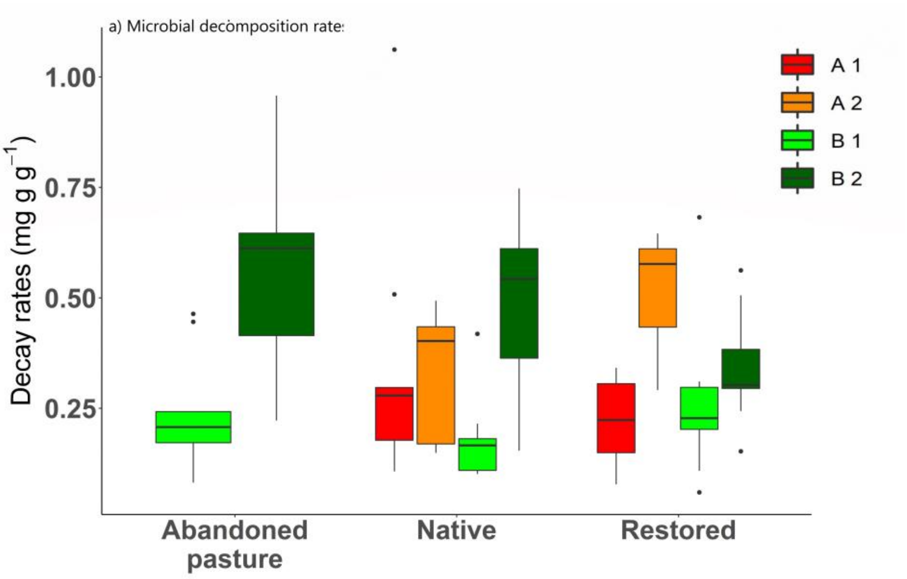


Figure 1. Soil microbial respiration; parameters of cover, aboveground and relative water content (RWC). a) Microbial Soil respiration measured CO^2 efflux; b) Microbial Carbon; c) Relative water content (RWC); d) Aboveground biomass (ABG); e) Exotic cover; f) Bare soil cover. A represents block A and B represents block B in each area. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present



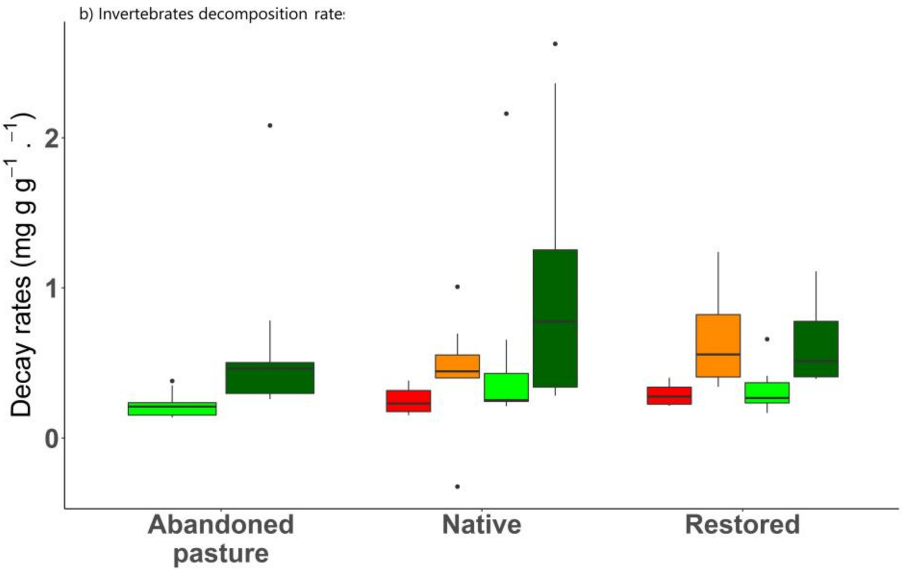


Figure 2. Decomposition rates. a) Wood decay rates by microorganisms b) Wood decay rates by invertebrates. A1 represents block A to first year of decomposition, A2 represents block A to second year of decomposition, B1 represents block B to first year of decomposition, B2 represents block B to second year of decomposition. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present.

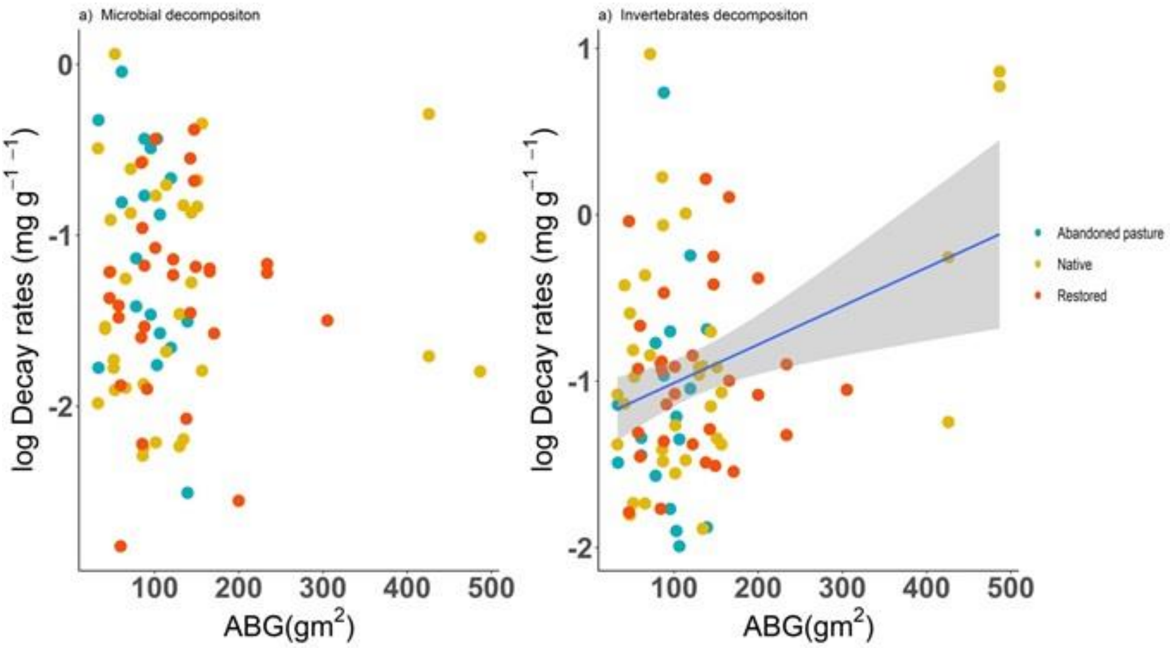


Figure 3. Relation between aboveground and decomposition rate year 1 and 2 in all vegetation type. a) Wood decay rates by microorganisms; b) Wood decay rates by microorganisms invertebrates. The points represent the wood blocks decomposition and colors represent each vegetation.

Supplementary material

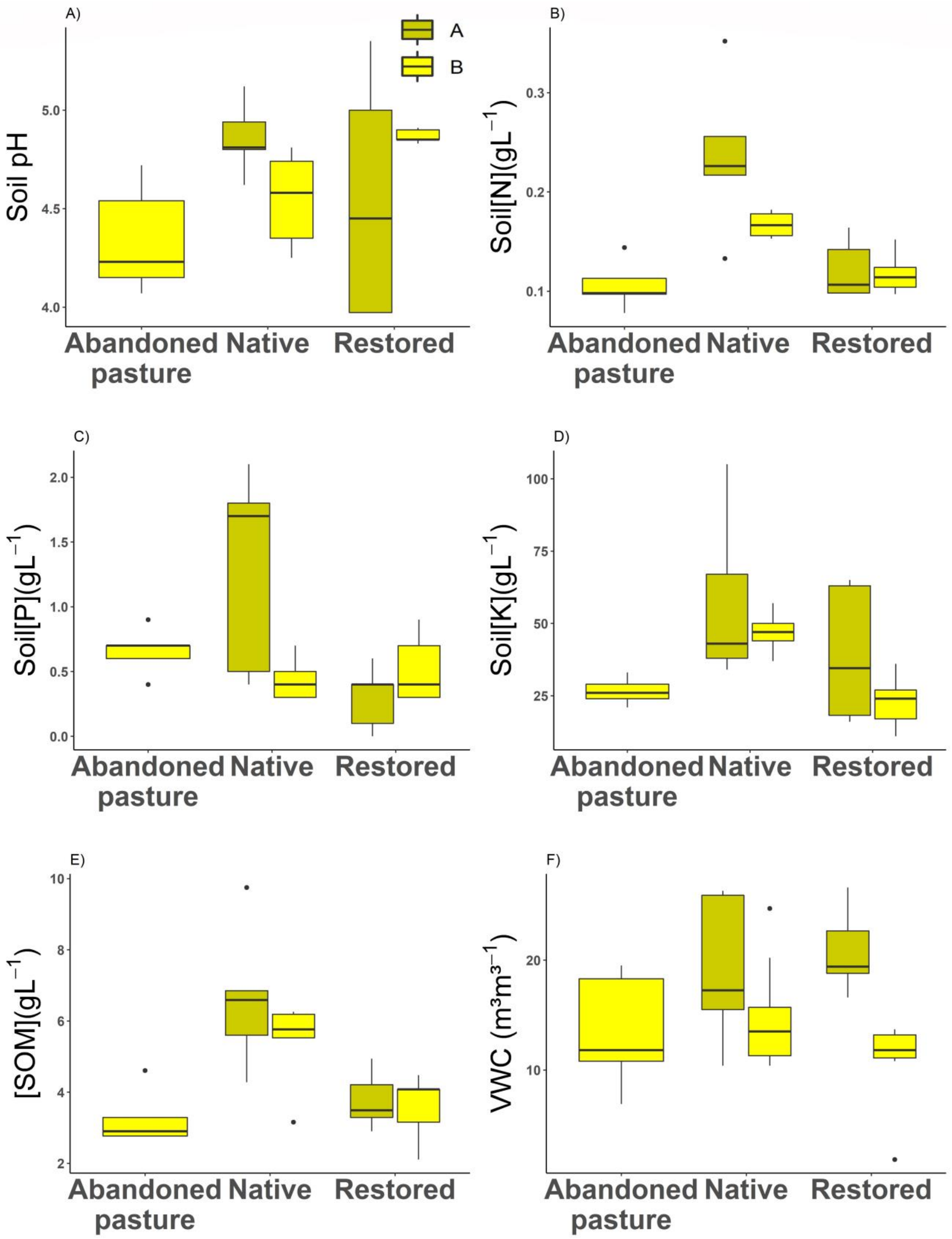


Figure S1. The relation of soil fertility parameters with vegetation. Different colors represents different blocks , site A and B. a) Soil pH; b) Soil Nitrogen concentration; c) Soil phosphorus concentration; d) Soil Potassium concnetration; e) Soil organic Matter; f) Volume water content in the soil.

Table 1: Results of the linear mixed effect model evaluating the relations of the vegetation type (abandoned pasture, restored and native), block (A or B) and decomposition year (year 1 and year 2) on wood decay rates by microorganisms and invertebrates.

	Degrees of Freedom	**F-value**	**p-value**
Wood decay rates by microorganisms			
Vegetation	2, 54	4.42	0.10
Block	1, 54	0.08	0.76
Year	1, 54	12.24	**<0.01**
Vegetation : Block	2, 54	3.52	**<0.01**
Vegetation : year	2, 54	2.01	0.36
Block : year	1, 54	1.67	0.19
Vegetation : Block: year	2, 54	11.32	**<0.1**
Wood decay rates by invertebrates			
Vegetation	2, 54	0.22	0.80
Block	1, 54	0.08	0.77
Year	1, 54	20.36	**<0.01**
Vegetation : Block	2, 54	1.00	0.31
Vegetation : year	2, 54	0.07	0.92
Block : year	1, 54	0.69	0.40
Vegetation type : Block: year	2, 54	2.69	0.10

Table 2: Results of the linear mixed effect model evaluating the relations of the vegetation type (abandoned pasture, restored and native), block (A or B) on soil microbial carbon and soil microbial respiration.

	Degrees of Freedom	**F-value**	**p-value**
Microbial Carbon Concentration			
Vegetation	2, 54	4.03	**0.02**
Block	1, 54	9.15	< **0.01**
Year	1, 54	0.57	0.45

Vegetation : Block	2, 54	3.52	**0.04**
Vegetation : Year	2, 54	0.11	0.89
Block : Year	1, 54	3.28	0.08
Vegetation : Block: Year	2, 54	2.74	0.07
Co2 microbial efflux			
Vegetation	2, 54	17.44	< **0.01**
Block	1, 54	5.93	**0.02**
Year	1, 54	18.57	< **0.01**
Vegetation : Block	2, 54	0.59	0.56
Vegetation : Year	2, 54	4.89	**0.01**
Block : Year	1, 54	1.73	0.19
Vegetation : Block: Year	2, 54	3.57	**0.03**

Table 3: Results of the linear mixed effect model evaluating the relations of the vegetation type (exotic, restored and native), block (A or B) on soil moisture, aboveground biomass, exotic cover and soil exposure.

	Degrees of Freedom	F-value	p-value
Relative water contente (RWC)			
Vegetation	2, 45	15.42	< **0.001**
Block	1, 45	6.67	< **0.001**
Vegetation : Block	2, 45	0.**44**	0.50
Exotic cover			
Vegetation	2, 45	10.55	< **0.001**
Exposed soil			
Vegetation	2, 45	13.82	**0.05**
Block	1, 45	42.44	< **0.001**
Vegetation : Block	2, 45	22.20	**0.86**
Aboveground biomass			
Vegetation	2, 45	5.27	**0.07**
Block	1, 45	1.27	0.25
Vegetation : Block	2, 45	9.59	<0.001

Table 4: Results of the linear mixed effect model evaluating the relations of the vegetation type (abandoned pasture, restored and native), block (A or B) on the soil fertility parameters.

	Degrees of Freedom	F-value	p-value
Soil pH			
Vegetation	2, 45	1.40	0.26
Block	1, 45	0.17	0.69
Vegetation : Block	2, 45	0.30	0.74
Block : Season	1, 45	0.02	0.90
Soil P concentration			
Vegetation Type	2, 45	10.55	**< 0.001**
Block	1, 45	21.12	**< 0.001**
Vegetation Type : Block	2, 45	8.24	**< 0.001**
Soil N concentration			
Vegetation Type	2, 45	13.82	**< 0.001**
Block	1, 45	42.44	**< 0.001**
Vegetation Type : Block	2, 45	22.20	**< 0.001**
Soil K concentration			
Vegetation Type	2, 45	6.93	**< 0.001**
Block	1, 45	7.33	**0.01**
Vegetation Type : Block	2, 45	1.55	0.22
Soil Organic Matter concentration			
Vegetation Type	2, 45	10.09	**< 0.001**
Block	1, 45	32.21	**< 0.001**
Vegetation Type : Block	2, 45	16.71	**< 0.001**

Table 5: Results of the linear mixed effect model evaluating the relations of the aboveground biomass and year with (year 1 and year 2) on wood decay rates by microorganisms and invertebrates.

	Degrees of Freedom	**F-value**	**p-value**
Wood decay rates by invertebrates			
Aboveground biomass	2, 54	20.77	<**0.01**
Final model: decay rates~ABG+ (ano|plot)			
Wood decay rates by microorganisms			
aboveground	2, 54	4.22	0.82
year	1, 54	4.7	**0.03**
Vegetation	1, 54	5.2	0.23
Aboveground:year	2, 54	0.22	0.28
Aboveground:Vegetation	2, 54	3.71	**0**.06
Year : Vegetation	2, 54	1.82	0.13
Aboveground : year:vegetation	2, 54	0.85	0.34
Final model: decay rates~ABG*Vegetation*Year (ano|plot)			

CONCLUSÃO GERAL

Os novos filtros mediados pelos eventos extremos globais são capazes de alterar o funcionamento de espécies e ecossistemas e mudanças na amplitude do nicho ecológico de espécies. Com intuito de prever e mitigar essas modificações e seus efeitos nas futuras trajetórias dos ecossistemas devemos seguir a abordagem baseada em atributos que sejam relacionados a estratégias ecológicas e a história evolutiva das espécies. Para ampliar essa abordagem são necessários estudos experimentais que envolvam mudanças nas condições ambientais associadas a medidas nas variações de atributos no nível do indivíduos e espécies e seus impactos na comunidade e ecossistema.

Aqui demonstramos que modificações na precipitação podem levar a aclimatação de árvores do subosque de florestas tropicais. Além disso, árvores pequenas são mais tolerantes à seca que árvores grandes presentes no dossel da floresta. Essa partição de nicho entre arvores pequenas e grandes, bem como o ajuste de arvores pequenas em respotas à luz e água podem se manter ao longo do desenvolvimento da espécies. A principal questão está em saber se a distinta capacidade de tolerar a seca irá se manter e gerar uma seleção de indivíduos mais baixos com maior tolerância à seca. Isso pode implicar em florestas mais baixas ou até mesmo desaparecimento de espécies que não atingem a estatura da maturidade adulta para produção de frutos. Por outro lado, espécies pequenas que estão experienciando a seca logo nos estágios iniciais do desenvolvimento poderão manter tolerância a seca pela manutenção dos atributos nos estágios ontogenéticos futuros.

Por fim, como forma de mitigar danos da era do antropoceno, a restauração ecológica de ecossistemas tem o potencial de restaurar a biodiversidade e o funcionamento de ecossistemas. Entretanto, ainda faltam técnicas adequadas que levem em conta a particularidade dos processos de filtragem ambiental em cada ecossistema. Em savanas neotropicais, a técnica de semeadura direta parece eliminar os filtros ecológicos comumente responsáveis por estruturar estas comunidades, gerando comunidades desacopladas do solo com abundância de espécies de estratégias rápidas e vulneráveis aos distúrbios, como o fogo por exemplo. Além disso, a alteração da estrutura edáfica pelo manejo do solo dificulta o processos de decomposição por microorganismos. Essa prática de restauração leva em conta a ideia da rápida cobertura do solo por espécies de crescimento rápido. Isso pode favorecer a decomposição mediada por invertebrados, mas não gera nenhum ganho em termos de funcionalidade do ecossistemas quando comparado ao estado degradado de pasto abandonado. Ao contrário do que imaginamos, a rápida cobertura do solo por espécies de crescimento rápido pode ter implicações para resiliência a distúrbios. Na década onde há aumento de risco de mega incêndios, o uso apenas da técnica semeadura direta em larga escala pode tornar todos os esforços e investimento monetário ineficientes em restaurar o cerrado.

É notável que a expansão da técnica de semeadura direta gera renda e emancipação para comunidades tradicionais locais. Contudo, talvez seja necessário uma nova abordagem que leve em conta as estratégias reprodutivas dominantes nas comunidades. Em sistemas propensos a distúrbios, a perturbação é um importante filtro estruturador da comunidade. Portanto, atributos de raízes são determinantes para aumentar a resiliência a distúrbios. Propomos que como forma de melhorar os resultados de restauração nesse ecossistema, utilizar o transplante de órgãos subterrâneos, como banco de gemas. Ainda, para dar continuidade à geração de renda a comunidades tradicionais, podemos combinar o conhecimento popular de manejo de roças desenvolvido pelas comunidades tradicionais para começar entender como manejar órgãos subterrâneos e utiliza-los em técnicas de restauração em larga escala.

Por fim, eventos extremos ainda persistirão e podem ser potenciais experimentos naturais para entender respostas das comunidadesde plantas a mudanças nos padrões de recursos e condições diferentes das quais evoluíram. Olhar para nicho além da abordagem correlativa, usando modelos mecanísticos da vegetação podem ajudar a mitigar problemas futuros que a humanidade possa enfrentar pela escassez de recursos. Contudo, há necessidade de aumentar o conhecimento dos limites do nicho das espécies nas comunidades. Para isso precisamos continuar e aumentar estudos com a abordagem experimental usando atributos funcionais como os experimentos de “secar as florestas” (ESECAFLOR) abordados aqui e outros ecossistemas. Somado a isso, a restauração ecológica representa um sistema experimental ideal para testar como a alteração de recursos afetam as espécies e a montagem de comunidades, consequentemente a provisão de serviços aos seres humanos. Entretanto, há necessidade de maior integração das técnicas de restauração com conhecimento ecológico e conhecimento tradicional de comunidades. Para isso precisamos entender questões evolutivas e de filtros ambientais dentro de cada ambiente, bem como da capacidade pratica da realização de novas técnicas. Assim, abordagens futuras que levem em conta a geração de renda para comunidades humanas vulneráveis as alterações climáticas, combinado com conhecimento ecológico embasado em teorias será a chave para diminuir danos mais drásticos no futuro.

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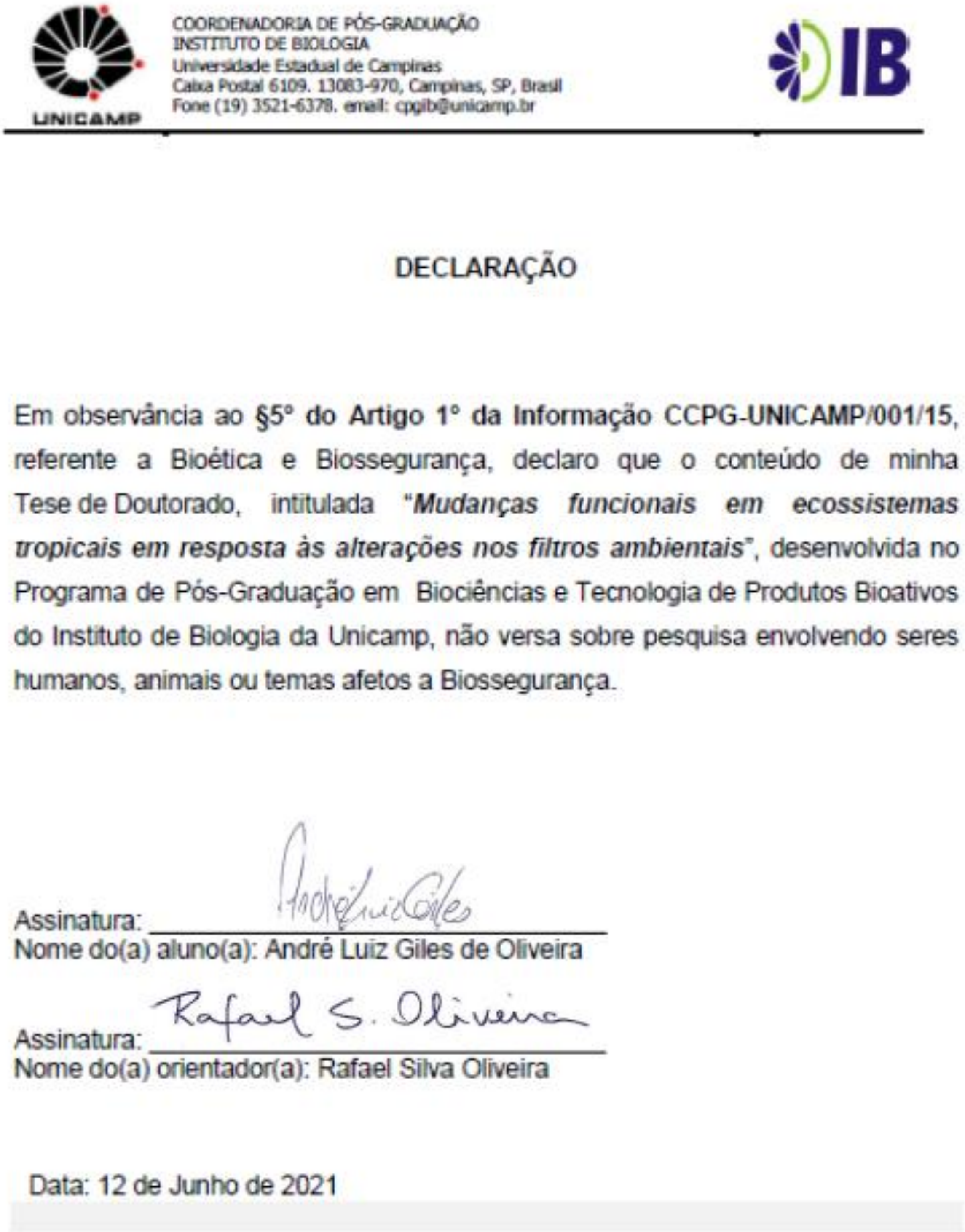
Anexo 1: Declaração de bioética e biossegurança



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Em observância ao **§5° do Artigo 1° da Informação** CCPG-UNICAMP/001/15, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "*Mudanças funcionais em ecossistemas tropicais em resposta às alterações nos filtros ambientais*", desenvolvida no Programa de Pós-Graduação em Biociências e Tecnologia de Produtos Bioativos do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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Data: 12 de Junho de 2021

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