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# How is forest restoration plantations' functioning affected by tree diversity?

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Thesis presented to obtain the degree of Doctor in Science. Area: Forest Resources. Option in: Conservation of Forest Ecosystems

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Bachelor in Biological Sciences

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versão revisada de acordo com a resolução CoPGr 6018 de 2011. -

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To my loving family

To my parents: my roots
To my sister, brothers, nieces and nephews: my light

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# O cântico da terra

Cora Coralina

*Eu sou a terra, eu sou a vida.*
*Do meu barro primeiro veio o homem.*
*De mim veio a mulher e veio o amor.*
*Veio a árvore, veio a fonte.*
*Vem o fruto e vem a flor.*

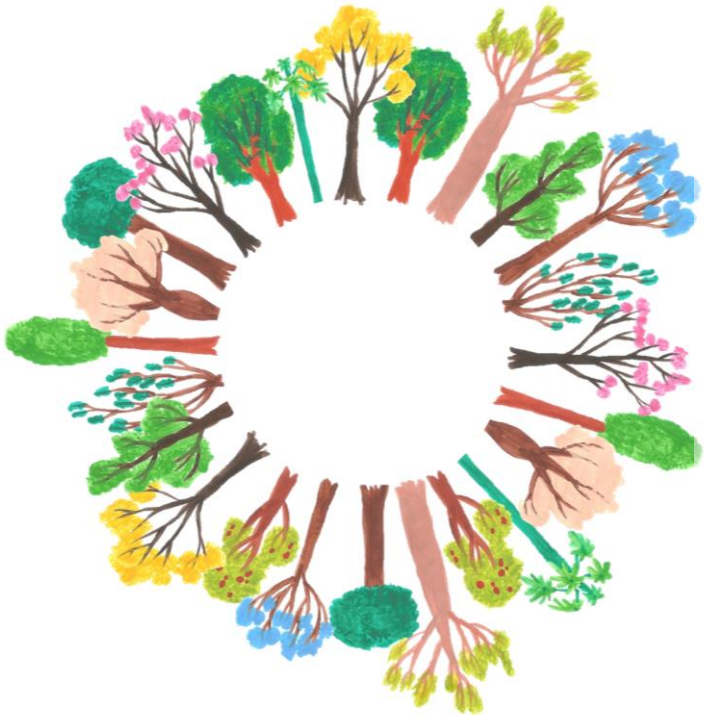
*Eu sou a fonte original de toda vida.*
*Sou o chão que se prende à tua casa.*
*Sou a telha da coberta de teu lar.*
*A mina constante de teu poço.*
*Sou a espiga generosa de teu gado*
*e certeza tranquila ao teu esforço.*
*Sou a razão de tua vida.*
*De mim vieste pela mão do Criador,*
*e a mim tu voltarás no fim da lida.*
*Só em mim acharás descanso e paz.*

*Eu sou a grande Mãe Universal.*
*Tua filha, tua noiva e desposada.*
*A mulher e o ventre que fecundas.*
*Sou a gleba, a gestação, eu sou o amor.*

*A ti, ó lavrador, tudo quanto é meu.*
*Teu arado, tua foice, teu machado.*
*O berço pequenino de teu filho.*
*O algodão de tua veste*
*e o pão de tua casa.*

*E um dia bem distante*
*a mim tu voltarás.*
*E no canteiro materno de meu seio*
*tranquilo dormirás.*

*Plantemos a roça.*
*Lavremos a gleba.*
*Cuidemos do ninho,*
*do gado e da tulha.*
*Fartura teremos*
*e donos de sítio*
*felizes seremos.*



# SUMMARY

## RESUMO

**Como o funcionamento de plantios de restauração florestal é influenciado pela riqueza arbórea?**

A restauração de florestas tropicais é uma importante ferramenta para a mitigação de mudanças climáticas e conservação de biodiversidade. Essas duas medidas podem ser aliadas, de acordo com a teoria de biodiversidade e funcionamento de ecossistemas (BEF, do inglês: *biodiversity and ecosystem functioning*), segundo a qual a diversidade pode favorecer funções do ecossistema, como a produtividade primária. Entretanto, a maior parte dos estudos de BEF até muito recentemente focaram em campos de gramíneas e não em ecossistemas tão complexos quanto florestas tropicais. É necessário entender tanto processos acima quanto abaixo do solo pelos quais a biodiversidade atua no funcionamento de ecossistemas. Este trabalho teve como objetivo verificar o efeito da riqueza de espécies arbóreas em processes ecológicos acima e abaixo do solo. Ele se baseou em duas áreas de estudo, em Sardinilla (Panamá) e em Anhembi (Brasil). A primeira foi especialmente projetada para estudos de BEF e permitiu destrinchar efeitos da biodiversidade em funções do ecossistema. A segunda possuía parcelas com mais de cem espécies, permitindo explorar os efeitos de altos níveis de riqueza. Tanto em Sardinilla quanto em Anhembi, investigamos se a riqueza de espécies arbóreas influenciou um processo ecológico acima do solo, a interceptação de luz, bem como mecanismos que podem estar associados a ele. A riqueza de espécies aumentou a interceptação de luz pelo dossel e estimulou mecanismos como a distribuição de luz ao longo do espaço (horizontal e vertical) e tempo. Ela promoveu tanto efeito de seleção quanto de complementaridade. Na área de Anhembi, investigamos se a riqueza de espécies influenciou processes abaixo do solo relacionados ao estoque de carbono nesse compartimento. A riqueza no dossel aumentou a produção e o estoque de raízes finas. Número de espécies do dossel teve efeito não linear sobre taxas de decomposição e estoque de serapilheira. A riqueza do conteúdo da serapilheira, contudo, não influenciou sua decomposição. O número de espécies do dossel também não influenciou a produção de serapilheira. As diferenças de produção e estoque de serapilheira e de produção de raízes finas, entre diferentes níveis de riquezas, não se alteraram ao longo do tempo. Contudo, o número de espécies arbóreas promoveu maior distribuição de raízes finas em diferentes camadas do solo. Concluímos que elevados níveis de riqueza não saturaram alguns processes ecológicos estudados. A diversidade foi capaz de atuar em processos tanto acima quanto abaixo do solo, por vários meios, muitas vezes em sentidos opostos, contando com *feedbacks* multidirecionais. É muito importante entender esses mecanismos para potencializar a conservação da biodiversidade e a provisão de funções ecossistêmicas, no processo de restauração de florestas tropicais, em um contexto internacional de necessidade de mitigação de mudanças climáticas. Estudos futuros devem focar em efeitos da diversidade em processos abaixo do solo (que são os menos abordados em estudos até o momento), em entender como altos níveis de diversidade podem afetar a regeneração natural em florestas e em explorar os atributos funcionais apresentados por cada espécie.


Palavras-chave: BEF; Biodiversidade e funcionamento de ecossistemas; Interceptação de luz; Mitigação de mudanças climáticas; Processos ecológicos; Raiz fina; Serapilheira

## ABSTRACT

### How is forest restoration plantations' functioning affected by tree diversity?

Tropical forests restoration is an important tool for climate change mitigation and biodiversity conservation. We can ally both of these elements, according to the biodiversity and ecosystem (BEF) functioning theory, which says that diversity enhances ecosystem functions, as primary productivity. Nevertheless, the greatest part of BEF studies up to very recently have focused on grasslands and not on as complex ecosystems as tropical forests. It is necessary to better understand above- and below-ground processes through which biodiversity acts on ecosystem functions. This work aimed to investigate effects of tree richness on both above- and below-ground ecological processes. It was based on two tropical forests undergoing restoration, in Sardinilla (Panama) and in Anhembi (Brazil). The former was especially designed for BEF studies and allowed to untangle effects of biodversity on ecosystem functions. The latter had more than a hundred species in plots and permitted investigation of the effects of high tree richness levels. In both Sardinilla and Anhembi, we investigated if tree richness levels affected an above-ground ecological process, light interception, and which mechanisms could be related to it. Richness could enhance light interception and mechanisms as spatial (horizontal and vertical) and temporal light distribution. It promoted both selection and complementarity effects. In Anhembi, we investigated if species richness influenced below-ground processes related to soil carbon stocks. Stand richness enhanced fine root production and stock. Effects of stand number of species on litter decomposition and stock were not linear. Richness of litter content, however, did not affect its decomposition rates. Number of stand species did not influence litter production. Differences of litter production, stock and fine root production among distinct richness levels did not change over the time. However, distribution of fine roots over the space, within different layers of soil, was affected by number of tree species. We concluded that even very high richness levels could not saturate some of the ecological processes studied. Diversity acted on both above- and below-ground processes, in various and sometimes opposite ways, counting on multi-direction feedbacks. It is very important to understand these mechanisms in order to potencialize biodiversity convervation and carbon sequestration by tropical forest restoration. Future studies may focus on untangling effects of diversity on below-ground processes (which have not been exhaustively explored in research), on understanding how high diversity levels affects natural regeneration and on investigationg functional traits provided by different species.


Keywords: BEF; Biodiversity and ecosystem functioning; Light interception; Climate change mitigation; Ecological processes; Fine root; Litter

# 1. INTRODUCTION

Diversity within plant communities has long been focus of attention in Ecology. By late 1950's, sparse observational studies led to the conclusion that higher diversity could increase ecosystem's stability, resistance to biological invasion and to disease. Nevertheless, around the 1960's, ecologists turned their attention to explaining which factors determined diversity levels of different ecosystems and coexistence of various species, rather than assessing which roles diversity could play (Tilman et al. 2014). By the 1980's, high rates of species extinction raised questions of what consequences they could have on ecosystems. Studies showed that diversity loss could affect ecosystems' structure and functions, as habitat provision ("ecosystem engineering"), nutrient cycling and primary productivity (Hooper et al. 2005, Cardinale et al. 2012). In the 1990's, research on the relationships between biodiversity and ecosystem functioning (BEF) gained ground and counted on incentives, as international programs, to boost it (Cardinale et al. 2012). By the beginning of the 2000's, numerous studies had already presented empirical evidence that diversity affected primary productivity (Tilman et al. 2014). Current BEF research focuses on not simply assessing whether biodiversity has any effect or not, but tries to untangle the mechanisms through which diversity acts on ecosystem functioning (Sapijanskas et al. 2014, Forrester & Bauhus 2016). BEF research has also shifted from studies restricted to simpler ecosystems, as grasslands, and expanded to include more complex ones, as forests (Trogisch et al. 2017). It also started to explore the mechanisms taking place not only above-ground, but also below-ground, since these compartments play mutual influences on each other and must be integrated for a more comprehensive view of an ecosystem (Wardle et al. 2004).

In the BEF concept, "biodiversity" is a very broad term, including from differences within species to differences among ecosystems (Hooper et al. 2005). It encompasses taxonomic, functional and genetic diversity, including phylogenetic distances between species (Cardinale et al. 2012). Numerous works have shown that number of species, taxonomic diversity, can affect ecosystem functioning (Potvin et al. 2011, Tilman et al. 2014). Functional diversity affects it as well (Ruiz-Jaen & Potvin 2011) and can even have stronger influence on ecosystems functions than number of species *per se* (Díaz & Cabido 2001). Simulations of different extinction scenarios showed considerable differences according to the functional traits that were maintained or lost with the extinctions of certain functional groups (Cardinale et al. 2012, Bello et al. 2015, Peres et al. 2016). Phylogenetic diversity may also be a relevant metric in predicting biomass accumulation by trees (Cadotte et al. 2008, Flynn et al. 2011), possibly as a consequence of the more intense competition between closely related species (Webb et al. 2002). In addition to those three metrics

presented above, studies can split diversity into its components: richness (related to number of species) and evenness (related to distribution of individuals among species). Even though species richness is the most often approached component (Forrester & Bauhus 2016), manipulating evenness can also lead to distinct ecosystem responses (Tilman et al. 2014).

The term "ecosystem functioning" includes a whole sort of mechanisms responsible for maintaining an ecosystem (Brockerhoff et al. 2017), which are related to energy, organic matter and nutrient fluxes, like primary productivity, nutrient cycling and decomposition (Cardinale et al. 2012). The interactions that support those fluxes are called ecosystem processes, such as photosynthesis and seed dispersal (Brockerhoff et al. 2017). Ecosystem processes' responses to diversity − the net biodiversity effect − can be grouped into two different types of effects: complementarity and selection. Complementarity effect happens when species take some advantage from the presence of others, being able to perform better in a diverse community than in a monoculture (Loreau & Hector 2001). It can happen through facilitation, when a species provides resources or conditions for others (*e.g.* nitrogen fixation), or through competitive reduction, when species partition resources, allowing interspecific competition to be not as strong as intraspecific competition (Forrester & Bauhus 2016). Selection effect takes place when diversity increases the chance of occurring one species responsible for increasing a certain ecosystem function (Loreau & Hector 2001). Some authors use the expression "sampling effect" to describe this (Forrester & Bauhus 2016). However, Loreau & Hector (2001) stated that the term "selection effect" would be more appropriate, since both complementarity and selection effects could count on sampling mechanisms. In the former, sampling a determined combination of species with complementary traits would enhance ecosystem functions. In the latter, sampling one single very productive species would lead to community overyielding (Fargione et al. 2007). Therefore, we will use hereafter only the term "selection effect" to describe the higher probability of occurrence of one species that performs better than the others, at higher diversity levels.

Complementarity and selection effects can also be negative. It may happen when a species hinders the existence of others, by inhibition (Loreau & Hector 2001) or competition (Forrester & Bauhus 2016), or when diversity increases the probability of occurrence of a weak performer species (Loreau & Hector 2001). However, there is theoretical background to support the hypothesis that coexistence of species would be beneficial for the ecosystem, through complementarity. According to the Lotka-Volterra model, intraspecific competition tends to be stronger than interspecific competition (Tilman et al. 2014). In addition to that, studies show that unrelated species, regardless if phylogenetic or ecologically, can perform better together, favoring diversity (Webb et al. 2002). There is also empirical evidence that, even though selection effect

can be positive, negative or neutral, complementarity effects tends to be positive (Loreau & Hector 2001).

The theoretical background provided by BEF knowledge has great potential to improve forest restoration projects (Naeem 2016). These projects are considered successful when they are able to reestablish ecological processes responsible for maintaining a forest ecosystem over the time. Therefore, high diversity could favor this restoration goal (Rodrigues et al. 2009), and this assumption was already transferred to legal norms regulating restoration activities (Aronson et al. 2011). In fact, besides ecological processes and ecosystem functions, restoring biodiversity itself is one of the goals of Restoration Ecology (Wright et al. 2009), although it is not clear if simply planting different tree species is enough for establishing diverse and self-sustaining forest communities in the long run. Use of high tree diversity in restoration, at the onset of a plantation, is still controversial and a focus of great discussion. On the one hand, authors defend that planting various tree species does not assure that a forest undergoing restoration will have a successful fate. They show examples of monoculture plantings that reached high diversity of natural regeneration during the process of ecological succession, overcoming mixed plantations and even secondary forests, thus evidencing that restoration success is more dependent on the landscape context than on the number of tree species employed at the moment of plantation (Durigan et al. 2010). On the other hand, researchers have assumed that restoration plantations counting on higher richness (above 50 species) were more successful in resulting in forests enduring natural disturbances and supporting ecosystem stability (Rodrigues et al. 2009, Brancalion et al. 2010). Even though studies show that natural regeneration poorly resembles the composition of canopy community in forests undergoing restoration, questioning the need of high diversity plantations (Suganuma et al. 2014), still each species can influence its environment (Naeem 2016), acting like an ecosystem engineer and potentially driving the success of restoration projects. Different species can create microhabitats and provide distinct filters for regeneration (Wright & Jones 2006). Therefore, theory supports that diversity could change the fate of forest succession (Naeem 2016) and consequently influence forest restoration results (Hulvey et al. 2013).

Nevertheless, even though it would be useful, the existing BEF knowledge cannot yet be directly transferred to tropical forest restoration. Most of BEF studies were carried out in grasslands and not in forests (Balvanera et al. 2006). More recently, BEF studies shifted to include as complex ecosystems as forests, but this requires equally complex experimental designs, in order to produce robust conclusions (Trogisch et al. 2017). Some forest biodiversity studies still make use of tree stands with different species numbers and compositions, established

independently from each other, without experimental control (Piotto et al. 2010, Kanowski & Catterall 2010), but today, BEF studies can already count on a network of experiments especially designed for this purpose, the TreeDivNet. These experiments, however, bear up to 24 tree species/plot (TreeDivNet 2017). There is supporting information to believe that adding higher diversity to forests undergoing restoration may influence their trajectory, since, when dealing with complex systems, high number of species is needed to saturate multiple ecosystem functions (Hector & Bagchi 2007). In Brazil, for instance, forest restoration projects are commonly carried out using over 80 species (Rodrigues et al. 2009), even though the need for this richness level is still debated (Aronson 2010). Therefore, BEF theory could also benefit from Restoration Ecology experiments, which count on high richness levels, are frequently monitored and can provide information on how diversity affects ecosystem functions in practical situations and at different spatial scales (Wright et al. 2009).

It is clear that there is still a lot to study in order to improve existing BEF knowledge and to apply it to forest ecosystems undergoing restoration. Having that in mind, in this work, we use two distinct experiments to explore this issue. One of them (in Sardinilla, Panama) is part of TreeDivNet and was specifically designed for understanding relationships between biodiversity and ecosystem functions. It permits to untangle mechanisms through which species identities and richness influence ecosystem functions. As it possesses species in both monocultures and distinct mixtures, it is possible to explore selection and complementarity effects, as well as results of different compositions. The other experiment (in Anhembi, Brazil) was designed for understanding the effects of high richness levels on forest carbon accumulation. It does not present species in monocultures, but in mixtures from 20 to 114 species. Therefore, it does not allow understanding effects of different compositions and species identities. Nevertheless, it permits to investigate how ecosystem functions and ecological processes change at very high richness levels. Working at these study sites, our main goals are:

1. To determine whether diversity may affect light interception, an above-ground ecological process, and which are the mechanisms related to it, in forest restoration plantations, in both Sardinilla and Anhembi (Chapter 2). We hypothesize that higher number of species will lead to higher light interception. One of the mechanisms related to that may be greater vertical stratification, as crowns of trees can present different forms of growth and occupy spaces of canopy. Another mechanism would more homogeneous horizontal light distribution in forests provided by higher diversity levels. Both selection and complementarity affects may also act on light interception, as well as seasonal variations.

2. To determine whether tree diversity may act on below-ground processes related to carbon pools within the soil: litter dynamics and fine root stocks and production, in Anhembi (Chapter 3). We hypothesize that species-richer forests may count on more intense litter production, decomposition and fine root production.

3. To present final considerations (Chapter 4), in which we discuss practical outcomes of our study and suggest further research in this theme.

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# 2. HIGH TREE RICHNESS LEVELS ENHANCE LIGHT INTERCEPTION AND DISTRIBUTION IN TROPICAL FORESTS


## Abstract

Experiments testing the biodiversity and ecosystem functioning (BEF) theory have shown that light interception by forest canopies is maximized with tree richness and that crown plasticity is a key trait driving this outcome. However, little is known about the impacts of very high tree diversity in canopy functioning and the role of vertical stratification as an ecological mechanism regulating light interception by forests. In this work, we investigated effects of diversity on light interception, an ecological process directly related to primary production and to natural regeneration, in two tropical forest plantations: the Sardinilla site (Panama), especially designed for BEF studies (containing from monocultures to 18-species mixtures), and the Anhembi site, which bore more than a hundred species in plots (which consisted of replicates of 20, 58 and 114-species combinations). We assessed whether higher tree richness levels were able to enhance light interception and investigated mechanisms related to this process. Light interception increased with richness, even from plots containing 58 to ones containing 114 species. At the Sardinilla site, fraction of intercepted photosynthetically active radiation (PAR) varied from 0.82 ± 0.06 (means and standard error) in monocultures to 0.96 ± 0.004 in 18-species mixtures. At the Anhembi site, from the least to the most diverse treatments (20 to 114 species), mean (and standard error) intercepted PAR varied from 0.71 ± 0.02 to 0.87 ± 0.01, during the dry season, and from 0.88 ± 0.01 to 0.94 ± 0.004, during the rainy season. We observed that tree richness also promoted more homogeneous horizontal distribution of light, at both study sites. Vertical light distribution was also enhanced by richness at the Anhembi site whereas, at the Sardinilla site, only richness levels up to five species were able to improve vertical distribution of light. Experiments at the Anhembi site showed that tree richness also promoted more homogeneous distribution of light interception over the time. At the Sardinilla site, we could detect both selection and complementarity effects, mechanisms that might be related to light interception. At both sites, we found unprecedented evidence of multi-layering effect caused by diversity. We concluded that high tree diversity may be needed to maximize canopy functioning in tropical forests, as a consequence of diverse mechanisms enhancing light interception over space and time.


Keywords: Biodiversity and ecosystem functioning; BEF; Complementarity effect; Ecological processes; Ecosystem services; Light partitioning; Restoration ecology; Selection effect

## 2.1. Introduction

During the last decades, there has been increasing interest in understanding how diversity can influence the way an ecosystem behaves (Balvanera *et al.* 2006; Tilman *et al.* 2014). Previous studies, instead, focused on explaining causes of biodiversity rather than its consequences (Tilman *et al.* 2014). After the 1980s, extinction has become a major issue of concern. Many ecology studies turned their attention into which roles species played in an

ecosystem and what would be the consequences of their loss (Cardinale *et al.* 2012). According to the biodiversity and ecosystem functioning (BEF) theory, when diversity increases in an ecosystem, the pathways for its processes are diversified and make the system more efficient to use resources across time and space, enhancing its functioning (Loreau et al., 2001). In recent years, this theory has gained ground to support the idea that distinct species, functional traits and genes are fundamental for the maintenance of an ecosystem (Cardinale *et al.* 2012).

Although most of BEF studies have been carried out on grasslands (Balvanera *et al.* 2006), research on how diversity affects forest ecosystems' functions has received growing attention (Brockerhoff *et al.* 2017). One of the major research challenges now is to expand these BEF studies in forest ecosystems, especially in tropical regions, in order to better comprehend the role of plant diversity to support functioning of ecosystems with a much more complex three-dimensional structure than grasslands and temperate forests (Trogisch *et al.* 2017). This research challenge has many practical implications, since tropical forests perform very important ecosystem services, for instance carbon storage, food provision, conservation of biodiversity and ecological interactions etc. (Costanza *et al.* 1997). They are by far the terrestrial ecosystems with the highest potential to store carbon as biomass (Beer *et al.* 2010). Maintenance of ecological processes and ecosystem functions is crucial for the stability of existing forests (Brockerhoff *et al.* 2017) and for the restoration of new ones (Rodrigues *et al.* 2009). In fact, forest restoration is included among policies for ecosystem services provisioning, especially those aimed at climate change mitigation strategies (Griscom *et al.* 2017).

Ecosystem functions (*e.g.* primary production) are a result of interactions between structures and processes within an ecosystem and are responsible for its long-term maintenance (Brockerhoff *et al.* 2017). BEF theory assumes that variables describing ecosystem functions present higher values, on average, as diversity increases. However, the growth rates of these variables decrease as diversity increases, yielding a curve that saturates upon reaching a particular number of species (Cardinale *et al.* 2011). In a meta-analysis considering various types of ecosystems, from aquatic to terrestrial, Cardinale *et al.* (2011) predict that maximum biomass would be on average 2.38 times higher than the average biomass of monocultures and that half of this yield would be reached with 1.35 species. The exact level of diversity required to saturate an ecosystem function is, however, still an unresolved issue (Cardinale *et al.* 2012). One of the great difficulties in determining this saturation level is due to spatial scale. Experimental plots are relatively small and cannot bear very large numbers of species, thus curves representing mathematical predictions may be misleading (Srivastava & Vellend 2005; Cardinale *et al.* 2011). TreeDivNet, a network that includes tree diversity experiments all over the world, for instance,

has a maximum of 24 tree species per plot (TreeDivNet 2017). Another difficulty in determining how many species saturate an ecosystem function is the influence of species identity: different species compositions may have distinct effects for the same richness levels (Srivastava & Vellend 2005). The subject gets even more complex when we see an ecosystem from a multifunctionality approach. A greater diversity is needed to maintain multiple ecosystem functions (Meyer *et al.* 2018) and services (Isbell *et al.* 2011), since there may be low overlap between species that influence each function (Hector & Bagchi 2007).

Despite these uncertainties regarding levels of diversity required to saturate ecosystem functioning, many BEF studies have already shown that the variety of species (Tilman et al. 2014), genes (Cadotte *et al.* 2008; Flynn *et al.* 2011) and functional traits (Ruiz-Jaen & Potvin 2011) do affect ecosystem functions. Nevertheless, only recently studies have begun to untangle mechanisms through which diversity acts on these functions (Pretzsch 2014; Forrester & Bauhus 2016). Therefore, studies with empirical evidence of these mechanisms are still scarce (Sapijanskas *et al.* 2014; Jucker *et al.* 2015; Williams *et al.* 2017), especially for the species-rich, highly productive, and structurally complex tropical forests (Trogisch *et al.* 2017).

Ecological processes are interactions between biotic and abiotic factors, responsible for supporting fluxes of energy, matter and information, which work to support ecosystem functions (Brockerhoff *et al.* 2017). One ecosystem function that has been focus of great attention, especially due to recent policies for carbon uptake and climate change mitigations, is primary production (Potvin *et al.* 2011; Ferez *et al.* 2015). It results from how much photosynthetic active radiation (PAR) reaches a plant, the amount of this incident PAR that is effectively absorbed by leaves and the plant's light-use efficiency (Forrester & Bauhus 2016). Hereafter, we will also use the term "light" to refer to PAR, as it is a fraction of light (Nouvellon *et al.* 2000). Therefore, enhancing light absorption (an ecological process) is one way to increase primary production (an ecosystem function) (Nouvellon *et al.* 2000). Also of great importance, the process of light interception through the canopy regulates the amount of light that reaches the understory, influencing the diversity of regenerating saplings (Poorter & Arets 2003; Tilman *et al.* 2014; Suganuma & Durigan 2015).

Variations in vertical arrangement of trees may lead to differences in the spatial distribution of light over the canopy (Montgomery & Chazdon 2001). Increasing diversity of a forest ecosystem would add a greater variety of structures to it, allowing crowns to intersperse and better occupy the canopy (Pretzsch 2014; Sapijanskas *et al.* 2014; Jucker *et al.* 2015; Williams *et al.* 2017). According to Sapijanskas et al. (2014), diversity promotes variations in crown shape, temporal niche differences and phenotypic plasticity, which enhances light absorption over time

and space and contributes to overyielding. Therefore, studies have already shown that diversity contributes to increasing complexity of canopies and light interception (Sapijanskas *et al.* 2014; Jucker *et al.* 2015; Williams *et al.* 2017). Nevertheless, they do not explore how richness levels above a dozen species change the ability of canopies to intercept light. In addition to that, empirical studies have not yet investigated whether multi-layering is really a cause of higher light interception (Sapijanskas *et al.* 2014).

The role species play on canopy packing can also be split into two distinct types of effects. On the one hand, selection effect takes place when a single species is responsible for playing a major role in certain ecosystem function in a plant community. On the other hand, complementarity effect takes place when interactions between species are responsible for higher ecosystem functioning (Loreau & Hector 2001). Distinct species can partition niches and count on reduced competition, being able to expand their crowns more than they would if they were in monocultures (Forrester & Bauhus 2016). Both selection and complementarity effects can act together in the same community, not being exclusive (Hooper *et al.* 2005a).

Here, we investigated the effects of tree diversity on PAR interception in tropical forests and explored the mechanisms explaining canopy functioning in distinct levels of diversity, including: 1) horizontal distribution of intercepted light over the forest; 2) vertical distribution of intercepted light over the canopy; 3) seasonal variation in light interception; 4) effects of selection and complementarity, as well as differences in species composition, which could influence light interception. Our motivation was to understand how tree diversity may potentially affect an important ecosystem process, which strongly influences understory regeneration (Poorter & Arets 2003; Tilman *et al.* 2014; Suganuma & Durigan 2015) and primary productivity (Sapijanskas *et al.* 2014), contributing not only to a forest's maintenance in the long run (Rodrigues *et al.* 2009) but also to the services it provides (Sapijanskas *et al.* 2014; Brockerhoff *et al.* 2017).

## 2.2. Material and methods

### 2.2.1. Study sites

We included in this study two experiments established through different methodological approaches to test the influence of tree diversity on light interception by tropical forests. The Sardinilla site, in Panama, is an experiment especially designed for BEF studies. It contains species both in monocultures and in different combinations of up to 18 species (TreeDivNet 2017), which allows for the separation of the selection and complementary effects of diversity on

ecosystem processes and functions (Loreau & Hector 2001). Its maximum richness level of plots was based on the diversity of natural, old-growth forests nearby the experimental areas (TreeDivNet 2017). The Anhembi site, in Brazil, was designed to assess effects of very high tree diversity levels (up to 114 species) on carbon storage. It does not present species in monoculture and thus does not permit splitting selection and complementarity affects, but its design allows for testing the effects of an unprecedented tree diversity level on ecosystem processes and functions.

The Sardinilla experiment is a long-term study site established to investigate relationships between biodiversity and ecosystem functioning (BEF) in tropical forests. It is one of the oldest diversity experiments in tropical forests (TreeDivNet 2017), located in an area maintained by the Smithsonian Tropical Research Institute (STRI). It was set up with two plantations at different times. The hereafter called "main plantation" was established in 2001, with 5,000 seedlings including *Luehea seemannii* Triana & Planch (Malvaceae), *Cordia alliodora* (Ruiz & Pav.) Oken (Boraginaceae), *Anacardium excelsum* (Bertero ex Kunth) Skeels (Anacardiaceae), *Hura crepitans* L. (Euphorbiaceae), *Cedrela odorata* L. (Meliaceae) and *Tabebuia rosea* (Bertol.) Bertero ex A.DC. (Bignoniaceae) planted in 24 45 x 45 m plots, containing one (12 plots: two replicates of each of the six monocultures), three (six plots: six different combinations of a fast-growing, a slow-growing and an intermediate species) and six species (six plots: six replicates of the mixture). A Latin-square design for seedlings distribution was used, within plots, and spacing between trees was 3 x 3 m. In this design, in the same plot, the four nearest neighbors of a tree always belonged to the same two species (Potvin & Dutilleul 2009). Almost all of the individuals of *C. alliodora*, however, died after plantation (Kunert *et al.* 2012). As a consequence, this species is not considered in our studies. Our effective plots are: two replicates of five distinct monocultures, three combinations of two species, three combinations of three species, and six replicates of a five-species combination (Figure 1).

Later in 2003, the hereafter called "high diversity plantation" was established, with 800 additional trees planted in 24 plots measuring 18 x 18 m. They were organized in eight blocks, each of them containing six, nine and 18 species plots (there were four different combinations, hereafter called groups, for each level of richness – two replicates of each – varying within a 28-species pool, see APPENDIX A. In the same block, the six species group was included in the nine species groups, which was included in the 18 species group) (Ruiz-Jaen & Potvin 2011).

Both experiments are located in the same area, in Sardinilla, Colon, Panama (9°19'N, 79°38'W), 70 m a.s.l.. The climate is classified as Am (equatorial monsoon) according to Köppen-Geiger (Kottek *et al.* 2006), presenting 2,289 mm of annual mean precipitation. The driest season usually ranges from January to April, however its duration and intensity vary as a consequence of

El Niño. The mean annual temperature is 25.2°C (Wolf *et al.* 2011b). The soils are Ultisols with patches of Vertisols, containing high percentage (above 50%) of clay (Wolf *et al.* 2011a). The area was originally covered by semi deciduous lowland forest. The native forest was converted to croplands around 1952 and, two years after cultivation, croplands were converted to extensive pastures, which was the main land use in the area before the establishment of tree plantations (Scherer-Lorenzen *et al.* 2007). According to the inventory carried out on an annual basis at the Sardinilla site, in 2016, each of the treatments had reached the following mean basal area values (and standard errors): 1) monocultures: 14.69 ±2.03 m²/ha; 2) two species: 14.29 ±1.57 m²/ha; 3) three species: 21.84 ±2.41 m²/ha; 4) five species: 19.35 ±1.17 m²/ha (at the main plantation); 5) six species: 26.79 ±2.39 m²/ha; 6) nine species: 23.50 ±1.55 m²/ha; 7) 18 species: 25.69 ±1.20 m²/ha (at the high diversity plantation).

The Anhembi project focused on comparing different forms of establishing and managing plantations of native species to find out the best strategies to improve carbon uptake by forests undergoing restoration. In this particular experiment, the goal was to assess the effect of increasing tree richness levels, reaching more than a hundred native species, on ecological processes and ecosystem functions associated to carbon sequestration. The experiment is located at the Experimental Station of Forest Sciences of the University of São Paulo, in Anhembi, São Paulo, Brazil (22°42' S, 48°10' W), 455 m a.s.l.. The plantation was established in May, 2006, in an area previously covered by pastures of exotic grasses, with no regeneration of native tree species. The area counted on ant and weed control, by using formicide and glyphosate. Land was subsoiled (at a 60 cm depth) and then limed. Native tree seedlings were planted in 45 x 48 m plots (3 x 1,5 m spacing - 480 individuals per plot), using different levels of richness: 20, 58 and 114 species (APPENDIX B), in a completely randomized design with four replicates (A-D) of each (Figure 1). The 20 species were included in the 58 species, which were included within the 114 species pool as well. The spatial distribution of species was randomly determined for the first replicate and then repeated in the other three replicates of the same treatment. After plantation, formicide and glyphosate were periodically applied to the area in the first three years and, in the following years, whenever pest control was necessary. Soil fertilization was promoted periodically for three years as well. In 2006, according to an annual inventory carried out in this plantation, basal area (and standard error) achieved was 29.16 ±3.97 m²/ha in 20 species plots, 31.19 ±1.43 in 58 species plots m²/ha and 29.73 ±1.89 m²/ha in 114 species plots.

The climate in Anhembi region is classified as Cfa (Köeppen). The mean annual precipitation, measured in several years is 1,288 mm and the driest period ranges from April to September, when the mean monthly rainfall is 47 mm. The mean annual temperature is 20.6°C,

ranging from averages of 16.8°C in July and 23.5°C in February (Alvares *et al.* 2013). The weather is warm and humid during the summer and dry and colder during the winter. Figure 2 displays monthly rainfall, minimum and maximum temperatures, during the year which encompassed our experiments, measured at the Anhembi site's meteorological station. The soil is deep and acid, with low organic matter and high proportion of sand (*c.* 80%) (Ferez *et al.* 2015). This region was once covered by the Seasonal Semi-deciduous Forest (Morellato & Haddad 2000), where 30-50% of species are deciduous or semi-deciduous (Gandolfi *et al.* 2009). It is one of the main forest physiognomies of the Atlantic Forest global biodiversity hotspot (Morellato & Haddad 2000).

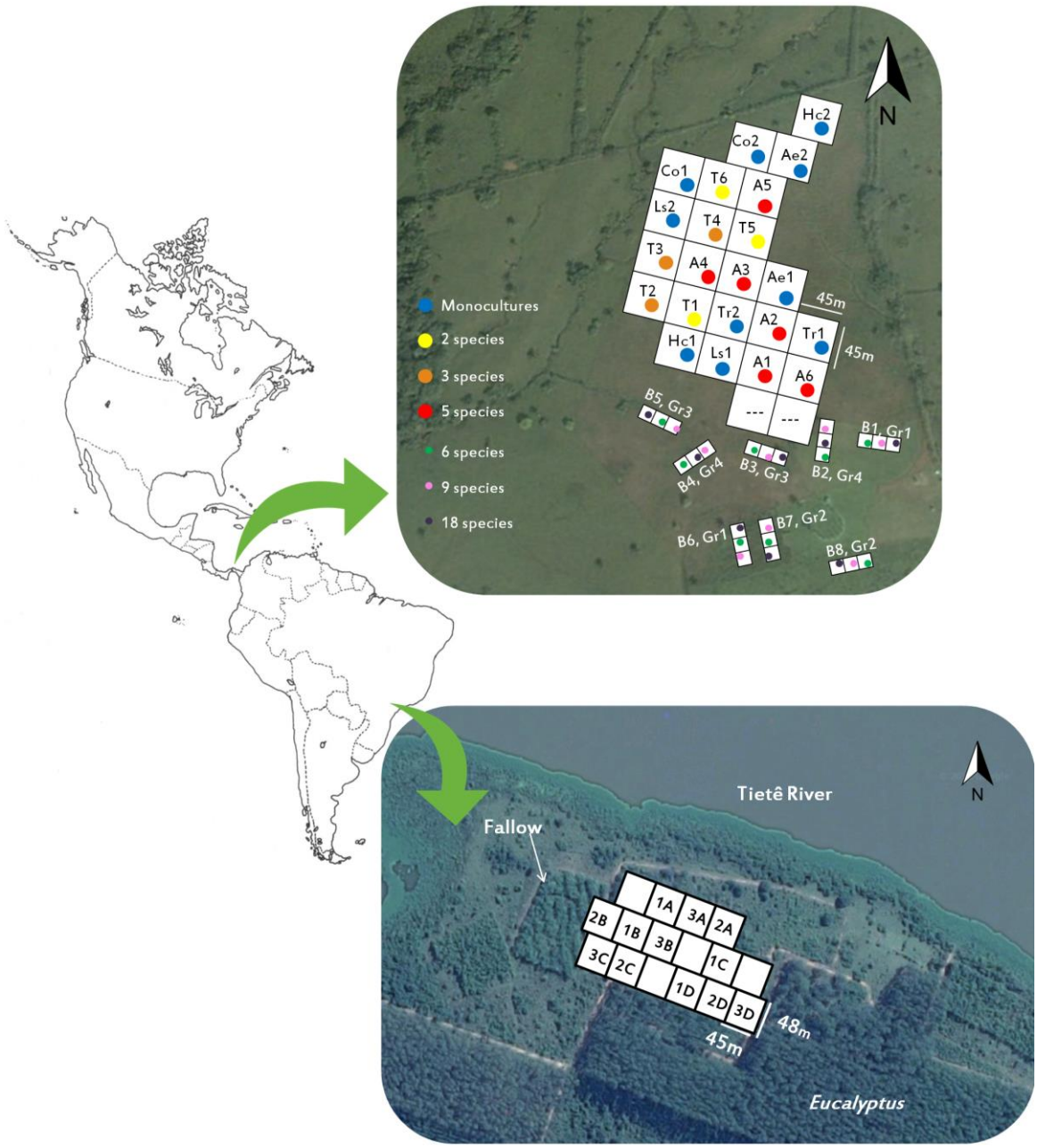


**Figure 1.** Study sites. Above, Sardinilla study site, located in Sardinilla, Colon, Panama (9°19'N, 79°38'W). Image from Google Earth Pro v. 7.3.0.3832 (from January, 2002, when the main experiment had been recently planted). 45 x 45 m squares correspond

to main plantation plots. Monocultures: Ae = *A. excelsum*, Co = *C. odorata*, Hc = *H. crepitans*, Ls = *L. seemannii*, Tr = *T. rosea*. Two-species combinations: T1 (Co + Hc), T5 (Hc + Tr), T6 (Ae + Tr). Three-species combinations: T2 (Ae + Ls + Tr), T3 (Ae + Co + Ls), T4 (Co + Hc + Ls). Five-species combination replicates: A1-A6. "High diversity" experiment is represented by blocks containing three adjacent plots each. B_ = number of block; G_ = Number of group. Each distinct group contains different combinations of species. Colored spots indicate the number of species contained in each plot. Below, Anhembi study site, located in Anhembi, Sao Paulo, Brazil. Image from Google Earth Pro v. 7.3.0.3832 (from November, 2017). Numbers correspond to level of richness: 1) 20 species, 2) 58 species, 3) 114 species. Letters A-D correspond to their replicates.

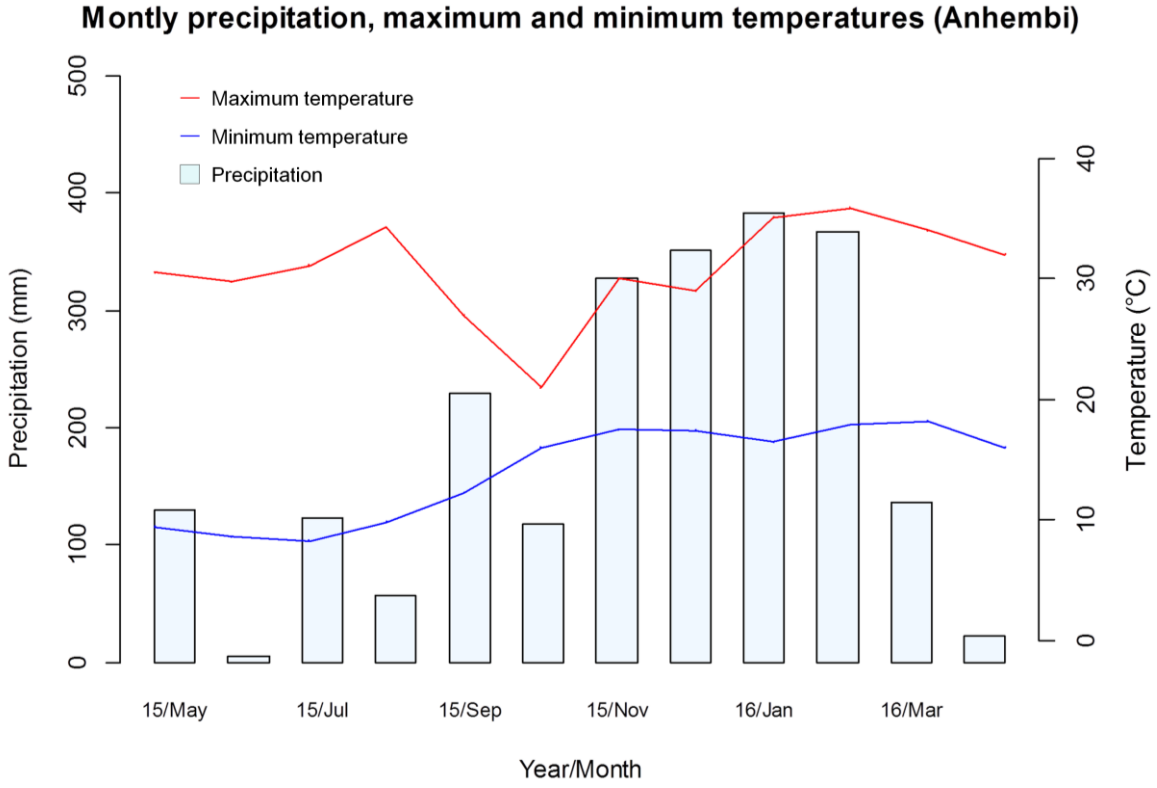


**Figure 2.** Monthly precipitation, maximum and minimum temperatures, at the Anhembi site, during the year that encompassed our experiments. Data were taken from the site's meteorological station in Anhembi, São Paulo, Brazil.

### 2.2.2. Horizontal light distribution

For our data collection, we chose to use intercepted photosynthetic active radiation (iPAR) as a proxy of absorbed PAR (aPAR). While aPAR is the fraction of the PAR that actually enters a leaf and can potentially be used for photosynthesis, iPAR is simply the difference between total PAR that reaches a canopy and the amount of this radiation that passes through it (Nouvellon *et al.* 2000). It considers neither the amount of light that reached the canopy and was not absorbed – but reflected by leaves and non-green parts of the canopy (Weiss *et al.* 2004) – nor the light reflected by the soil – which could be absorbed by the abaxial surface of leaves. Due to practical difficulties in directly measuring aPAR at the tree plantation level, it is commonly predicted from iPAR. Nevertheless, the relationship between them is variable, especially between early and late stages of vegetation (Nouvellon *et al.* 2000). Since we compared in each site forests

of the same age and with closed canopies, iPAR should maintain a reasonably high correlation with aPAR and be a reliable surrogate to compare the potential effects of different tree diversity levels on aPAR.

At the Anhembi site, we divided each plot into 98 subplots established by a 3 x 6 m grid (APPENDIX C). We measured, in the center of each subplot, the incident PAR at the height of 1 meter using a leveled Decagon AccuPAR LP-80 ceptometer. We used another identical ceptometer, calibrated with the first one, leveled and headed to the same direction, to record total incident PAR at every minute, outside the forest. The ratio between the measurements of the ceptometers inside and outside the forest, at the same time, corresponded to the fraction of total incident PAR that reached the sample point. By subtracting this fraction from 1, we obtained the fraction of PAR intercepted by vegetation (hereafter iPAR). We measured iPAR in each subplot at the peak of the dry season (August 2015), when iPAR was expected to be at its lowest level due to canopy deciduousness, and repeated the measures at the end of the wet season (March 2016), when iPAR was expected to be at its highest level. Measurements were taken from 10 A.M to 2:20 PM, under stable weather conditions. We made sure that there was no cloud moving in front of the sun during our measurements, which could preclude ceptometers inside and outside the forest to be under the same irradiance conditions.

We also repeated this procedure at the Sardinilla site, in July and August 2016 (wet season). We established 49 subplots by 6 x 6 grids in all plots of the main plantation and 17 subplots in all plots of the high diversity experiment (APPENDIX D). The predominance of unstable cloudy days during this period of the year in Sardinilla precluded us from using a ceptometer. Therefore, we used a leveled Skye SKR 110 sensor to measure the ratio of red:far-red light spectra (hereafter R:FR) (Figure 3). Measurements were taken at 1.7 meter high and from 9:50 AM to 2:15 PM, always under diffuse light conditions. Under cloudy conditions and diffuse light, R:FR ratio can be a fair proxy of iPAR. However, their relationship changes according to vegetation structure, thus being site-specific (Capers & Chazdon 2004).

For data taken both by the R:FR sensor and by the ceptometer to be comparable, we fit a regression equation to convert R:FR ratio into iPAR, specific for the study site, as recommended by Capers & Chazdon (2004). We established 25 points under different canopy cover conditions at the Sardinilla experiment (from an open to a densely closed canopy) and measured R:FR ratio at 1 meter high at each point, on a cloudy day, from 10 AM to 1:20 PM. At the exact same times, we measured iPAR at the same points, both on a cloudy and on a sunny day. A quasi-binomial model was fitted to iPAR data including different intercepts and slopes per each sky condition over the R:FR values. The significance of the effects was assessed using F-

tests for nested models. As the behavior of the curve did not significantly change for different sky conditions ($P>0.05$), we used all data to fit the following equation:

$$\ln [iPAR/(1-iPAR)] = 6.76 - 6.85 * R:FR,$$

where iPAR corresponds to the percentage of intercepted PAR measured with a ceptometer under either cloudy or sunny (but always stable) weather conditions and R:FR is the ratio between red and far-red light spectra, measured under diffuse light using an R:FR sensor.

### 2.2.3. Vertical light distribution

We established 12 systematically-distributed vertical transects, equidistant to the four nearest neighbor trees, in each plot of the Anhembi site in the dry season (August 2015; APPENDIX E). With the use of a ladder, we measured incident PAR every 1 meter along the vertical transects, from 0 m to 4 m (Figure 4). We used a ceptometer outside the forest, logging PAR measurements every minute, to determine iPAR for each sample point, as already described in section 2.2.2. The experiment was repeated during the wet season (February 2016). Measurements were always taken from 10 AM to 2 PM, under stable weather conditions.

The evaluation of iPAR over the vertical transects had, however, the limitation of assessing vertical iPAR distribution only up to 4 meters high, in tree plantations taller than 15 m. We thus complemented this evaluation by using a ground LiDAR to analyze vertical structure of vegetation in Anhembi, from the ground up to the peak of the tallest tree of the plot (Stark *et al.* 2012), as an alternative methodological approach to assess light vertical distribution in the forest. This equipment emits vertical laser pulses and records at what height they were intercepted by vegetation or if pulses did not hit any surface at all – "sky hits" (Parker *et al.* 2004). As the instrument cannot assess vegetation that lies beyond the point where pulse was intercepted, corrections are necessary for determining vertical structure of canopy (for instance, considering that pulses are extinguished at an exponential rate) (Stark *et al.* 2012). Subsequently, under certain assumptions, canopy structure can work as a surrogate of absorbed PAR. There are nevertheless some issues in determining aPAR from canopy structure measures. This vertical profile of vegetation does not distinguish leaves, which in effect absorb PAR, from non-green material (Weiss *et al.* 2004). In addition to that, as LiDAR pulses hit surfaces (Parker *et al.* 2004), they work as if any structure was opaque, not considering light transmittance through leaves (Nouvellon *et al.* 2000). We therefore recognize that LiDAR data provide a rougher surrogate of aPAR compared to iPAR measurements, but an excellent overview of the potential interception of PAR over the entire vertical profile of the forest.

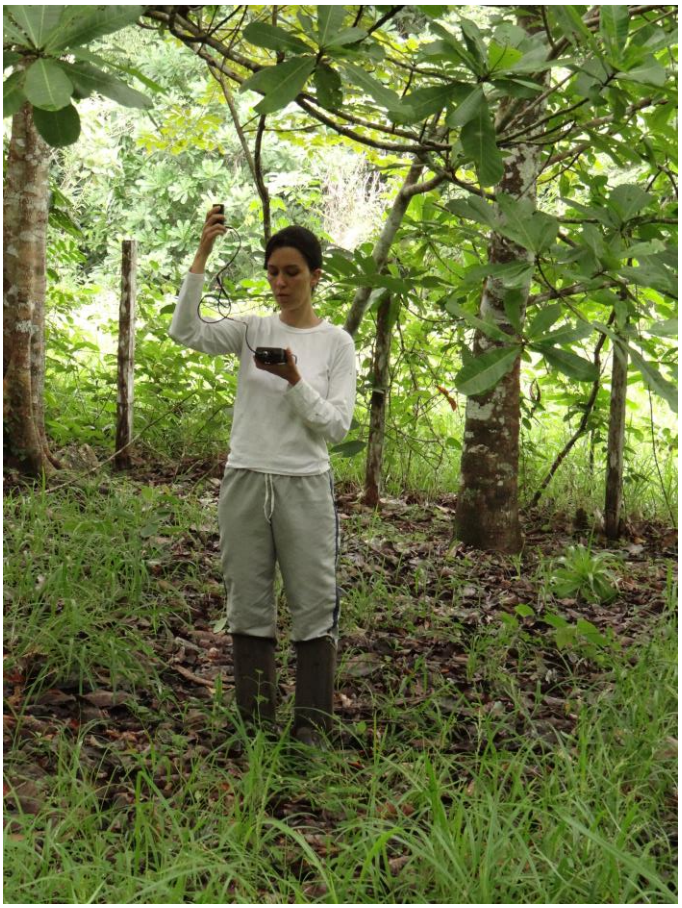

**Figure 3.** Measurement of R:FR on a horizontal 6 x 6 m grid, in Sardinilla, Colon, Panama, July and August 2016.

**Figure 4.** Measurements of PAR at heights 0, 1, 2, 3 and 4 m, respectively, using a ceptometer. Anhembi, Sao Paulo, Brazil.

We obtained the leaf area density (LAD) for each plot using a portable canopy profiling LiDAR (PCL) system, model Riegl LD90-3100VHS-FLP (Horn, Austria). It consists in a profiling range-finder type laser. The instrument was held one meter above ground, maintaining vertical aim by an operator who walked along a horizontal linear transect, at a constant pace (0.5 $m \cdot s^{-1}$), using an electronic metronome. In each plot, we established three parallel linear transects

of 43.5 m each, 12 m apart from each other (APPENDIX E). We carried this procedure out during both dry (August 2015) and rainy (February 2016) seasons. The LAD was determined from 2-D return clouds (produced by PCL) using the MacArthur-Horn equation (MacArthur & Horn 1969), following the method proposed by Almeida *et al.* (2016). In summary, this method accounts for the percentage of pulses that cross the canopy (compared to the amount of pulses that hit vegetation and are reflected), which is used to determine the density of vegetation in different layers of the canopy from optical transmission rates (MacArthur & Horn 1969; Stark *et al.* 2012). To apply the MacArthur-Horn equation, each 43.5-meter PCL transect (LiDAR return cloud) was previously divided into twenty 2.175-meter columns and subdivided every one meter along the vertical profile, from 2-meter high on. We obtained each transect's leaf area index (LAI) from the sum of all horizontal layer's LAD above 4 meters high (Almeida *et al.* 2016), height which could not be reached with the ceptometer.

At the Sardinilla site, we took R:FR measurements every meter, from the heights of 1 to 5 m, using a similar method as described above for Anhembi. Six vertical transects were regularly allocated in each plot of the main plantation and four vertical transects in each plot of the high diversity plantation (APPENDIX F), in which R:FR measurements were taken in the rainy season (July and August 2016), from 9:30 AM to 14:40 PM, under diffuse sunlight.

### 2.2.4. Crown volume (Sardinilla)

At the Sardinilla site, in July 2016, we measured crown volume of each of the main experiment's species, both in monocultures and in the five-species mixtures, in order to account for selection and complementarity effects. Within each monoculture plot, we chose the five individuals that were the closest to the center of the plot, assuming border effects would be weaker there. Within each of the five-species mixture plot, we sampled two individuals of each species, also the ones closest to the center. Using a Haglöf distance measurer (DME), we measured the projection of the larger diameter of the crown, as well as its perpendicular diameter and the two diagonals (Figure 5). We calculated the crown radius from the mean of those four diameters divided by two. We used a Haglöf vertex to determine the crown and tree heights, and to calculate crown depth from their difference. We calculated crown volume as a cylinder (Sapijanskas *et al.* 2014) (crown volume = $(\text{crown radius})^2 * \pi *$ crown depth).

**Figure 5.** Measurement of crown radius, using a DME, in Sardinilla, Colon, Panama, in July 2016.

### 2.2.5. Data analysis

#### 2.2.5.1. Light interception

Firstly, we analyzed whether levels of richness enhanced light interception, for both the Sardinilla and Anhembi experiments. For the analyses, we did not convert R:FR data into iPAR, since it could embed unwanted error to the results. Those two variables present a negative relationship, according to the equation shown in section 2.2.2.

At the Sardinilla site, to analyze if mean R:FR within plots (data from horizontal grids, section 2.2.2) varied between different levels of richness (one, two, three and five species for the main experiment and six, nine or 18 species for the high diversity experiment), we fitted multivariate covariance generalized linear models (Bonat & Jørgensen 2016), separately for the main and high diversity experiments. These models are useful in this case, because they allow for the simultaneous modeling of the mean and dispersion, with covariates. It is possible to separate the variation induced by the mean-variance relationship from the extra-variability that may be accounted for with regressors. Because the data from the Sardinilla site are continuous and not bounded, a normal model with identity link function was a reasonable assumption, coupled with a constant mean-variance assumption. We included the effects of richness, percentage of survival and coordinates (x and y) of subplots in the linear predictors for both the mean and dispersion

parameters. It was important to include the percentage of survival within each plot as a covariate, since it was known that there was high mortality in some plots and the density of individuals is a factor that can influence light interception. Wald tests were used to assess significance of effects. Here, the Wald test statistic follows asymptotically a chi-squared distribution. We obtained, for the final selected model, estimated means and 95% confidence intervals.

At the Anhembi site, we also fit multivariate covariance generalized linear models (Bonat & Jørgensen 2016) to analyze if iPAR means (data from horizontal grid, section 2.2.2), varied with richness (20, 58 and 114 species). However, since iPAR is bounded between 0 and 1, we used a logit link for the mean linear predictor, and assumed a mean-variance relationship analogous to the one for the binomial model, since we are modeling continuous proportions. Hence, by making these first and second moment assumptions, the non-gaussianity of the data is accounted for by the mean-variance relationship, whereas the extra-variability is then modeled via the extra dispersion parameter, which depends on the covariate effects we want to study. The factors replicate, coordinates within the plot (x and y) and percentage of survival were included in the linear predictors for the mean and dispersion parameters. Wald tests were used to assess significance of effects. For the final selected model, we obtained the estimated means and 95% confidence intervals.

#### 2.2.5.2. Horizontal light distribution

At the Sardinilla site, we used the same multivariate covariance generalized linear models (Bonat & Jørgensen 2016) described in section 2.2.5.1 to analyze not only means, but also dispersion of iPAR, which was a proxy of heterogeneity in light distribution (the higher is dispersion parameter, the higher is the heterogeneity in iPAR).

Similarly for the Anhembi site, we used the model described in section 2.2.5.1 to account for dispersion effects of iPAR (Bonat & Jørgensen 2016). We also constructed interpolation maps to graphically analyze grids of iPAR distribution over the Anhembi plots, shown in APPENDIX I, using the software GS+. For that purpose, kriging was performed using exponential isotropic variogram models (active lag distance = 26.54 m, lag class distance = 3 m).

#### 2.2.5.3. Vertical light distribution

For the Sardinilla site, to analyze vertical transects data (from section 2.2.3), we also used multivariate covariance generalized linear models (Bonat & Jørgensen 2016) to determine

whether means and dispersion of R:FR varied among richness levels and heights. For the main experiment, effects accounted for, in both the mean and dispersion linear predictors, were richness level (one, two, three and five species), percentage of survival, subplot, height (1 to 5 m) and interaction between richness and height. For the high diversity experiment, effects accounted for were richness level (six, nine or 18 species), percentage of survival, height (1 to 5 m) and interaction between richness and height. Wald tests were used to assess significance of effects. We obtained estimates and 95% confidence intervals for R:FR means and dispersion.

For the vertical iPAR distribution at the Anhembi site (data from section 2.2.3), we fit multivariate covariance generalized linear models (Bonat & Jørgensen 2016) including as effects richness level (20, 58, and 114 species), height, interaction between richness level and height, subplot and percentage of survival in both the linear predictors for the mean and dispersion parameters. Wald tests were used to assess significance of effects. For the final selected model, we obtained the estimated means and 95% confidence intervals.

To analyze vegetation structure at heights we could not reach using a ceptometer, at the Anhembi site, we assessed if LAI above 4 m high (data from section 2.2.3) differed between richness levels by carrying out an ANOVA, considering each plot's LAI (obtained from LiDAR data) as dependent variable and number of species (20, 58 and 114 species) as independent variable.

#### 2.2.5.4. Seasonal variation in light interception

As iPAR means at the Anhembi site was measured both in dry and wet seasons, we used its analyses (section 2.2.5.1) to observe if differences between richness levels were maintained in distinct seasons.

In addition to that, we analyzed temporal stability, at the Anhembi site, between dry and wet seasons. Even though "stability" may be a controversial term and its definition is not a consensus (Grimm *et al.* 1997), we chose to use a straightforward temporal stability metric: the means of a community's property divided by its standard deviation over time ($S=\mu/\sigma$) (Tilman *et al.* 2006). We determined each plot's iPAR means and standard deviation between dry and wet seasons, using data obtained from horizontal 3 x 6 m grids. We considered stability the ratio between each plots iPAR mean and standard deviation within this period. To compare these values among treatments, we carried out an ANOVA using log-transformed stability values as the dependent variable and richness level as independent variable.

#### 2.2.5.5. Selection, complementarity and composition effects

The Sardinilla site's design permitted analyses among monocultures, among different compositions within the same richness level and between monocultures and mixtures, helping to split selection and complementarity effects. We carried out an analysis of variance to compare mean R:FR within horizontal grids of the main experiment (data from section 2.2.2) among different species compositions (12 levels: 5 monocultures, 3 combinations of two species, 3 combinations of three species and 1 combination of five species). Since data was continuous and not bounded, a normal model was a reasonable assumption. We used percentage of survival within each plot as a covariate. We used Tukey test at a 95% confidence level for multiple comparisons, with Bonferroni correction for the global confidence level. We established specific contrasts to compare mean R:FR: 1) Between monocultures; 2) Between each monoculture and their combinations of two or three species; 3) Amongst two or three species mixtures; 4) Between five species mixture and each of the other treatments (monocultures, two and three species mixtures).

To account for complementarity effects, we also compared crown volume among the five main species (data from section 2.2.4) using a two-way ANOVA, with log-transformed crown volume as dependent variable and tree species and plot richness level (1 or 5 species) as independent variables. All analyses were carried out in the R environment (R Core Team 2018).

## 2.3. Results

### 2.3.1. Light interception

Richness enhanced PAR interception at both sites (Table 1). Nevertheless, light interception was not influenced by tree richness levels above six species in Sardinilla, whereas iPAR increased with every richness level, even from 58 to 114 species, in Anhembi, in the dry season (APPENDIX H).

At the Sardinilla site's main experiment, mean light interception significantly increased (since R:FR decreased, and it maintains a negative relationship with iPAR, according to the equation in section 2.2.2) with species richness ($\chi^2$=310.34, d.f.=3, $P$<0.0001; Figure 6). According to the model, plots containing 5 species presented higher light interception than the ones containing 2 or 3 species, which did not differ from each other ($P$<0.05), and all mixtures significantly intercepted more light than monocultures ($P$<0.0001). At the high diversity experiment, light interception was not influenced by richness levels from six to 18 species

($\chi^2$=1.54, d.f.=2, *P*=0.46). Estimates, standard-errors and significances, according to multivariate covariance generalized linear models are shown in the APPENDIX G.

**Table 1.** Means and standard errors of photosynthetically active radiation intercepted (iPAR) by the canopy of tree plantings with different richness levels (n species) in the Sardinilla (Panama) and Anhembi (Brazil) sites.

| **Sardinilla** | | **Anhembi** | | |
|---|---|---|---|---|
| n species | iPAR wet season | n species | iPAR dry season | iPAR wet season |
| 1 | 0.815 ± 0.055 | 20 | 0.706 ± 0.023 | 0.883 ± 0.012 |
| 2 | 0.879 ± 0.027 | 58 | 0.804 ± 0.026 | 0.935 ± 0.007 |
| 3 | 0.931 ± 0.006 | 117 | 0.874 ± 0.012 | 0.941 ± 0.004 |
| 5 | 0.941 ± 0.006 | | | |
| 6 | 0.956 ± 0.013 | | | |
| 9 | 0.936 ± 0.031 | | | |
| 18 | 0.960 ± 0.004 | | | |

At the Anhembi site, increasing levels of richness enhanced PAR interception in both seasons ($\chi^2$=108.07, d.f.=2, *P*<0.0001 for the dry and $\chi^2$=57.91, d.f.=2, *P*<0.0001 for the wet season) (Figure 7). In the dry season, according to the estimates and standard errors of the model (APPENDIX G), all treatments showed significantly different PAR interception (*P*<0.05), which is evident in the graphical representation of the model (APPENDIX H). In the wet season, PAR interception also increased with tree richness ($\chi^2$=57.91, d.f.=2, *P*<0.0001), but 58 and 114 species did not differ from each other (*P*>0.05; APPENDIX H). APPENDIX G shows estimates, standard-errors and significances, according to multivariate covariance generalized linear models.

### 2.3.2. Horizontal light distribution

Horizontal light dispersion decreased with richness at the Sardinilla site, both in the main ($\chi^2$=258.41, d.f.=3, *P*<0.0001) and in the high diversity experiments ($\chi^2$=8.08, d.f.=2, *P*<0.05). In the main experiment, horizontal dispersion was significantly higher for monocultures, intermediate for 3 species and lower for 2 and 5 species mixtures (*P*<0.05). In the high diversity experiment, horizontal light dispersion was lower for 9 and 18 species than for 6 species (*P*<0.05; see APPENDIX G for estimates and standard errors of the model).

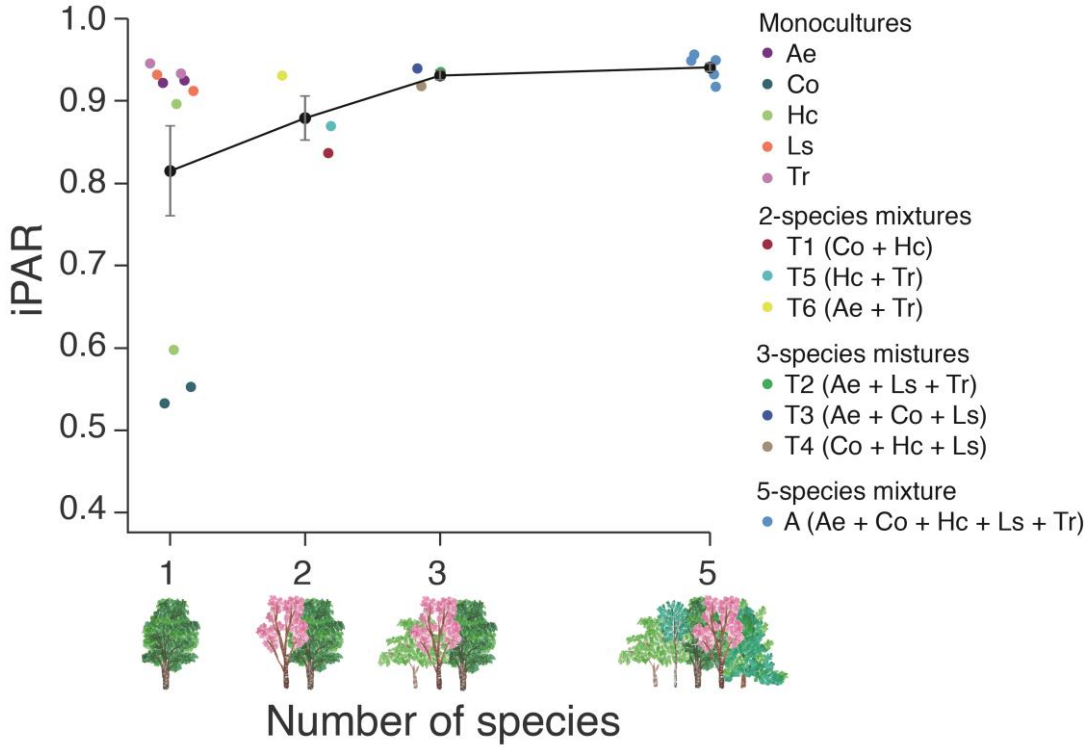


**Figure 6.** Mean (black dots) and standard error (bars) of iPAR at each richness level ($\chi^2$=310.34, d.f.=3, *P*<0.0001), measured during the rainy season, in Sardinilla (Panama). Colored dots represent all plots measured. Each different color represents a different composition, within each richness level.

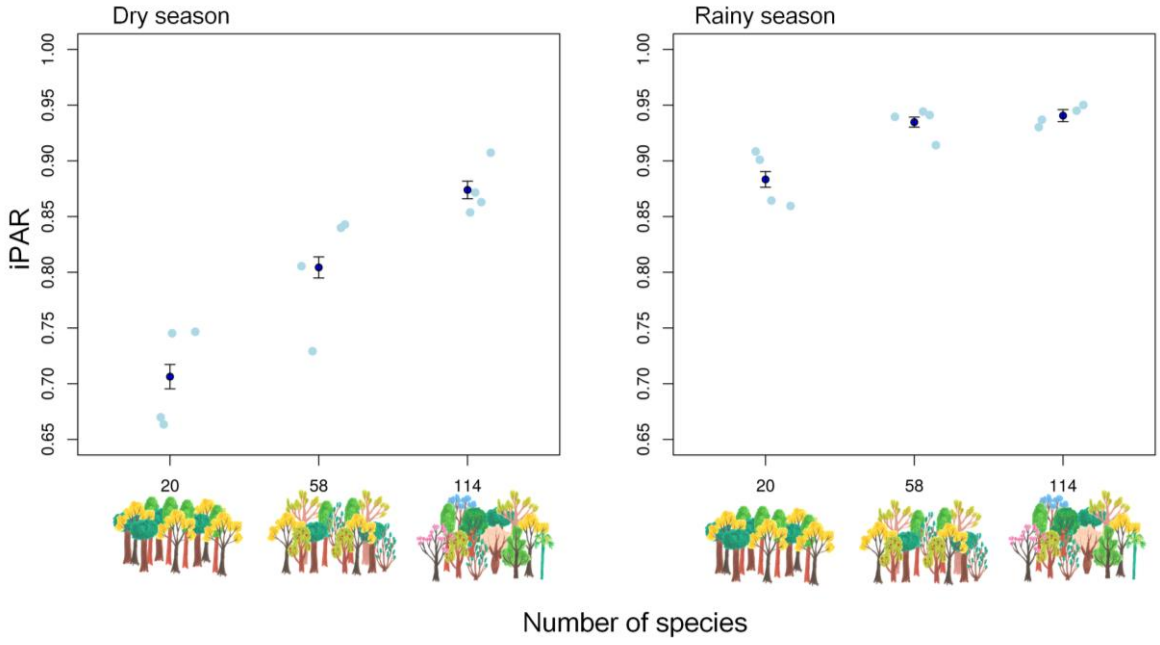


**Figure 7.** Mean iPAR (dark blue points) and standard errors (bars) measured for different species richness levels at the Anhembi site (Brazil), in dry ($\chi^2$=108.07, d.f.=2, *P*<0.0001 ) (A) and rainy ($\chi^2$=57.91, d.f.=2, *P*<0.0001) (B) seasons. Light blue points represent every plot measured within each richness level.

At the Anhembi site, the horizontal variance in iPAR interception decreased with richness, differing between all richness levels during the dry season and not differing only from

58 to 114 species in the rainy season, following the same pattern of mean iPAR interception, from section 2.3.1 (see APPENDIX I to visualize iPAR horizontal distribution at the Anhembi site). That is due to the quadratic mean-variance relationship, with maximum variance value at 50% of PAR interception. The further data are from 50%, the lower is their variance (McCullagh & Nelder 1989). Therefore, stands with higher richness, which maintained iPAR values further from 50% than stands with fewer species, accounted for lower iPAR variance. In addition to the variance, the distribution of intercepted PAR on a horizontal grid was also analyzed through the dispersion parameter. It refers to an extra source of variation, independent of the mean (Bonat & Jørgensen 2016). At the Anhembi site, during the dry season, this extra source of variation did not vary according to richness level ($\chi^2$=0.9364, d.f.=2, *P*=0.63). During the wet season, the dispersion parameter slightly varied according to richness level, with lower dispersion values (thus higher homogeneity) for 58 species, when compared to 20 species ($\chi^2$=6.7155, d.f.=2, *P*=0.03). Estimates, standard errors and significances of dispersion parameters, according to multivariate covariance generalized linear models are available in the APPENDIX G.

### 2.3.3. Vertical light distribution

At both sites, higher richness levels were able to better distribute light interception over different layers of the canopy. At the Sardinilla site's main experiment, light interception at different heights increased with richness, significantly differing from monocultures to mixtures ($\chi^2$=77.2180 , d.f.=3, *P*<0.0001) but, between mixtures, decreasing only from three to five species mixtures (Figure 8). As effects of interaction between richness and height were not significant ($\chi^2$=4.1200, d.f.=3, *P*=0.25), this means that mixtures could maintain higher light interception at every layer of the canopy, compared to monocultures. Dispersion effects over the vertical transects also decreased from monocultures to mixtures ($\chi^2$=68.1179, d.f.=3, *P*<0.0001). APPENDIX G shows estimates, standard errors and significances for means and dispersion of all richness levels analyzed. In the high diversity experiment, light interception over vertical transects was not influenced by richness ($\chi^2$=5.5849, d.f.=2, *P*=0.0613) or by its interaction with height ($\chi^2$=3.6841, d.f.=2, *P*=0.1585). The dispersion parameter of intercepted light over vertical transects did not vary among richness levels either ($\chi^2$=1.4887, d.f.=2, *P*=0.4750).

At the Anhembi site, in the dry season, PAR interception over the vertical transects significantly increased with richness ($\chi^2$=97.2058, d.f.=2, *P*<0.0001), differing between every richness level at a 95% confidence level (Figure 9). There was no interaction between the number of species and height ($\chi^2$=4.0268, d.f.=2, *P*=0.1335). Increasing richness showed decreasing

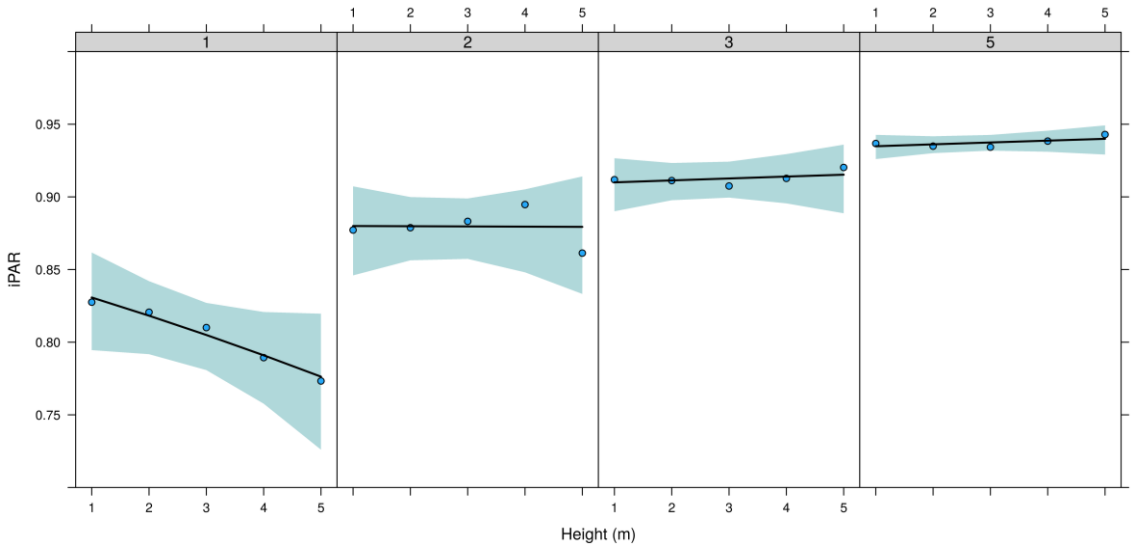


**Figure 8.** Mean iPAR (solid curves) and 95% confidence intervals (shaded areas) measured for different species richness levels (indicated above each curve, highlighted in gray), at different heights, at Sardinilla (Panama) site's main experiment, according to multivariate covariance model. Observed means and standard errors are represented by solid points and vertical bars.

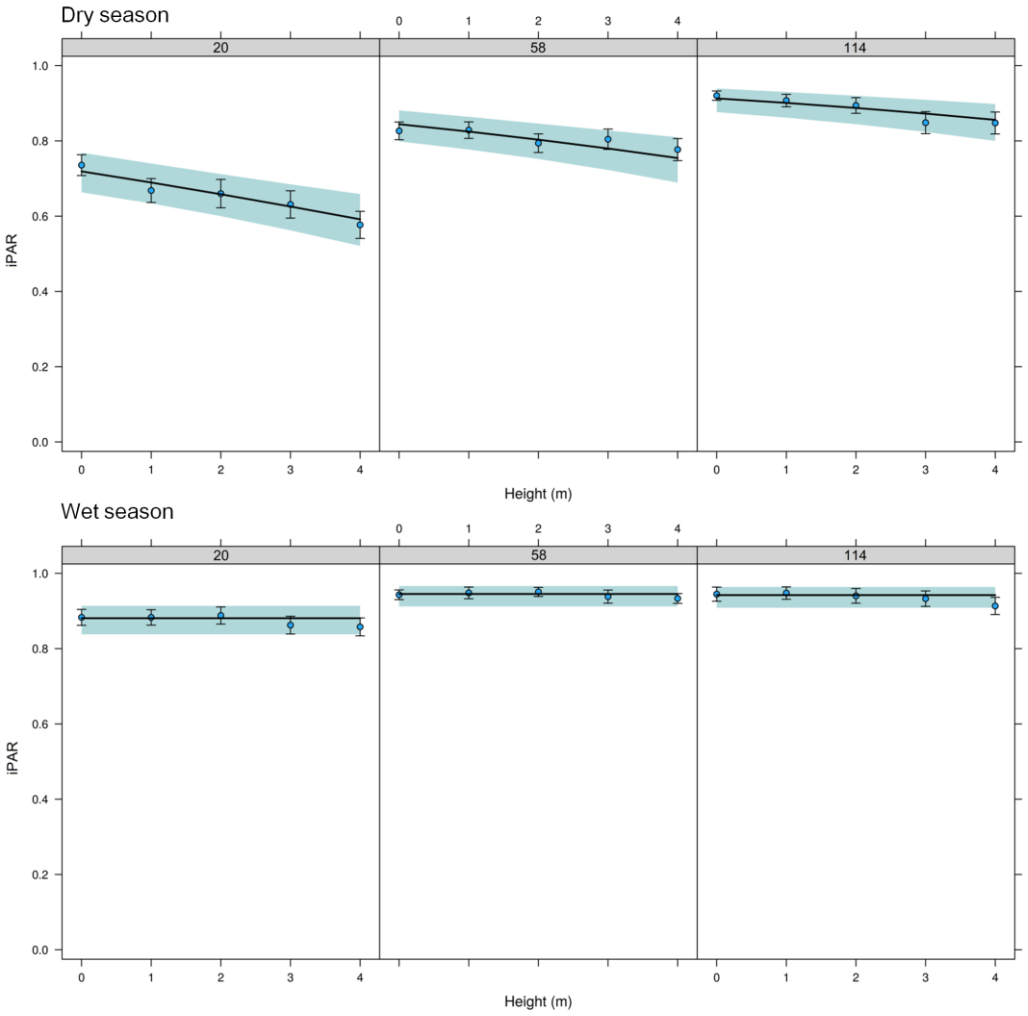


**Figure 9.** Intercepted PAR (solid curves) and 95% confidence intervals (blue shaded areas) according to height and richness level (indicated above each curve, highlighted in gray), measured during the dry and wet seasons, at the Anhembi (Brazil) site. Observed means and standard errors are represented by solid points and vertical bars.

dispersion of iPAR ($\chi^2$=12.3733, d.f.=2, *P*=0.0021). Interaction between height and species richness did not influence iPAR dispersion over the vertical transect ($\chi^2$=4.7651, d.f.=2, *P*=0.09).

During the wet season, in Anhembi, PAR interception over the vertical transects was higher in plots containing 58 and 114 species ($\chi^2$=39.6361, d.f.=2, P<0.0001) and did not respond to interaction between height and richness level ($\chi^2$=1.5535, d.f.=2, P=0.4599) (Figure 9). Dispersion effects did not significantly differ according to richness level ($\chi^2$=2.5248, d.f.=2, P=0,2830) or to its interaction with height ($\chi^2$=0.5655, d.f.=2, P=0.7537). Estimates, confidence intervals and significances of means and dispersion over different heights, for all levels of richness, are available in the APPENDIX G.

In addition to PAR interception on different layers of the canopy, vegetation structure over 4 meters high was also denser in the dry season at higher richness levels. LAI above 4 meters high increased with richness during this season ($F_{2,9}$=12.336, *P*=0.003), significantly differing only from 20 species to 58 and 114 species (*P*<0.05). In the wet season, number of species did not influence LAI ($F_{2,9}$= 0.9738, d.f.=2. *P*=0.414). Analyzing different layers over the whole canopy, LAD profiles (Figure 10) display confidence intervals overlapping, especially up to 8 meters high, showing that LiDAR data could not detect differences in vegetation structure at lower heights. Beyond 8 meters high, LAD measured at 20-species plots tended to be lower than in other richness levels, showing a tendency of richer plots to better explore the canopy at higher heights, especially for 58-species plots during the wet season.

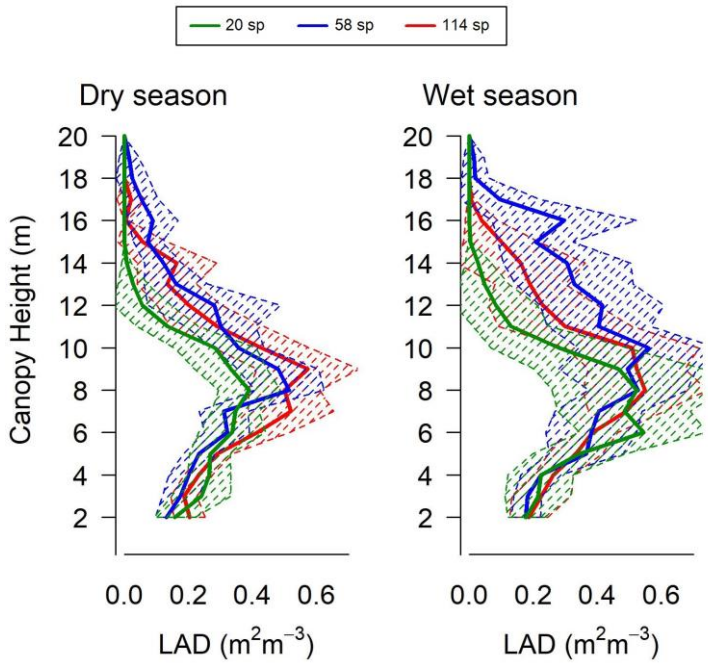


**Figure 10.** Mean leaf area density (LAD) (solid curves) and 95% confidence intervals (shaded areas) according to number of species, at each height of Anhembi (Brazil) site's plots. Dry season measurements were carried out in August, 2015, whereas rainy season measurements were taken in February, 2016. Anhembi, Sao Paulo, Brazil.

### 2.3.4. Seasonal variation in light interception

At the Anhembi site, light interception presented variation in different seasons, since it behaved differently between richness levels from the winter to the summer (APPENDIX I). During the dry season, the richest plots (114 species) could maintain higher light interception than the others (20 and 58 species), while during the wet season, the 58 and 114 species plots did not differ regarding PAR interception (results from section 2.3.1). In addition to that, seasonal stability values increased with number of species ($F_{2,9}$=10.6, $P$=0.0043). Plots containing 114 species had higher stability between seasons than plots containing 20 or 58 species ($P$<0.05).

### 2.3.5. Selection, complementarity and composition effects

Analyzing different compositions within each richness level, some monocultures differed between each other regarding light interception, as well as two species mixtures. Great part of mixtures maintained light interception at least equivalent to their best monocultures, except for one. Although significant in only some cases, five species mixtures tended to have the highest iPAR values, compared to monocultures and other mixtures (Figure 11).

Crowns presented higher volume in five species mixtures than in monocultures ($F_{1,110}$=19.9248, $P$<0.0001). They also varied according to species ($F_{4,110}$=6.8671, $P$<0.0001) but were not influenced by the interaction between species identity and richness level ($F_{4,110}$=2.2641, $P$=0.0668) (APPENDIX J).

## 2.4. Discussion

Light interception - an ecosystem process related to functions as primary production and biomass accumulation (Binkley *et al.* 2013; Sapijanskas *et al.* 2014; Jucker *et al.* 2015) and to processes like natural regeneration (Poorter & Arets 2003; Tilman *et al.* 2014; Suganuma & Durigan 2015) - was influenced by tree diversity, both at low (one to five species) and very high (58 to 114 species) diversity levels. Theory proposes that ecosystem functions should saturate at some point, as diversity increases (Cardinale *et al.* 2012). For instance, according to Hector & Bagchi (2007), the number of grassland species responsible for maintaining each ecosystem function varied between three and seven. In an entire ecosystem, though, these key species that are able to influence a single process are mixed with others, within a larger pool of species that

can vary across time and space. Therefore, the design of an experiment can result in distinct compositions and deeply influence its results on the relationship between levels of diversity and ecosystem functioning. There are innumerous ways of choosing community assemblages and which species are maintained or lost (Srivastava & Vellend 2005). Nevertheless, in our work, even a level as high as 58 tree species was not able to saturate light interception, since 114 species showed greater levels of iPAR in the dry season, in Anhembi, an unprecedented result for a BEF experiment.

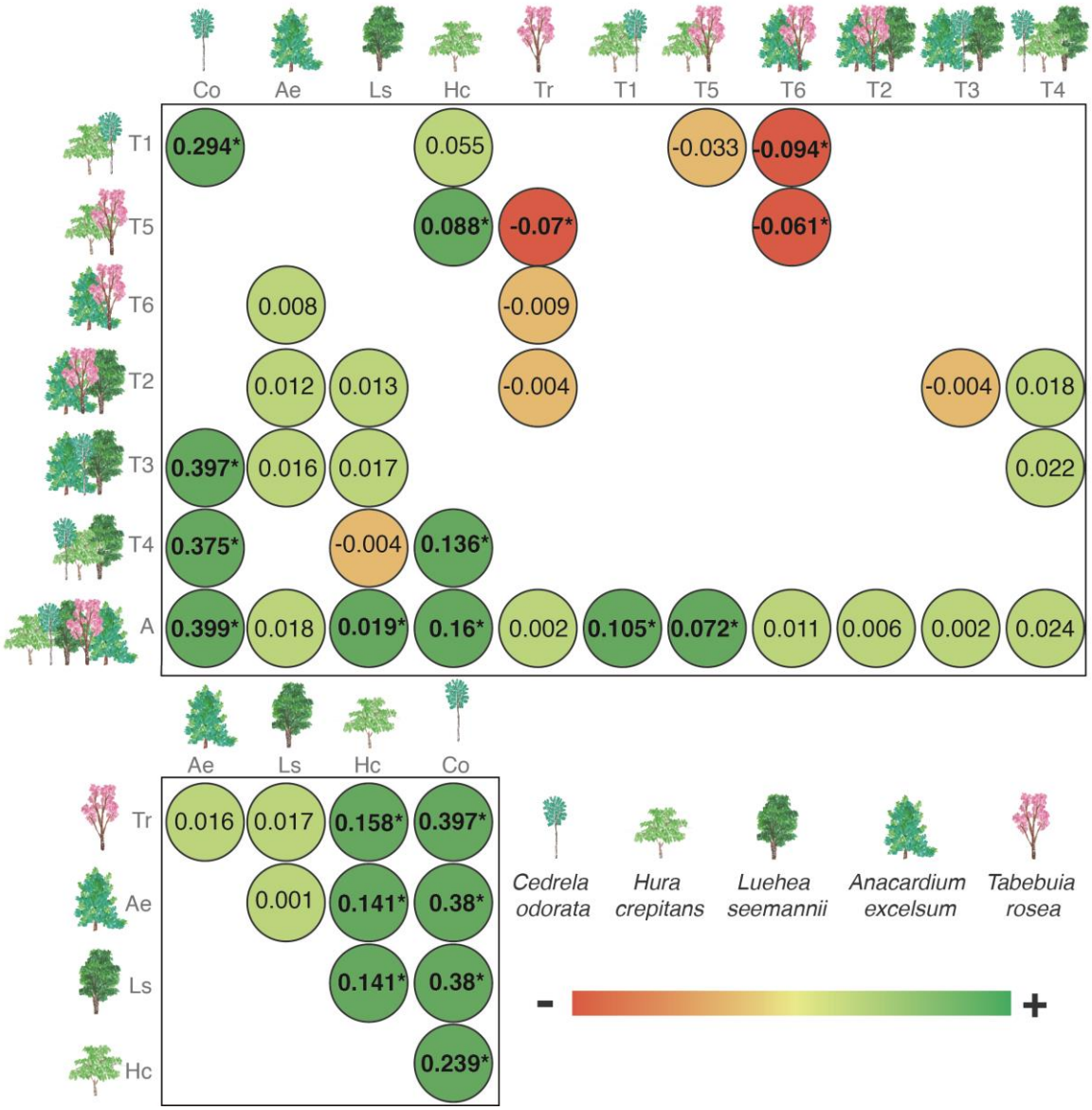


**Figure 11.** Comparisons of contrasts between different treatments' mean iPAR. Numbers within the cells represent the difference between mean iPAR from the treatment in the row and from the treatment in the column. Treatments: 1) monocultures: Ae = *A. excelsum*, Co = *C. odorata*, Hc = *H. crepitans*, Ls = *L. seemannii*, Tr = *T. rosea*; 2) two species combinations: T1 (Co + Hc), T5 (Hc + Tr), T6 (Ae + Tr); 3) three species combinations: T2 (Ae + Ls + Tr), T3 (Ae + Co + Ls), T4 (Co + Hc + Ls) and 4) five-species combination: A. Positive differences are colored in green, while negative differences are colored in red. Intensity of color is related to magnitude of difference between means. Significant contrasts at 95% confidence level are in bold and contain an asterisk. Analyses were performed using R:FR data and values were converted to iPAR only for better visualization.

At the Sardinilla site, light interception increased from monocultures up to five species mixtures. Nevertheless, at the high diversity experiment, light interception did not change from six to nine or to 18 species. It makes one question if these ecosystem processes were already saturated at six species, while in Anhembi they were not saturated at 58 species yet. Nevertheless, there are some considerations to make. Different responses on ecological processes may be due to Sardinilla and Anhembi being different sites and forest types, in different regions and containing distinct compositions (Hooper *et al.* 2005a; Hector & Bagchi 2007; Jucker *et al.* 2015). In addition to that, the differences between species levels is much more abrupt in Anhembi (38 species, between 20 and 58 species levels, and 56 species, between 58 and 114 species levels) than at Sardinilla's high diversity experiment (difference of 12 species between six and 18 species levels). Thus, at the former experiment, compositions of species are more distinct among richness levels, comparing to the latter, which can lead to differences in ecological processes (Naeem 2016). Outcomes of Ecology experimental plantations, even when they present the same species compositions, may vary according to site and time when the experiment was set up (wetter versus drier years, for instant) (Stuble *et al.* 2017). Our different results at distinct study sites highlight that careful must be taken when making conclusion about levels of species saturation in BEF, especially when there are not replicates in distinct areas.

Although at different levels in distinct experiments, the improvement of canopy functioning promoted by diversity, observed in our experiments, may lead to various practical consequences. High diversity, by intercepting more light, favors regeneration of late-successional (shade-tolerant) species (Suganuma & Durigan 2015) rather than light-demanding invaders (Tilman *et al.* 2014), such as grasses, strong competitors with tree seedlings in forest gaps (Hooper *et al.* 2005b), contributing for forest succession. It also promotes photosynthesis and consequent carbon stock in vegetation (Sapijanskas *et al.* 2014), contributing to climate change mitigation (Grassi *et al.* 2017; Griscom *et al.* 2017). In the face of an impending scenario that predicts extreme climate events, biodiversity is important not only to promote, but also to maintain high levels of light interception. High variability may allow the existence of certain species able to overcome changing conditions and to contribute to a forest's persistence under unstable conditions (Aerts & Honnay 2011).

One of the mechanisms that might be related to the positive influence of tree richness on light interception is horizontal spatial variation of intercepted light. Different species can provide distinct features and conditions to a forest (Loreau 2001), diversifying the forms to exploit the environment (Tilman *et al.* 2014). Among these features are duration and intensity of deciduousness (Gandolfi *et al.* 2009), crown shapes and arrangements (Pretzsch 2014; Sapijanskas

*et al.* 2014; Jucker *et al.* 2015), growth speed (Poorter & Arets 2003), maximum height and leaf traits (Ruiz-Jaen & Potvin 2011). Of course the roles played by species depend on their identity (Naeem 2016). Nevertheless, chances are that increasing diversity will lead to higher ecosystem functioning (Loreau *et al.* 2001; Meyer *et al.* 2018). Diversification may be even more intense than what would be expected if only species identity were considered, since the traits mentioned have plasticity and can change in monocultures or mixtures, presenting complementarity (Ruiz-Jaen & Potvin 2011; Sapijanskas *et al.* 2014; Jucker *et al.* 2015). Trees of distinct species have the ability to intersperse their crowns and better occupy empty spaces throughout the canopy (Pretzsch 2014), thus enhancing light capture (Sapijanskas *et al.* 2014). That may explain the results of our both experiments, in which higher levels of richness provided more regular light distribution, possibly a consequence of a better occupation of the canopy by tree branches and leaves, a process of competitive reduction (Forrester & Bauhus 2016). In fact, at the Sardinilla site, previous study have shown that biomass allocation to branches was higher in three-species mixtures than in monocultures (Potvin & Dutilleul 2009). Therefore, better canopy occupation might be one of the mechanisms explaining higher PAR interception at higher species richness (Sapijanskas *et al.* 2014).

Higher tree richness has also provided a more regular vertical distribution in light interception over the canopy. This was expected since, as the crowns grow and touch branches from other trees, they tend to change direction of growth (Pretzsch 2014). There is a degree of crown plasticity within species. When distinct species are combined, the diversity of forms of occupying the canopy at different heights is enhanced, thus resulting in a denser packing (Sapijanskas *et al.* 2014; Jucker *et al.* 2015). According to Sapijanskas et al. (2014), however, up to date studies fail to show whether multi-layering is really a cause of higher light interception or not. At both experiments investigated here (except for Sardinilla site's high diversity experiment, which has a particular behavior, as already mentioned), higher levels of diversity could intercept more light on all of the different heights sampled. In addition to that, vertical dispersion of intercepted light was lower at higher richness levels both at Sardinilla's main experiment and during the dry season at Anhembi. This is an evidence of multi-layering effect caused by diversity.

Using ground LiDAR measurements as a surrogate of iPAR was indeed a rougher method than using a ceptometer, since LAD profiles could not detect difference between treatments up to 4 meters high, as cepetometer did. Nevertheless, above 4 meters high, LAI was higher for 58 and 114 species in the dry season, showing that, despite the method's limitations, it could still detect some level of higher packing throughout the canopy

Richness promoted more homogeneous distribution of light interception not only across space, but also over time. At Anhembi, light interception was more similar among treatments during the wet season, when compared to the dry season, when the plots containing 114 species maintained highest iPAR. The richest stands also showed higher temporal stability of iPAR, according to the definition of stability proposed in Tilman *et al.* (2006). Species present seasonality on the roles they play on ecosystem functions (Wright *et al.* 2009). Phenological differences among species, such as not coincident deciduous periods, for instance, may enhance light interception on plot level (Sapijanskas *et al.* 2014; Forrester & Bauhus 2016). That may be explained by the portfolio effect, which proposes that, when an ecosystem has various species, it can buffer fluctuations of each species' functions over the time (Srivastava & Vellend 2005). Besides portfolio effect, facilitation processes may take place as well. Studies show that individuals can receive more light and grow more intensely when they have deciduous neighbors (Pretzsch 2014). Thus, a stand can enhance its resource use over the time when it bears species presenting distinct leaf phenology patterns (Sapijanskas *et al.* 2014).

Even though at the Anhembi experiment it is not possible to isolate complementarity mechanisms as facilitation (Forrester & Bauhus 2016), as mentioned before, Sardinilla's site design permits the conclusion that selection and complementarity effects, as well as distinct species compositions, play their role in higher interception of light. Analyzing contrasts among species and mixtures of Sardinilla's main experiment (Figure 11), some monocultures differed between each other regarding light interception. That indicates that some species are better performers than others in intercepting PAR, what may lead to selection effect (Loreau & Hector 2001). The greater part of mixtures had PAR interception levels superior or equivalent to their best monocultures, with the exception of only one of the two-species mixtures that had intermediate iPAR, comparing to its two monocultures. Mean iPAR measured in five species mixtures was in no case inferior to monocultures and two or three-species mixtures, and in many cases it was significantly superior. Complementarity effects (Loreau & Hector 2001) might be taking place in this process, which is reinforced by our data on crown plasticity, that showed that species in mixtures developed larger crown volumes than in monocultures. Reduction of competition may have taken place to allow crowns to grow larger and better occupy canopy spaces in mixtures (Forrester & Bauhus 2016). Two-species mixtures, compared among each other, had significantly different PAR interception, which shows that not only richness matters, but also species composition influences ecosystem processes (Naeem 2016).

At Sardinilla, mean PAR interception per plot according to species richness (Figure 6) behaved very similarly to what theory proposes. Light interception means increased with

richness, while variability among different compositions of the same species level decreased (Wright *et al.* 2009). Some of the five species mixtures' could not surpass the best monocultures regarding PAR interception (see light blue dots on Figure 6), as shown by other BEF works (Wright *et al.* 2009). We may argue whether high diversity is really necessary to maintain ecosystem functions, when some monocultures are good performers. Nevertheless, authors defend that the advantage of holding more species, even though one of them can perform well in providing determined ecosystem function, is their ability to keep multifunctionality (Hector & Bagchi 2007; Wright *et al.* 2009; Cardinale *et al.* 2012) and to maintain functional redundancy (Hooper *et al.* 2005a). Ecosystems containing higher diversity will likely present better overall functioning than less diverse ones (Aerts & Honnay 2011). In fact, in this study, the five-species mixture was in no case outperformed by any of the monocultures. Although it gathered both good and bad performers in the same plot, this mixture could maintain levels of light interception as high as the ones of the best monocultures.

We conclude that when diversity increases, light interception, which is an important process related to primary production (Sapijanskas *et al.* 2014), tends to increase as well. Mechanisms of light distribution over space (horizontally and vertically) and time take place and are likely influencing light interception performance (Forrester & Bauhus 2016). We gathered evidence that both effects of selection and complementarity (Sapijanskas *et al.* 2014), as well species composition (Naeem 2016), contribute to canopy occupation and light interception. High tree richness levels can be an important tool to contribute to various ecological processes responsible for the persistence of a forest and to ecosystem services that it can provide to humans (Aerts & Honnay 2011).

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## APPENDICES

**APPENDIX A.** List of species planted at the Sardinilla site, in 2003 (Flora do Brasil 2020 2018; The Plant List 2010 2018). Sardinilla, Colon, Panama.

| Family | Species |
|---|---|
| Anacardiaceae | *Anacardium excelsum* (Bertero ex Kunth) Skeels |
| | *Astronium graveolens* Jacq. |
| | *Spondias mombin* L. |
| Annonaceae | *Xylopia aromatica* (Lam.) Mart. |
| Bignoniaceae | *Handroanthus guayacan* (Seem.) S.O.Grose |
| | *Tabebuia rosea* (Bertol.) Bertero ex A.DC. |
| Boraginaceae | *Cordia alliodora* (Ruiz & Pav.) Oken |
| Burseraceae | *Bursera simaruba* (L.) Sarg. |
| Combretaceae | *Terminalia amazonia* (J.F.Gmel.) Exell |
| Euphorbiaceae | *Hura crepitans* L. |
| Fabaceae | *Albizia adinocephala* (Donn.Sm.) Record |
| | *Dalbergia retusa* Hemsl. |
| | *Diphysa americana* (Mill.) M.Sousa |
| | *Dipteryx oleifera* Benth. |
| | *Enterolobium cyclocarpum* (Jacq.) Griseb. |
| | *Erythrina fusca* Lour. |
| | *Gliricidia sepium* (Jacq.) Walp. |
| | *Hymenaea courbaril* L. |
| | *Inga punctata* Willd. |
| | *Ormosia macrocalyx* Ducke |
| | *Pseudosamanea* sp |
| Malvaceae | *Guazuma ulmifolia* Lam. |
| | *Luehea seemannii* Triana & Planch |
| | *Pachira quinata* (Jacq.) W.S.Alverson |
| Meliaceae | *Cedrela odorata* L. |
| Rhamnaceae | *Colubrina glandulosa* G.Perkins |
| Rubiaceae | *Calycophyllum candidissimum* (Vahl) DC. |
| Vochysiaceae | *Vochysia ferruginea* Mart. |

**APPENDIX B.** List of species planted at the Anhembi site, in 2006, in each of the treatments: 1) 20 species; 2) 58 species; 3) 114 species (Flora do Brasil 2020 2018; The Plant List 2010 2018). Anhembi, Sao Paulo, Brazil.

**Treatment 1 - 20 species**

| Family | Species |
|---|---|
| Anacardiaceae | *Schinusterebinthifolia* Raddi |
| Bignoniaceae | *Handroanthusimpetiginosus* (Mart. ex DC.) Mattos |
| | *Jacaranda cuspidifolia* Mart. |
| Euphorbiaceae | *Croton urucurana* Baill. |
| Fabaceae | *Enterolobium contortisiliquum* (Vell.) Morong |
| | *Erythrina falcata* Benth. |
| | *Hymenaea courbaril* L. |
| | *Myroxylon peruiferum* L.f. |
| | *Peltophorum dubium* (Spreng.) Taub. |
| | *Poecilanthe parviflora* Benth. |
| | *Pterogyne nitens* Tul. |
| | *Senegalia polyphylla* (DC.) Britton & Rose |
| Lecythidaceae | *Cariniana estrellensis* (Raddi) Kuntze |
| Lythraceae | *Lafoensia pacari* A.St.-Hil. |
| Malvaceae | *Ceiba speciosa* (A.St.-Hil.) Ravenna |
| | *Guazuma ulmifolia* Lam. |
| | *Heliocarpuspopayanensis* Kunth |
| | *Luehea divaricata* Mart. & Zucc. |
| Meliaceae | *Cedrela fissilis* Vell. |
| Moraceae | *Ficusguaranitica* Chodat |

**Treatment 2 - 58 species**

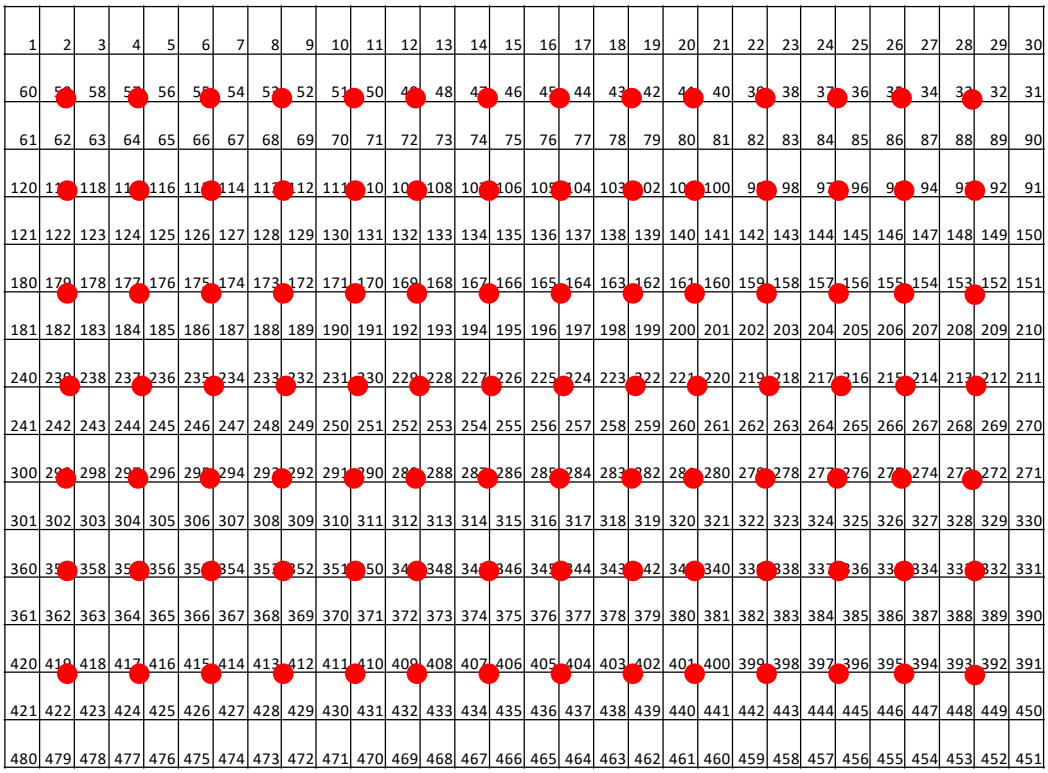
| Family | Species |
|---|---|
| Anacardiaceae | *Lithraea molleoides* (Vell.) Engl. |
| | *Myracrodruon urundeuva* Allemão |
| | *Schinusmolle* L. |
| | *Schinusterebinthifolia* Raddi |
| Apocynaceae | *Aspidosperma polyneuron* Müll.Arg. |
| | *Aspidosperma subincanum* Mart. |
| Bignoniaceae | *Handroanthusimpetiginosus* (Mart. ex DC.) Mattos |
| | *Jacaranda cuspidifolia* Mart. |
| | *Jacaranda mimosifolia* D.Don |
| | *Zeyheria tuberculosa* (Vell.) Bureau ex Verl. |
| Boraginaceae | *Cordia myxa* L. |
| Caricaceae | *Jacaratia spinosa* (Aubl.) A.DC. |
| Celastraceae | *Maytenusgonoclada* Mart. |
| Clusiaceae | *Clusia criuva* Cambess. |
| Euphorbiaceae | *Croton urucurana* Baill. |
| Fabaceae | *Albizia niopoides* (Spruce ex Benth.) Burkart |
| | *Anadenanthera colubrina* (Vell.) Brenan |
| | *Bauhinia forficata* Link |
| | *Cassia grandis* L.f. |
| | *Centrolobium tomentosum* Guillem. ex Benth. |
| | *Copaifera langsdorffii* Desf. |
| | *Enterolobium contortisiliquum* (Vell.) Morong |
| | *Erythrina falcata* Benth. |
| | *Hymenaea courbaril* L. |
| | *Inga vera* Willd. |
| | *Libidibia ferrea* (Mart. ex Tul.) L.P.Queiroz |
| | *Myroxylon peruiferum* L.f. |
| | *Parapiptadenia rigida* (Benth.) Brenan |
| | *Peltophorum dubium* (Spreng.) Taub. |
| | *Poecilanthe parviflora* Benth. |
| | *Poincianella pluviosa* var. peltophoroides (Benth.) L.P.Queiroz |
| | *Pterocarpusrohrii* Vahl |
| | *Pterogyne nitens* Tul. |
| | *Schizolobium parahyba* (Vell.) Blake |
| | *Senegalia polyphylla* (DC.) Britton & Rose |
| | *Senna macranthera* (DC. ex Collad.) H.S.Irwin & Barneby |
| Lecythidaceae | *Cariniana estrellensis* (Raddi) Kuntze |
| Lythraceae | *Lafoensia glyptocarpa* Koehne |
| | *Lafoensia pacari* A.St.-Hil. |
| Magnoliaceae | *Magnolia ovata* (A.St.-Hil.) Spreng. |
| Malvaceae | *Ceiba speciosa* (A.St.-Hil.) Ravenna |
| | *Guazuma ulmifolia* Lam. |
| | *Heliocarpuspopayanensis* Kunth |
| | *Luehea divaricata* Mart. & Zucc. |
| | *Pseudobombax grandiflorum* (Cav.) A.Robyns |
| Meliaceae | *Cedrela fissilis* Vell. |
| Moraceae | *Ficusguaranitica* Chodat |
| Myrtaceae | *Myrciaria tenella* (DC.) O.Berg |
| | Myrtaceae Juss. |
| | *Psidium cattleyanum* Sabine |
| | *Psidium guajava* L. |
| Phytolaccaceae | *Gallesia integrifolia* (Spreng.) Harms |
| Polygonaceae | *Triplarisamericana* L. |
| Rutaceae | *Esenbeckia leiocarpa* Engl. |
| | *Helietta apiculata* Benth. |
| Sapindaceae | *Sapindussaponaria* L. |
| Urticaceae | *Cecropia hololeuca* Miq. |
| Verbenaceae | *Citharexylum myrianthum* Cham. |

**Treatment 3 - 114 species**

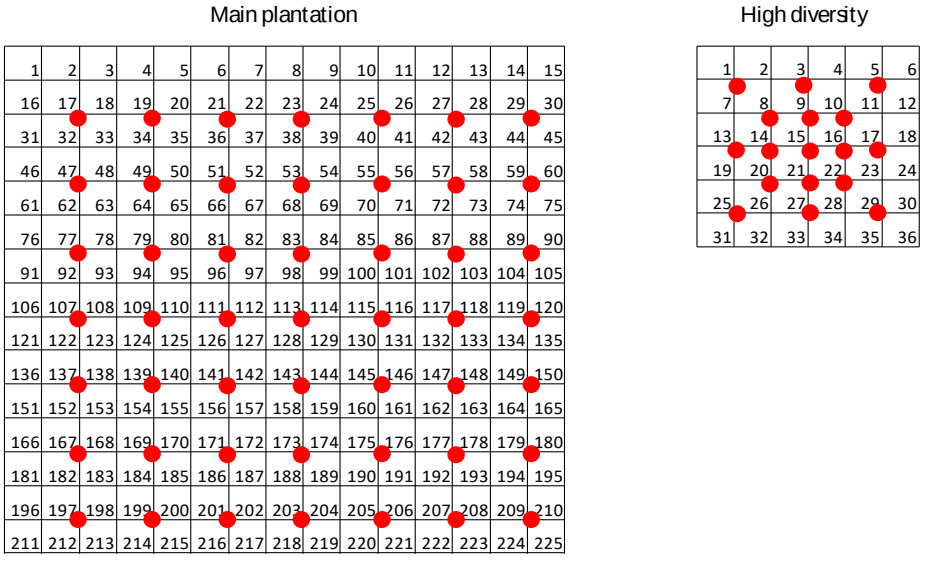
| Family | Species |
|---|---|
| Anacardiaceae | *Astronium fraxinifolium* Schott |
| | *Astronium graveolens* Jacq. |
| | *Lithraea molleoides* (Vell.) Engl. |
| | *Myracrodruon urundeuva* Allemão |
| | *Schinus molle* L. |
| | *Schinus terebinthifolia* Raddi |
| | *Tapirira guianensis* Aubl. |
| Apocynaceae | *Aspidosperma cylindrocarpon* Müll.Arg. |
| | *Aspidosperma parvifolium* A.DC. |
| | *Aspidosperma polyneuron* Müll.Arg. |
| | *Aspidosperma subincanum* Mart. |
| | *Tabernaemontana catharinensis* A.DC. |
| Bignoniaceae | *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos |
| | *Handroanthus heptaphyllus* (Vell.) Mattos |
| | *Handroanthus impetiginosus* (Mart. ex DC.) Mattos |
| | *Handroanthus ochraceus* (Cham.) Mattos |
| | *Handroanthus vellosoi* (Poorter et al.) Mattos |
| | *Jacaranda cuspidifolia* Mart. |
| | *Jacaranda mimosifolia* D.Don |
| | *Tabebuia insignis* (Miq.) Sandwith |
| | *Tabebuia roseoalba* (Ridl.) Sandwith |
| | *Zeyheria tuberculosa* (Vell.) Bureau ex Verl. |
| Boraginaceae | *Cordia americana* (L.) Gottschling & J.S.Mill. |
| | *Cordia myxa* L. |
| | *Cordia sellowiana* Cham. |
| | *Cordia trichotoma* (Vell.) Arráb. ex Steud. |
| Cannabaceae | *Trema micrantha* (L.) Blume |
| Caricaceae | *Jacaratia spinosa* (Aubl.) A.DC. |
| Celastraceae | *Maytenus gonoclada* Mart. |
| Clusiaceae | *Clusia criuva* Cambess. |
| Combretaceae | *Terminalia argentea* Mart. |
| Euphorbiaceae | *Alchornea glandulosa* Poepp. & Endl. |
| | *Alchornea triplinervia* (Spreng.) MÃ¼ll.Arg. |
| | *Croton floribundus* Spreng. |
| | *Croton urucurana* Baill. |
| | *Mabea fistulifera* Mart. |
| Fabaceae | *Albizia niopoides* (Spruce ex Benth.) Burkart |
| | *Anadenanthera colubrina* (Vell.) Brenan |
| | *Bauhinia forficata* Link |
| | *Cassia grandis* L.f. |
| | *Centrolobium tomentosum* Guillem. ex Benth. |
| | *Chloroleucon tortum* (Mart.) Pittier |
| | *Copaifera langsdorffii* Desf. |
| | *Enterolobium contortisiliquum* (Vell.) Morong |
| | *Erythrina falcata* Benth. |
| | *Hymenaea courbaril* L. |
| | *Inga edulis* Mart. |
| | *Inga laurina* (Sw.) Willd. |
| | *Inga marginata* Willd. |
| | *Inga vera* Willd. |
| | *Leucochloron incuriale* (Vell.) Barneby & J.W.Grimes |
| | Libidibia ferrea (Mart. ex Tul.) L.P.Queiroz |
| | *Machaerium brasiliense* Vogel |
| | *Mimosa bimucronata* (DC.) Kuntze |
| | *Myroxylon peruiferum* L.f. |
| | *Parapiptadenia rigida* (Benth.) Brenan |
| | *Peltophorum dubium* (Spreng.) Taub. |

| Family | Species |
|---|---|
| Fabaceae | *Poecilanthe parviflora* Benth. |
| | *Poincianella pluviosa* var. peltophoroides (Benth.) L.P.Queiroz |
| | *Pterocarpus rohrii* Vahl |
| | *Pterogyne nitens* Tul. |
| | *Schizolobium parahyba* (Vell.) Blake |
| | *Senegalia polyphylla* (DC.) Britton & Rose |
| | *Senna macranthera* (DC. ex Collad.) H.S.Irwin & Barneby |
| Lamiaceae | *Aegiphila integrifolia* (Jacq.) Moldenke |
| Lecythidaceae | *Cariniana estrellensis* (Raddi) Kuntze |
| | *Cariniana legalis* (Mart.) Kuntze |
| Lythraceae | *Lafoensia glyptocarpa* Koehne |
| | *Lafoensia pacari* A.St.-Hil. |
| Magnoliaceae | *Magnolia ovata* (A.St.-Hil.) Spreng. |
| Malvaceae | *Apeiba tibourbou* Aubl. |
| | *Bastardiopsis densiflora* (Hook. & Arn.) Hassl. |
| | *Ceiba speciosa* (A.St.-Hil.) Ravenna |
| | *Guazuma ulmifolia* Lam. |
| | *Heliocarpus popayanensis* Kunth |
| | *Luehea divaricata* Mart. & Zucc. |
| | *Pseudobombax grandiflorum* (Cav.) A.Robyns |
| Melastomataceae | *Pleroma granulosa* (Desr.) D. Don |
| Meliaceae | *Cabralea canjerana* (Vell.) Mart. |
| | *Cedrela fissilis* Vell. |
| | *Cedrela odorata* L. |
| | *Guarea guidonia* (L.) Sleumer |
| Moraceae | *Ficus guaranitica* Chodat |
| | *Ficus obtusifolia* Kunth |
| | *Maclura tinctoria* (L.) D.Don ex Steud. |
| Muntingiaceae | *Muntingia calabura* L. |
| Myrtaceae | *Calyptranthes clusiifolia* O.Berg |
| | *Eugenia uniflora* L. |
| | *Myrcia tomentosa* (Aubl.) DC. |
| | *Myrciaria tenella* (DC.) O.Berg |
| | Myrtaceae Juss. |
| | *Psidium cattleyanum* Sabine |
| | *Psidium guajava* L. |
| Phytolaccaceae | *Gallesia integrifolia* (Spreng.) Harms |
| | *Seguieria langsdorffii* Moq. |
| Polygonaceae | *Triplaris americana* L. |
| Primulaceae | *Myrsine parvifolia* A.DC. |
| Rhamnaceae | *Colubrina glandulosa* Perkins |
| Rosaceae | *Prunus myrtifolia* (L.) Urb. |
| Rubiaceae | *Amaioua intermedia* Mart. ex Schult. & Schult.f. |
| | *Coutarea hexandra* (Jacq.) K.Schum. |
| | *Genipa americana* L. |
| Rutaceae | *Balfourodendron riedelianum* (Engl.) Engl. |
| | *Esenbeckia leiocarpa* Engl. |
| | *Helietta apiculata* Benth. |
| | *Metrodorea stipularis* Mart. |
| Sapindaceae | *Diatenopteryx sorbifolia* Radlk. |
| | *Sapindus saponaria* L. |
| Sapotaceae | *Chrysophyllum gonocarpum* (Mart. & Eichler ex Miq.) Engl. |
| Urticaceae | *Cecropia hololeuca* Miq. |
| | *Cecropia pachystachya* Trécul |
| Verbenaceae | *Aloysia virgata* (Ruiz & Pav.) Juss. |
| | *Citharexylum myrianthum* Cham. |

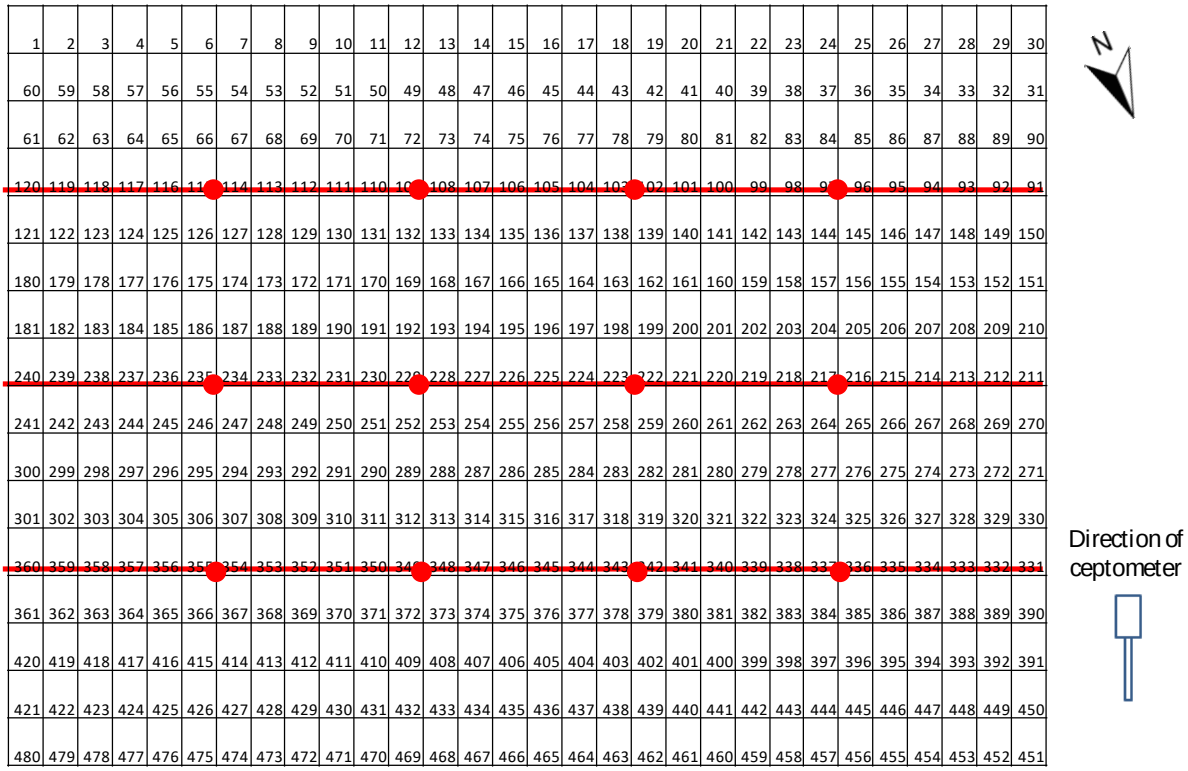
**APPENDIX C.** Subplots established by 3 x 6 m grid, to assess light horizontal distribution within plots at the Anhembi site, Anhembi, Sao Paulo, Brazil. Each numbered square from 1 to 480 represents a tree. On each of the 98 sub-plots (round points), which were placed equidistant to the four nearest neighbor trees, incident PAR was measured at height 1 m. All measurements were taken with both internal and external ceptometers headed to the same direction.




**APPENDIX D.** 49 sub-plots established by 6 x 6 m grid to assess light horizontal distribution within main plantation plots (left) and 17 subplots to assess light horizontal distribution at the high diversity experiment (right), in Sardinilla, Colon, Panama. Each numbered square represents a tree. On each subplot (round point), which was placed equidistant to the four nearest neighbor trees, red:far-red light ratio was measured at height 1.7 m.



**APPENDIX E.** Systematic established vertical transects (round points) in each plot of the Anhembi site, Anhembi, Sao Paulo, Brazil. Each numbered square from 1 to 480 represents a tree. Each spot, placed equidistant to the four nearest trees, represents a vertical transect. Along each vertical transect, iPAR was measured every meter, from 0 to 4 meters high. Thick lines represent transects, on which LiDAR was positioned at one meter high. All measurements were taken with both internal and external ceptometers headed to the same direction.




**APPENDIX F.** Systematic established vertical transects (round points) in each plot of the main plantation (left) and high diversity experiment (right), at the Sardinilla site, Sardinilla, Colon, Panama. Each numbered square represents a tree. Each spot, placed equidistant to the four nearest trees, represents a vertical transect. Over each vertical transect, R:FR was measured every meter, from 1 to 5 meters high.

Main plantation

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 |
| 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
| 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 |
| 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 |
| 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | 103 | 104 | 105 |
| 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 |
| 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 |
| 136 | 137 | 138 | 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 |
| 151 | 152 | 153 | 154 | 155 | 156 | 157 | 158 | 159 | 160 | 161 | 162 | 163 | 164 | 165 |
| 166 | 167 | 168 | 169 | 170 | 171 | 172 | 173 | 174 | 175 | 176 | 177 | 178 | 179 | 180 |
| 181 | 182 | 183 | 184 | 185 | 186 | 187 | 188 | 189 | 190 | 191 | 192 | 193 | 194 | 195 |
| 196 | 197 | 198 | 199 | 200 | 201 | 202 | 203 | 204 | 205 | 206 | 207 | 208 | 209 | 210 |
| 211 | 212 | 213 | 214 | 215 | 216 | 217 | 218 | 219 | 220 | 221 | 222 | 223 | 224 | 225 |

High diversity

| | | | | | |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |
| 7 | 8 | 9 | 10 | 11 | 12 |
| 13 | 14 | 15 | 16 | 17 | 18 |
| 19 | 20 | 21 | 22 | 23 | 24 |
| 25 | 26 | 27 | 28 | 29 | 30 |
| 31 | 32 | 33 | 34 | 35 | 36 |

**APPENDIX G.** Estimates (estim.), with standard errors in parentheses, for R:FR means and dispersion over horizontal grids and vertical transects and P-values, in Sardinilla (Panama) and for iPAR means and dispersion over horizontal grids and vertical transects and P-values, in Anhembi (Brazil) according to multivariate covariance generalized linear models.

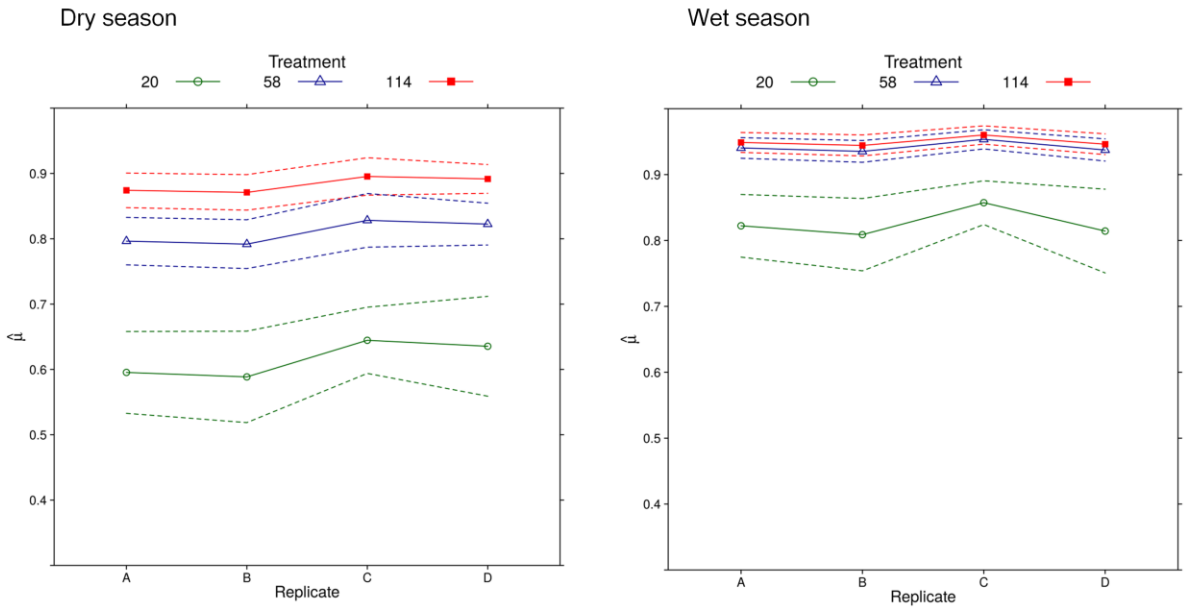
**Sardinilla (Panama):**

| | Horizontal | | | | Vertical | | | |
|---|---|---|---|---|---|---|---|---|
| n species | means estim. | *P*-value | dispersion estim. | *P*-value | means estim. | *P*-value | dispersion estim. | *P*-value |
| 1 (intercept) | 0.99 (0.03) | | 0.08 (0.01) | | 1.08 (0.04) | | 0.08 (0.006) | |
| 2 | -0.10 (0.02) | < 0.0001 | -0.03 (0.003) | < 0.0001 | -0.16 (0.03) | < 0.0001 | -0.04 (0.005) | < 0.0001 |
| 3 | -0.10 (0.01) | < 0.0001 | -0.02 (0.002) | < 0.0001 | -0.09 (0.03) | < 0.01 | -0.02 (0.004) | < 0.0001 |
| 5 | -0.18 (0.01) | < 0.0001 | -0.03 (0.002) | < 0.0001 | -0.20 (0.02) | < 0.0001 | -0.03 (0.004) | < 0.0001 |
| 6 (intercept) | 0.50 (0.06) | | 0.06 (0.01) | | 0.73 (0.05) | | 0.05 (0.008) | |
| 9 | 0.01 (0.01) | 0.30 | -0.004 (0.001) | < 0.01 | 0.04 (0.02) | 0.03 | -0.001 (0.002) | 0.74 |
| 18 | $8.9*10^{-6}$ (0.02) | 0.99 | -0.007 (0.003) | 0.02 | -0.004 (0.02) | 0.88 | -0.004 (0.004) | 0.22 |

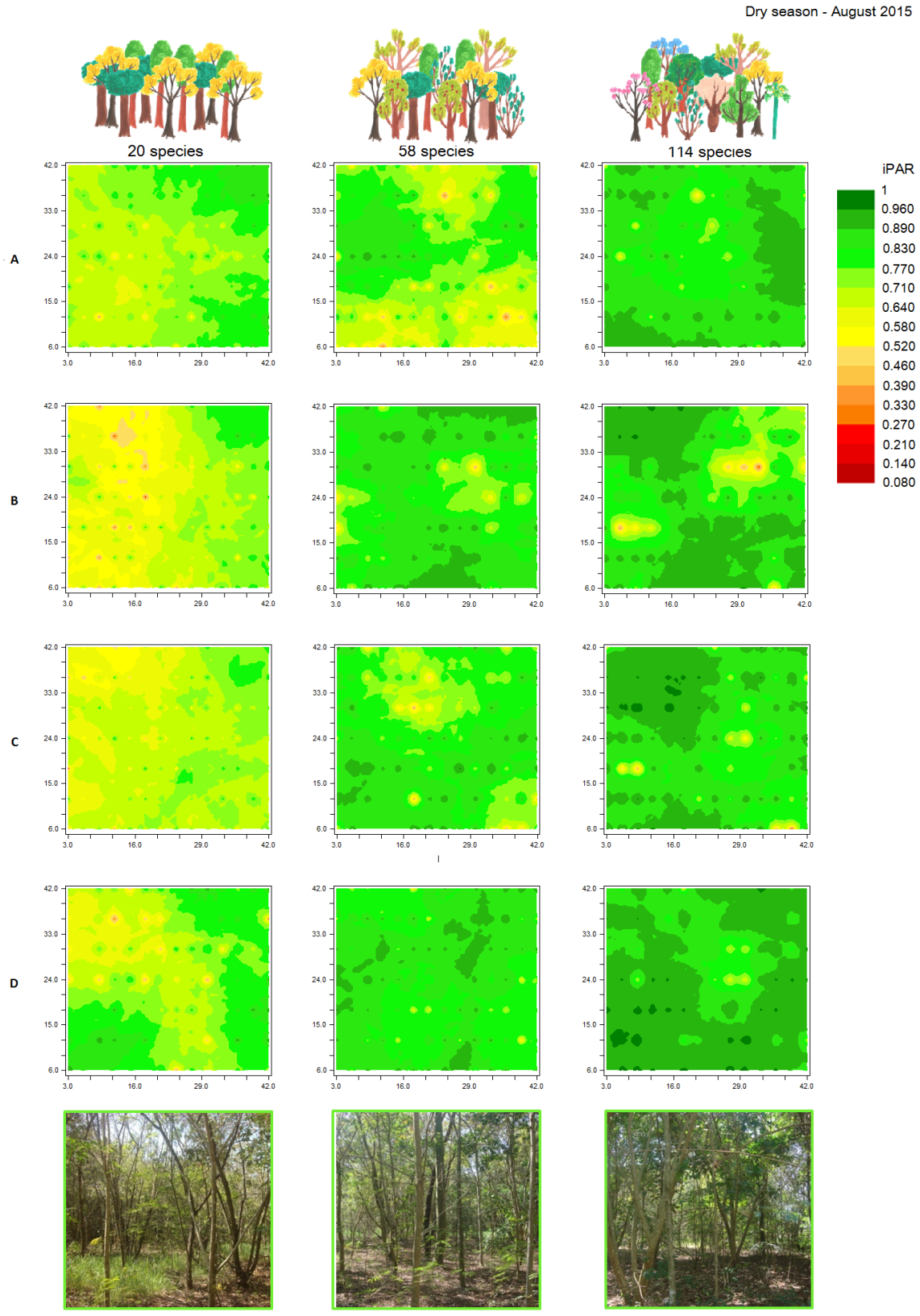
**Anhembi (Brazil):**

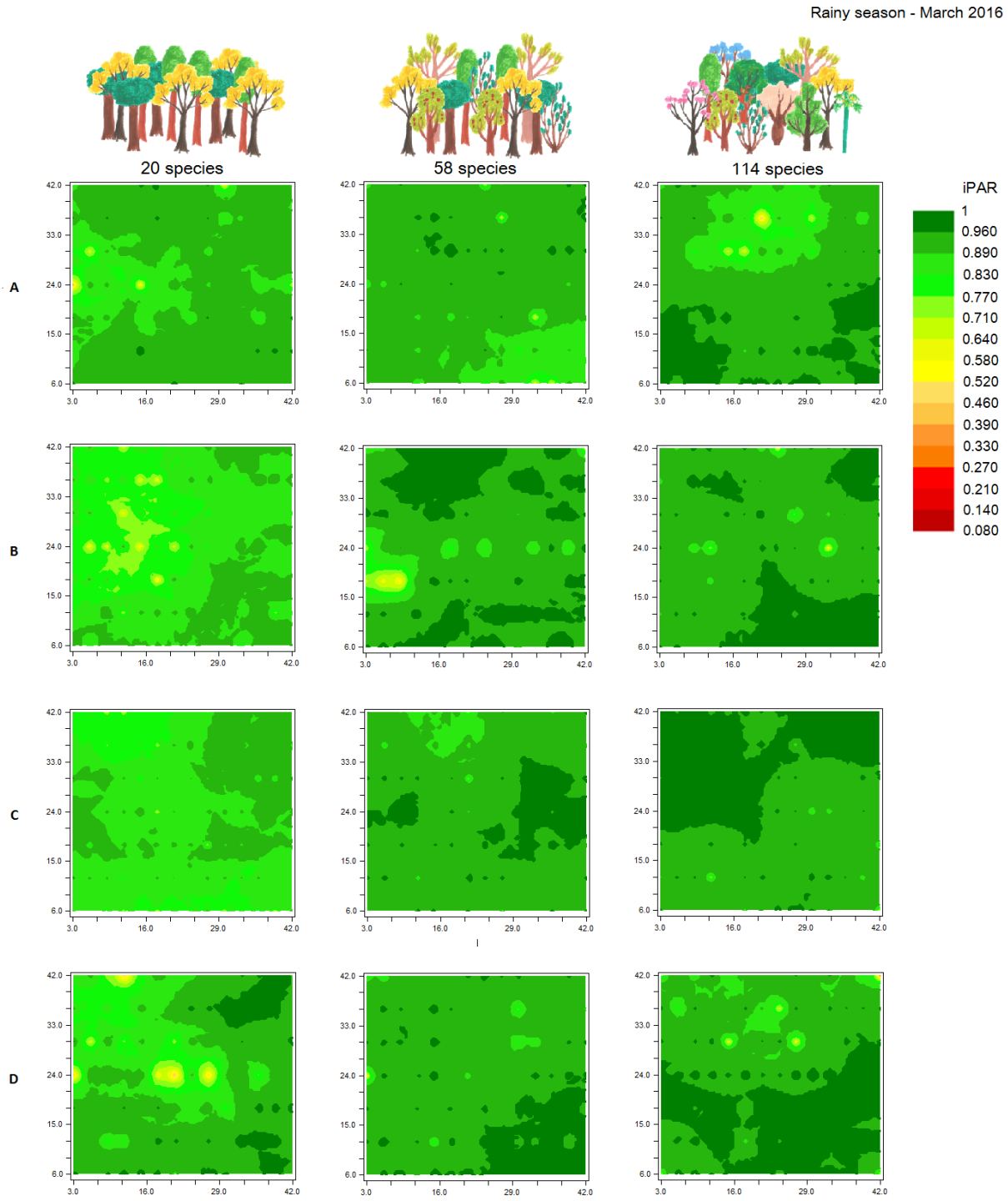
| | | Horizontal | | | | Vertical | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n species | mean estim. | *P*-value | dispersion estim. | *P*-value | mean estim. | *P*-value | dispersion estim. | *P*-value |
| Dry season | 20 (intercept) | -3.35 (1.10) | | -3.37 (1.77) | | -6.26 (1.39) | | 5.14 (2.10) | |
| | 58 | 0.98 (0.14) | <0.0001 | 0.21 (0.22) | 0.34 | 1.36 (0.21) | <0.0001 | -1.03 (0.33) | 0.002 |
| | 114 | 1.55 (0.15) | <0.0001 | 0.20 (0.29) | 0.49 | 2.29 (0.23) | <0.0001 | -1.33 (0.40) | <0.001 |
| Wet season | 20 (intercept) | -3.88 (1.42) | | 1.68 (3.47) | | -6.84 (2.11) | | 6.46 (3.98) | |
| | 58 | 1.23 (0.17) | <0.0001 | -0.76 (0.37) | 0.04 | 1.68 (0.36) | <0.0001 | -0.25 (0.87) | 0.78 |
| | 114 | 1.39 (0.19) | <0.0001 | -0.30 (0.48) | 0.53 | 2.03 (0.34) | <0.0001 | -0.98 (0.65) | 0.13 |

**APPENDIX H.** Mean iPAR and confidence intervals for different richness levels (lines with different colors and styles of points) according to multivariate covariance models (Bonat & Jørgensen 2016), from wet and dry seasons, at the Anhembi (Brazil) site. When lines of one treatment and confidence intervals of other treatment do not overlap, treatments are considered significantly different.

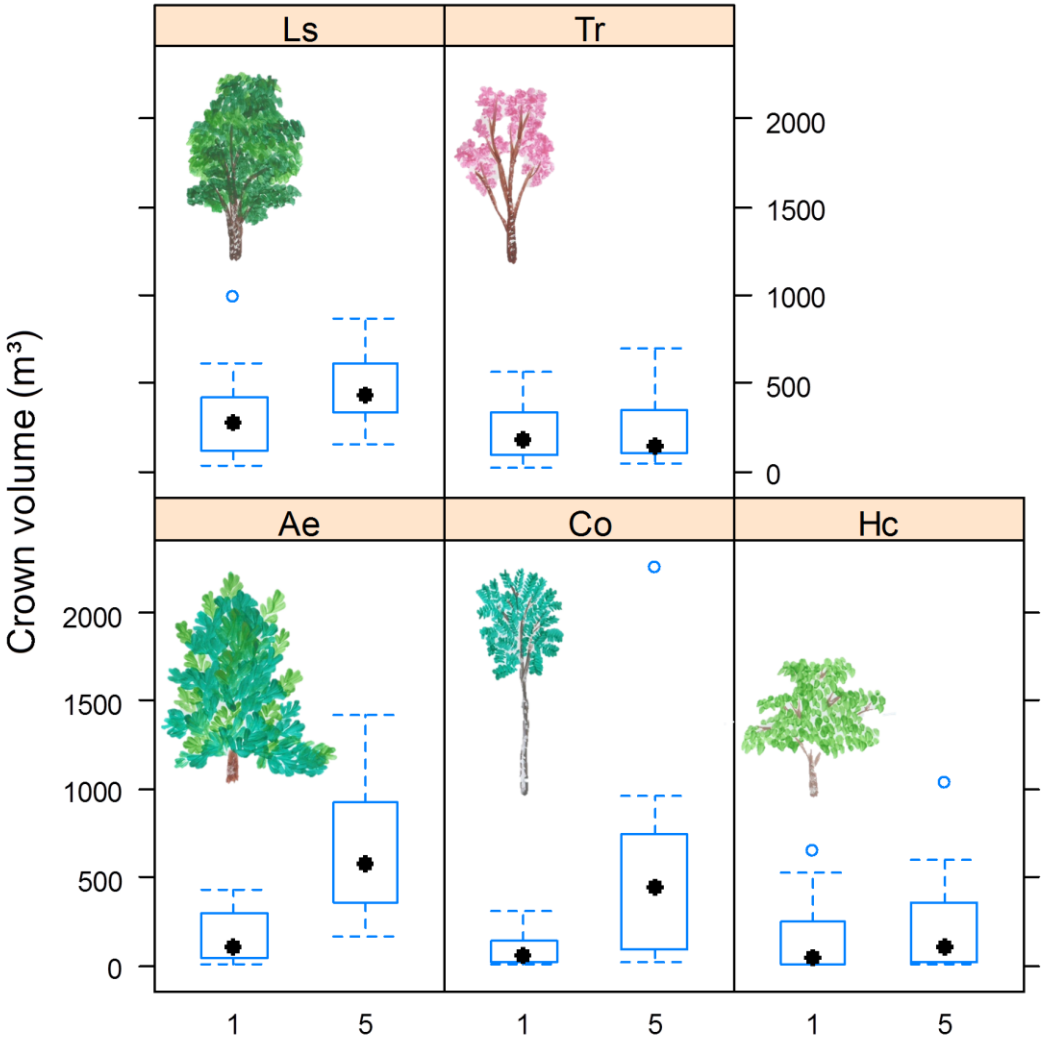


**APPENDIX I.** iPAR distribution in 45 x 48 m plots containing 20, 58 and 114 species (four replicates A-D of each), in Anhembi-SP, Brazil, during the dry season, August 2015. Map was obtained by kriging procedure, using exponential isotropic variogram models.




Rainy season - March 2016
20 species
58 species
114 species
A
B
C
D
iPAR
1
0.960
0.890
0.830
0.770
0.710
0.640
0.580
0.520
0.460
0.390
0.330
0.270
0.210
0.140
0.080
42.0
33.0
24.0
15.0
6.0
3.0
16.0
29.0
42.0

**APPENDIX J.** Crown volume (m³) for different species (Ae = A. excelsum, Co = C. odorata, Hc = H. crepitans, Ls = L. seemannii, Tr = T. rosea) in monocultures or five species mixtures, at Sardinilla site's main experiment. Sardinilla, Colon, Panama.



# 3. TREE RICHNESS EFFECTS ON FINE LITTER DYNAMICS AND FINE ROOT PRODUCTION IN TROPICAL FOREST


## Abstract

Manipulating plant diversity has been suggested to be a central strategy to enhance ecosystem functioning and the services associated to it, according to the biodiversity and ecosystem functioning (BEF) theory. In order to maximize carbon uptake, it is necessary to approach both above and below-ground ecological processes, and to understand how soil carbon pools are influenced by plant diversity. In this work, we investigated how tree richness levels influence fine litter dynamics and fine root production in tropical forest, as a means for understanding the impacts of tree diversity on soil carbon storage. We assessed a 10-year-old native tree plantation experiment established with 20, 58, and 114 tree species in the Atlantic Forest region of Brazil. Annual fine litter fall ranged from 6.56 ± 0.42 $Mg.ha^{-1}$ (20-species plots) to 7.53 ± 0.08 $Mg.ha^{-1}$ (58-species plots) and litter stocks (average of four measurements in one year) ranged from 8.46 ± 0.24 $Mg.ha^{-1}$ (20-species mixtures) to 9.94 ± 0.56 Mg.ha-1 (58-species mixtures). Fine root stocks within a 30 cm depth ranged from 5.38 ± 0.69 $Mg.ha^{-1}$ (20 species plots) to 6.44 ± 0.43 $Mg.ha^{-1}$ (114 species plots) and its quarterly production, measured quarterly in one year, varied from 1.07 ± 0.14 $Mg.ha^{-1}$ (58 species plots) to 6.44 ± 0.43$Mg.ha^{-1}$ (114 species plots). While stands' tree richness levels enhanced fine root production and stock, they had non-linear effects on litter decomposition and stock. Richness permitted fine roots to better occupy different layers of soil, enhancing their spatial distribution. However, richness did not enhance temporal distribution of any below-ground process analyzed, since differences between richness levels were not influenced by the time of collection. We conclude that diversity can affect below-ground processes, but that its effects are not always straightforward and may act in different directions. It is fundamental to try to untangle these mechanisms in order to use biodiversity as a tool for climate change mitigation.


Keywords: BEF; Below-ground carbon; Biodiversity and ecosystem functioning; Carbon uptake; Climate change mitigation; Ecological processes; Restoration

## 3.1. Introduction

Manipulating plant diversity has been suggested to be a central strategy to enhance ecosystem functioning and the services associated to it, such as climate change mitigation through enhanced forest productivity (Potvin & Gotelli 2008; Díaz *et al.* 2009). In a practical context, however, it is not usual to ally diversity protection and carbon sequestration solutions in the same policies (Díaz *et al.* 2009), despite its potential. According to the biodiversity and ecosystem functioning (BEF) theory and its practical applications, higher diversity (of species, genes and functional traits) allows more efficient functions within an ecosystem, such as primary productivity, nutrient cycling, decomposition (Cardinale *et al.* 2012). Higher plant diversity may not only increase overall ecosystem functioning and biodiversity protection (Alexander *et al.*

2011), but may also enhance their resistance to disturbances, thus preventing major biomass collapse under extreme climatic events (Isbell *et al.* 2015) and representing climate insurance (Aerts & Honnay 2011).

BEF research has focused on above-ground rather than on below-ground processes, even though soils shelter a considerable part of the world's biodiversity and carbon pools (Bardgett & van der Putten 2014; Powers & Marín-Spiotta 2017). Soils receive half of the carbon content incorporated by terrestrial plants in ecosystems (Pausch & Kuzyakov 2018) and store the greatest part of terrestrial carbon (Lange *et al.* 2015), twice as much as the atmosphere does (Paterson *et al.* 2009). Therefore, soil carbon is a very important component of ecosystems' carbon pools and should be considered in BEF studies as means of maximizing the use of biodiversity to mitigate climate change. It is fundamental to ally both above- and below-ground processes in order to understand total biomass storage within a forest, including its plants and soils (Wardle *et al.* 2004; Trogisch *et al.* 2017).

Studies show that plant diversity can maintain higher soil carbon stocks, in grasslands (Lange *et al.* 2015). To our knowledge, controlled BEF studies assessing effects of diversity in soil carbon are rare in tropical forests (Potvin *et al.* 2011). According to Díaz *et al.* (2009), in productive ecosystems as forests, diversity can, at the same time, enhance processes contributing to carbon inputs to soils (as litter production) and processes that take carbon out of soil (as decomposition). These ecosystems, therefore, sometimes count on higher above-ground than below-ground carbon storage. In fact, Potvin *et al.* (2011) observed that topsoil carbon pools decreased over the process of land use conversion from pastures to forests, and that effects of stand richness on carbon pools were not linear. It illustrates that there is a large array of complex, interconnected mechanisms that may take part carbon storage, requiring further elucidation (Díaz *et al.* 2009; Lange *et al.* 2015). Above- and below-ground diversity-mediated processes can mutually influence each other and make this relationship even more complex (Fujii *et al.* 2017).

Soil carbon pools depend on 1) inputs, as from plant biomass; 2) outputs, as through decomposition and respiration; 3) the size of equilibrium carbon stocks and 4) the probability of disturbances that may drastically displace pools from their equilibrium. Plant diversity can differently affect those inputs and outputs (Díaz *et al.* 2009), as well as the probability of vegetation to resist to extreme events (Isbell *et al.* 2015). Therefore, influence of plant diversity on soil carbon stocks will depend on its influence on various processes combined, which may present multi-directional effects (Díaz *et al.* 2009). It usually takes a long time for carbon stocks in soil to change (Lange *et al.* 2015; Powers & Marín-Spiotta 2017), but some mechanisms that influence them may act in a shorter time span (Bardgett & van der Putten 2014).

One of the carbon inputs to soil is plant litter production (Facelli & Pickett 1991; Lange *et al.* 2015). It is an important link between above and below-ground ecological processes (Huang *et al.* 2017). It is expected that, in forests containing higher diversity levels, the same above-ground mechanisms that permit overyelding (Sapijanskas *et al.* 2014) and denser canopy cover also enhance litter production (Jucker *et al.* 2015), since this process is closely related to primary production (Facelli & Pickett 1991). Studies show that higher diversity increases litter fall (Peh *et al.* 2012; Huang *et al.* 2017) and also influences its chemical composition (Huang *et al.* 2017). Litter fall can also be related to seasonal or stochastic factors, as water shortage, strong winds or storms (Facelli & Pickett 1991).

A considerable output for soil carbon is decomposition (Díaz *et al.* 2009), a process through which litter loses carbon (Scherer-Lorenzen *et al.* 2007). Tree diversity can influence this ecosystem function both by providing different litter components to be decomposed and by providing distinct environmental conditions for decomposition (Seidelmann *et al.* 2016). Litter constitution, which is highly dependent on plant species identity, can influence decay rates (Hättenschwiler *et al.* 2005; Scherer-Lorenzen *et al.* 2007; Seidelmann *et al.* 2016). In addition to that, it is common that combinations of different species in litter present synergistic effects and elevated decomposition rates, compared to what was predicted from monocultures (Hector *et al.* 2000; Hättenschwiler *et al.* 2005; Peh *et al.* 2012). This can be attributed to interactions among different chemical compounds, to reduced nutrient limitation due to the presence of different types of litter, or to the ability of this diverse content to provide facilitation among decomposers (Hättenschwiler *et al.* 2005). Still, studies predominantly show no trend provided by litter content richness on decomposition (Wardle *et al.* 1997; Scherer-Lorenzen *et al.* 2007).

Studies show that effects of plant diversity are stronger when it comes to providing differential environmental conditions for the decomposition, rather than providing differential litter content to decay (Srivastava *et al.* 2009). Tree species may influence microclimatic conditions in forests (Seidelmann *et al.* 2016), and since temperature and humidity are underlying factors for decomposition, plant richness can influence this function at a fine scale (Facelli & Pickett 1991). In addition to creating distinct canopy conditions, tree diversity produces chemical (Huang *et al.* 2017) and structural (Scherer-Lorenzen *et al.* 2007) differences in litter and fine roots (Bardgett & van der Putten 2014), which provide distinct microenvironments for detritivores and decomposer organisms (Facelli & Pickett 1991), which play a major role in nutrient cycling (Srivastava *et al.* 2009; Trogisch *et al.* 2017). On the one hand, distinct types of decomposers can modify litter conditions for the next consumers in the food chain (Facelli & Pickett 1991) and present facilitation among each other, enabling higher matter decay at higher diversity levels of

consumers (Srivastava *et al.* 2009). On the other hand, they can compete for resources and impair each other's activity (Hättenschwiler *et al.* 2005). Soil biota can also act as ecosystem engineers and influence diversity of plants, since they can alter nutrient availability for them and provide different types of interactions, as mutualism, herbivory, parasitism, or diseases (Mangan *et al.* 2010). The relationship plants maintain with soil fauna is able to influence factors that account for their fitness, as reproduction, facilitation, competition, defense from herbivores and tolerance to stress (Bardgett & van der Putten 2014). It is clear that decomposition is a complex ecosystem function and it depends on a large array of interactions among organisms and abiotic factors that can exert multi-directional influences (Facelli & Pickett 1991), and thus be influenced by plant diversity levels.

A direct product of the interaction between litter production and decomposition is litter stock. It is the result of an equation that involves litter production (input), transportation (to and from adjacent areas, thus meaning either inputs or outputs) and destruction (output). Different rates of litter production and decomposition can create large variability in litter accumulation (Facelli & Pickett 1991). Increase in soil carbon pools are related either to high inputs or to slow decomposition (Lange *et al.* 2015). Thus, all the processes already mentioned to influence litter production and litter decomposition act in opposite ways to determine litter stocks in soil.

Besides litter, plant roots are also able to influence carbon inputs to soils (Bardgett *et al.* 2014). Roots play significant role in putting carbon into soil, through exudates, dead tissues and mucilage, which contain organic compounds that feed microbial community and support nutrient cycling (Pausch & Kuzyakov 2018). The advantage of carbon inputs from roots is that, by growing underground, they permit the distribution of this element through different layers of soil (Díaz *et al.* 2009). Fine roots, the ones with less than 2 mm of diameter (Brassard *et al.* 2013), maintain a whole sort of interactions with soil plants, animals, water and nutrients, being the most dynamic part of the root (Trogisch *et al.* 2017). Forests containing higher tree diversity may present niche partitioning and facilitation among fine roots (Brassard *et al.* 2013). Differences in fine root architecture may permit their growth in distinct layers and their better occupation of space and time, reducing competition for nutrient uptake, under high diversity levels (Forrester & Bauhus 2016). Roots can chemically influence the presence of other life forms around them (Bardgett & van der Putten 2014), thus, their diversity enhances microbial activity and nutrient release (Bardgett *et al.* 2014; Lange *et al.* 2015).

It is clear that interactions among litter, roots and soil microorganisms are tangled and feedbacks may act in various directions (Hättenschwiler *et al.* 2005), being influenced by tree diversity in many different ways and also influencing above-ground processes (Díaz *et al.* 2009;

Seidelmann *et al.* 2016; Fujii *et al.* 2017). Biodiversity thus can both be a driver and outcome from ecosystem functioning (Díaz & Cabido 2001).

In this work, we investigated how tree richness levels influence fine litter dynamics and fine root production in tropical forest, as means of understanding the impacts of tree diversity on soil carbon storage. In particular, we investigated if tree richness affects fine litter and fine roots production and stocks, and fine litter decomposition.

## 3.2. Material and methods

### 3.2.1. Study site

This study was carried out at the Experimental Station of Forest Sciences of the University of São Paulo, in Anhembi, Sao Paulo, Brazil. The land was previously used for pastures, occupied by exotic grasses. The area's original forest cover was Semi-deciduous Seasonal Forest (Morellato & Haddad 2000), which counts on 30 to 50% of deciduous or semi-deciduous species (Gandolfi *et al.* 2009). In 2007, the pasture was cleared (by the use of herbicides) and an experiment to assess effects of high diversity levels on carbon storage was established. Previously to plantation, the land was subsoiled, limed and received formicide, for ant control. Seedlings were then planted on the study site to restore a Semi-deciduous Seasonal Forest. In 45 x 48 m plots, the following treatments were randomly assigned: 1) 20 species; 2) 58 species (which included the 20 species from treatment 1) and 3) 114 species (which included the 58 species from treatment 2), with four replicates (A-D) of each (Figure 12). Each treatment had 50% of species and individuals classified as fast-growers and the other 50% classified as intermediate to slow-growers. Their sequence was randomly assigned, equally for all four replicates of the same treatment. Spacing between individuals was 3 x 1.5 m. Lists of species within each treatment are available in the APPENDIX B. Plantations were periodically manured and counted on grass control (either manually or by using glyphosate) and on ant control (by the use of formicide) for the period of three years. In 2016, tree basal area for distinct treatments was: 1) 20 species: 29.16 ± 3.97 $m^2$/ha; 2) 58 species: 31.19 ± 1.43 and 3) 114 species: 29.73 ± 1.89 $m^2$/ha, measured in annual inventory.

The experiment is located under the coordinates 22°42' S and 48°10' W, 455 m a.s.l., where climate is Cfa (Köeppen). Mean temperature is 20.6°C over the year, being July the coldest month (16.8°C on average) and February the warmest (23.5°C on average). Rainfall throughout the year is on average 1.288 mm. The driest months occur during the winter, from April to

September, when mean precipitation is below 50 mm (Alvares *et al.* 2013). Figure 13 shows temperature and precipitation during the period that encompassed our experiments, according to the study site's meteorological station. Soil is classified as Typic Hapludox. It presents low pH (around 4) and contains more than 80% of sand, around 13% of clay and 5% of silt. In the superficial layer (45 cm), organic matter is below 2% (Ferez *et al.* 2015).

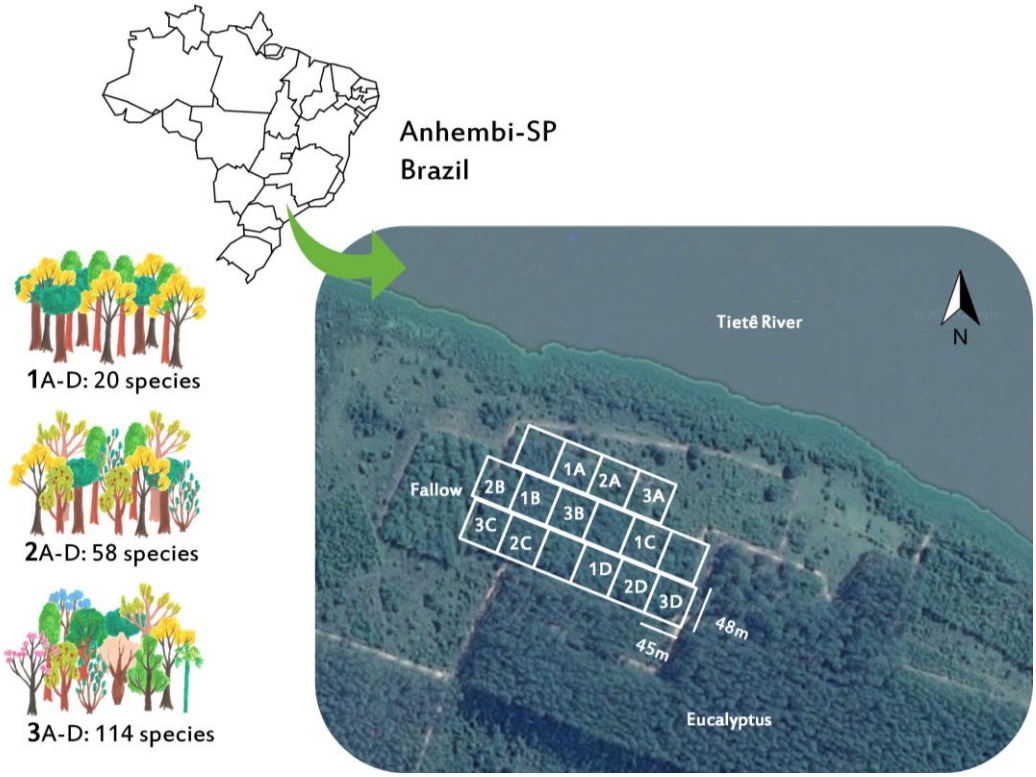



**Figure 12.** Study site located in Anhembi, Sao Paulo, Brazil. There are four replicates (A-D) of each richness level: 1) 20 species, 2) 58 species, 3) 114 species. Image from Google Earth Pro v. 7.3.0.3832 (November, 2017).

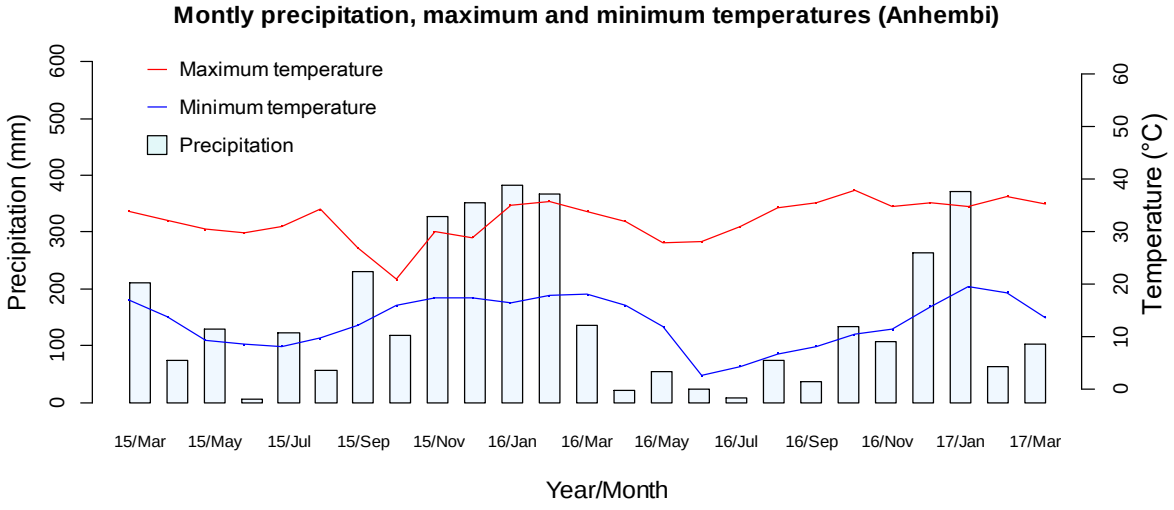



**Figure 13.** Monthly precipitation, maximum and minimum temperatures, at the study site, during the two years that encompassed our experiments. Data were taken from the site's meteorological station in Anhembi, São Paulo, Brazil.

### 3.2.2. Fine litter fall

We randomly placed 10 litter traps in each plot (their distance to nearest neighbor trees was random as well), excluding eight-meter large bands on each side of the plots, as borders. Litter traps were made of plastic circles (63 cm of diameter) and plastic mesh (70% of coverage, 0.6 x 0.125 cm aperture) at least 20-cm deep, placed 50 cm above the ground (Figure 14).

We started, in August 2015, monthly collections of fine litter fall (leaves, flowers, fruit and branches measuring up to 2 cm of diameter, hereafter called "litter", for simplification), until July 2016. We dried each trap's content at 60°C (until its weight became stable) and weighted it. Since collection dates were slightly different, we divided each monthly measurement by the number of days passed since the last collection, to determine daily litter fall. We then multiplied it by 30 to establish corrected monthly litter fall (hereafter only "monthly litter fall").

**Figure 14.** Litter traps placed on each plot (10 traps per plot) in Anhembi, Sao Paulo, Brazil, in February, 2015.

### 3.2.3. Fine litter decomposition

We based our decomposition experiment on the method used by Wardle *et al.* (2006). We used the 10 litter traps randomly placed on each plot (as described in section 3.2.2) and periodically collected fine litter, from February to July, 2015. The content of litter traps was dried (60° C) and mixed among all four replicates of the same richness level (20, 58 and 114 species separately), thus constituting three different contents, according to litter's species richness. We mixed litter collected from February to the end of July, so that we would have leaves from trees presenting different deciduous periods.

We used 10 g (dry weight) of each of the three different contents (20, 58 and 114 species) to fill 20 x 20 cm litter bags. These bags were made of 2 mm plastic mesh on the side that would face forest floor and 5 mm plastic mesh on the side facing up. The option to use mixed mesh (Trogisch *et al.* 2017) was due the intention to, on the one hand, avoid losing material (from using a large mesh) and, on the other hand, permit entrance of meso- and macro-fauna (Bradford et al. 2002).

In January 2016, we systematically placed one of each type of litterbags (20, 58 and 114 species) on 12 regularly spaced subplots within each plot (Figure 15). Thus, there were 36 litterbags per plot, 12 of each type (Figure 16). After 2, 4, 8 and 12 months, 3 bags of each different type were harvested from every plot, and their content was oven dried right after collection (60°C). Dry litter was then washed, had fine roots and soil removed, dried again, and weighted.

**Figure 15.** Three litter bags containing 10 g of litter (one of each distinct type of content: 20, 58 and 114 species) placed on each of the 12 sample points per plot, in Anhembi, Brazil, in January 2016. Double sized mesh was used, with larger aperture facing upside and smaller aperture facing forest floor.

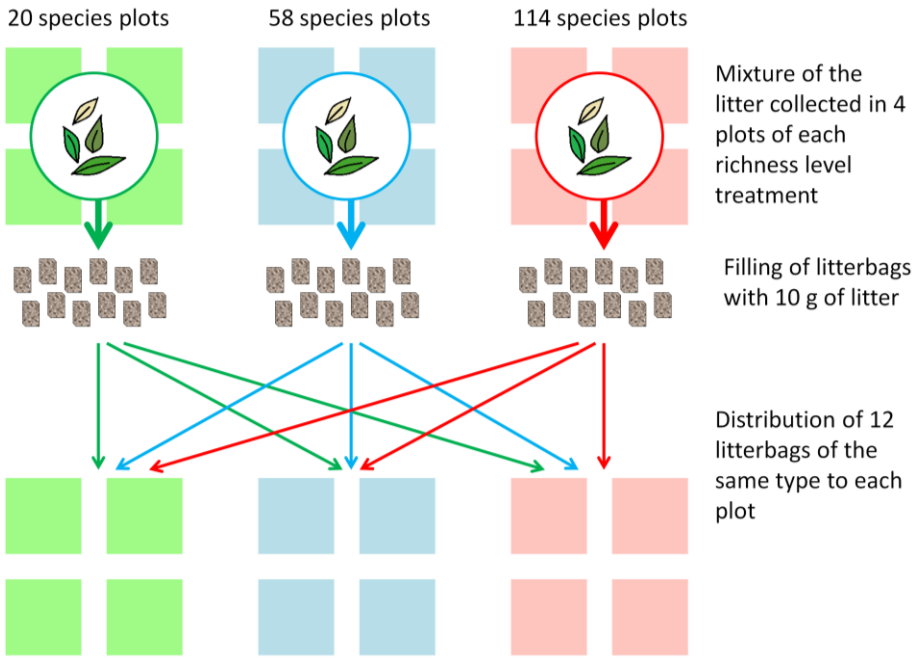



**Figure 16.** Decomposition experiment design. We collected litter from the four replicates of each richness level from February to July 2015, mixed and used it to fill litterbags. 12 litterbags of the same content (20, 58 and 114 species) were placed in each plot, in January 2016, in Anhembi, Brazil.

### 3.2.4. Fine litter stock

In order to analyze litter stock, for one year, we randomly chose five points per plot (not including the 8 meters wide bands nearest to the boarders), every three months, from August 2015 to June 2016 (4 collection periods) and used a wood frame to collect fine litter within a 25 x 25 cm area (material that touched the frame was cut before being included) (Figure 17). We dried the content at 60°C (until weight was stable) and weighted it, to determine fine litter stock.

**Figure 17.** 25 x 25 cm frame that was used to collect litter from the forest floor in Anhembi, Brazil. August 2015.

### 17.1.1. Fine root collection

We analyzed fine root stock and their quarterly growth over one year. We considered fine roots the ones with diameter up to 2 mm (Brassard *et al.* 2013). In June 2015, we chose four subplots per plot, equidistant to their four nearest neighbor trees, but randomly located within the plots (disregarding the 5 m wide bands nearest to the boarders). We used an auger (10 cm of diameter) to dig and separate soil portions from distinct layers in different recipients: 0-10 cm, 10-20 cm and 20-30 cm deep. In each recipient, we screened fine roots for eight minutes (split into four intervals of two minutes), and kept them in paper bags. We placed an ingrowth core inside each hole and returned the soil that previously occupied its space, respecting original order of layers (Figure 18). Paper bags were kept in freezer until their content was washed, dried at 60°C (until their weight was stable) and fine roots were weighted. The method we used was a modification of the one proposed by Metcalfe *et al.* (2007). However, for data analyses, we chose not to make predictions of the amount of roots that would be collected in 120 min, as proposed by the method, since: 1) our goal was just to compare different treatments within the same experiment and not to predict the amount of fine roots in the ecosystem; 2) our plots were located adjacently, at the same study site and the same people carried out measurements of all treatments, reducing unwanted variation among our samples; 3) we considered that it would be more accurate to use raw data, since using predictions from regressions would embed larger amount of errors in our analyses. We repeated the same method of fine root collection to determine quarterly fine root growth within the ingrowth core for one year (collections from September 2015 to June 2016). To display in our results regarding fine roots content (for both stock and production), we used a logarithmic curve to fit the values of amounts of fine roots collected in four series of two minutes and predicted the amount of roots that would have been collected in a 120 minutes, as proposed by Metcalfe *et al.* (2007).

As some plots presented substantial cover of exotic grasses and of *Senegalia polyphylla* (DC.) Britton & Rose plantlets, we considered that their roots could be an important source of noise that could mask responses from planted trees' fine root data. We used a 1 x 1 m frame to collect above-ground parts of grasses and *S. polyphylla* plantlets around all of our sample points, in December 2015. We dried and weighted this material.


**Figure 18.** Fine root collection in Anhembi, Brazil, in June, 2015. A) A 10 cm diameter auger was used to remove soil from three different depths and placing them in three distinct recipients. B) Hole made after removing soil from the depth 0-10 cm. C) Soil containing fine roots was placed in different trays and paper bags were used for keeping fine roots. D) Ingrowth core. E) After fine root collection, ingrowth cores were placed within the holes and soil was returned, respecting its original order of layers.

### 3.2.5. Data analyses

To analyze if litter fall varied among the three richness levels over the year, we used linear mixed-effect model considering the monthly litter fall (we used log (x+1) transformation in order to make the residuals with a normal distribution and to avoid –Inf values when we log transformed our data) as a function of stand richness (fixed effect), month of collection (fixed effect), their interaction and the error of litter trap (random effect) within plot (random effect), and both within month of collection. We used the function "lmer" from the "lme4" package (Bates *et al.* 2015). We used Wald tests to estate significance of variables.

For decomposition analysis, we used a three-way ANOVA (Gotelli & Ellison 2004), being the logarithm of litter bag dry mass the dependent variable and richness level of plot (categorical), number of species of litter (categorical), number of days since beginning of experiment (numeric) and the interaction among all of these factors. This model presented higher Akaike information criterion (AIC=-364.69) compared with a two-way ANOVA that did not include number of species of litter content (AIC=-384.28). We therefore used the latter, with higher likelihood, for *post hoc* means comparisons. We carried out a Tukey test to compare means of litter mass (logarithmically transformed) among richness levels of plots, all over the time, at a 95% confidence level, using the package "lsmeans". We also performed Tukey tests to compare litter mass (logarithmically transformed) among richness levels in each of the four collection

dates, also at a 95% confidence level. Finally, we used the function "lstrends" of the package "lsmeans" to compare the curves of litter decay under each plot's richness (Lenth 2016).

To analyze litter stock over the time, we also used linear mixed-effects model, also using the function "lmer" from the "lme4" package (Bates *et al.* 2015), carrying out Wald tests to determine which variables significantly influenced the response. The model was the same as for litter fall, but considering litter stock instead. We used the "lsmeans" package (Lenth 2016) to compare means through Tukey test at a 95% confidence level.

To analyze fine root production over the time, we used linear mixed models, as in Defrenet *et al.* (2016), using the package "lme4" (Bates *et al.* 2015). We considered collection date, stand richness and depth (0-10, 10-20 and 20-30 cm) as fixed effects and collection date, plot and subplot as random effects, due to the repeated measurement and nested design of the experiment. The model consisted in produced fine root dry mass as a function of stand richness, collection date, depth and the interaction among them, including the error of subplot within plot, within collection date. We performed Wald tests to identify variables that significantly influenced fine root production. We also built linear mixed models using fine root production from the each layer separately, as a function of stand richness, collection date and the interaction between them, including the error of subplot within plot, within collection date. We used the function "lsmeans" and "lstrends" (Lenth 2016) to compare means of different richness levels and rate across time (slopes), through Tukey test at a 95% confidence level.

We carried out a multivariate analysis of variance (MANOVA) to determine if fine root stock (dry mass) in different soil layers (0-10, 10-20 and 20-30 cm) varied according to plot's richness level (Gotelli & Ellison 2004). For that purpose, we used the "jmv" package (Selker *et al.* 2018). We used Tukey tests to carry out *post hoc* comparisons. We compared fine root stock between layers within each one of stand richness levels. We also compared fine root stock between richness levels within the 0-10 cm layer of soil, using the function "lsmeans" and "lstrends" (Lenth 2016).

To assess if the presence of grasses and *S. polyphylla* differed among stands' richness levels (which could be a noise to fine root measurements), we carried out three paired Fisher's exact tests, comparing the three levels of stands' richness

For all data analyses carried out, we used the R environment (R Core Team 2018).

## 3.3. Results

### 3.3.1. Fine litter fall

Total litter fall over the period of one year was 6.56 ± 0.42 Mg.ha$^{-1}$ for 20-species plots, 7.53 ± 0.08 Mg.ha$^{-1}$ for 58-species plots and 7.41 ± 0.46 Mg.ha$^{-1}$ for 114-species plots. It was not influenced by richness levels ($\chi^2$=1.57, d.f.=2, *P*=0.46) or by their interaction with time ($\chi^2$=3.74, d.f.=2, *P*=0.15), and was only affected by the month of collection ($\chi^2$=53.60, d.f.=1, *P*<0.0001; Figure 19).

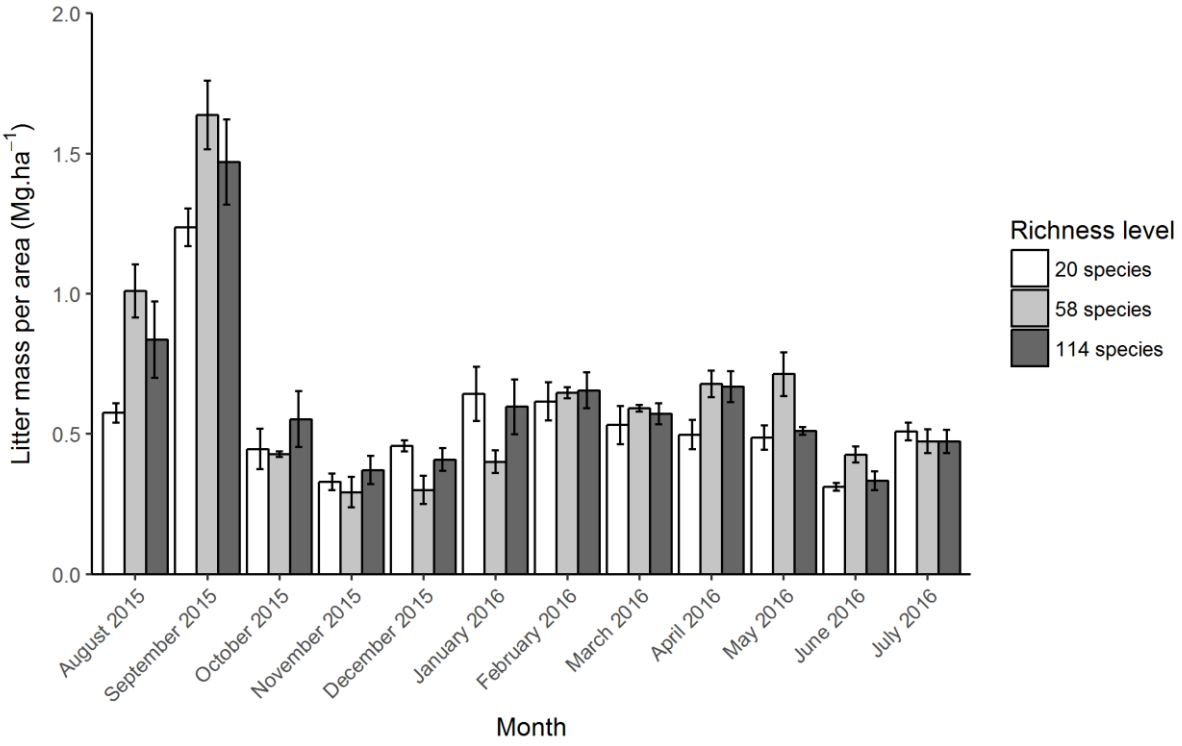



**Figure 19.** Mean monthly litter fall (and standard error), over the period of one year, for plots containing 20, 58 and 114 species, in Anhembi, Brazil.

### 3.3.2. Fine litter decomposition

Litter decomposition responded only to richness of plot (F=20.25, d.f.=2, *P*<0.0001), date of litter bag collection (F=119.48, d.f.=1, *P*<0.0001) and to their interaction (F=11.03, d.f.=2, *P*<0.0001). It did not respond to litter bag content and neither to its interaction with any other factor (*P*>0.10). It means that tree stand richness influenced decomposition, but that plots containing distinct numbers of species did not behave similarly over the time (Figure 20). Tree stands containing 114 species showed higher total litter decomposition, over the experiment duration (Tukey test at 95% confidence level, using the model with higher likelihood, excluding litter content).

Analyzing each collection date separately, on the first collection date (March 2016), plots containing 114 species had significantly decomposed more litter than those containing 20 species ($P<0.01$), while the ones with 58 species had intermediate levels of decomposition, not differing from any of the others. We found a similar pattern on the second collection date (May 2016), as the richest plots significantly differed from the ones with the lowest richness level ($P<0.0001$), while plots containing intermediate number of species marginally differed from the others, at 95% significance level. On the third collection date (September 2016), however, the decomposition trend shifted. Plots with intermediate number of species had decomposed the lowest amount of litter, significantly differing from the richest plots ($P<0.05$). Plots with the lowest richness level had decomposed intermediate amount of litter. Finally, at the last collection period (January 2017), both the plots with highest and lowest species richness had decomposed the highest amounts of litter, not differing from each other ($P>0.10$), but both differing from stands with intermediate richness ($P<0.01$). When analyzing the shape of the decomposition curve, plots with 20 and 114 species produced similar curves, significantly differing from 58 species plots at a 95% confidence level.

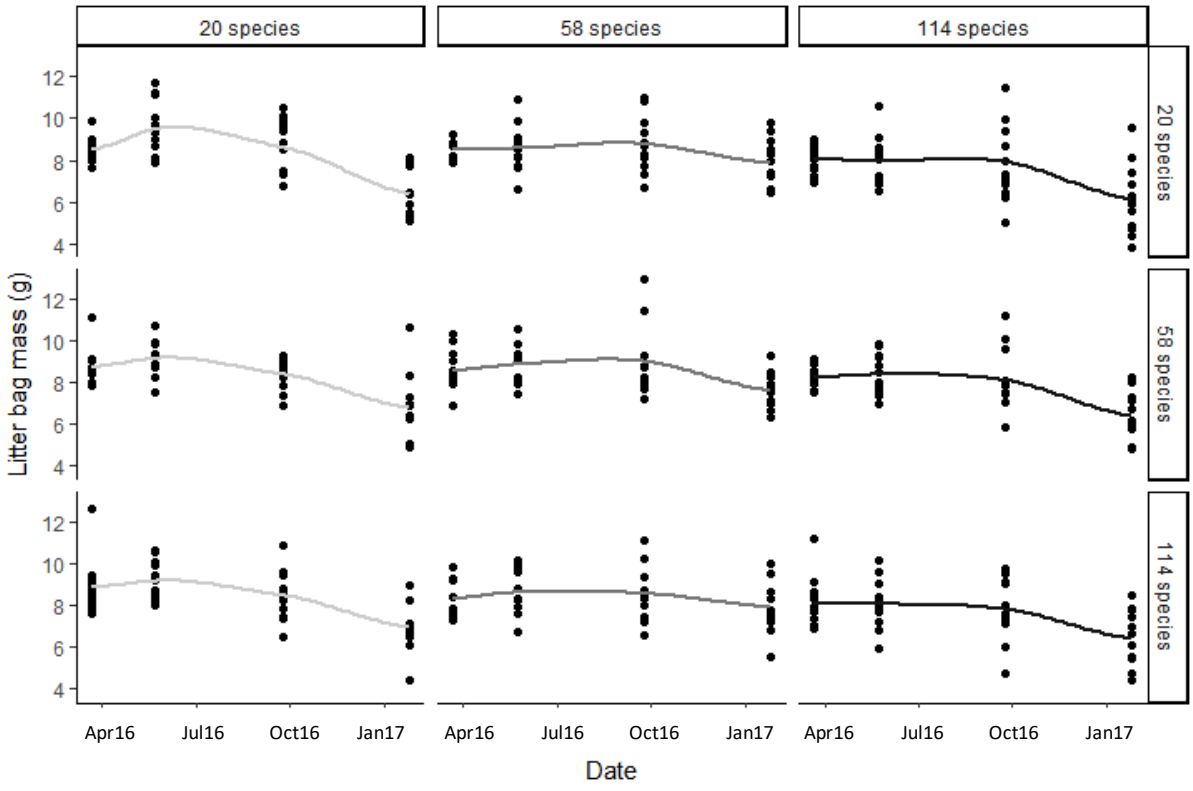



**Figure 20.** Dry mass of litter bags (after content was washed), after each collection, according to stand richness (columns) and richness of litter (rows), at different dates from April 2016 to January 2017, in Anhembi, Brazil. Initial dry wheight of each bag was 10 g.

### 3.3.3. Fine litter stock

Mean fine litter stock (and standard error), measured quarterly over a year, was 8.46 ± 0.24 Mg.ha$^{-1}$ for 20-species mixtures, 9.94 ± 0.56 Mg.ha$^{-1}$ for 58-species mixtures and 8.81 ± 0.39 Mg.ha$^{-1}$ for 114-species mixtures. It varied according to richness levels ($\chi^2$=7.86, d.f.=2, *P*=0.02), but not to collection date ($\chi^2$=0.53, d.f.=1, *P*=0.47) or to their interaction ($\chi^2$=0.91, d.f.=2, *P*=0.63). Stands containing 58 species had the highest litter stock, significantly differing from stands containing 20 species. Plots with the highest richness levels had intermediate litter stocks and did not differ from the others (*P*>0.05; Figure 21).

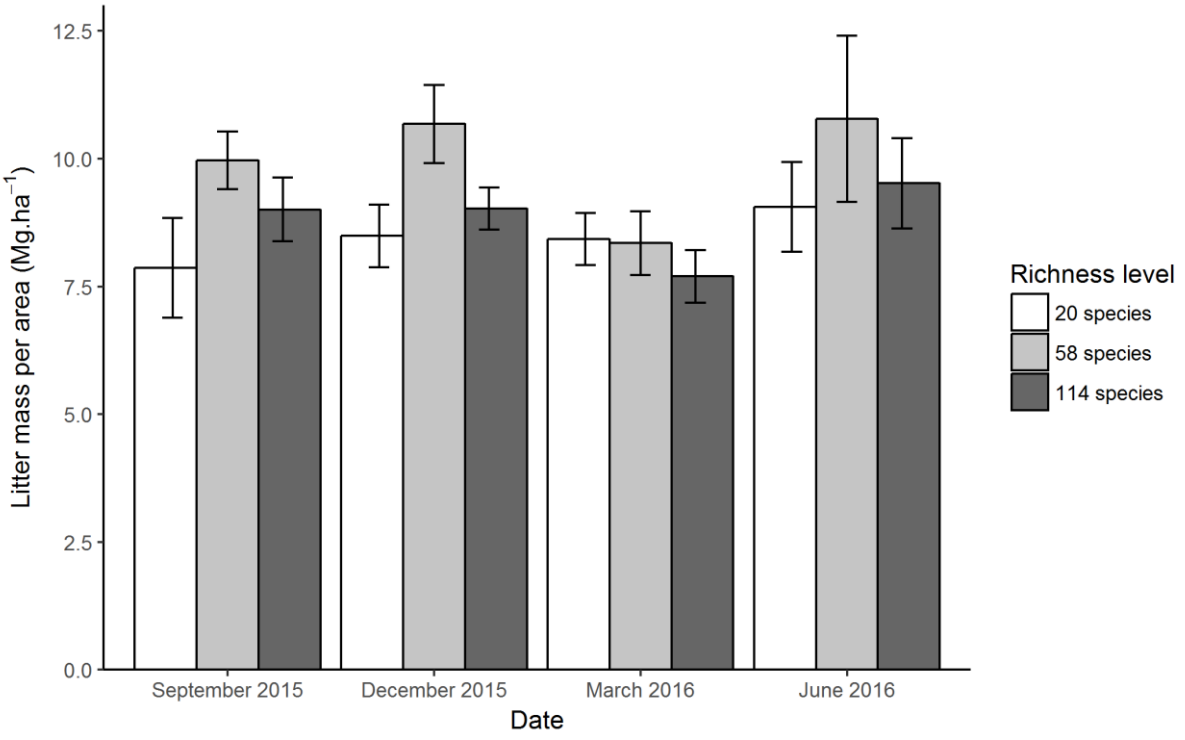



**Figure 21.** Mean litter stock (and standard error) collected quarterly, over one year, in Anhembi, Brazil. Plots containing different richness levels (20, 58 and 114 species) are represented by distinct colors.

### 3.3.4. Fine root stock

Fine root stock within a 30 cm depth was 5.38 ± 0.69 Mg.ha$^{-1}$ in 20-species plots, 5.41 ± 0.20 Mg.ha$^{-1}$ in 58-species plots and 6.44 ± 0.43Mg.ha$^{-1}$ in 114-species plots. Among the four statistics generated in a MANOVA, only the Roy's Largest Root was significant for fine root stock by different richness levels within each soil depth (F=2.95, d.f.1=3, d.f.2=44, *P*=0.04). In univariate tests, treatments marginally differed within the 20-30 deph (F=2.91, d.f.=2, *P*=0.065). *Post hoc* tests (lsmeans) show that the 114-species plots had significantly higher fine root stock

than 20-species plots ($P$<0.05), while intermediate richness levels (58 species) provided intermediate fine root stock, not differing from other stands (**Erro! Fonte de referência não encontrada.**).

Comparing different depths within each richness level, in stands containing 20 and 58 species, all the layers contained different fine root mass ($P$<0.01). Only in stands with 114 species the decrease of fine root biomass in deeper layers was not so sharp, since 10-20 and 20-30 cm layers did not present significantly different amounts of fine roots ($P$=0.18).

Plots containing the lowest species richness level were the ones that contained higher load of grasses an dominant *S. polyphylla* plantlets ($\chi^2$=20.13, d.f.=2, $P$<0.0001), significantly differing from both types of richer plots ($P$<0.01).

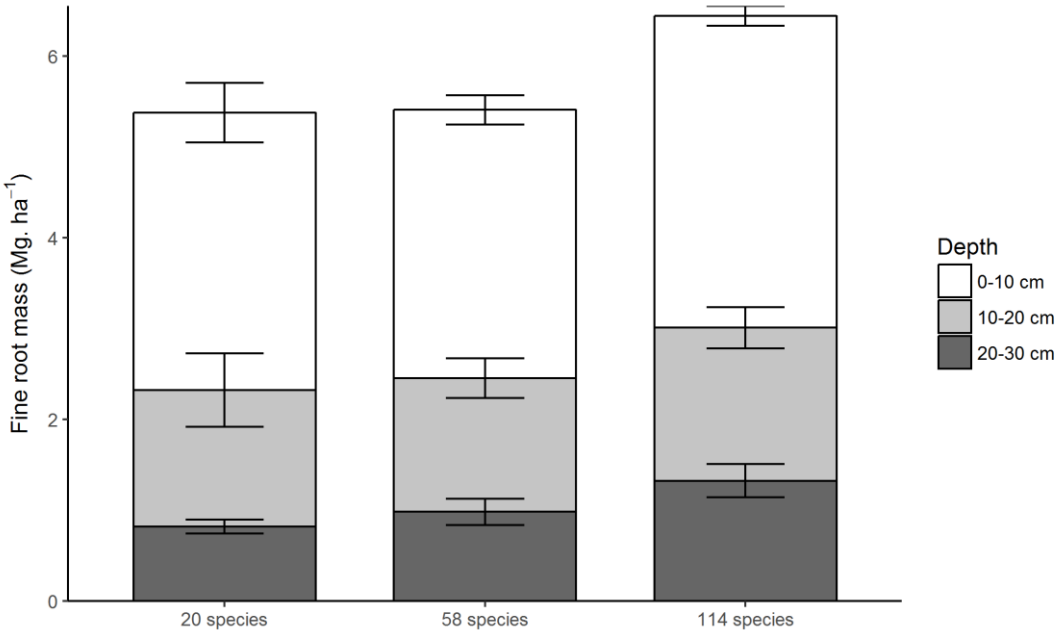



**Figure 22.** Mean fine root stock (and standard error), in distinct soil depths, collected in May, 2015, in Anhembi, Brazil. Plots containing different richness levels (20, 58 and 114 species) are represented by distinct colors.

### 3.3.5. Fine root production

Mean fine root quarterly production, over one year, was 1.16 ± 0.14 Mg.ha$^{-1}$ in 20 species plots, 1.07 ± 0.14 Mg.ha$^{-1}$ in 58 species plots and 1.49 ± 0.08 Mg.ha$^{-1}$ in 114 species plots. It responded only to stand richness ($\chi^2$=29.80, d.f.=2, $P$<0.0001) and depth ($\chi^2$=70.14, d.f.=2, $P$<0.0001; Figure 23), not significantly responding to collection date or interactions between any of the factors accounted for. In the two most superficial layers, stands containing the highest

richness level had significantly higher root production than the others, which did not differ between each other. In the deepest layer, there was no difference in fine root production.

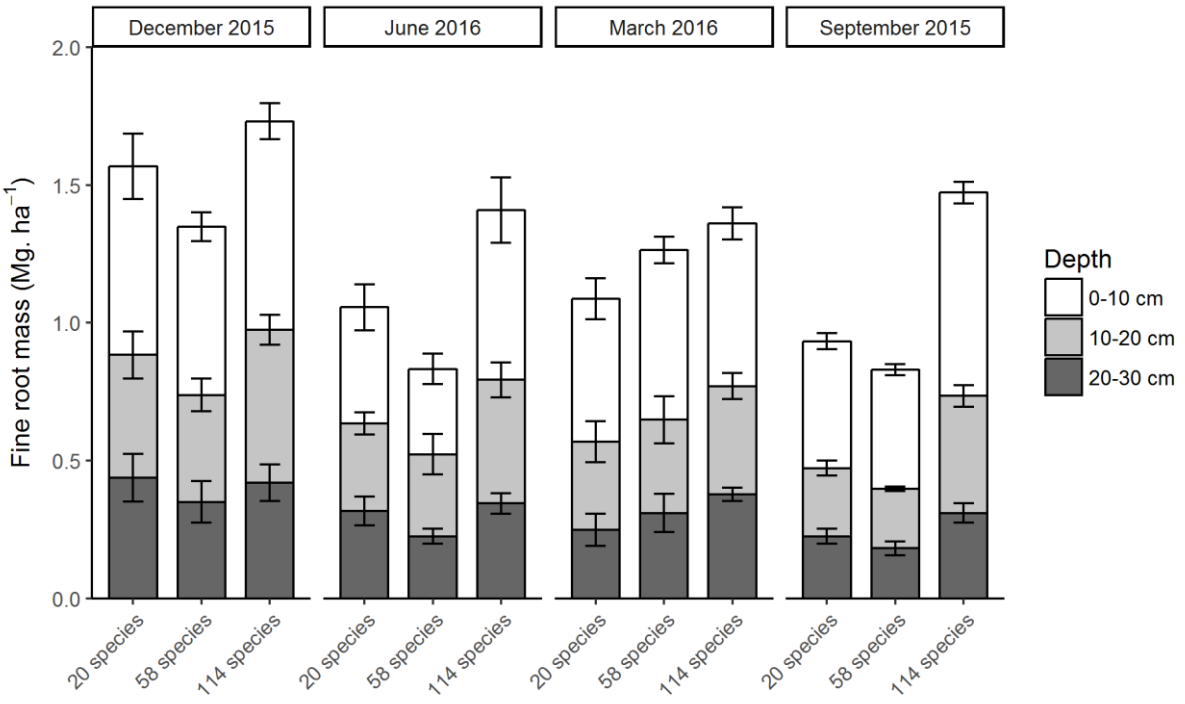



**Figure 23.** Fine root mass produced quarterly, over one year, in different layers (0-10, 10-20 and 20-30 cm) of ingrowth cores placed in stands containing 20, 58 or 114 species, in Anhembi, Brazil.

### 3.4. Discussion

Effects of diversity on below-ground processes analyzed in this work were not as conspicuous as its effects on above-ground processes (for instance, light interception and spatial and temporal distribution, shown in chapter 2). We found effects of tree species richness on litter decomposition and stock and fine root dynamics, while there were no effects of it on litter production. Some of those differences were marginally significant and diversity effects were not always linear. This might contribute for the slow pattern of increase in soil carbon pools (Lange *et al.* 2015), in opposition to above-ground yields, which can be more evident (Potvin & Gotelli 2008). This study is based on a 10-year old forest, which does not yet present a developed understory structure (pers. obs.), is considered young and is not expected to present the structure of an old growth forest (Garcia *et al.* 2016). Below-ground stocks and fluxes are variable over the first years after a restoration plantation (Potvin *et al.* 2011) and might change over the development of a forest.

Litter production was not affected by tree richness. According to Lange *et al.* (2015), high inputs, rather than low outputs, would be the major causes for increasing soil carbon pools at high diversity levels. We expected tree richness to increase litter fall, since this process is related to primary productivity (Facelli & Pickett 1991) and can be boosted by plant diversity (Peh *et al.* 2012; Huang *et al.* 2017). In chapter 2, we observed, for the same study site, higher light interception at the richest plots. This could be related to higher leaf area index and could have provided higher litter production. This did not happen, however. Other studies showed, as well, that this relationship between diversity and litter production is not always straightforward. According to Scherer-Lorenzen et al. (2007), in young restoration forests, litter production was maximum at intermediate richness levels (three-species mixtures), compared to monocultures and six-species mixtures.

Over the time, litter fall differed in distinct months, due to the study site being a semi-deciduous forest (Morellato & Haddad 2000), in which 30 to 50% of the species lose at least part of their leaves during the drier months (Gandolfi *et al.* 2009). At higher richness levels, theory predicts that species could distribute their deciduousness over the time (Sapijanskas *et al.* 2014; Forrester & Bauhus 2016), decreasing temporal variance of litter fall within the whole community, what is called "portfolio effect" (Srivastava & Vellend 2005). Nonetheless, it was not observed in our study, since every richness level showed the same behavior over the time.

On the equation of fine litter inputs and outputs (Díaz *et al.* 2009), decomposition was a factor affected by tree diversity, even though the experiment took place in an unusually dry year (Figure 13), which could have slowed down decomposition rates (Seidelmann *et al.* 2016) and masked differences between treatments. Effects of diversity on decomposition did not happen through providing differential litter content. Richness of litter did not affect decomposition rates, which was also found in other studies (Wardle *et al.* 1997; Scherer-Lorenzen *et al.* 2007; Srivastava *et al.* 2009), even though it is known that species provide distinct litter physical and chemical compositions, capable of decaying at different speeds (Hättenschwiler *et al.* 2005; Scherer-Lorenzen *et al.* 2007). It is not common to find works containing mixed-species litter bags. In general, great part of decomposition studies use bags containing litter of a single species (Hättenschwiler *et al.* 2005; Scherer-Lorenzen *et al.* 2007). They do not faithful represent real ecosystems though, where litter from various species is mixed and may interact, either synergistically or antagonistically, influencing decomposition rates of every species present (Hättenschwiler *et al.* 2005; Peh *et al.* 2012). Decomposition bags containing litter from various species, such as the ones we used, can better represent the diversity of tropical forests (Scherer-Lorenzen *et al.* 2007). Nevertheless, at our experiments, the mixture of a high number of species

(from 20 to more than 100) may have masked identity of plots, since one bag containing only 10 g of litter could hardly represent the diversity within plots containing more than 20 species. Therefore, despite the advantage of better representing litter from tropical forests, mixed bags have the constraint of not permitting good distinction among high levels of richness.

On the other hand, richness of plots did affect decomposition over the time studied, by providing differential environmental conditions for litter decay. Other studies show that features of the environment where decomposition takes place (as diversity of consumers, for instance) are more relevant than litter diversity, in influencing decomposition rates (Wardle *et al.* 1997; Srivastava *et al.* 2009). Still, mechanisms through which plant diversity may provide differential environments to affect litter decomposition are not completely unveiled. Direct effects of plant species richness on decomposition were idiosyncratic, sometimes slowing down (Seidelmann *et al.* 2016) and sometimes slightly enhancing this ecosystem function (Hector *et al.* 2000). Nevertheless, indirect effects of tree diversity on decomposition were observed, since it influenced understory conditions and increased diversity of decomposers, which, in turn, enhanced decomposition rates (Fujii *et al.* 2017). The fact that diversity effect is not straightforward and that it can act in multiple directions when influencing below-ground processes (Wardle *et al.* 2004; Hättenschwiler *et al.* 2005) can explain the non linear relationships we found over the time between species richness and decomposition. In the first two collections of litter bags, decomposition tended to increase according to plot richness level, reasserting that diversity can contribute to increase this ecosystem function (Cardinale *et al.* 2012). After the third collection of litter bags, coincident with the period of the year when trees shed leaves (see chapter 2), this pattern started to change. In the last collection, plots containing intermediate number of tree species showed the lowest decay. Various factors can be leading to this pattern (Seidelmann *et al.* 2016; Fujii *et al.* 2017) and we cannot determine exactly which one is acting. Our hypothesis is that tree richness did favor processes that could enhance decomposition. However, during the coldest months of the year in that region (from May to September) (Alvares *et al.* 2013), this pattern was blurred by the fact that plots with lowest richness levels maintained higher solar incidence (see chapter 2). This could have caused photodegradation (Pan *et al.* 2015) or an increase of temperature that favored decomposer activity (Seidelmann *et al.* 2016). This initial decay could have turned litter into smaller parts and facilitated its process of decomposition through the next season. Seidelmann *et al.* (2016) also found faster decomposition rates under higher solar incidence, in the forest BEF-China experiment.

Interaction between richness within litter content and richness within plots where decomposition took place did not influence litter decay. It means that each plot decomposed

each type of litter bag similarly. Since some specific organisms can better decompose specific litter contents (Hättenschwiler *et al.* 2005), authors believe that there may be an interaction between content of litter and environment of decomposition (Hector *et al.* 2000). The "home-field advantage" hypothesis states that certain litter content is more easily decomposed under a canopy of its same species, rather than under a heterospecific one. It tends to be stronger as the distinction between canopy and litter content increases (Bardgett & van der Putten 2014). At our study, we did not observe this pattern. This could be due to the presence of a large number of species in each litter bag type we used (from 20 to 114 species). There was a small chance that a bag containing litter from a certain species would be placed close to a co-specific neighbor. Another hypothesis more recently proposed, the "substrate quality–matrix quality interaction" is broader than the "home-field advantage". It proposes that, in complex ecosystems, the composition of decomposers should be more strongly influenced by the mainly representative species in litter. Therefore, this community will maintain a positive relationship with part of the litter and a negative relationship with other portion of the same litter content (Freschet *et al.* 2012). Our results do not have the capacity to support this theory either, since no interaction between plot diversity and litter content was found.

Litter stock was also influenced by richness levels. In highly productive ecosystems, compared to poorer ones (*e.g.* pastures), diversity favors mechanisms that enhance both litter production and decomposition. Therefore, it is common that diverse stands, despite having high below-ground carbon inputs, store more carbon above-ground, due to fast decomposition rates (Díaz *et al.* 2009). In this work, since there was no difference in litter fall among plot richness levels, we infer that higher litter stock in stands with intermediate number of species (58) was due to its lower decay, as demonstrated by decomposition curves. Litter stocks, on the other hand, did not change over time, even though some periods of the year clearly showed higher litter fall. That shows that, in our experiment, changes in litter stocks were slower than its inputs and outputs.

Both fine roots stock and production were somehow increased in richer plots, showing the ability of diversity to enhance these processes, which account for below-ground productivity (Brassard *et al.* 2013). These data add information to the discussion on to what extent biodiversity could increase ecosystem functioning, before reaching a saturation plateau (Cardinale *et al.* 2012, and see chapter 2 for more details on this discussion). The richest stands showed smaller distinction between soil layers than the other treatments. Therefore, in plots containing more species, fine roots could probably partition niches (Forrester & Bauhus 2016) and better occupy vertical spaces (Brassard *et al.* 2013), taking higher amount of carbon to distinct layers of soil

(Díaz *et al.* 2009). It is interesting that this pattern was observable even though the poorest stands had a significantly higher amount of grasses and dominant herbs, which may have contributed for total fine roots biomass, since grasses allocate a third of the assimilated carbon to their below-ground parts (Pausch & Kuzyakov 2018). As a consequence of that, richer plots probably counted on higher amount of fine roots from trees.

High levels of richness were also able to enhance fine root production over the time. When considering only the two most superficial layers of soil, it was higher in plots containing 114 species, showing that the level of 58 species had not yet saturated this process. Still regarding fine root production, we could detect no partitioning of it in time (Forrester & Bauhus 2016), since there was no interaction between plot richness and collection date, showing that fine root production within each richness level behaved similarly over the seasons. We also observed that date of collection did not affect fine root production, in opposition of what was found by Brassard *et al.* (2013), who had hypothesized that the growing season would boost root biomass.

Among all ecosystem functions and ecological processes we studied in this work, litter decomposition and stock, as well as fine root stock and production, were related to stand tree species. There is still a lot to find out about the mechanisms underlying below-ground processes, which are very complex (Wardle *et al.* 2004; Lange *et al.* 2015). Diversity can enhance both carbon inputs and outputs, being able to influence its pools in opposite ways, in different situations (Díaz *et al.* 2009). Interactions among above- and below-ground processes are multidirectional and context-dependent (Wardle *et al.* 2004). They influence not only carbon sequestration from the atmosphere, but also cycling of nutrients that are responsible for the maintenance of terrestrial ecosystems (Pausch & Kuzyakov 2018). There lies the importance of increasing the amount of BEF studies considering below-ground processes, in order to better understand how diversity contributes to them in different ecosystems over the world. This information is very relevant in a context of climate change (Wardle *et al.* 2004; Aerts & Honnay 2011) and loss of biodiversity (Cardinale *et al.* 2012), to support conservation and restoration practices.

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# 1. FINAL CONSIDERATIONS

This work showed that very high forest richness levels could enhance ecological processes both above- and below-ground, thus favoring ecosystem functioning. Species richness influenced different processes in distinct compartments of forests and in two different parts of the world. It is an evidence of the importance of conservation and restoration of forest diversity.

In ecological restoration, it is a consensus that achieving desirable diversity levels, according to the ecosystem restored, is an important target to aim at, even for groups that hold distinct points of view (Aronson *et al.* 2011). There is, nevertheless, relevant discussion regarding the way of choosing species to plant, on the onset of a restoration project.

The community approach focuses on reproducing biotic elements of an ecosystem. It is not completely realistic, though, since trajectories of ecosystems are not predictable and it is impossible to follow a recipe to build a forest. Another problem of that approach is that, by reestablishing plant species, it is not guaranteed that ecosystem functions will be restored as well (Wright et al. 2009). However, some authors defend that planting high diversity of species increases the chances of adding functional traits to the ecosystem and enhancing its ecological processes (Rodrigues *et al.* 2009). Other authors criticize this approach, claiming that natural regeneration, a process that is responsible for the constitution of the forest canopy in the future, do not represent richness of the current canopy. Thus planting high richness of seedlings will not necessarily result on a diverse forest, in the future (Durigan *et al.* 2010).

The ecosystem approach focuses on reestablishing certain ecosystem functions, providing basic conditions for taxonomic diversity to naturally increase over the time (Aerts & Honnay 2011). An advantage of concerning about functions which species can play is that they are related to ecological processes responsible for the maintenance of forests that are being restored (Rodrigues *et al.* 2009). Nevertheless, it might be a dangerous proceeding to promote forest restoration focusing only on a group of species that might provide certain ecosystem functions or services. Ecosystems count on multiple functions which, in general, need a high number of species for their maintenance (Hector & Bagchi 2007; Meyer et al. 2018). It is improbable that a limited number of species will be able to provide all of the functions an ecosystem needs (Aerts & Honnay 2011). In addition to that, species may change their provision of functions over the time, thus the presence of higher diversity would add insurance to this provision over the process of forest succession (Wright et al. 2009; Aerts & Honnay 2011). Species also respond differently to disturbances (Wright *et al.* 2009), thus functional redundancy is important to hold ecological processes under climate change and extreme events (Aerts &

Honnay 2011). Another aspect commonly overviewed in the ecosystem approach is that focusing on restoring ecosystem services will not guarantee biodiversity conservation, since there are species that are not relevant for provisioning services, but their existence *per se* is valuable (Meyer et al. 2018). Authors add that there is no evidence that adding high diversity on the onset of a restoration project causes any negative consequences to an ecosystem's stability. It, therefore, may be a sheer economical choice whether or not to perform ecological restoration with high diversity (Wright *et al.* 2009).

The growing biodiversity-ecosystem function approach was proposed by (Naeem 2006). It recognizes that biotic elements can alter other biotic and abiotic elements in the ecosystem and thus influence ecosystem functioning (Aerts & Honnay 2011). This is the point of view adopted in our work. Here, we show that elements from both the community (as planting high number of species) and the ecosystem approach (as focusing on functions that species may provide) can be allied in the biodiversity-ecosystem function approach and become powerful tools for forest restoration.

Some of our results show that specific combinations of species (and even some monocultures) were more effective in providing certain ecosystem processes. Choosing sets of functional traits of interest could be an effective way for forest restoration to recover target ecosystem processes. Knowing which ecosystem processes are performed by each species could be applied to maximize the number of ecosystem functions provided by a forest undergoing restoration.

Our study also shows that, despite those differences in composition, richness levels are able to boost ecological processes. In addition to that, species can benefit from the presence of others, through complementarity effects. High numbers of species were able to provide different understory conditions (regarding light and litter, for instance), which could influence regeneration niches in a forest (Poorter & Arets 2003) and influence the canopy in the future. However, it is only a possibility, since we did not test regeneration under canopies containing distinct richness levels, in this work.

Future research which can contribute to enhancing ecosystem functions in forest restoration may consist in:

- Determining sets of species which contribute to different ecological processes. This will enable forest restoration projects to include as much ecosystem functions as possible, and to distribute species among these functions.

- Studying how provisioning of functions changes over the process of forest succession, in order to assure they will be provided over the time.
- Analyzing effects of high richness levels on natural regeneration, in experiments that control for other factors (as distance from forest fragments, for instance).
- Exploring below-ground ecological processes, which are not considered in great part of BEF studies. In order to understand an ecosystem, it is necessary to consider that above and below-ground parts are integrated and influence each other (Fujii *et al.* 2017). For instance, ecological restoration may be more effective when attention is given to recover not only plants, but also the soil they lie on (Wright *et al.* 2009).

Biodiversity conservation and ecosystem functions are two important goals of forest restoration. This work contributed to understand how they are connected and can be a tool to restore and conserve tropical forests.

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