**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**The impact of environmental and silvicultural factors on the facilitation
and competition processes in pure and mixed plantations of *Eucalyptus* and
*Acacia mangium***

Ivanka Rosada de Oliveira

Thesis presented to obtain the degree of Doctor in
Science. Area: Forest Resources. Option in: Silviculture
and Forest Management

**Piracicaba
2020**

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Forest Engineer

The impact of environmental and silvicultural factors on the facilitation and competition process in pure and mixed plantations of *Eucalyptus* and *Acacia mangium*

versão revisada de acordo com a resolução CoPGr 6018 de 2011

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DEDICATION

To my father, Mario (*in memoriam*), who left us in the middle of this journey and who dreamed of this achievement the most.

To my mother Marilene and my sister Bianca, for always supporting me.

To my life and research companion Bruno, for all the partnership, support and love.

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EPIGRAPH

*“Life is made of moments,
moments that we have to go through,
good or not, for our learning.
Nothing happens by chance.
We need to do our part,
play our role on the stage of life,
remembering that life doesn't always follow our will,
but it's perfect in what it has to be”*

Chico Xavier

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RESUMO

O impacto dos fatores ambientais e silviculturais nos processos de facilitação e competição em plantações puras e mistas de *Eucalyptus* e *Acacia mangium*

Plantações mistas de árvores fixadoras de N_2 (NFT) e árvores não fixadoras de N_2 (não- NFT) tem sido propostas para aumentar a produtividade e os serviços ecossistêmicos em regiões com solos deficientes em N. Essas plantações podem aumentar a disponibilidade, e a eficiência do uso dos recursos naturais (luz, água, nutrientes) quando comparados às monoculturas. No entanto, plantações de espécies mistas no Brasil ainda cobrem uma área muito pequena em comparação às monoculturas. Para mudar esta realidade, estudos sobre as relações entre espécies, condições do solo, do clima e das técnicas de gestão caracterizando as funções e interações com os ecossistemas devem ser realizados a fim de aumentar a produtividade dessas plantações, de uma forma ecológica e eficiente. Nosso estudo teve como objetivo obter informações sobre os processos de facilitação e competição em plantações puras e mistas de *Eucalyptus* e *Acacia mangium*. A tese está estruturada em três partes principais nas quais procuramos compreender melhor os processos ecológicos de plantação puras e mistas sob diferentes condições silviculturais e edafoclimáticas. Utilizamos o traçador isotópico ^{15}N , o modelo MAESTRA, e dados de inventário e biomassa para avaliar os efeitos de competição e de facilitação em plantações puras e mistas de *Eucalyptus* e *Acacia mangium*. Condições contrastantes de fertilização, diferentes densidades de plantio, arranjos espaciais, e condições edafoclimáticas foram consideradas. Na primeira parte, nossos resultados mostraram que o N liberado da decomposição do solo florestal foi 31% maior em parcelas fertilizadas do que em parcelas não fertilizadas, e que houve uma transferência a curto prazo de N abaixo do solo da acácia para os eucaliptos perto da idade da colheita, sendo maior nos tratamentos não fertilizados. Na segunda parte, mostramos que as diferenças entre os tratamentos no crescimento da biomassa do lenho (CBL) do eucalipto foram principalmente explicadas pelas diferenças na absorção de luz, enquanto que as diferenças no CBL de acácia foram explicadas pela absorção de luz e eficiência na utilização de luz. A maior produtividade do povoamento foi observada em 100E com 1666 árvores ha⁻¹. Contudo, o eucalipto no arranjo 33A:67E com 1111 árvores ha⁻¹ teve um CBL maior do que em 50A:50E e uma produção semelhante ao 100E. Além disso, o CBL, a absorção de luz e a eficiência do uso da luz de *Acacia* resultaram dos efeitos combinados da densidade do povoamento e da presença de *Eucalyptus*, enquanto que para os *Eucalyptus* estas variáveis foram fortemente dependentes dele mesmo, com baixo ou nenhum impacto da *Acacia*. Na terceira parte, os nossos resultados mostraram que condições estressantes, como secas mais quentes, levaram à uma maior mortalidade dos eucaliptos em Sinop-MT e das acácias em Colinas-TO. O maior crescimento e maior produção de biomassa dos eucaliptos foi encontrado em Itatinga-SP, enquanto que o maior crescimento e produção da *Acacia* ocorreu em Sinop-MT. Nós mostramos como o manejo florestal pode impactar o comportamento das plantações de espécies puras e mistas de *Eucalyptus* e *A. mangium* e como as mudanças no clima e nas propriedades do solo podem afetar a sobrevivência e desenvolvimento de cada espécie. Neste projeto, evidenciamos o interesse de associar a acácia aos eucaliptos em plantações florestais para limitar as deficiências de N do eucalipto quando os povoamentos são estabelecidos em solos de baixa fertilidade e/ou quando não são aplicados fertilizantes. Destacamos a importância de definir o arranjo e densidade de plantio ótima para melhorar a produção florestal, bem como a importância das características edafoclimáticas em condições de stress para evitar uma grande taxa de mortalidade e promover a gestão sustentável das plantações florestais.

Palavras-chave: Plantações mistas, Facilitação, Competição, Fertilização, Absorção de luz, Secas quentes

ABSTRACT

The impact of environmental and silvicultural factors on facilitation and competition processes in pure and mixed plantations of *Eucalyptus* and *Acacia mangium*

Mixed forest plantations of N₂-fixing trees (NFT) and non-N₂-fixing trees (non-NFT) have been proposed to increase productivity and ecosystem services in regions with N-deficient soils. These intercropped systems can increase availability, use, and efficiency of natural resources (light, water, nutrients) when compared to monocultures. However, mixed-species plantations in Brazil cover a very small area in comparison to monocultures. To overcome this situation, studies must be carried out on the relationships between species, soil, and climate conditions, and management techniques by characterizing ecosystem functions and interactions to increase productivity of mixed-species plantations compared to monocultures, in an ecologically friendly and efficient way. Our study aimed to gain insights into facilitation and competition processes in pure and mixed-species plantations of *Eucalyptus* and *Acacia mangium*. The thesis is structured in three main parts where we aimed to better understand the ecological processes of pure and mixed-species plantations under contrasting silvicultural and edaphoclimatic conditions. We used ¹⁵N isotopic tracer, MAESTRA model, and inventory and biomass data to assess the effects of competition and facilitation process in pure and mixed-species plantations of *Eucalyptus* and *Acacia mangium* under contrasting nutrient supply, different planting densities, and spatial arrangements, as well as different climatic and soil conditions. In the first part, our results showed that the N released from forest floor decomposition was 31% higher in fertilized than in unfertilized plots, and that short-term belowground N transfer from *Acacia* to *Eucalyptus* was found close to harvest age and higher with lower nutrient supply. In the second part, we showed that the differences between treatments in the stem biomass growth (SBG) of *Eucalyptus* was mainly explained by differences in light absorption, while the differences in SBG of *Acacia* was explained by light absorption and light use efficiencies. The highest stand productivity was observed in 100E with 1666 trees ha⁻¹. However, *Eucalyptus* in the arrangement 33A:67E with 1111 trees ha⁻¹ had a greater SBG than in 50A:50E and a similar production than in 100E. Moreover SBG, light absorption and light use efficiency of *Acacia* resulted from the combined effects of stand density and presence of *Eucalyptus*, while for *Eucalyptus* these variables were strongly dependent on *Eucalyptus* stand with low or no impact of *Acacia*. In the third part, our results showed that stressful conditions, such as hotter droughts, led to higher *Eucalyptus* mortality in Sinop-MT and higher *Acacia* mortality rates in Colinas-TO. The greatest growth and highest biomass production of *Eucalyptus* trees was found in Itatinga-SP, while the highest tree growth and stand production for *Acacia* occurred in Sinop-MT. Our results provided insights into how silvicultural practices may impact the behavior of pure and mixed-species plantations of *Eucalyptus* and *Acacia mangium* and how changes in climate and soil properties may affect the survival and development of each species. In this project, we stressed the interest to associate *Acacia* to *Eucalyptus* in forest plantations to limit N eucalypt deficiencies when stands are established in low-fertility soils and/or fertilizers are not applied. We highlighted the importance to define optimal planting design and planting density to improve forest production, as well as account for climatic, and soil characteristics under stressful conditions to avoid large mortality rate and promote sustainable forest plantation management.

Keywords: Mixed plantation, Facilitation, Competition, Fertilization, Light absorption, Hotter droughts

RÉSUMÉ

L'impact des facteurs environnementaux et sylvicoles sur les processus de facilitation et de compétition dans les plantations pures et mixtes d'*Eucalyptus* et d'*Acacia mangium*

Les plantations mixtes d'arbres fixateurs N₂ (NFT) et d'arbres N₂ non fixateurs (non NFT) sont capables d'augmenter la productivité et les services écosystémiques dans les régions où les sols sont pauvres en N. Ces plantations peuvent augmenter la disponibilité, et l'efficacité de l'utilisation des ressources naturelles (lumière, eau, nutriments) par rapport aux monocultures. Cependant, les plantations mixtes au Brésil couvrent encore une très faible superficie par rapport aux monocultures. Pour changer cette réalité, des études portant sur les relations entre les espèces, le sol, les conditions climatiques, des techniques de gestion caractérisant les fonctions et des interactions avec les écosystèmes doivent être menées afin d'augmenter la productivité de ces plantations, de manière écologique et efficace. Notre étude a pour objectif d'obtenir des informations sur les processus de facilitation et de compétition dans les plantations pures et mixtes d'*Eucalyptus* et d'*Acacia mangium*. La thèse est structurée en trois parties principales dans lesquelles nous cherchons à mieux comprendre les processus écologiques qui ont lieu en plantation pures et mixtes et dans différentes conditions sylvicoles et édaphoclimatiques. Différentes techniques ont été utilisées comme le traceur isotopique ¹⁵N, le modèle MAESTRA et les données d'inventaire et de biomasse afin d'évaluer les effets de compétition et de facilitation dans les plantations pures et mixtes d'*Eucalyptus* et d'*Acacia mangium*. Conditions contrastées de fertilisation, différentes densités de plantation, d'arrangements spatiaux et de conditions édaphoclimatiques ont été considérées. Dans la première partie, nos résultats ont montré que le N libéré par la décomposition du sol forestier était plus élevé de 31 % dans les parcelles fertilisées par rapport aux parcelles non fertilisées et qu'il y avait un transfert à court terme de N sous le sol, des *Acacia* vers les *Eucalyptus* lorsque l'on approche de l'âge de récolte et étant plus élevé dans les traitements non fertilisés. Dans la deuxième partie, nous montrons que les différences entre les traitements dans la croissance en biomasse des tiges (CBT) d'*Eucalyptus* sont principalement dues à l'absorption de lumière, tandis que les différences de CBT des *Acacia* résultent de l'absorption de la lumière et de l'efficacité d'utilisation de la lumière. La plus forte productivité a été observée dans le traitement 100E avec une densité de 1666 arbres ha⁻¹. Cependant, l'*Eucalyptus* dans l'arrangement 33A: 67E avec 1111 arbres ha⁻¹ avait un CBT supérieur à celui dans le traitement 50A: 50E et une production similaire aux *Eucalyptus* dans le traitement 100E. En outre, le CBT, l'absorption de la lumière et l'efficacité de l'utilisation de la lumière des *Acacia* résultent des effets combinés de la densité de plantation des arbres et de la présence d'*Eucalyptus*, tandis que pour l'*Eucalyptus*, ces variables sont fortement dépendantes d'elles-mêmes, avec peu ou pas d'impact de la présence des *Acacia*. Dans la troisième partie, nos résultats ont montré que des conditions stressantes, telles que des sécheresses couplées avec des fortes températures ont conduit à une forte mortalité des *Eucalyptus* à Sinop-MT et des *Acacia* dans le site de Colinas-TO. La plus forte croissance et la production de biomasse la plus élevée des *Eucalyptus* ont été observées au site de Itatinga-SP, tandis que la croissance et la production la plus importante pour les *Acacia* ont eu lieu à Sinop-MT. Nous avons montré comment la gestion forestière peut avoir un impact sur le comportement des espèces en plantations pures et mixtes d'*Eucalyptus* et *A. mangium* et comment les changements de climat et des propriétés du sol peuvent affecter la survie et le développement de chaque espèce. Dans ce projet, nous avons voulu mettre en évidence l'intérêt d'associer des arbres d'*Acacia* et d'*Eucalyptus* en plantation forestière dans le but de limiter les carences en N des *Eucalyptus* lorsque les peuplements sont implantés sur des sols à faible fertilité et / ou lorsque les engrais ne sont pas appliqués. Nous soulignons l'importance de définir la densité et les

arrangements optimaux entre les espèces pour améliorer la production forestière, ainsi que l'importance des caractéristiques édaphoclimatiques dans des conditions de stress pour éviter un taux de mortalité élevé et promouvoir la gestion durable des plantations forestières.

Mots clés: Plantations mixtes, Facilitation, Compétition, Fertilisation, Absorption de lumière, Sécheresse

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and 2 m x 3 m). Different letters indicate significant differences ($P < 0.05$): A, B, C for differences between treatments; a, b, c for differences between *Eucalyptus* trees; α and β for differences between *Acacia* trees. Black bars represented total LUE in 33A:67E and 50A:50E.

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1. INTRODUCTION

For several years, forest plantations have been managed without concerns with their sustainability (Forrester et al., 2006). However, this scenario has changed due to increased costs of fertilizers (Brunelle et al., 2015), reduced biodiversity, productivity losses due to pest and disease attacks (Gonçalves et al., 2013), and increased in tree mortality caused by climate changes (i.e. increases in temperature and droughts) (Allen et al., 2015; Gonçalves et al., 2017). In addition, most tropical forest plantations are restricted to regions with nutrient depleted soils (Laclau et al., 2010; Stape et al., 2010; Gonçalves et al., 2013; Keenan et al., 2015). In Brazil, monocultures of *Eucalyptus* have stood out in relation to native species, showing faster growth and good wood quality with multiple uses (Gonçalves et al., 2013).

One of the main limiting factors to tree growth is the nutritional deficiency of N (Vitousek et al., 2010). Without N fertilization, negative N balances in *Eucalyptus* plantations could drastically reduce the levels of N availability in the soil for trees (Bouillet et al., 2014). The main problems related to N are the high rates required by plants and their high cost. Due to phosphorus (P) markets and tensions over fossil energy, the increase in fertilizer prices (+ 9% per year on average) observed in recent decades may persist in the future (Brunelle et al., 2015). A solution to meet this nutritional demand and ensure sustainability of forest plantations is to introduce fast-growing N₂-fixing trees (NFT) with non-N₂ fixing trees (non- NFT). Mixed plantations of NFT and non-NFT could improve soil fertility and nutrient cycling, reducing fertilization costs (Binkley, 2003; Forrester, et al., 2006; Laclau et al., 2008).

Mixed-planted forests can improve the provision of ecosystems services (Balieiro, et al. 2020). The introduction of NFT to forest plantations could improve the soil nutritional status by increasing the potential mineralizable organic N (Fisher and Binkley, 2000), improving N soil availability (Paula et al., 2015; Tchichelle et al., 2017; Santos et al., 2017; Voigtlaender et al., 2019) and increasing P availability (Koutika, 2019; Aleixo et al., 2020). NFT may enhance P availability through rhizosphere acidification (Hinsinger et al., 2011), increasing the amount of P in intercropped systems than in monocultures (Forrester et al., 2005). NFT can also increase the soil carbon stocks (Balieiro et al., 2008; Koutika et al., 2014), fine roots biomass (Laclau et al. 2013; Germon et al., 2018; Bordron et al, 2020 in press), soil fauna, and microbial diversity (Rachid et al., 2013; Pereira et al., 2018). These intercropped systems can improve biomass production (Forrester et al, 2004; Bouillet et al., 2013; Santos et al., 2016), light absorption (le Maire et al., 2013), light use efficiency of the

canopy (Forrester et al., 2012), and water use efficiency (Forrester et al., 2010), in comparison to monocultures.

Among the main N₂-fixing species *Acacia mangium* is an interesting option for the production of timber and non-timber products. *Acacia mangium* is a fast-growing species (first cut at 4 years), providing a diversification of products (Khanna, 1997; Montagnini, 2000). In Southwest Asia, *A. mangium* stands out for the large commercial plantations for pulp production, besides other products, such as charcoal for energy (Yamashita et al., 2008). *Acacia mangium* has also a capacity to shade competitors quickly, capacity to absorb large amounts of rainwater associated to essential nutrients, and high tolerance to compacted, acidic, and nutrient-poor soils (Lorenzi et al. 2003; Midgley and Turnbull 2003; Balieiro et al. 2007). In Asia, it has been widely used in reforestation programs (Midgley and Turnbull 2003).

Acacia mangium can fix N₂ from the atmosphere when it is in symbiosis with rhizobium (Galiana et al., 2002; Bouillet et al., 2008; Balieiro et al., 2010; Paula et al, 2018), with values ranging from 250 to 400 kg N ha⁻¹ during a rotation (Voigtlaender et al., 2019). This species can also transfer N through belowground processes (direct or indirect contact between fine roots with two species), providing significant amounts of N to Eucalyptus trees (Paula et al., 2015). *Acacia mangium* has potential to deposit annually between 5 to 12 dry tons of litter per hectare, which represent 150 to 300 kg of N inserted into the soil, increasing the rate of N mineralized into the soil (Voigtlaender et al., 2012). Litterfall accumulation under *A. mangium* also benefits the recovery of degraded areas by protecting the soil from erosion and temperature fluctuation, increasing nutrient reserves thus used in reforestation to recover degraded areas (Griffin et al., 2011). In addition, studies have shown that *A. mangium* can affect P cycling and bioavailability (Waithaisong et al., 2020) by increasing phosphatase release from the roots, stimulating soil organic matter (SOM) decomposition (Forrester et al., 2005) and changing microbial communities (Santos et al., 2017; Pereira et al., 2019) due to higher P inputs through harvest residue and litterfall (Koutika et al., 2019). Nevertheless, the potential invasion of *Acacia mangium* has been observed in some regions (Attias et al., 2014; Moura et al., 2020). These environments are normally open and altered, with little or no forest cover, favoring dispersion and germination of species propagules (Pysek and Richardson 2007; Moura et al., 2020). In forest areas and pastures, commercial plantations of exotic species are rarely associated to biological invasions, due to the high competitive capacity of native species (Moura et al., 2020) and the short crop rotation (6 to 8 years) (Richardson 1998).

Studies have shown that intercropped systems with NFT can be more productive than monospecific forest plantations (Binkley et al., 2003; Forrester et al., 2006; Bouillet et al., 2013; Santos et al., 2016; Marron and Epron, 2019). Complementarity of above and belowground resource use is one of the causes that lead to increased productivity of mixed forest plantations, favouring greater efficiency in the use of natural resources (Kelty, 2006). Nonetheless, there are cases of neutral or reduced productivity when NFT are mixed with Eucalyptus plantations (Forrester et al., 2006; Firn et al., 2007; Laclau et al., 2008; Bouillet et al., 2013; Epron et al., 2013; Koutika et al., 2014). The species interactions in mixed plantations depend on the balance between competition and facilitation processes (Vandermeer, 1989). Competition processes can be observed in the aboveground compartment through competition for light use (Hunt et al., 2006) and belowground as competition for water and nutrients (Boyden et al., 2005; Silva et al., 2009). Facilitation can include direct and indirect process. In the direct process, one species increases availability of a resource for another species, improving harsh environmental conditions or increasing resource availability, whereas in the indirect process, the facilitating plant changes environmental condition of the other (Callaway, 1995; Little et al., 2002; le Maire et al., 2013). Another beneficial process is the complementarity of resource use, which can lead to a more efficient capture of resources when compared to monocultures, as well as the reduction of competition (Bauhus et al., 2000).

Depending on the balance between competition and facilitation, intercropping can be 'underyielding', when the mixture produces less than the sum of the proportions of species production in monoculture, 'overyielding', when the mixture produces more than the sum of the proportions of species production in monoculture, but less than the most productive monoculture, or 'transgressive overyielding', when the mixture production is greater than the most productive monoculture (Pretzsch and Schütze, 2009). This balance may change with time and environment effects and can be explained by the Stress Gradient Hypothesis (SGH) (Maestre et al., 2009; Holmgren and Scheffer, 2010; Kikvidze et al., 2011; Forrester, 2014). The SGH proposals indicate that the frequency and importance of facilitation should increase with increasing environmental severity (Callaway and Walker, 1997). However, studies indicated that facilitation between species would be greater under moderate stress than under extreme conditions (Callaway, 1995; Castanho et al., 2015). Facilitative and competitive interactions between NFS and non-NFS in mixed-species forests could be influenced by soil fertility (Boyden et al., 2005; Bordron et al., 2020 in press). There are two viewpoints about this question. The first predicts that, in soils with low fertility, neighboring

plants have a smaller competitive effect (Bertness and Callaway 1994; Baribault and Kobe, 2011; Coates et al., 2013). The second viewpoint predicts that in soils with lower fertility, there is greater competition for soil resources (Trinder et al., 2012). Studies have shown the positive influence interaction (e.g. facilitation, complementary, transgressive overyielding) from NFT to non-NFT in soils with poor N availability (Boyden et al., 2005; Pretzsch et al., 2010; Bouillet et al., 2013; Santos et al., 2016). On the other hand, Boyden et al. (2005) showed high facilitation with higher P availability in the soil.

Facilitation for N can be studied by the use of ^{15}N isotopic tracers. The stable isotope ^{15}N has been used in recent studies (Paula et al., 2015; Bordron et al., 2019; Pinheiro et al., 2019; Yao et al., 2019). The ^{15}N tracer allows marking the soil or via direct injection into the xylem to obtain estimates of the rate of N transfer from NFT to non-NFT (%Ndft), based on differences in ^{15}N enrichment in NFT and non-NFT tissues (used as reference for fixation). The ^{15}N tracer allowed estimating N transfer between plants (Chalk et al., 2014) through belowground mechanisms (Paula et al., 2015).

Another factor that affects facilitation processes is the design and density trees in intercropped systems, which could be adapted to local conditions to maximize positive interactions (Forrester et al. 2006; Marron and Epron, 2019). In recent years, studies have been carried out on mixed *Eucalyptus* and *Acacia mangium* plantations, used different designs and environmental conditions (Laclau et al., 2008; Bouillet et al., 2013; le Maire et al., 2013). An important issue for designing mixed plantations is the definition of the optimal spatial arrangement for the study of interspecific and intra-specific interactions and, at the same time, applicable at the level of rural enterprises and producers. Planting arrangement is one of the main factors that influences tree sizes (Gerrand et al., 1997) and it determines the wood quality and applications (Persson et al., 1995). Tree spacing allows inferring the number of trees in a given area and determining the best management and harvesting procedures (Scolforo, 1997).

Le Maire et al. (2013) showed that in mixed plantations with 3 m x 3 m spacing, Eucalypts trees quickly dominate *Acacia* trees. The authors also found that canopy stratification led to an increase in the leaf area index (LAI) and light absorption, important for carbon and water balance of mixed stands. Bouillet et al. (2013) studied pure and mixed plantations of *Eucalyptus* and *Acacia mangium* in Southeast Brazil and Congo. The results showed that mixed plantation had a relatively higher productivity compared to eucalyptus monoculture when inserted in areas characterized by nutrient-poor soils, with hot and humid climates and with low water limitation, as is the case in Congo. These conditions are

favorable to *Acacia* growth (Catie, 1992; Rossi et al., 2003). However, they are not optimal for eucalyptus plantations, explaining the success of the intercropped systems. Santos et al. (2016) studied the *Eucalyptus* and *Acacia* mixed plantation in Seropédica-Rio de Janeiro (Brazil), in a region with sandy N-deficient soil and favourable climate for *Acacia*, and found higher productivity in mixture than in pure *Eucalyptus* plantations. A recent meta-analysis identified that site condition is the main factor influencing the success of mixed plantations (Marron and Epron, 2019). Therefore, the introduction of mixed plantations of *A. mangium* and *Eucalyptus* presents several ecological and economic advantages, as it provides diversification and reduced costs and risks for forest production. The intercropped system is also an alternative for the recovery of degraded areas and may boost the economic activity for small and medium rural producers, as observed in other countries (e.g. Vietnam, India) (Griffin et al., 2011).

Studies should be developed on the relationships between species, soil, and climate conditions, and management techniques through the characterization of ecosystem functions and interactions in order to increase productivity of mixed plantations compared to monocultures, in an ecologically correct and efficient way (Binkley et al., 2003; Jackson et al., 2008). Our research project aimed to better understand inter and intra-specific interactions of NFT and non-NFT. The study analyzed the processes of facilitation and competition in mixed plantations of *Eucalyptus* and *A. mangium* trees, submitted to different silvicultural treatments and planted in different regions of Brazil. This thesis was structured in three parts.

First, we investigated the effect of soil nutrient deficiencies on short-term belowground N transfer from NFTs to non-NFTs in mixed-species plantation of *E. grandis* x *E. urophylla* and *A. mangium*. We hypothesized that (1) N litter deposition, N stocks in the forest floor, and rate of N release from forest floor decomposition are higher in fertilized than non-fertilized plots, (2) short-term belowground N transfer occurs between *Acacia* and *Eucalyptus* close to harvest age, regardless of the N content in the soil, and (3) the rate of belowground N transfer is higher in non-fertilized than in fertilized plots.

The second part assessed how design and tree spacing affect tree growth and wood productivity in pure and mixed-species plantation of *E. grandis* and *A. mangium*, disentangling the effect of light competition (analysis of tree and stand absorbed photosynthetically active radiation, APAR) from other effects (analysis of tree and stand Light Use Efficiency, LUE). We aimed to answer four specific questions APAR and LUE in

pure and mixed *Eucalyptus* and *Acacia mangium* plantations: (1) How do APAR and LUE of *Eucalyptus* vary among planting designs? (2) What is the correlation between APAR, LUE and stem biomass growth (SBG)? (3) What is the impact of tree densities on APAR and LUE, and their effects on wood production? and, (4) Is there an optimal tree density and NFT- non-NFT ratio for maximizing total wood production or non-NFT wood production?

The third part investigated the effects of climatic and soil conditions on the mortality rate, tree growth and stand production of pure and mixed plantations (1:1) of *Eucalyptus* and *Acacia mangium* established in three regions of Brazil, South-East (São Paulo), North (Tocantins), and Central-West (Mato Grosso), with contrasting soil and climatic conditions. We tested the hypotheses that (1) in Colinas-TO, characterized by high temperatures and harsh dry seasons, the mortality rate will be higher for *Acacia* than *Eucalyptus* and higher in mixture than in pure stands, (2) in Sinop-MT, where the climate conditions are favorable for the development of *Acacia* trees and unfavorable for *Eucalyptus* trees, the mortality rate of *Eucalyptus* will be higher than *Acacia* and higher in mixture than in pure stands, and (3) *Eucalyptus* have a higher tree growth and stand biomass production in Itatinga than in Colinas, while for *Acacia* tree growth and stand production will be higher in Sinop than in Itatinga.

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2. LOWER N SUPPLY ENHANCED THE RATE OF SHORT-TERM BELOWGROUND TRANSFER OF NITROGEN FROM *ACACIA MANGIUM* TO *EUCALYPTUS* TREES IN MIXED-SPECIES PLANTATION

ABSTRACT

Short-term belowground N transfer from Nitrogen Fixing Tree (NFT) may provide significant amounts of N requirements of associated non-NFT in mixed forests, as previously shown at 26 months in a mixed-species plantation of *Acacia mangium* and *Eucalyptus*. However, there was no *in situ* information on the magnitude of this facilitation process depending on tree age and nutrient supply. Our study aimed to assess the effect of fertilization on the rate of N transfer from *A. mangium* trees to *Eucalyptus* trees (%NDFT) in a mixed-species plantation with 50% of each species at 2.5 m x 2.5 m spacing, in a complete randomized block design with three replicates. The collection of litterfall between 46 and 58 months after planting and on forest floor at 46 and 58 months after planting allowed to estimate annual N release from forest floor decomposition. $^{15}\text{N-NO}_3^-$ was injected into the stem of a dominant *Acacia* tree in each plot, 58 months after planting. The $x(^{15}\text{N})$ of fine roots of *Acacia* and *Eucalyptus* sampled within 1.8 m radius from the labeled *Acacia* tree was measured at 7, 14, 30 and 60 days after labeling. The $x(^{15}\text{N})$ was also estimated in the wood, bark, branches, and leaves of the 6 labeled *Acacia* trees and 12 *Eucalyptus* neighbors, before and at 60 days after labeling. N release from forest floor decomposition was 31% higher in fertilized (F+) than in unfertilized (F-) plots. Sixty days after labeling, the aboveground compartments of *Eucalyptus* were ^{15}N enriched in both treatments. Fine *Acacia* roots had higher $x(^{15}\text{N})$ values than $x(^{15}\text{N})$ background values from 7 and 30 days after labeling onwards in F+ and F-, respectively. Fine *Eucalyptus* roots had higher values of $x(^{15}\text{N})$ than $x(^{15}\text{N})$ background values in both treatments, from 30 days after labeling onwards. The average value of %NDFT was 18.0 % in F+ and 33.9 % in F- during the study period, and 22.8 % in F+ and 67.7 % in F- accounting for the last 30 days of the experiment. Belowground N transfer from *Acacia* to *Eucalyptus* was then observed for trees close to harvest age in both treatments, with higher value of %NDFT with lower nutrient supply. This result reinforces the interest to associate *Acacia* to *Eucalyptus* in forest plantations to limit N eucalypt deficiencies when stands are established in low-fertility soils and/or fertilizers are not applied.

Key-words: ^{15}N , fertilization, facilitation, mature trees, nitrogen fixing trees, Brazil.

2.1. Introduction

Most of tropical forest plantations are established in nutrient depleted soils (Laclau et al., 2010; Stape et al., 2010; Mareschal et al., 2011; Keenan et al., 2015). Large amounts of biomass harvested every 6-7 years in commercial *Eucalyptus* plantations are likely to lead to unbalanced input-output nutrient budgets, such as nitrogen (N) (Laclau et al., 2010; Voigtlaender et al., 2019). N fertilizers are commonly applied to meet the nutrient tree requirements, maintain high production of stands, and ensure long-term sustainability of *Eucalyptus* plantations (Gonçalves et al., 2013; Koutika et al., 2014). However, the use of N

fertilizers may be limited in the future because of their rising costs (Brunelle et al., 2015) and environmental impacts through nitrate leaching, NH_3 volatilization, or nitrogen oxide emissions (Binkley and Fisher, 2019).

Acacia mangium (Willd.) is a fast-growing tree species largely planted in South-East Asia for the pulp industry that stands out among the Nitrogen Fixing Tree species (NFT) of high silvicultural interest (Yamashita et al., 2008). The association of *A. mangium* with *Eucalyptus* can be an alternative to the use of N fertilizers. *A. mangium* can fix large amounts of atmospheric N_2 , as in Brazil, with 250 kg N ha^{-1} fixed in mixed-species plantations of *A. mangium* and *Eucalyptus grandis* W.Hill ex Maiden during a 6-year stand rotation (Voigtlaender et al., 2019). Comparable amounts of fixed N were found in Congo, in a 2-year-old mixed-species plantation of *A. mangium* and *E. urophylla* S.T. Blake x *E. grandis* (Tchichelle et al., 2017a). The N input from N_2 fixation by NFT can improve the N status of companion species through the decomposition of aboveground N-rich litter (Munroe and Isaac, 2014; Santos et al., 2017; Tchichelle et al., 2017b) and fine roots (Bachega et al., 2016). Moreover, non-NFT may benefit from short-term belowground transfer of N from NFT, as observed in pot experiments (He et al., 2004; 2005; Yao et al., 2019) and in the field with N transfer from *A. mangium* to *Eucalyptus* trees within a radius of 6.2 m around *Acacias* trees (Paula et al., 2015). However, to the best of our knowledge, there is no information on the change in the magnitude of this facilitation process depending on soil fertility.

Facilitation occurs when at least one species benefits from another. The balance between facilitation and competition between plant species depends on resource availability. According to the stress-gradient hypothesis, competitive interactions decrease and facilitation increases under stressful environmental conditions (Callaway and Walker, 1997; Maestre et al., 2009; Holmgren and Scheffer, 2010; Kikvidze et al., 2011). Studies have consistently shown that competition between species decreased at low soil fertility (Baribault and Kobe, 2011; Coates et al., 2013). However, opposite results were also found with high interspecific competition in low-fertility site (Newmann, 1973; Trinder et al., 2012). Another concern is the target species, as tree growth was reduced by *Eucalyptus* neighbors on high-phosphorus soils and facilitated on low-phosphorus soils in a mixed-species plantation of *Falcataria mollucana* and *Eucalyptus saligna* (Boyden et al., 2005). Conversely, *Eucalyptus* tree growth was reduced by *Falcataria* neighbors on low-phosphorus soils, but increased in high-phosphorus soils. In fertilized plots, *A. mangium* growth was highly depressed by the competition of associated *Eucalyptus* trees, whilst the competition of *Acacia* on *Eucalyptus* trees was low (Bordron et al., 2020 in press).

The fixation rate of N_2 by *Acacia mangium* decreased when soil fertility increased in *Acacia* monocultures (Galiana et al., 2002), and was higher in association with *Eucalyptus* than in *Acacia* monocultures, possibly due to *Eucalyptus* competition for soil N (Paula et al., 2018). In pot experiments, N application reduced the N_2 fixation of *Dalbergia odorifera* and led to lower N transfer from *Dalbergia* to *Eucalyptus* seedlings (Yao et al., 2019). Between 16 and 34 months, soil nutrient deficiency could promote belowground facilitation in mixed-species plantations of *Eucalyptus* and *A. mangium* through an increase in the density of *Eucalyptus* fine roots close to *Acacia* trees where *Eucalyptus* trees possibly took advantage of higher soil N availability (Bordron et al., 2020 in press). Another facilitation process should be short-term belowground transfer of N from *Acacia* trees to *Eucalyptus* trees, as observed at 26 months after planting in a nearby experiment (Paula et al., 2015). However, no experimental evidence supported the hypothesis of higher belowground transfer of N in non-fertilized than in fertilized plots.

Our study, conducted in the same trial of Bordron et al. (2020 in press), aimed to investigate the effects of soil nutrient deficiencies on short-term belowground N transfer from NFTs to non-NFTs in mixed-species plantation of *E. grandis* x *E. urophylla* and *A. mangium*. We estimated the short-term belowground transfer of N from *Acacia* to *Eucalyptus* neighbors under two contrasting levels of fertilization. Following the methodology of Paula et al. (2015), $^{15}N-NO_3^-$ was injected into the stem of a dominant *Acacia* tree in three blocks of a completely randomized design, 58 months after planting. The $x(^{15}N)$ values of fine roots of the labeled *Acacia* and four *Eucalyptus* neighbors were measured at 7, 14, 30 and 60 days after labeling. At the end of the study period, labeled *Acacia* and *Eucalyptus* neighbors were harvested to measure the ^{15}N enrichment in the aboveground components of the trees. Litterfall was collected monthly from 47 months to 58 months and forest floor was collected at 47 and 58 months. We hypothesized that: (1) N litter deposition, N stocks in the forest floor and rate of N release from forest floor decomposition are higher in fertilized than non-fertilized plots, (2) short-term belowground N transfer occurs between *Acacia* and *Eucalyptus* close to harvest age, and (3) the rate of belowground N transfer is higher in non-fertilized than fertilized plots.

2.2 Material and methods

2.2.1 Study Site

The study was carried out at the Itatinga experimental station of São Paulo University, Brazil (23°02'S, 48°38'W), at 860 m above mean sea level. The total rainfall was 2300 mm

during the study period from April 2017 to May 2018 and the mean temperature was 19.6 °C, with an average of 13.6 °C for the coldest month (July 2017) and 22.9 °C for the hottest month (March 2018). The soils were deep Ferralsols (FAO classification), acidic and of low fertility. In the 0-1.0 m layer, soil pH was around 4.0, clay content ranged from 18 to 24% and CEC from 3.2 to 7.6 cmol_c kg⁻¹, the sum of base cations was around 0.4 cmol_c kg⁻¹, organic matter varied from 6 to 16 g kg⁻¹ and total N from 0.6 to 0.9 g kg⁻¹ (Bordron et al., 2020 in press).

2.2.2 Experimental layout

In May 2013, a complete randomized block design was set up with three replicates of *E. grandis* x *E. urophylla* trees mixed with *A. mangium* trees, with (F+) or without (F-) fertilization at planting. Each plot consisted of 10 x 10 plants at a spacing of 2.5 m x 2.5 m with two buffer rows. Mixed-species plantation was established at a proportion of 1:1 between *Eucalyptus* and *A. mangium*, with the two species planted alternately in the row, and between adjacent rows. Seeds of *A. mangium* from Papua New Guinea and were inoculated with *Rhizobium* strains (BR 3609T and BR6009 provided by EMBRAPA Agrobiologia, Seropédica - Rio de Janeiro state) selected for their high levels of nodulation in nursery and high N₂ fixation efficiency. *Eucalyptus* cuttings (H13 clone) were provided by the Instituto de Pesquisa e Estudos Florestais (IPEF - São Paulo State).

In F+, 2000 kg ha⁻¹ of dolomite limestone was applied at planting, as well as 32.0 kg ha⁻¹ of FTE-BR (Fritted Trace Element, micronutrients and Borogran), 150 kg ha⁻¹ of K, 35 kg ha⁻¹ of P, and 24 kg ha⁻¹ of N only for the *Eucalyptus* trees to prevent reduction of N₂ fixation by *Acacia* trees (Paula et al., 2019).

2.2.3 Litterfall and forest floor

Litterfall was collected every month from April 2017 (47 months after planting) to March 2018 (58 months after planting). Leaf litterfall were collected in 12 traps (50 cm x 50 cm) per plot installed at different distances from the trees (Figure 1). Bark and dead branches were collected in an area of 6.25 m² delimited between four trees in each plot in all blocks. All compartments were separated by species and dried at 65°C to constant weight. For a given species, the components were gathered depending on the seasons April to June 2017 (autumn), July to September 2017 (winter), October to December 2017 (spring), January to March 2018 (summer), and ground for the N analysis.

The forest floor was sampled in all plots at the beginning (April 2017) and at the end of the study period (March 2018). Twelve 50 cm x 50 cm positions, different from the litterfall traps, were sampled in each plot to represent the spatial variability within the plot (Figure 1). For each position, the forest floor was divided into two components: Lf (no fragmental material or coarse fragments) and Hf (highly fragmented material). For each component and each plot, the samples were manually homogenized and dried at 65°C to constant weight. A composite sample was passed through a 2-mm mesh stainless sieve screen for the N analysis.

Figure 1. Litterfall and forest floor sampling positions. In each plot, six positions were sampled close to six different *A. mangium* trees and six positions were sampled close to six different *Eucalyptus* trees.

2.2.4 Rate of N release from forest floor decomposition

The N release (in kg N ha⁻¹ yr⁻¹) from forest floor decomposition was estimated as:

$$N_{\text{Release}} = N_{\text{Forest-floor}_{2017}} + N_{\text{Litterfall}_{2017-2018}} - N_{\text{Forest-floor}_{2018}} \quad (\text{Eq. 1})$$

Where:

- $N_{\text{Forest-floor}_{2017}}$ is the amount of N in the forest floor in April 2017 (in kg N ha⁻¹).
- $N_{\text{Forest-floor}_{2018}}$ is the amount of N in the forest floor in April 2018 (in kg N ha⁻¹).

- $N_{\text{Litterfall}_{2017-2018}}$ is the amount of N in the litterfall between April 2017 and March 2018 (in $\text{kg N ha}^{-1} \text{ yr}^{-1}$).

Eq. (1) includes N inputs by throughfall, and by stemflow for the forest floor samples near *Acacia* and *Eucalyptus* trees. Therefore, N_{Release} corresponds to the net flux of N released from forest floor decomposition. However, we used N release instead of net N release for convenience.

2.2.5 ^{15}N labelling of *Acacia* trees

In March 2018, in each plot, a ^{15}N labelled solution was injected into the stem of one *Acacia* tree 58 months after planting, following the methodology of Paula et al. (2015). The labeled *Acacia* trees had a single stem (3) or two stems (3), were dominant trees, and did not show any disease or damage. The mean height of the *Acacia* trees across the three blocks was 16.0 m and 15.6 m in F+ and F-, respectively. No tree mortality was observed within a radius of 2.5 m around the labeled trees. A hole was drilled into the stem (6 mm in diameter and 20 mm in depth). The trees with a single stem were drilled at 1.30 m in height and the trees with two stems were drilled below the fork. The drill was lubricated using distilled water to prevent damage to the xylem vessels. After removing the drill, a polyethylene tube (6 mm of diameter), attached to a bottle containing 500 ml of distilled water, was pushed 20 mm into the drilled hole. As a preliminary experiment had shown possible reflux of the injected solution, 3 ml of acetic acid was added to the distilled water to prevent vascular clogging (Johansen, 1940). The bottle was then connected to a second one, containing 2.0 g of N (98 atom% $^{15}\text{N}\text{-NO}_3$) as potassium nitrate, dissolved in 500 ml of distilled water. ^{15}N contamination was avoided by packing the bark around the tube with non-toxic mineral putty (Terostat®) and placing a plastic bag around the trees before labelling (Figure 2). The solution was absorbed by the stem between 28 and 60 days after labeling, depending on the treatments and trees. During 60 days, the few *Acacia* leaves that fell down were removed within 24 h of falling.

Figure 2. *Acacia* trees after labeling and fixing the bottles containing the solutions of distilled water and ^{15}N , and distilled water and acetic acid.

2.2.6 Sampling

In each plot, samples of leaves, living branches, stem wood, stem bark and fine roots of one acacia and one eucalypt tree were collected before labeling to measure the corresponding ^{15}N background values. At 7, 14, 30, and 60 days after the first day of ^{15}N labelling, four soil samples were collected from each plot using a PVC tube (5 cm in diameter and 10 cm in length), at four positions randomly within 1.8 m radius from the ^{15}N -labeled *Acacia* (Figure 3). The mean height of all four neighbor *Eucalyptus* trees of the labeled *Acacia* tree across the three blocks was 20.6 m and 18.7 m, in F+ and F-, respectively. For each plot, the soil samples were then bulked and rapidly brought to the laboratory. Living fine roots (diameter < 2 cm) of acacia and eucalypt were then separated. The color, thickness and branching patterns were good indications of the species to which they belonged and *Acacia* roots were brighter, rougher, thicker and less branched than *Eucalyptus* roots (Germon et al., 2018). At 60 days after labeling, the labeled *Acacia* tree and two *Eucalyptus* trees around the labeled *Acacia*, in the row and in the inter-row (Figure 3), were harvested in each plot (6 *Acacia* trees and 12 *Eucalyptus* trees in total). The N concentration and $\chi(^{15}\text{N})$ values of leaves, living branches, stem wood, stem bark, and fine roots were determined for all the sampled trees.

Figure 3. Sampling plan. Fine roots of *A. mangium* and *Eucalyptus* trees were sampled at 0, 7, 14, 30 and 60 days after ^{15}N -labelling of *Acacia* trees. At 60 days after labelling, the labelled *Acacia* tree and two neighboring *Eucalyptus* trees (in the row and in the inter-row) were harvested in each plot (6 *Acacia* trees and 12 *Eucalyptus* trees in total).

2.2.7 Isotopic analyses

The fine roots were gently washed in tap water. All samples were dried at 65°C to constant weight. The bark, wood, leaves, and living branches were ground in a Retsch mill Zm 200 (120 micra) and the fine roots were ground in a porcelain mortar. The $x(^{15}\text{N})$ value and N concentration of the samples were determined using a Hydra 20-20 mass spectrometer coupled to an automatic N analyzer (ANCA-GSL, SERCON Co., Crewe, UK), using 10 mg of dry mass of dry plant material, with a precision of 0.0001 ^{15}N atom%. The $x(^{15}\text{N})$ value of a given sample was expressed as:

$$x(^{15}\text{N})_{\text{sample}} (\text{‰}) = \left(\left[\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{sample}} - \left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{air}} \right] / \left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{air}} \right) * 1000 \quad (\text{Eq. 2})$$

2.2.8 N derived from transfer

The proportion of *Eucalyptus* N derived from transfer from *Acacia* based on $x(^{15}\text{N})$ fine roots was estimated at the collection date using the equation (Jalonon et al., 2009; Isaac et al., 2012; Paula et al., 2015):

$$\% \text{NDFT}_{\text{-roots}} = \left(x(^{15}\text{N})_{\text{Euca}}(0) - x(^{15}\text{N})_{\text{Euca}}(t) \right) / \left(x(^{15}\text{N})_{\text{Euca}}(0) - x(^{15}\text{N})_{\text{Acacia}}(t) \right) * 100 \quad (\text{Eq. 3})$$

Where:

- $x(^{15}\text{N})_{\text{Euca}}$ is the $x(^{15}\text{N})$ value of fine *Eucalyptus* roots collected at 0 - 1.8 m from the ^{15}N labeled *Acacia*.
- $x(^{15}\text{N})_{\text{Acacia}}$ is the $x(^{15}\text{N})$ value of fine *Acacia* roots collected at 0 - 1.8 m from the ^{15}N labeled *Acacia*.
- $x(^{15}\text{N})_{\text{Euca}}(0)$ and $x(^{15}\text{N})_{\text{Euca}}(t)$ are the $x(^{15}\text{N})$ values of fine *Eucalyptus* roots before *Acacia* labeling and at the end of each collection date.
- $x(^{15}\text{N})_{\text{Acacia}}(t)$ is the $x(^{15}\text{N})$ of fine *Acacia* roots at the end of each collection date.

2.2.9 Statistical analyses

For each collection date and treatment, the ^{15}N enrichment of *Acacia* and *Eucalyptus* material was tested against ^{15}N background values using one-tailed paired t test. Differences between treatments and blocks in dry matter, N concentration and N content in litterfall and forest floor, $x(^{15}\text{N})$ of *Acacia* and *Eucalyptus* material and %NDFT were tested using the two-way ANOVA. The homogeneity of variances was tested using the Levene test. When the variances were unequal, the values were log-transformed. When the ANOVA indicated significant effects, the means were compared with the Bonferroni's multiple range test. Statistical analyses were carried out using R 3.5.2 (R Core Team 2018). The significance level was 0.05.

2.3 Results

2.3.1 Litterfall and forest floor

Fertilization greatly influenced litterfall in our mixed-species stands (Figure 4). Litterfall dry matter was 38% higher in F+ than in F- with values of 7.8 and 5.6 Mg ha⁻¹ yr⁻¹, respectively. The proportion of *Eucalyptus* dry matter in the total litterfall was 81.9 % on average in F+ and 70.3 % in F-. Leaves accounted for 92.4% of the dry matter of *Acacia* litterfall and 45.3% of that of *Eucalyptus* litterfall. Mean N concentrations in litterfall were 17.3 g kg⁻¹ in F+ and 14.8 g kg⁻¹ in F- for *Acacia* leaves, and 6.5 g kg⁻¹ in F+ and 6.4 g kg⁻¹ in F- for *Eucalyptus* leaves (data not shown). On average, branches accounted for 7.6% of the dry matter of *Acacia* litterfall and 50.0% of *Eucalyptus* litterfall. Mean N concentrations in branch litterfall were 6.6 g kg⁻¹ in F+ and 8.1 g kg⁻¹ in F- for *Acacia*, and 2.0 g kg⁻¹ in F+ and 1.9 g kg⁻¹ in F- for *Eucalyptus* (data not shown). The total amount of N in litterfall was 26% higher in F+ than in F- with values of 49 and 39 kg N ha⁻¹ yr⁻¹, respectively (Figure 4). *Acacia* material accounted for 46.3% of the amount of N in litterfall in F+ and 62.4% in F-. On

average, leaves accounted for 72% the total N content in *Eucalyptus* litterfall and 97% of the total N content in *Acacia* litterfall.

The dry matter of the forest floor amounted to 6.7 Mg ha⁻¹ in F+ and 6.0 Mg ha⁻¹ in F- on average for the two sampling dates (Table 1). For a given layer, N contents in the forest floor were not significantly different between treatments, except for the Hf layer in 2017 with significantly higher N content in F- than in F+. The amount of N in the forest floor was not significantly influenced by the treatments with average values of 65 kg N ha⁻¹ in F+ and 62 kg N ha⁻¹ in F-. Between 46 and 58 months after planting, the amount of N released from forest floor decomposition was 31% higher in F+ than in F-, with values of 43 and 33 kg N ha⁻¹ yr⁻¹, respectively (Table1).

Figure 4. Litterfall dry matter (a), (b) and N content in litterfall (c), (d) in bark, leaves and branches collected from April 2017 to March 2018 near Acacia trees (Acacia) and near Eucalyptus trees (Eucalyptus) in mixed-species stands (50A:50E) with (F+) or without fertilisation (F-). For each treatment, total dry matter and total N content in litterfall of each species (Acacia or Eucalyptus) are shown. Standard errors between blocks are indicated (n=3).

Table 1. Dry matter and N content in the Lf and HF components of forest floor collected in April 2017 and March 2018 in mixed-species stand (50A:50E) with (F+) and without (F-) fertilization. Dry matter and N content in the litterfall collected between April 2017 and March 2018 in the same treatments, and corresponding N release between April 2017 and March 2018. Standard errors between blocks are indicated (n=3). Different letters indicate significant differences ($P < 0.05$): A, B for differences between treatments for each species; a, b for differences between *Acacia* and *Eucalyptus* in each treatment; α and β for differences between treatments.

	Near <i>Acacia</i>	F+ Near <i>Eucalyptus</i>	50A:50E	Near <i>Acacia</i>	F- Near <i>Eucalyptus</i>	50A:50E
Forest floor 2017						
Dry matter (Mg ha ⁻¹)						
Lf	4.44±0.34 Aa	4.08±0.54 Aa	4.26±0.35 α	3.46±0.32 Aa	3.61±0.19 Aa	3.53±0.25 α
Hf	1.75±0.05 Ba	1.80±0.09 Aa	1.77±0.05 β	2.37±0.21 Aa	1.94±0.16 Aa	2.15±0.03 α
Total	6.19±0.39 Aa	5.89±0.46 Aa	6.04±0.34 α	5.83±0.34 Aa	5.55±0.28 Aa	5.69±0.31 α
N content (kg N ha ⁻¹)						
Lf	46.81±1.08 Aa	37.20±6.79 Aa	42.01±3.80 α	36.52±1.45 Ba	30.43±5.23 Aa	33.47±3.14 α
Hf	19.93±0.27 Ba	20.02±3.00 Aa	19.97±1.41 β	27.26±1.17 Aa	24.35±2.76 Aa	25.81±1.56 α
Total	66.74±1.35 Aa	57.22±3.81 Aa	61.98±2.46 α	63.78±2.54 Aa	54.78±2.73 Aa	59.28±2.43 α
Forest floor 2018						
Dry matter (Mg ha ⁻¹)						
Lf	4.38±0.39 Aa	5.21±0.29 Aa	4.80±0.24 α	4.25±0.57 Aa	3.97±0.19 Ba	4.11±0.34 α
Hf	2.54±0.12 Aa	2.66±0.27 Aa	2.60±0.19 α	2.08±0.27 Aa	2.33±0.25 Aa	2.20±0.26 α
Total	6.92±0.36 Aa	7.88±0.56 Aa	7.40±0.37 α	6.32±0.37 Aa	6.30±0.29 Aa	6.31±0.33 α
N content (kg N ha ⁻¹)						
Lf	48.90±4.58 Aa	36.89±2.59 Aa	42.89±3.19 α	44.22±8.17 Aa	35.33±0.99 Aa	39.78±4.18 α
Hf	26.42±1.94 Aa	23.54±1.64 Aa	24.98±1.76 α	22.94±2.60 Aa	27.90±1.05 Aa	25.42±1.82 α
Total	75.32±3.39 Aa	60.42±3.71 Ab	67.87±3.12 α	67.16±7.11 Aa	63.23±0.36 Aa	65.20±3.38 α
Litterfall						
Dry matter (Mg ha ⁻¹ yr ⁻¹)			7.76±0.62 α			5.62±0.65 α
N content (kg N ha ⁻¹ yr ⁻¹)			48.81±2.96 α			38.73±3.00 α
N release (kg N ha ⁻¹ yr ⁻¹)			42.92±7.34 α			32.82±4.18 α

2.3.2 $x(^{15}\text{N})$ in labeled *Acacia* trees

In F+ from 7 days after labeling onwards, fine *Acacia* roots had higher $x(^{15}\text{N})$ mean values than the mean background value 2.73 ‰, with average values of 10.78 ‰ over the four sampling dates (Figure 5a). In F- from 30 days after labeling onwards, fine *Acacia* roots had higher values of $x(^{15}\text{N})$ than the mean background value of 1.91 ‰, with average values of 4.18 ‰ over the study period. Fine *Acacia* roots were significantly ^{15}N -enriched at 30 days in F- (Figure 5a).

Sixty days after labeling, the aboveground compartments of labeled *Acacia* exhibited higher $x(^{15}\text{N})$ values than background values (Table 2), with significant differences for wood, branches, and foliage in F+, and for bark and branches in F-.

2.3.3 $x(^{15}\text{N})$ in *Eucalyptus* trees

For both treatments from 30 days after labeling onwards, fine *Eucalyptus* roots had higher $x(^{15}\text{N})$ mean values than mean background values of 1.27 ‰ in F+ and 0.36 ‰ in F-, with significant ^{15}N -enrichment at 60 days in F+. In F-, P values were 0.051 and 0.058 at 30 and 60 days, respectively. Over the four sampling dates, the average values of $x(^{15}\text{N})$ of fine *Eucalyptus* roots were 1.73 ‰ and 1.41‰ in F+ and F-, respectively (Fig. 4b). The highest $x(^{15}\text{N})$ of fine *Eucalyptus* roots were found at 60 days with values 2.82 ‰ and 4.28 ‰ in F+ and F-, respectively. Fine *Eucalyptus* roots had significant higher $x(^{15}\text{N})$ in F+ than in F- at 7 days.

Sixty days after labeling, the aboveground compartments of *Eucalyptus* exhibited higher $x(^{15}\text{N})$ values than background values (Table 2), with significant differences for all compartments, except for bark in F+.

Figure 5. Mean $x(^{15}\text{N})$ values during the 60-day period of fine roots of *A. mangium* (a) and *Eucalyptus* (b) collected within 1.8 m from the ^{15}N -labelled *A. mangium*. Vertical bars indicate standard errors between blocks ($n=3$). At a given collection date, * indicates significantly higher $x(^{15}\text{N})$ values than background values ($P < 0.05$) and different letters indicate significant differences between treatments ($P < 0.05$).

Table 2. Mean $x(^{15}\text{N})$ values in aboveground compartments of *A. mangium* and *Eucalyptus* neighbors before (background values) and 60 days after *Acacia* ^{15}N -labelling. Standard errors between blocks are indicated (n=3). For a given tree compartment and a given treatment (F+ and F-), * indicates significantly higher $x(^{15}\text{N})$ values than background values ($P < 0.05$).

Compartment s	<i>A. mangium</i>				<i>Eucalyptus</i>			
	Background		60 days		Background		60 days	
	F+	F-	F+	F-	F+	F-	F+	F-
Bark	1.91±0.16	1.09±0.55	274.91±85.65	219.77*±58.71	-0.73±0.40	-1.27±0.09	2.40±0.79	2.28*±0.66
Wood	2.73±0.16	2.00±0.33	287.20*±37.0 1	347.07±197.41	-0.27±0.42	-0.36±0.24	4.31*±0.78	2.87*±0.57
Branches	1.82±0.64	0.91±0.64	156.52*±29.0 7	168.90*±32.93	-1.37±0.42	-1.46±0.18	1.53*±0.41	0.77*±0.09
Leaves	3.00±0.55	2.09±0.74	165.89*±18.8 4	109.75±38.46	-0.09±0.46	-0.18±0.36	0.96*±0.49	0.96*±0.08

2.3.4 Rate of N transfer from *Acacia* to *Eucalyptus* trees

The percentage of N of *Eucalyptus* trees derived from transfer (%NDFT) from *Acacia* trees using Eq. (3) ranged from 9.5% to 29.5% in F+ and 0 to 68.7% in F- depending on the sampling dates. Over the study period, the average values of %NDFT were 18.0% and 33.9% in F+ and F-, respectively (Figure 6).

Figure 6. Estimates of the percentage of *Eucalyptus* nitrogen derived from *A. mangium* (%NDFT) at 7, 14, 30 and 60 days after *Acacia* labelling. Vertical bars indicate standard errors between blocks (n=3). The average %NDFT value during the 60-day study period was 17.96% for F+ and 33.85% for F-.

2.4 Discussion

2.4.1 Fertilization increases N availability for *Eucalyptus* trees

In agreement with our first hypothesis, the amount of N released from forest floor decomposition was higher in fertilized than in non-fertilized plots. Soil N mineralization was not measured in our study, but could be higher in the fertilized than in non-fertilized plots (Silver et al., 2005), leading to higher soil N availability in F+ than in F-. The first 3 years after planting in the same experiment, fertilization increased *Eucalyptus* fine root foraging strategy for nutrients in the top soil (Bordron et al., 2020). The competition with *Eucalyptus* fine roots led to a partial exclusion of *Acacia* fine roots from the topsoil more marked in F+ than in F-, and a higher proportion of *Eucalyptus* fine roots at the vicinity of *Acacia* trees in F- (Bordron et al., 2020).

Litterfall dry matter in F+ was 10% lower than in a nearby fertilized mixed-species stand (Voigtlaender et al., 2012). This discrepancy could be partly explained by lower Eucalyptus growth in the present experiment (Bouillet et al., 2013). N concentrations in litterfall were lower for Eucalyptus than Acacia material. Therefore, higher percentage of Eucalyptus than Acacia material in litterfall in F+ than in F- led to only 26% larger N content in F+, while the amount of dry matter was 38% higher in fertilized plots. Nitrogen contents in litterfall of 39 kg N ha⁻¹ yr⁻¹ in F- and 49 kg N ha⁻¹ yr⁻¹ in F+ are lower than in other mixed-species plantations of Eucalyptus and *A. mangium* in Brazil (from 55 to 85 kg N ha⁻¹ yr⁻¹) (Voigtlaender et al., 2012, 2019; Santos et al., 2017) which could be consistent with low soil nutrient availability in this experiment, especially in non-fertilized stands.

The higher proportion of N-poor Eucalyptus litterfall in F+ than in F- did not prevent the decomposition of the forest floor. Nutrient concentrations and stoichiometry and C quality are major drivers of litter decomposition and nutrient release in forest ecosystems (Hobbie, 2000; Hättenschwiler and Jørgensen 2010; Hättenschwiler et al., 2011). The decomposition rates are commonly positively correlated with the N concentrations in litter fractions (Enríquez et al., 1993; Cornwell et al., 2008). However, some negative correlations are also reported (Berg, 2000). Consistently, negative correlations are commonly found between litter decomposition rates and C:N ratios (Melillo et al., 1982), but positive correlations have been also reported (Michel and Matzner, 2002; Craine et al., 2007). Lignin was also found to reduce the decomposition of forest residues (Freschet et al., 2012; Butenschoten et al., 2014), and negative correlations between lignin:N ratio and litter decomposition rate are often recorded (Melillo et al., 1982; Hobbie, 2000). However, high N concentrations can delay the processes of degrading lignin and lignified carbohydrates and lower the rates of litter decomposition (Santos et al., 2017). In a nearby experiment, decomposition rates in litterbags were faster for Eucalyptus leaves than Acacia leaves, with initial higher water-soluble carbon and lower lignin concentrations in Eucalyptus leaves (Bachega et al., 2016). Fertilization can increase litter decomposition rates and nutrient availability in forest ecosystems (Aslam et al., 2015; Keuskamp et al., 2015; Moore et al., 2019). Litter decomposition can be stimulated at low levels of N addition (Zhang et al., 2018). However, opposite results can be observed at high levels of N application (Zhang et al., 2018; Bonner et al., 2019). The small amounts of N applied at planting might therefore have contributed to increase the rates of forest floor decomposition and N release in F+ relative to F-.

2.4.2 Belowground N transfer from *Acacia* to *Eucalyptus*

In agreement with our second hypothesis, belowground N transfer from *Acacia mangium* to *Eucalyptus* was observed for trees close to harvest age. At the end of the study period, all components of *Eucalyptus* neighbors at 2.5m from the labeled *Acacia* were significantly enriched in ^{15}N , in both treatments, except for bark in F+. *Eucalyptus* trees farther away from the labeled *Acacia* may have also benefited from lower belowground N transfer with a decreasing gradient of ^{15}N enrichment to *Eucalyptus* trees located from 1.5 m to 6.2 m of *Acacia* tree, according to Paula et al. (2015).

The increase in ^{15}N enrichment of *Acacia* and *Eucalyptus* fine roots was lower than the findings of Paula et al. (2015) in 26-month-old trees. Here, the ^{15}N solution was absorbed between 28 and 60 days after labeling, while Paula et al. (2015) found 12 to 36 hours. This difference could be partly explained by a double volume of solution to be absorbed in the present study, as well as by the difference in tree age between the two experiments. In the ecological conditions of São Paulo State, *Acacia* trees are more suppressed by *Eucalyptus* trees at the end of stand rotation than in the early stand stage (Bouillet et al., 2013; le Maire et al., 2013), leading to lower transpiration of mature than young acacias, as observed in a nearby experiment (unpublished data). Other concerns could be partial clogging of xylem vessels after stem drilling or narrow conductive sapwood width in mature trees (Pallardy, 2008; Debell and Lachenbruch, 2009), which required to drill the holes into the stem less than 20 mm. However, this slow ^{15}N absorption possibly reduced the risk of increase in N root exudates after labeling (Paula et al., 2015). The limited number of replicates may have accounted for the high $x(^{15}\text{N})$ variability in the fine roots of both *Acacia* and *Eucalyptus* between blocks and the low number of significant differences with background values over the study period.

Short-term belowground N transfer may occur directly via common mycorrhizal networks (CMN) (Simard and Durall, 2004; Selosse et al., 2006; He et al., 2019) or root exudates of N compounds (Marschner and Dell, 1994; Fustec et al., 2010), or indirectly through rapid decomposition of very fine roots and microbial tissues (May and Attiwill, 2003; Staddon et al., 2003). Montesinos-Navarro et al. (2016) found that short-term belowground N transfer between adult plants was more effective by CMN than via roots exudates. CMN can be formed by arbuscular mycorrhizal fungi (AMF) (Montesinos-Navarro et al., 2012) or ectomycorrhizal fungi (He et al., 2005). Mycorrhizal roots of both *Eucalyptus* and *A. mangium* were observed in the present experiment (Bordron et al., 2020 in press) and both species may potentially form CMN. Arbuscular mycorrhizal fungi are closely associated to *A.*

mangium (Tawaraya et al., 2003; Dhar and Mridha, 2012), as well as *Eucalyptus* species (Adjoud-Sadadou and Halli-Hargas, 2000; da Silva et al., 2011; Bini et al., 2018). Intercropping *A. mangium* and *Eucalyptus* enhanced AMF colonization of *Eucalyptus* roots in the 0-10 cm layer (Bini et al., 2018) in a nearby mixed species stand, where 16 species of six AMF genera were found down to 8 m deep (Pereira et al., 2018). Ectomycorrhizas are associated to *Eucalyptus* (Horton et al., 2017; Robin et al., 2019) and *A. mangium* (Founoune et al., 2002; Diagne et al., 2013). *Pisolithus*, *Scleroderma*, *Thelephora* and *Boletellus* genera were found under *A. mangium* and *E. urophylla* (Aggangan et al., 2015), and *E. grandis* and *A. mangium* roots can be both colonized by *Pisolithus* sp. and *Scleroderma* sp. (Founoune et al., 2002; Duponnois and Plenchette, 2003; Ducousso et al., 2012).

2.4.3 Nutrient deficiency increased belowground transfer of N from *Acacia* to *Eucalyptus*

In agreement with our third hypothesis, the rate transfer values of N belowground from *Acacia* to *Eucalyptus* trees were higher in non-fertilized than in fertilized mixed-species stands, with average values of %NDFT 33.9 and 18.0%, respectively. Moreover, the differences in %NDFT between the two treatments could be underestimated due to faster enrichment in ^{15}N of *Acacia* roots in F+ than in F-. Fine *Acacia* roots had higher $x(^{15}\text{N})$ values than $x(^{15}\text{N})$ background values from 7 days onwards, and 30 days onwards, in F+ and F-, respectively. Equation (3) was therefore applicable at 7 and 14 days in F+, but not in F-. Taking into account the last 30 days of the experiment, when $x(^{15}\text{N})$ values of fine roots of both *Acacia* and *Eucalyptus* were higher than $x(^{15}\text{N})$ background values, the average values of %NDFT were 67.7% in F- and 22.8% in F+.

At 26 months, the average value of %NDFT was 43% in a nearby fertilized mixed-species plantation of *Eucalyptus* and *A. mangium* (Paula et al., 2015). This higher rate of belowground transfer of N could be explained by high N demand of *Eucalyptus* in the young stand stage to elaborate canopy (Laclau et al., 2010). Mature fertilized *Eucalyptus* trees could fulfil most of their N requirement from forest floor decomposition and soil N mineralization (Laclau et al., 2010; Voigtlaender et al., 2012; 2019) and internal remobilization (Laclau et al., 2001). In non-fertilized plots, *Eucalyptus* trees might offset lower N release from forest floor decomposition through higher rate of N belowground transfer from *Acacia*. This facilitation process could have led to the same N concentration in *Eucalyptus* leaves in F- and in F+, as observed at 34 months (Bordron et al., 2020 in press), whilst foliar N concentration was lower in non-fertilized than in fertilized *Eucalyptus* monocultures in nearby *E. grandis* plantations (Silva et al., 2013). In F-, *Eucalyptus* trees could proportionally benefit from

higher belowground N transfer from *Acacia* than in F+ through higher fine roots exploration near *Acacia* trees, whose roots were mainly restricted close to the *Acacia* stem (Bordron et al., 2020 in press). At 34 months, *Eucalyptus* fine Root Mass Density and Specific Root Length (ratio between length of fine roots and their dry mass) near *Acacia* trees was higher in F- than in F+, at the 0-0.15 m and 0-1 m layer, respectively (Bordron et al., 2020 in press).

2.4.4 Ecological and forest management perspectives

Higher values of rate of belowground transfer of N from *A. mangium* to *Eucalyptus* in non-fertilized than in fertilized mixed-species stands suggests that this facilitation process of *Acacia* on *Eucalyptus* increases under harsh conditions. This finding reinforces the interest to associate *Acacia* to *Eucalyptus* in forest plantations to limit N eucalypt deficiencies when stands are established in low-fertility soils and/or fertilizers are not applied, as usually observed in small-holder forests (Verhaegen et al., 2014).

Facilitation and complementarity between species can lead mixed-species plantations of *Eucalyptus* and *Acacia* to outperform *Eucalyptus* monocultures, as observed in Brazil (Santos et al., 2016), Congo (Bouillet et al., 2013; Tchitchelle et al., 2017a) or Australia (Forrester et al., 2006). These mixtures could be a credible alternative to monocultures, for both rural and commercial plantations, in particular in marginal zones, which will be largely cropped by *Eucalyptus* plantations in the future (ABRAF, 2013). In addition to stand production, mixed-species plantations of *Eucalyptus* and *Acacia* can increase soil organic carbon (C) (Forrester et al., 2013; Hoogmoed et al., 2014), improve N soil status (Rachid et al., 2013, Voigtlaender et al., 2019), soil P availability (Koutika, 2019), and enhance litter and soil microbial diversity (Bini et al., 2013, 2017; Pereira et al., 2019) contributing to the ecological sustainability of these short-rotation forest plantations (Pereira et al., 2018).

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3. EFFECTS OF PLANTING DESIGN AND TREE DENSITY ON BIOMASS PRODUCTION AND LIGHT USE EFFICIENCY OF MONOCULTURES AND MIXED-TREE PLANTATIONS OF *EUCALYPTUS* AND *ACACIA MANGIUM*

ABSTRACT

Competition for light in mixed-species plantations is a major limiting factor for tree growth. Understanding light absorption and light use efficiency for each species at different planting densities and spatial arrangement of trees is essential for improving management of these plantations. A complete randomized block design was set up with 3 blocks and 10 treatments per block with pure and mixed plantations of *Eucalyptus grandis* (E) and *Acacia mangium* (A), a N₂-fixing species, composed of different planting densities (3mx6m, 3mx3m and 2mx3m) and arrangements (100% A, 100% E, 50% A and 50% E, 33% A and 67% E) between species. Tree growth and biomass were monitored at 38, 45 and 52 months after planting. The absorbed photosynthetically active radiation (APAR) for each tree was simulated with the MAESTRA model. The leaf area of each tree, leaf angle distributions, and leaf area density were estimated *in situ* to parameterize the model. Hemispherical photos were taken during the same period (every 2 months) and used to test MAESTRA simulations and validate light interception simulations. Computed Light Use Efficiency (LUE) for stem wood production was estimated as the wood growth ratio. The APAR of *Eucalyptus* did not significantly differ between 100E and 33A:67E, compensating the reduction of number of its trees by increasing individual light absorption. The LUE of *Eucalyptus* did not change much among monoculture and mixtures in densities with 1111 trees ha⁻¹; however, in densities with 1666 trees ha⁻¹, the replacement of *Eucalyptus* by *Acacia* decreased the stand scale LUE. The stem biomass growth (SBG) of *Eucalyptus* was mainly explained by differences in APAR, while both APAR and LUE explained the SBG of *Acacia* trees. The highest productivity was reached in the design 100E with 1666 trees ha⁻¹; however, *Eucalyptus* in the arrangement 33A:67E with 1111 trees ha⁻¹ had greater SBG than in 50A:50E and similar production in 100E. The *Acacia* behavior resulted from the combined effects of density and presence of *Eucalyptus*, while the *Eucalyptus* behavior was strongly dependent on its own density with low or no impact of *Acacia*.

Key-words: Eucalypt, N₂-fixing tree, MAESTRA, light absorption, light use efficiency, planting design, planting density.

3.1 Introduction

The success of mixed plantations with nitrogen fixing trees (NFT) and non-nitrogen fixing trees (non-NFT) depends on the combination of species with different growth rates and functional traits, promoting a complementarity for resources acquisition (Kelty, 2006). Complementarity between species may lead to a more efficient exploitation and utilization of resources (Binkley et al., 2004; Richards et al., 2010; Forrester, 2014), such as reduced competition for light through the partitioning niche by stratification of the crown (Hunt et al., 2006; Laclau et al., 2008), and/or for water and nutrients with better soil exploration (Germon et al., 2018; Bordron et al., 2020 in press), and/or N fixation from NFT benefiting to the non-

NFT.

Among the nitrogen fixing trees, *Acacia mangium* and its combinations with *Eucalyptus* spp. have been proposed to improve N soil availability (Paula et al., 2015; Tchichelle et al., 2017; Santos et al., 2017; Voigtlaender et al., 2019), increasing soil carbon stocks (Koutika et al., 2014), fine roots biomass (Laclau et al., 2013; Germon et al., 2018; Bodron et al., 2020), and soil fauna and the microbial diversity (Rachid et al., 2013; Pereira et al., 2018). These intercropped system could increase biomass production (Bouillet et al., 2013; Santos et al., 2016; Marron and Epron, 2019) and light absorption (le Maire et al., 2013), compared to their respective monocultures.

Forest growth depends on different parameters, as plant supply, resources (light, water and nutrients) capture, and use efficiency (Binkley et al., 2004; Richards et al., 2010). In mixed plantations, intra- and inter-specific competition for light is one of the main processes that influence the biomass partitioning between tree components (Bauhus et al., 2004; le Maire et al., 2013). Light competition could be managed using variations of planting designs, following the consideration of height growth dynamics (Forrester et al., 2006a). For instance, in mixed *Eucalyptus* and *Acacia mangium* plantations in southern Brazil, canopy stratification starts at 2-3 years after planting, when *Acacia* trees are already completely dominated by the *Eucalyptus* canopy leading to a higher light capture at the ecosystem level (le Maire et al., 2013).

Tree growth in mixed plantation is affected by planting arrangements (Laclau et al., 2008; Nouvellon et al., 2012; le Maire et al., 2013; Marron and Epron, 2019). There are two types of mixing design for field testing the effect of increasing the number or proportion of NFT on productivity: (i) additive series, where non-NFT density is constant and NFT density varies, and (ii) replacement series, where the total density of the mixed plantation is constant and NFT replaces certain non-NFT. Both designs could be planted in alternating rows of each species, or with both species inter-planted in the same row. These types of series allow evaluating the effect of density support species interactions and stratification patterns on mixed forest plantation growth (Garber and Maguire, 2005).

Another important forest practice that affects tree growth rate is tree spacing (Lin et al., 2013) changing the stocking density and species interactions in mixed-forest plantations (Forrester, 2014). The optimal planting density promotes higher growth rates, determines the amount of resources available for each species, crown attributes, and time for eventual tree crown closure (Akers et al., 2013). Moreover, the spacing choice between trees is an important factor to evaluate costs of planting in mixed plantations (Grant et al., 2006).

Our study aimed to understand how design and tree spacing affect tree growth and wood productivity in pure and mixed-species plantation of *E. grandis* and *A. mangium*, and disentangling the effect of light competition (analysis of tree and stand absorbed photosynthetically active radiation, APAR) from other effects (analysis of tree and stand Light Use Efficiency). We simulated the APAR for each tree and at the stand level using the three-dimensional model MAESTRA (Norman and Welles, 1983) from 38 to 52 months after planting. The LUE was estimated at tree and plot scales by the ratio of measured stem biomass increment divided by the APAR.

We aimed to answer four specific questions regarding light absorption and light use efficiency in pure and mixed *Eucalyptus* and *Acacia mangium* plantations:

- How do APAR and LUE of Eucalyptus vary among planting designs?
- What is the correlation between APAR, LUE, and stem biomass growth (SBG)?
- What is the impact of tree densities on APAR and LUE and their effects on wood production?
- Is there an optimal tree density and NFT- non-NFT ratio for maximizing total wood production or non-NFT wood production?

We performed a joint modeling and experimental approach of pure and mixed plantations of *A. mangium* and *E. grandis* in different replacement series and planting densities in a middle rotation. The MAESTRA model was used to estimate the absorbed radiation for each tree and at stand level. The effects of replacement series and planting density on intra and inter-specific light competition were analyzed by separating the influence of leaf area index (LAI), stem biomass growth, absorbed radiation, and light use efficiencies. We expect to identify the interactions between the replacement of eucalypt by acacia and investigate planting densities that could result in a better production.

3.2 Material and methods

3.2.1 Study site

The study was carried out at Itatinga Experimental Station (São Paulo, Brazil, 23° 06' S, 48° 36' W) at 857 m above mean sea level. During the study period, the mean annual rainfall was 2022 mm, with the dry season from June to September, the rainy season from October to May and the mean annual temperature was 20 °C (climate Cfa, Köppen classification). The mean annual rainfall of the year of the study period was 63 %, 8 % and 3 % higher than the first, second, and third year of planting study, respectively. The mean

annual temperature was similar for all ages. The site relief is flat to soft wavy, typical of the plateau of western São Paulo. The soil was classified as Red-Yellow Latosol, dystrophic, medium texture. Soil texture is characterized by 77% sand, 18% clay and 5% silt in the first 3 m of soil. The pH is acid, ranging from 3 to 4, with low amount of available nutrients. The natural vegetation of the region is the Cerrado and, in the last 30 years, the experiment site was cropped in rotations of *Eucalyptus grandis* monocultures. The residues (branches, leaves and bark) from the harvest were uniformly left in the field, only the trunks were removed from the plot.

3.2.2 Experimental design

A complete randomized block design was set up in December 2013 with 10 treatments and 3 blocks. The development of monospecific stands of *Acacia mangium* and *Eucalyptus grandis* was compared with mixed-species plantations in different replacement series, varying in terms of spacing and proportions between species. Each plot was established in an area of 1.296 m² with two buffer rows (inner plot of 576 m²) (Figure 7). The treatments were as follows:

- T1: 100A 3m x 6m - *A. Mangium* at spacing 3m x 6m (555 acacias ha⁻¹);
- T2: 100E 3m x 6m - *E. grandis* at spacing 3m x 6m (555 eucalyptus ha⁻¹);
- T3: 100E 3m x 3m - *E. grandis* at spacing 3m x 3m (1111 eucalyptus ha⁻¹);
- T4: 100A 3m x 3m - *A. mangium* at spacing 3m x 3m (1111 acacia ha⁻¹);
- T5: 33A:67E 3m x 3m - 33% of *A. mangium* and 67% of *E. grandis* at spacing 3m x 3m (744 eucalyptus ha⁻¹ and 367 acacias ha⁻¹);
- T6: 50A:50E 3m x 3 m - 50% of *A. mangium* and 50% of *E. grandis* at spacing 3m x 3m (555 acacias ha⁻¹ and 556 eucalyptus ha⁻¹);
- T7: 100E 2m x 3m - *E. grandis* at spacing 2m x 3m (1666 eucalyptus ha⁻¹);
- T8: 33A67E 2m x 3m - 33% of *A. mangium* and 67% of *E. grandis* at spacing 2m x 3m (550 acacias ha⁻¹ and 1116 eucalyptus ha⁻¹);
- T9: 50A:50E 2m x 3m - 50% of *A. mangium* and 50% of *E. grandis* at spacing 2m x 3m (833 acacias ha⁻¹ and 833 eucalyptus ha⁻¹);
- T10: 100E+N 3m x 3m - *E. grandis*, with nitrogen fertilization, at spacing 3m x 3m (1111 eucalyptus ha⁻¹).

Seedlings were planted in alternating rows. When the proportion is 50% *A. mangium* and 50% *E. grandis*, the arrangement is made of two rows of eucalypt followed by two rows of acacia. When the proportion is 33% *A. mangium* and 67% *E. grandis*, the arrangement is one row of acacia by two rows of eucalypt. Seedlings of *E. grandis* were supplied by the company "Suzano Papel e Celulose" and *A. mangium* seedlings were produced in the Itatinga Experimental Station nursery from seeds from Vietnam (origin supplied by IPEF). *Acacia mangium* seedlings were inoculated with Rhizobium strains (supplied by EMBRAPA Agrobiologia) for their high capacity to fix atmospheric N and their high nodulation rate presented in nurseries. Dolomitic limestone was applied at 2 Mg ha⁻¹ at planting to supply Ca and Mg. For the first 9 treatments, 250 kg ha⁻¹ of triple superphosphate, 50 kg ha⁻¹ of potassium chloride, 30 kg ha⁻¹ of FTE and 45 kg ha⁻¹ Borogran® were applied. For treatment 10, which was a 100E treatment with additional N fertilization, all the fertilization described was applied and 50 kg ha⁻¹ of ammonium sulphate. After 6 months, 200 kg ha⁻¹ of potassium chloride in all treatments and 45 kg ha⁻¹ of ammonium sulphate were applied in the treatment 10.

Figure 7. One block scheme of the trial representing each tree position, including those on the borders. The treatment numbers are labeled from T1 to T10, and the letters indicate the positions of *Acacia* (a) and *Eucalyptus* (e). *Eucalyptus* and *Acacia* proportions and within-line and inter-line distances of trees are given below each treatment.

3.2.3 MAESTRA model

MAESTRA, or its new version MAESPA, is a 3D tree-level model that was used to predict light absorption by each tree based on a study by Norman and Welles (1983) (Wang and Jarvis, 1990; Medlyn, 2004; Duursma and Medlyn, 2012; le Maire et al., 2013; Vezy et al., 2018). It is a model that represents each tree in 3D, with simplified shapes, and calculates light interception and distribution between the trees and within the crown. The description of each tree (position, sizes, shapes, etc.) and their eventual evolution through time are inputs of the model, generally obtained from field measurements.

For leaves interception and absorption of Photosynthetically Active Radiation (PAR), MAESTRA sub-model uses half-hourly incoming radiation. APAR (MJ ha^{-1} or MJ tree^{-1}) is the radiation absorbed by the leaves proportion in the PAR domain. It is calculated every half hour for each m^2 of leaf area throughout the crown of each tree and summed up for all trees in each plot. Radiation scattering, proportion of direct and diffuse radiant energy, and the sun position are obtained using the methodology of Norman and Welles (1983). In this study, direct and diffuse radiation was treated separately and the transmission of diffuse PAR was modelled by the method of Norman (1979).

3.2.4 MAESTRA model parameterization

3.2.4.1 Inventories

Circumference at breast height and tree height were measured on all trees in the inner plots (Figure 7) for all treatments and blocks at 38 (February 2017), 45 (September 2017) and 52 (April 2018) months after plantation. For acacias with multi-stem, all the stems with a diameter at breast height >2 cm were measured and an “equivalent circular circumference” for each stem was calculated from the sum of the basal areas of each stem tree.

3.2.4.2 Aboveground biomass

Aboveground biomass were estimated by sampling 8 trees of each species (i.e. 16 trees in plurispecific stands) in each of treatments 1, 2, 3, 4, 6, 7 and 9. The cut trees were selected in the internal border rows to avoid disturbing the inner area of the treatment. The trees sampled per treatment were chosen to be distributed over the range of tree basal areas of inner trees (Nouvellon et al., 2012). The measurements were repeated at 38, 45 and 52 months after planting, leading to 216 cut trees in total. The trees were separated into leaves, living branches, stem wood, and stem bark and the masses were measured in the field. The foliage was collected and weighed from three different third sections of the trees crown (top, middle

bottom). Sub-samples of ~25 leaves for each crown level were weighed for fresh mass, scanned for surface measurements, and dried at 65 °C to obtain constant dry weight. This allowed to convert fresh leaves weighed in the field into dry weight and into leaf surface, for each crown level. Similarly, branches, bark and stem sub-samples were weighed before and after drying to measure the water content and convert field measurements from fresh to dry weights. Biomass equations were established for all species and trees compartments at 38 and 52 months and only for branches and leaves at 45 months after planting (Appendix A). The equations obtained in treatments 3, 6 and 9 for each species were applied in similar treatments 10, 5 and 8, respectively. The treatment-specific allometric relationships established at 38, 45 and 52 months of age, which relate tree density breast height (DBH) with component biomasses (Figure S1), were applied to the DBH of the inventories performed at the same ages. All biomass values reported in this study refer to dry mass (Figure S2 and Table S1). The tree height was not applied due to lower relationship.

3.2.4.3 Stand canopy and structural variables

The individual tree leaf area ($\text{m}^2 \text{ tree}^{-1}$) was calculated from leaf biomass estimations. The leaf area index (LAI) ($\text{m}^2 \text{ m}^{-2}$) was predicted using the allometric leaf area equations for each treatment and species. Leaf angle distributions (LAD) were measured for biomass at 45 and 52 months of age, as follows. On each tree, at three heights in the canopy (upper, middle and lower), axillary branches were randomly selected among the four azimuthal quarters in the row and in the inter-row direction. Six leaves were randomly selected between the bottom and the end of each branch. On each of these leaves, the vertical component of the leaf blade inclination was measured. The LAD was obtained at 10° intervals from the 72 leaves measured for each species and treatment. It was measured the tree height (H), the crown diameter in both directions (row and inter-row), and the initial height to the live crown tree (H_i) for each tree harvested for biomass. The crown length (CL, m) was calculated as the difference between H and H_i . Allometric relationships were calibrated relating tree DBH with H_i , CL, total tree leaf area, and crown diameter in the row and inter-row directions.

3.2.5 Hemispherical photographs

For the validation of light interception simulated with MAESPA, and for the time-interpolation of LAI, hemi-photos were taken below the canopy with a hemispherical camera in order to obtain measurements of the directional gap fractions. Digital hemispherical photographs were taken in all treatments, in block B2, every two months from April 2017

(month 40) to April 2018 (month 52), using a Canon EOS 7D camera (18 megapixels) equipped with a Canon Fish-Eye. In each plot, six locations were sampled, located in two diagonal transects. The measurements were taken in a systematic grid to cover the central part of the plot and sample points at different distances from the trees. The camera was placed at 60 cm aboveground using a tripod, oriented, and leveled. Photographs were taken in the early morning in low-wind conditions and exposition was adjusted to obtain maximum contrast between the leaves and the sky. Three pictures were taken at each location, that is, 1260 pictures were taken (10 treatments*6 locations*3 pictures*7 dates) (Figure 8).

Figure 8. Hemispherical photos of each treatment taken at June 2017.

3.2.5.1 Analysis of hemispherical photographs

First, the camera and fish-eye lens system were calibrated according to the method proposed by CAN_EYE V6.4.91 User Manual in order to determine its optical center and projection function. CAN_EYE is an imaging software program used to extract features of the canopy structure from true color images taken with a fish-eye lens. Through a classification process, the image is converted from RGB to a binary image, separating green elements from the sky and the ground. It allows masking undesirable parts of the images and a "gamma"

factor could be changed to illuminate or darken the images and provide better visual discrimination between green elements and the background. From the zenithal gap fraction, CAN_EYE computes the Plant Area Index (PAI), Mean Leaf Leaning Angle (ALA), Photo-Active Absorbed Radiation Fraction (FAPAR), Plant Cover Fraction, and Bidirectional and Mono-Directional (FCOVER) using the classical light extinction model with the homogeneous turbid medium radiative transfer model (Table S2). Batch treatment for all 18 images per plot was carried out using only the zenith angle down to 70° due to the high trunk density seen at higher angles, having large confounding effect on gap fractions. Close-by tree trunks obtained at some images location were masked.

3.2.6 LAI time interpolation

The LAI was interpolated between the destructive measurements using the hemi-photos. Among the different estimations of PAI provided by CAN_EYE, PAIv5 provided the highest R^2 compared to destructive measurements, but with significant slopes and intercepts (Table S2). A linear regression between PAIv5 obtained by the CAN_EYE software and the destructive measurements was performed to correct CAN_EYE estimations based on destructive measurements. This correction is due to the presence of branches and different clumping of the canopies. A different linear regression was used for each tree spacing (3m x 6m, 3m x 3m and 2m x 3m). These corrections were applied on CAN_EYE PAIv5 of each date, allowing to obtain intermediary values of LAI, coherent with the LAI estimated from destructive measurements which are more precise.

Individual tree leaf area is estimated at the three destructive measurement date. Following the procedure detailed in le Maire et al. 2013, its contribution to the total Leaf Area (LA, m²) of the plot is computed and interpolated with time. The time-interpolated Leaf Area contributing fraction of each tree is then multiplied by the time interpolated stand-level LAI obtained using destructive measurements and hemispherical photographs estimations.

3.2.7 APAR modeling with MAESTRA along the year

In each plot, light absorption was simulated for each tree of the inner plot considering the shading effects of trees in the plots surrounding and/or six surrounding rows of buffer trees (i.e. trees in T3 (block 2) were modeled considering trees of T1 (block 1), T4 (block2), T8 (block 3) and six surrounding rows of buffer trees) (Figure 9). Block 2 was used for the simulations. The APAR of each treatments and species was calculated as the sum of the APAR of all trees in the plot. Daily individual tree APAR (MJ tree⁻¹ day⁻¹) was calculated

along one year, from February 1st, 2017 to April 30th, 2018. The complete description of APAR estimation could be found in le Maire et al. (2013).

Figure 9. Representation of the trial at 38 months after planting, an input of the MAESTRA model. Absorbed photosynthetically active radiation (APAR) was calculated for each *Eucalyptus* tree (in red) and *Acacia* tree (in green). X and Y are measured in meters (refer to Figure 7 for the orientation). This figure was created with the R package Maeswrap (R. Duursma, <http://cran.r-project.org/web/packages/Maeswrap/index.html>).

3.2.8 Light use efficiency computation and statistical analyses

Stem biomass (stem and bark) was calculated for each inner tree of each treatment at the beginning and end of the 1st year period of growth between 38 and 52 months after planting. The difference between these dates gave the Stem Biomass Growth (SBG gDM tree⁻¹ year⁻¹). In these fast-growing stands, the one-year period is long enough to have large tree growth, with variability among trees and species (see the Results section, with a tree biomass increase of 59% on average). Tree scale Light Use Efficiency (LUE, gDM MJ⁻¹) was computed as SBG divided by tree-scale APAR. The plot-scale APAR was computed as the sum of APAR of all trees within each inner plot divided by the area of that inner plot in order to test differences between the treatments and the effect of mixture. The two-way ANOVA was used to analyze differences between treatments and blocks for all variables ($P < 0.05$). The homogeneity of variances was assessed using the Levene test. When the variances were unequal, the values were transformed. The Bonferroni's multiple range test was used to compare the treatments when there was significant differences between treatments. The effects of density and mixing (M1), and density and neighbors (M2) on SBG, APAR and LUE of *Eucalypts grandis* and *Acacia mangium* were analyzed using a linear model (Eq.4). Statistical analyses were carried out using R 3.5.2 (R Core Team 2018).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon \quad (\text{Eq. 4})$$

Where:

X_1 =density of *Eucalyptus* (dE) and X_2 =density of *Acacia* (dA) (M1)

X_1 = dE or dA and X_2 = presence of other species (yes/no factor) (n) (M2)

3.3 Results

3.3.1 Monospecific stands

Since there was no significant difference between 100E and 100E+N for all variables analyzed, as observed in other studies (le Maire et al, 2013; Laclau et al., 2008), 100E was retained for further analysis. The mean LAI values over the study period were significantly higher in the monospecific *Acacia* stands than in the *Eucalyptus* stands (Table 3). This results in higher average APAR values for *Acacia* than for *Eucalyptus*, with LAI being the main explicative factor of APAR (compared to leaf angles, crown clumping, etc.). Despite the smaller amount of radiation absorbed, production of stem biomass was slightly higher for 100E than 100A (Table 3) for both densities: with 2758

and $2697 \text{ g m}^{-2} \text{ year}^{-1}$ for density of $555 \text{ trees ha}^{-1}$, and 2898 and $2713 \text{ g m}^{-2} \text{ year}^{-1}$ for density of $1111 \text{ trees ha}^{-1}$, respectively. The mean LUE values were on average 13% higher in 100E than 100A, meaning that for the same amount of light absorbed, *Eucalyptus* produced more stem, offsetting the lower absorption of radiation. An important result of monospecific trees was the tree height 100E on average 44% higher than 100A (Table 3).

Table 3. Leaf area index (LAI), absorbed photosynthetically active radiation (APAR), stem biomass growth (SBG), and light use efficiency (LUE) mean values $\pm$ standard error (n=3 for the 3 blocks) between 38 and 52 months after planting. Different letters indicate significant differences ($P < 0.05$): a, b, etc. for differences between treatments; A, B for differences between *Eucalyptus* trees; α , β and γ for differences between *Acacia* trees

Design	Spacing	Species	Density (tree ha ⁻¹)	LAI (m ² m ⁻²)				APAR (MJ m ⁻² year ⁻¹)				SBG (g m ⁻² year ⁻¹)				LUE (g MJ ⁻¹)				Height (m)							
				Mean	Std	Test		Mean	Std	Test		Mean	Std	Test		Mean	Std	Test		Mean	Std	Test					
100A	3m x 6m	A	555	4.80	0.12	bc		α	3189	103	d	$\alpha\beta$		2697	43	c	α		0.84	0.04	abc		α	10.69	0.43	β	
100E	3m x 6m	E	555	3.57	0.02	f	AB		2963	63	d	AB		2758	76	bc	AB		0.91	0.03	ab	AB		20.55	0.49	A	
100E	3m x 3m	E	1111	3.33	0.02	f	AB		2972	382	d	AB		2898	75	bc	AB		0.95	0.13	a	A		20.59	0.24	A	
100A	3m x 3m	A	1111	5.34	0.04	a		α	3414	111	cd		α	2713	98	c		α	0.78	0.01	abcd		α	12.34	0.58	$\alpha\beta$	
33A:67E	3m x 3m	E	744	3.09	0.06		AB		3218	56		AB		2630	71		AB		0.84	0.02		AB		20.69	0.68	A	
33A:67E	3m x 3m	A	367	0.97	0.05			β	806	26			γ	422	10			β	0.52	0.01			β	12.19	0.99	$\alpha\beta$	
33A:67E	3m x 3m	A+E	1111	4.06	0.02	e			4023	58	bc			3052	65	bc			0.76	0.02	abcd						
50A:50E	3m x 3m	E	556	2.25	0.01		B		2264	49		B		1938	73		B		0.87	0.03		AB		20.59	0.44	A	
50A:50E	3m x 3m	A	555	2.23	0.05			$\alpha\beta$	1652	17			$\alpha\beta\gamma$	875	17			$\alpha\beta$	0.52	0.01			β	12.87	0.21	α	
50A:50E	3m x 3m	A+E	1111	4.48	0.06	d			3915	66	bc			2813	89	bc			0.70	0.02	bcd						
100E	2m x 3m	E	1666	3.88	0.02	e	A		4150	69	b	A		3961	109	a	A		0.93	0.01	a	AB		20.07	0.33	A	
33A:67E	2m x 3m	E	1116	3.59	0.06		AB		4034	75		A		2677	52		AB		0.67	0.00		B		19.91	0.46	A	
33A:67E	2m x 3m	A	550	0.97	0.06			β	1016	49			$\beta\gamma$	533	16			β	0.51	0.01			β	11.88	0.29	$\alpha\beta$	
33A:67E	2m x 3m	A+E	1666	4.56	0.05	cd			5050	45	a			3210	38	b			0.63	0.01	cd						
50A:50E	2m x 3m	E	833	2.62	0.04		AB		2934	27		AB		1944	74		B		0.66	0.02		B		20.08	0.24	A	
50A:50E	2m x 3m	A	833	2.26	0.02			$\alpha\beta$	2311	15			$\alpha\beta\gamma$	1115	45			$\alpha\beta$	0.49	0.02			β	12.84	0.36	α	
50A:50E	2m x 3m	A+E	1666	4.88	0.02	b			5245	37	a			3099	119	bc			0.57	0.02	d						
100E+N	3m x 3m	E	1111	3.35	0.04	f	AB		3477	84	bcd	AB		2943	141	bc	AB		0.78	0.03	abcd	AB		20.47	0.46	A	

3.3.2 Effect of replacement series

Average values of LAI, APAR, SBG, LUE, and Height for each treatment and their associated statistics are given in Table 3. The total mean LAI values was significantly higher in 50A:50E and 33A:67E than 100E, and significantly higher in 50A:50E than 33A:67E, when comparing the plots with the same tree densities (Figures 10a and 10b). In 50A:50E, the species-specific LAI values were similar for both *Eucalyptus* and *Acacia* and in 33A:67E, the values were on average 30% for *Acacia* and 70% for *Eucalyptus*. It is interesting to note that *Eucalyptus*-specific LAI values were not significantly different in monospecific plot and in 33A:67E at the same density, meaning that for LAI, the smaller number of *Eucalyptus* (replaced by *Acacias*) was totally offset by an increase of the average leaf area per *Eucalyptus* trees. This compensation effect does not hold for 50A:50A, with lower *Eucalyptus*-specific LAI for both densities (Figures 10a and 10b).

The average APAR values followed the same pattern of LAI. APAR was significantly higher in mixtures than in 100E and did not differ between 33A:67E and 50A:50E in the plots with the same tree density (Figures 10c and 10d). In both replacement series, *Eucalyptus* trees absorbed a larger part of PAR than *Acacia* trees did. The APAR of *Acacia* trees in 33A:67E and 50A:50E (1111 trees ha⁻¹) represented 23% and 48% of the value measured in 100A. The APAR of *Eucalyptus* trees showed a different pattern with 108% and 72% (1111 trees ha⁻¹) in 33A:67E and 50A:50E and with 97% and 71% (1666 trees ha⁻¹) of the value of 100E, respectively. While APAR of *Acacia* was reduced almost proportionally to its tree proportion within the stand, it was not the case for *Eucalyptus*, which offset its reduction of number of trees by increasing the individual light absorption, keeping high APAR values even with 50% less trees.

The stand-level mean SBG values did not vary significantly between 100E, 33A:67E and 50A:50E in the stand with 1111 trees ha⁻¹ (Figure 10e), while it was significantly higher in 100E than 33A:67E and 50A:50E in the stand with 1666 trees ha⁻¹ (Figure 10f). The *Eucalyptus* mean SBG values were significant between 100E and 33A:67E did not differ in the stand with 1111 trees ha⁻¹, despite a surface of soil proportionally reduced in 33A:67E. However, *Eucalyptus* SBG was reduced in 50A:50E, but not proportionally to the reduction of *Eucalyptus* tree number (Figure 10e). In the stands with 1666 trees ha⁻¹, the effect of reducing the number of *Eucalyptus* trees was less offset by an increase of production per tree (Figure 10f). In 50A:50E with 1111 trees ha⁻¹, the SBG of *Acacia* was only 32% of 100A with the same density. To summarize, replacement of *Eucalyptus* by *Acacia* was equivalent for total SBG at low planting density, but with clear changes on average species-specific SBG which

increased for *Eucalyptus* and decreased for *Acacias*. At higher density, where *Eucalyptus* monocultures were more productive, there was still this change of species-specific SBG in replacement tests, but it did not offset the reduction in number of *Eucalyptus* trees replaced by *Acacias*, resulting in a high reduction of stand-level SBG.

The mean LUE values were on average 21% higher in 100E than in 50A:50E and 33A:67E in the stands with 1111 trees ha⁻¹. In the stands with 1666 trees ha⁻¹, the mean LUE values were also significantly higher in 100E than 33A:67E and 50A:50E (Figures 10g and 10h). The LUE of *Acacia* was significantly higher in 100A than 33A:67E and 50A:50E with 1111 trees ha⁻¹ (Figure 10g) and did not vary between 33A:67E and 50A:50E for both densities 1111 and 1666 trees ha⁻¹. The mean LUE values of *Eucalyptus* did not vary between 100E, 33A:50E, and 50A:50E in the stands with 1111 trees ha⁻¹ (Figure 10g), while it was significantly higher in 100E than in 33A:67E and 50A:50E in the stands with 1666 trees ha⁻¹ (Figure 10h). In mixtures, the LUE of *Eucalyptus* was on average 30% higher than LUE of *Acacia* showing that *Acacia* trees, which already intercepted less light as observed above, also used this light less efficiently than *Eucalyptus* trees did. Replacement of more *Eucalyptus* by *Acacia* decreased further the stand scale LUE. However, it was interesting to note that species-specific LUE of *Eucalyptus* and *Acacias* did not change much between the levels of mixtures. It implies a major change in trees functioning when passing from a monoculture to a mixture at the levels of mixtures studied; however, such changes did not occur when only the proportion of the mixture was changed.

Figure 10. The effect of 100A, 100E, 33A:67E, and 50A:50E treatments and species on leaf area index (LAI; a, b), absorbed photosynthetically active radiation (APAR; c, d), stem biomass growth (SBG; e, f) and light use efficiency (LUE; g, h) at each spacing (3 m x 3 m and 2 m x 3 m). Different letters indicate significant differences ($P < 0.05$): A, B, C for differences between treatments; a, b, c for differences between *Eucalyptus* trees; α and β for differences between *Acacia* trees. Black bars represented total LUE in 33A:67E and 50A:50.

At tree scale, 33A:67E and 50A:50E showed a better correlation between stem production and light absorbed compared to 100E in both densities (Figures 11a and 11b). On the other hand, SBG per tree and LUE was better correlated in 100E than in 33A:67E and 50A:50E at both densities (Figures 11c and 11d).

Figure 11. The effect of treatments 100E, 33A:67E, and 50A:50E on *Eucalyptus* stem biomass growth (SBG) in function of *Eucalyptus* absorbed photosynthetically active radiation (APAR, Fig. a, b) and *Eucalyptus* light use efficiency (LUE, Fig. c, d) at each spacing (3m x 3m and 2m x 3m). The Pearson's correlation coefficient (R^2) is given with its significance $p < 0.0001$ marked as *.

For *Acacia*, the correlation between SBG and APAR, and SBG and LUE was significant for all treatments 100A, 33A:67E and 50A:50E at both densities, except for 33A:67E with 1666 trees ha⁻¹, which showed no significant correlation between SBG and APAR (Figure 12).

Figure 12. The effect of treatments 100A, 33A:67E, and 50A:50E on *Acacia* stem biomass growth (SBG) in function of *Acacia* absorbed photosynthetically active radiation (APAR, Fig. a, b) and *Acacia* light use efficiency (LUE, Fig. c, d) at each spacing (3m x 3m and 2m x 3m). The Pearson's correlation coefficient (R^2) is given with its significance $p < 0.0001$ marked as *.

In summary, for *Eucalyptus*, inter-tree variability of SBG was mostly explained by differences in light absorption, driven by differences in tree size (Figure 13) but was less or not at all explained by differences of LUE (Figure 14). For *Acacias*, the inter-tree variability was explained both by APAR and by LUE, with both variables linked. However, these tree-scale values of APAR and LUE were less explained by tree height than for *Eucalyptus* (Figures 13 and 14).

Figure 13. Absorbed photosynthetically active radiation (APAR) as a function of tree height for all trees in *Acacia* monocultures (100A), *Eucalyptus* monocultures (100E) and replacement series (33A:67E and 50A:50E) for both species at 3 m x 3 m and 2 m x 3 m spacing. The Pearson's correlation coefficient (R^2) is given with its significance $p < 0.0001$.

Figure 14. Light use efficiency (LUE) as a function of tree height for all trees in *Acacia* monocultures (100A), *Eucalyptus* monocultures (100E) and replacement series (33A:67E and 50A:50E) for both species at 3 m x 3 m and 2 m x 3 m spacing. The Pearson's correlation coefficient (R^2) is given with its significance $p < 0.0001$.

3.3.3 Effects of stand density

The mean LAI values in 100E were significantly higher with 1666 trees ha⁻¹ than 1111 and 555 trees ha⁻¹. This result reflected in the mean values of APAR and SBG that followed the same pattern (Table 3); however, there was no significant difference for mean LUE values and tree height between these densities, despite the large differences it created in terms of inter-row gaps and tree structure. In 100A, the mean LAI, APAR, SBG, and LUE values were

not significantly different between each other when the planting density was divided by two, from 1111 trees ha⁻¹ to 555 trees ha⁻¹, meaning that there were large changes in individual leaf areas and growth (Table 3).

The total mean values of LAI and APAR in 33A:67E and 50A:50E were significantly higher with 1666 trees ha⁻¹ than with 1111 trees ha⁻¹ and in 33A:67E and 50A:50E, SBG did not vary between densities. The LUE value in mixtures was an average 18% higher with 1111 trees ha⁻¹ than with 1666 trees ha⁻¹ (Table 3). For *Eucalyptus*, the LAI and APAR in 33A:67E and 50A:50E were on average 14% and 21% higher with 1666 trees ha⁻¹ than with 1111 trees ha⁻¹, while the LUE was 20% and 24% higher at densities with 1111 trees ha⁻¹ than with 1666 trees ha⁻¹. The SBG and tree height did not vary between densities for both mixtures (Table 3). For *Acacia*, the LAI, LUE and tree height in 33A:67E and 50A:50E did not vary between densities, while the APAR and SBG in 33A:67E and 50A:50E were on average 21% and 25% higher with 1666 trees ha⁻¹ than with 1111 trees ha⁻¹ (Table 3). Although changes in mixtures densities affected LAI, APAR, and LUE of *Eucalyptus*, they did not affect its productivity. Contrary to *Eucalyptus*, high densities lead to a higher APAR and SBG of *Acacia*. It seems that, in mixture, changes in densities on the stand-scale have more effect on *Acacia* than on *Eucalyptus*.

At densities with 1111 trees ha⁻¹, the SBG of an average *Eucalyptus* tree (dividing the stand value by the number of trees) was 1.3 (50A:50E) and 1.4 (33A:67E) times higher than in the monoculture stand, while at densities with 1666 trees ha⁻¹, the SBG of an average *Eucalyptus* tree was 1.0 (50A:50E) and 0.98 (33A:67E). This finding implies that individual *Eucalyptus* trees improved their productivity in the mixtures with lower densities, while there was no effect at higher densities.

3.3.4 Disentangling the effects of species density, proportion of mixing, and their interactions on SBG, APAR and LUE (M1 model)

The results of the linear modeling are presented in Table 4. For instance, the SBG of *Eucalyptus* was highly affected by its own density at plot scale, with a positive and significant effect. *Acacia* density had a small negative effect on *Eucalyptus* SBG in this model, that is, higher density of *Acacia* led to a decrease of *Eucalyptus* SBG and this negative effect was not dependent on *Eucalyptus* density itself since there was no significant interaction (Table 4 and Figure 15a). For *Acacia*, the SBG was highly negatively affected by *Eucalyptus* density. Interestingly, *Acacia* SBG was affected by *Acacia* density, but this greatly depends on which *Eucalyptus* density was associated (dA effect was significant only through the interaction

factor). This is clearly visible in Figure 15b.

The *Eucalyptus* APAR was positively and highly affected by *Eucalyptus* planting density. *Acacia* density had a slightly significant negative effect on *Eucalyptus* APAR, and such effect depends on the level of interaction between *Acacia* and *Eucalyptus* densities. (Table 4 and Figure 15c). The APAR of *Acacia* followed the same pattern of SBG with a significant effect of *Acacia* density, but only in interaction with *Eucalyptus*, and as the number of *Eucalyptus* increased, *Acacia* showed a great loss of APAR (Table 4 and Figure 15d).

Interestingly, the model showed no significant effects of *Eucalyptus* or *Acacia* density, alone or in interaction, on stand-level *Eucalyptus* LUE. This means that when densities increased, the *Acacia* seemed to affect negatively the LUE of *Eucalyptus* (Figure 15e). The model itself was significant ($p=0.005$), but with a lower R^2 . The LUE of *Acacia* was highly and negatively affected by *Eucalyptus* density. *Acacia* LUE depended also on *Acacia* density in interaction with *Eucalyptus* (Table 4 and Figure 15f).

Table 4. Parameter estimates, standard error (std error), t-value and P for models describing the SBG, APAR and LUE of *E. grandis* and *A. mangium*. M1 models test the effect of mixture and M2 models test the effect of a neighbor; where, for M1: dE=density of *Eucalyptus*; dA=density of *Acacia*; dExdA=the interaction between density of *Eucalyptus* and density of *Acacia*; for M2: n=presence of other species (yes/no factor), d is the density of the species under consideration, and nxd means their interaction.

	Parameter	Estimate	<i>E. grandis</i>				<i>A. mangium</i>				
			std error	t-value	P	R ²	Estimate	std error	t-value	P	R ²
M1											
SBG (g m ⁻² year ⁻¹)	Intercept	1928	263.7	7.309	***	0.79	2416	273.7	8.826	***	0.96
	dE	1.090	0.224	4.864	***		-7.563	0.788	-9.595	***	
	dA	-1.268	0.665	-1.907	.		0.316	0.313	1.008	n.s.	
	dExdA	0.001	0.001	1.128	n.s.		0.007	0.001	7.105	***	
	Intercept	2158	292.8	7.370	***	0.74	2684	363.6	7.383	***	0.93
APAR (MJ m ⁻² year ⁻¹)	dE	1.105	0.249	4.442	***		-8.172	1.047	-7.803	***	
	dA	-1.691	0.738	-2.290	*		0.707	0.416	1.698	n.s.	
	dExdA	0.002	0.001	2.833	*		0.009	0.001	6.530	***	
	Intercept	0.887	0.087	10.157	***	0.46	0.857	0.050	17.050	***	0.94
LUE (g MJ ⁻¹)	dE	0.000	0.000	0.092	n.s.		-0.001	0.000	-7.181	***	
	dA	0.000	0.000	0.105	n.s.		0.000	0.000	-1.094	n.s.	
	dExdA	0.000	0.000	-1.036	n.s.		0.000	0.000	4.437	***	
M2											
SBG (g m ⁻² year ⁻¹)	Intercept	1414	368.4	3.837	**	0.77	-167.5	139.4	-1.202	n.s.	0.98
	n	524.3	454.9	1.153	n.s.		2847.8	222.1	12.821	***	
	d	1.088	0.440	2.471	*		1.586	0.232	6.824	***	
	nxd	-0.005	0.495	-0.011	n.s.		-1.556	0.305	-5.109	***	
APAR (MJ m ⁻² year ⁻¹)	Intercept	689.5	399.7	1.725	.	0.76	-441.3	240.1	-1.838	.	0.96
	n	1515	493.5	3.070	**		3405	382.7	8.899	***	
	d	2.983	0.478	6.247	***		3.275	0.400	8.181	***	
	nxd	-1.915	0.537	-3.566	**		-2.870	0.525	-5.470	***	
LUE (g MJ ⁻¹)	Intercept	1.068	0.120	8.875	***	0.48	0.558	0.035	16.056	***	0.96
	n	-0.196	0.149	-1.320	n.s.		0.335	0.055	6.055	***	
	d	0.000	0.000	-2.663	*		0.000	0.000	-1.453	n.s.	
	nxd	0.000	0.000	2.488	*		0.000	0.000	-0.225	n.s.

Figure 15. Effect of mixture (M1) and effect of a neighbor (M2) as a function of planting density on stem biomass growth (SBG), on absorbed photosynthetically active radiation (APAR) and on light use efficiency (LUE) for *Eucalyptus grandis* (a,c and d) and for *Acacia mangium* (b, d and f) where: dE = density of *Eucalyptus*; dA = density of *Acacia*; dExdA/dAx dE = the interaction between density of *Eucalyptus* and density of *Acacia*; n = presence of other species and dExn/dAxn = the interaction between the presence of other species and the density of *Eucalyptus*/*Acacia*. All models are presented in Table 4.

3.3.5 Disentangling the effect of stand density and presence of another species in the plot (M2 model)

The results obtained above showed a change of behavior of *Eucalyptus* and *Acacias* when they were mixed. This behavior was also highlighted with model M2, where the presence of the other species was placed as function of the model (yes/no variable). The results showed that *Eucalyptus* SBG per unit of plot area was affected only by *Eucalyptus* tree density (Table 2 and Figure 8a). The fact that *Eucalyptus* was planted on monospecific plantation or not statistically does not change its productivity, only the number of *Eucalyptus* trees influenced its productivity for the density ranges and mixture proportion *Eucalyptus* and *Acacia* tested. *Acacia* density significantly impacted *Acacia* SBG; however, contrary to *Eucalyptus*, the fact that *Acacia* was associated with *Eucalyptus* provided significant changes in *Acacia* SBG. The interaction was also important, that is, effects of *Acacia* density were not the same when *Acacia* was mixed or not (Figure 8b, $p < 0.0001$).

In turn, APAR presented direct effects of density and presence of the other species, for both *Acacias* and *Eucalyptus*. LUE, however, displayed a contrasted behavior: *Eucalyptus* LUE depended on *Eucalyptus* density and this dependence was affected by *Acacia* presence, while *Acacia* LUE was highly influenced by the presence of *Eucalyptus*, but not by its own density.

3.4 Discussion

3.4.1 Light absorption, light use efficiency and stem biomass growth

Our results show that the APAR of *Eucalyptus* did not significantly differ between 100E and 33A:67E. The smaller number of *Eucalyptus* in 33A:67E was totally offset by an increase of APAR by *Eucalyptus* trees; however, in 50A:50E this compensation effect occurred only in stands with 1111 trees ha^{-1} . Similar to our study, le Maire et al. (2013) showed that individual *Eucalyptus* light absorption was higher than in 100E in a replacement series of 50A:50E with 1111 trees ha^{-1} and in additive series of 25A:100E, 50A:100E and 100A:100E located in the same experimental site. In mixed plantations the stratified canopy can lead to a better *Eucalyptus* light absorption due to lower inter than intra-specific competition for light (Bauhus et al., 2004; Forrester et al., 2004; le Maire et al., 2013; Forrester et al., 2014). LUE of *Eucalyptus* did not change much between monoculture and mixtures at densities with 1111 trees ha^{-1} . However, at densities with 1666 trees ha^{-1} when replacing *Eucalyptus* by *Acacia* trees (33A:67E and 50A:50E), LUE decreased significantly in relation to 100E. Le Maire et al. (2013) showed that the LUE of *Eucalyptus* decreased with

the increase of *Acacia* trees density in a comparison of 100A:100E and 100E. Moreover, LUE of *Acacia* decreased even more, reducing by 70%, in comparison to 100A (le Maire et al., 2013). In our study, the smaller size of *Acacia* trees (height 40% lower than *Eucalyptus*) in both mixtures (33A:67E and 50A:50E) had lower APAR, but also lower LUE than in 100A. This result could be explained by the high sensitivity of *Acacia* to *Eucalyptus* trees competition. Others studies have shown that LUE of *Eucalyptus* increased in mixed plantations in relation to their respective monocultures (Blinkey et al., 1992; Forrester et al., 2012). Higher LUE could result from higher rates of photosynthesis, or lower allocation to other sinks, such as respiration and belowground production (Binkley et al., 2010). Interactions that improve APAR and LUE may increase with increasing soil resources availability, or more favorable climatic conditions (Kelty, 1992; Forrester et al., 2014).

Total SBG at stand level was equivalent when replacing *Eucalyptus* by *Acacia* trees at low planting density (1111 trees ha⁻¹) showing that individual *Eucalyptus* trees improved their productivity in the mixtures. At higher density (1666 trees ha⁻¹), total SBG of *Eucalyptus* was lower in mixtures than in 100E. In mixtures, SBG of individual *Eucalyptus* trees also increased its productivity in relation 100E, but not enough to offset the loss of *Eucalyptus* trees replaced by *Acacias* at a stand level. An increase in tree density could have or not a positive effect among species (Condes et al., 2013). For *Acacia*, SBG was highly and negatively affected by the increase of *Eucalyptus* trees density. Le Maire et al. (2013) also found that the increase of the number of *Eucalyptus* trees impact negatively SBG of *Acacia* trees. The authors suggested that the inter-specific competition was lower than the *Eucalyptus* intra-specific competition, but higher than the *Acacia* intra-specific competition. In addition, after the closure of the canopy, *Acacia* trees invested more biomass in organs to capture resources than in stem wood production, due to the stronger inter-specific competition for water (le Maire et al., 2013). In our study, the variability of *Eucalyptus* SBG was mainly explained by differences in APAR. Conversely, SBG of *Acacia* trees was explained by both APAR and LUE. In mixed plantations of *Eucalyptus* and *Acacia mangium* (50A:50E), the inter-specific dominance of *Eucalyptus* trees strongly enhanced their stem growth and APAR in comparison to the 100E treatment, showing that the differences in growth between the stands were attributed to the quantity of radiation absorbed by the trees (le Maire et al., 2013).

Several studies have shown that the increases in wood production result from greater LUE and/or a shift in biomass allocation (Blinkey et al., 2004; Bauhus et al., 2004; Stape et al., 2010; Ryan et al., 2010; Binkley et al., 2010; Campoe et al., 2013). The complementary effect of light capture between species in mixed plantation, due to canopy stratification, may

not lead to an increase in stem biomass if availability of other important resources is limiting the plant growth (Binkley et al., 2004; le Maire et al., 2013) and/or if competition is intense (Marron and Epron, 2019). The relationship between complementary effects and tree density depends on which resource is the most limiting factor to growth (Forrester 2014). Forrester et al. (2013) showed that higher tree density increased complementarity between *Picea abies* and *Abies alba* in areas where mixtures were more productive than monocultures. Competitive interaction are more current when belowground resources, such as water and nutrients, are limiting factors rather than light (Forrester 2014, Pretzsch and Biber, 2010). Belowground competition for water between *Eucalyptus* and *Acacia* (le Maire et al. 2013) and changes in nutrient allocation (Nouvellon et al., 2012) possibly contribute to a decrease in LUE and SBG between species at higher tree densities. In summary, *Eucalyptus* and *Acacias* changed their behaviors when they were mixed and changes in trees density could increase or reduce the complementary effects on mixed plantations (Garber and Maguire, 2004; Amoroso and Turnblom, 2006; Río and Sterba, 2009; Condés et al., 2013).

3.4.2 Mixtures and neighbor effects x densities

The two models (M1 and M2) used in our study allowed understanding the behavior of each species in relation to tree density and mixture or presence of other species. Our study revealed that the changes in SBG, APAR, and LUE of *Acacia* resulted from the combined effects of density and presence of *Eucalyptus*, while changes in SBG and APAR of *Eucalyptus* were strongly dependent on its own density. Changes in LUE for *Eucalyptus* trees varied between models. The first model showed no significant effects of *Eucalyptus* or *Acacia* density, while the second model showed that *Eucalyptus* LUE depends on its own density. Mathematical models for the impact of frequency and density dependence could be used to predict the condition under which additional effects could change ecological processes (Underwood et al., 2014). Vila et al. (2013), using a structural equation modeling, showed that the increase in timber production with tree species richness was largely mediated by a positive association between the basal stand area and tree species richness. Obriang et al., (2012) showed that the growth model was useful for forest management using a growth model to study the effects of tree size derangement and local competition on tree growth. Growth strongly responded to local stand attributes, such as tree density and basal area. Our study indicated the importance of disentangling the effect of stand density and presence of another species or mixture for a better understanding of the impact of associative effects on species behavior.

3.4.3 Forest management

Our last objective was to investigate an optimal tree density and planting design for maximizing total wood production. Although stratified canopies increased light absorption in mixtures, the highest productivity was reached in *Eucalyptus* pure stand with the highest tree density. Our study shows that the choice for less productive *Eucalyptus* is an option to maximize wood stand production in *Eucalyptus* and *Acacia mangium* mixed plantation. A less productive *Eucalyptus* species or clones, with less water consumption could also decrease inter-specific competition. The greater production of mixed plantations also depends on compatibility between height growth rates (Forrester et al., 2006a). Increasing the productive potential of *Acacia* is necessary to improve its competitiveness against *Eucalyptus* in mixed stands (Santos et al., 2016). Furthermore, we showed that *Eucalyptus* in the arrangement 33A:67E with 1111 trees ha⁻¹ had greater SBG at stand level than in 50A:50E and a similar production to 100E. Planting two rows of *Eucalyptus* followed by one row of *Acacia* at densities with 1111 trees ha⁻¹ seems a good option to facilitate the harvesting of trees and reduce the implantation costs. Despite productivity and financial returns of mixed plantations, they are not likely to reach returns in *Eucalyptus* monocultures in more productive sites, such as in our study, other advantageous aspects should be taken into account, such as increasing biodiversity (Pereira et al., 2019), reduction in pest attacks (Kelty, 2006), and droughts resistance when planting in regions with water stress (Gong et al., 2020).

Further studies should be developed to assess these planting arrangements and densities along a climatic gradient with better climatic condition for *Acacia mangium* growth (i.e. higher temperatures and air humidity), reducing inter-specific competition, promoting better growth and production in mixed plantations.

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4. TREE MORTALITY, TREE GROWTH AND BIOMASS PRODUCTION OF *EUCALYPTUS* AND *ACACIA MANGIUM* IN PURE AND MIXED-SPECIES PLANTATIONS ALONG AN ECOLOGICAL GRADIENT IN BRAZIL

ABSTRACT

Severe droughts, especially during the hottest months, are responsible for tree mortality and reduction of tree growth in tropical regions. Over the last years, *Eucalyptus* plantations in Brazil are expanding to tropical regions, such as Mato Grosso and Tocantins States. The new frontiers of *Eucalyptus* expansion in Brazil are located in stressful environments, such as low nutrient soils. Mixed-species plantations could be a solution to reduce water stress and improve soil fertility. Our study aimed to assess the impact of soil and climatic conditions on tree mortality, tree growth, and stand production in pure and mixed stands of *Eucalyptus* and *Acacia mangium* along an environmental gradient in Brazil. The experiments were established at Itatinga, in the state of São Paulo (December 2013), in Colinas, in the state of Tocantins (January 2014), and in Sinop, in Mato Grosso State (January 2015). The same experimental design was replicated in all the sites with pure *Eucalyptus* stand with and without N fertilization, pure stand of *Acacia mangium*, and a mixed-species plantation with 50% of *Acacia mangium* and 50% of *Eucalyptus*, without N fertilization. The rate of tree mortality and tree growth (i.e. height and circumference) were measured at 12, 24, 38, and 52 months after planting in Itatinga-SP; at 12, 36, and 54 months after planting in Colinas-TO; and at 15, 26, 36, and 52 months after planting in Sinop-MT. Aboveground biomass equations were established and soil samples were collected in Itatinga at 52 months of age, in Colinas at 54 months of age, and in Sinop at 52 months of age. *Eucalyptus* mortality ranged from 3-6 % in Itatinga, 1-3 % in Colinas, and 27-56 % in Sinop. In Sinop, mortality of *Eucalyptus* trees was higher in 50A:50E than in 100E. The mortality rates of *Acacia* vary from 10-17 % in Itatinga, 49-58 % in Colinas, and 4-23 % in Sinop. In Colinas, mortality of *Acacia* trees was higher in 50A:50E than in 100A. *Eucalyptus* exhibited the greatest tree growth and the highest biomass production in Itatinga-SP, while the highest growth and stand production of *Acacia* were observed in Sinop-MT. Stemwood production of *Eucalyptus* and of *Acacia* was higher in pure stand than in mixed-species stand. These results highlight the importance of accounting for climatic and soil conditions under stressful conditions, such as severe droughts, to avoid large tree mortality and promote sustainable forest plantation management.

Key-words: Hotter droughts, *Eucalyptus* plantations, N₂-fixing trees, tree mortality.

4.1 Introduction

Climate forecasts predict an increase of droughts frequency and severity in tropical and subtropical regions with global temperature increasing by 2-4°C, on average (Sterl et al., 2008; Solomon et al., 2009; Allen et al., 2010; Dai, 2011; He and Soden, 2017). Droughts occurring during the hot periods of the year are called “hotter droughts” (IPCC, 2007; Sterl et al., 2008; Allen et al., 2010; Allen et al., 2015) and are characterized by soil and atmospheric drought (Williams et al., 2013). Hotter droughts can lead to massive tree mortality that could

be caused by physiological disorders as well as pest and pathogen issues (Anderegg et al., 2013; Allen et al., 2010; Allen et al., 2015).

In the recent years, climate change has been a major concern for tree growth and tree survival (Grossiord et al., 2018). In Brazil, due to its large variability of edaphoclimatic and topographic conditions, impacts of climate change may vary regionally (Ambrizzi et al., 2007; Nobre et al., 2011). The occurrence of extreme events and their frequency have caused significant economic losses (IPCC, 2007), mainly to the agricultural and forestry sectors, such as increase of tree mortality (Marengo et al., 2016). Furthermore, climate change projections predict an increase in mean annual air temperature throughout the country (Elli, 2019). As an example, in the summer of 2014, the air temperature was up to 2.5°C higher than normal and monthly rainfall was 150 mm below normal in the Cantareira region of the state of São Paulo (Marengo et al., 2016).

Forest plantations composed by the *Eucalyptus* genus have high demands for water (Christina et al., 2011) and present a great sensitivity to prolonged droughts (Marengo et al., 2016). In Brazil, *Eucalyptus* plantations cover an area of about 7.4 million hectares and represent 75% of the country's total planted forest area (IBGE-PEVS, 2017). Between 2013 and 2017, Mato Grosso do Sul State, characterized by high temperatures and low air humidity, expanded its *Eucalyptus* plantations with an increase of 460.000 hectares (IBGE-PEVS 2017). *Eucalyptus* plantations are also expanding to tropical areas, such as Mato Grosso and Tocantins States, and to equatorial zones such as Maranhão and Piauí States (Gonçalves et al., 2017). Gonçalves et al. (2017) reported that 200,000 ha of *Eucalyptus* plantations died during the long drought of 2014 in the northern Minas Gerais State, the region with most *Eucalyptus* plantations in the central region of Brazil (IBGE-PEVS 2017). In the last years, climate abnormalities have caused stress to many *Eucalyptus* plantations, as a consequence of increase in mean annual temperatures, intense rainfall events, severe water stress, and frequent and extreme weather events (Gonçalves et al., 2017).

Mixed forest plantations could be an alternative to monocultures for timber and biomass production, as well as for the recovery of degraded lands (Amazonas et al., 2018). In the context of global warming and frequent droughts, mixed-species plantations could be more conducive to alleviating water stress than monocultures (Gong et al., 2020). Mixtures of *Acacia mearnsii* and *Eucalyptus globulus* in Australia exhibited higher water use efficiency than monocultures, leading to significant increases in aboveground biomass production (Forrester et al., 2010). Mixed-species plantations of *Eucalyptus* and *Acacia mangium* could be established under various conditions of temperatures and precipitations (Bouillet

et al., 2013). Mixing species could enhance the resilience of tropical planted forests to climate changes by increasing their access to water and nutrients stored in very deep soil layers (Germon et al., 2018), since interspecific competition for water and nutrients may force root exploration of deep soil layers (Cardinael et al., 2015). Furthermore, mixed plantations of *Eucalyptus* and *A. mangium* could improve soil quality by intensifying biogeochemical cycles (Tchichelle et al., 2017; Santos et al., 2017; Voigtlaender et al., 2019) and reduce the susceptibility to pests and diseases (Petit and Montagnini, 2006).

Our study investigated the effects of climatic and soil conditions on tree mortality rate, tree growth, and stand production of pure and mixed-species plantations (1:1) of *Eucalyptus* and *Acacia mangium* established in three regions of Brazil, South-East (São Paulo), North (Tocantins) and Central-West (Mato Grosso), with contrasting soil and climatic conditions. Studies have shown that mixed-species plantations of *Acacia mangium* and *Eucalyptus* could be more productive than *Eucalyptus* monoculture on nutrient-poor soils with low water limitation, when hot and humid climatic conditions are favorable for *Acacia* growth but are not optimal for *Eucalyptus* plantations (Bouillet et al., 2013, Santos et al., 2016). Our hypotheses are: (1) in Colinas-TO, characterized by high temperatures and harsh dry seasons, the mortality rate will be higher for *Acacia* than *Eucalyptus* and it will be higher in mixture than in pure stands, (2) in Sinop-MT, where the climate conditions are favorable for the development of *Acacia* trees and unfavorable for *Eucalyptus* trees, the mortality rate of *Eucalyptus* will be higher than *Acacia* and it will be higher in mixture than in pure stands and (3) *Eucalyptus* have a higher tree growth and stand biomass production in Itatinga than in Colinas, while for *Acacia* tree growth and stand production will be higher in Sinop than in Itatinga.

4.2 Material and methods

4.2.1 Study sites

The first site was located at the Experimental Station of Itatinga-São Paulo (SP - southeastern Brazil). Over the 10 years prior to this study, the mean annual rainfall was 1476 mm. During the study period, the mean annual rainfall was 1732 mm and the mean annual relative humidity was 72.3%. A dry season occurs between June and September. The mean air temperature was 20.3 °C, with lower temperatures between May and August (Table 5). The soil is Red-Yellow Latosol (Table 6). The second site is located at the International Paper Experimental Field in Colinas-Tocantins (TO - northern Brazil). Over the 10 years prior to this study, the mean annual rainfall was 1798 mm. A dry season occurs between May and

September. During the study period, from January 2014 to July 2018, the mean annual rainfall and the relative humidity were 1654 mm and 75%, respectively. The mean air temperature was 25.8 °C, with little variation throughout the year (Table 5). The soil is a Quartzarenic Neossol (Table 7). The third site is located at the Embrapa Agrossilvipastoril Experimental Field in Sinop-Mato Grosso (MT - central-western Brazil). Over the 10 years prior to this study, the mean annual rainfall was 2193 mm. During the study period (from January 2014 to July 2018), the mean annual rainfall and the relative humidity were 1910 mm and 82.5%. A dry season occurs between May and September. The mean air temperature was 25.5 °C, with few variations throughout the year (Table 5). The soil is a Red-Yellow Latosol (Table 8). In all sites, the pH was acidic varying between 3.4 and 4.6. The amounts of available P and K were low at all sites. The amount of Soil Organic Matter (SOM) was also low, with the highest values found in Itatinga-SP (24-72 mg kg⁻¹), followed by Sinop-MT (19-28 g kg⁻¹), and Colinas-TO (8-12 g kg⁻¹). Nutrient contents decreased with soil depth (0-5 and 5-15cm). The soils differed in texture, with clay content varying from 156-204 g kg⁻¹ in Itatinga-SP, 66-87 g kg⁻¹ in Colinas-TO, and 218-263 g kg⁻¹ in Sinop-MT (Tables 6, 7 and 8).

Table 5. Climatic characteristics of each experimental site.

Site	Koppen Classification	Mean annual rainfall prior 10 years (mm)	Rainfall accumulated over study period (mm)	Mean annual rainfall over each year of the study period (mm)	Dry season (months)	RH (%)	Tmax (°C)	Tmin (°C)	Tmean (°C)
Itatinga SP	Cfa - with hot summer	1476	7449	2014 - 747	April-August	72.3	22.3	16.9	20.3
				2015 - 1866					
				2016 - 1970					
				2017 - 2303					
				2018 (until April) - 562					
Colinas TO	Aw - with dry winter	1798	7611	2014 - 2012	May-September	75	32.6	20.7	25.8
				2015 - 1153					
				2016 - 1571					
				2017 - 1801					
				2018 (until July) - 1074					
Sinop MT	Am - monsoon	2193	8597	2015 - 1610	May-September	82.5	32.7	20.1	25.5
				2016 - 2029					
				2017 - 1995					
				2018 - 2032					
				2019 (until June) - 932					

Koppen Classification according to Alvares et al. (2013)

RH=Mean relative humidity

Tmax=Mean maximum air temperature

Tmin=Mean minimum air temperature

Tmean=Mean air temperature

Table 6. Main soil characteristics in the experiment located in Itatinga-SP.

Treatment	Soil layer (cm)	P	O.M	pH	K	Ca	Mg	H+Al	Al	SB	T	V	Sat. Al ³⁺	Clay	Silt	Sand
		mg kg ⁻¹	g kg ⁻¹	CaCl ₂				mmolc+ kg ⁻¹					%		g kg ⁻¹	
100E	0-5	8	72	3.6	1	8	24	196	36	33	229	17	53	170	76	754
	5-15	4	24	3.8	1	4	7	70	19	11	81	14	62	156	34	809
100E+N	0-5	9	72	3.4	1	6	25	200	43	33	233	14	58	183	75	742
	5-15	4	28	3.7	1	4	6	102	26	11	113	10	69	204	28	768
100A	0-5	7	59	3.6	1	12	23	171	37	36	207	17	58	183	64	753
	5-15	4	25	3.8	1	5	8	80	20	14	94	15	59	197	27	776
50A:50E	0-5	6	59	3.6	1	8	13	142	29	22	164	15	54	183	50	766
	5-15	4	24	3.8	1	4	7	78	20	12	90	14	60	197	28	775

Table 7. Main soil characteristics in the experiment located in Colinas-TO.

Treatment	Soil layer (cm)	P	O.M	pH	K	Ca	Mg	H+Al	Al	SB	T	V	Sat. Al ³⁺	Clay	Silt	Sand
		mg kg ⁻¹	g kg ⁻¹	CaCl ₂				mmolc+ kg ⁻¹					%		g kg ⁻¹	
100E	0-5	3	11	4.0	1	5	5	35	8	11	45	24	44	81	10	909
	5-15	3	10	4.0	1	4	4	31	8	9	40	22	48	87	25	888
100E+N	0-5	4	11	4.0	1	5	5	33	8	10	43	23	45	66	24	910
	5-15	3	9	4.0	1	4	5	31	8	10	41	25	43	71	31	898
100A	0-5	4	12	4.0	1	5	7	32	7	12	44	28	38	66	16	918
	5-15	3	10	4.0	1	4	5	30	8	10	39	25	43	76	17	907
50A:50E	0-5	3	11	4.0	1	4	6	29	7	10	40	26	41	71	19	910
	5-15	3	8	4.1	1	4	5	28	7	9	37	25	42	76	31	892

Table 8. Main soil characteristics in the experiment located in Sinop-MT.

Treatment	Soil layer (cm)	P	O.M	pH	K	Ca	Mg	H+Al	Al	SB	T	V	Sat. Al ³⁺	Clay	Silt	Sand
		mg kg ⁻¹	g kg ⁻¹	CaCl ₂				mmolc+ kg ⁻¹					%		g kg ⁻¹	
100E	0-5	5	26	4.6	1	12	7	44	3	21	65	32	15	218	55	727
	5-15	4	23	4.5	1	11	6	43	4	18	60	29	19	253	36	710
100E+N	0-5	5	28	4.5	1	13	8	46	4	22	68	32	18	249	66	685
	5-15	4	21	4.3	1	8	5	48	7	14	62	23	33	263	31	705
100A	0-5	5	28	4.4	1	13	9	46	4	23	70	33	17	223	50	727
	5-15	4	21	4.4	1	9	8	43	5	18	61	29	22	258	27	715
50A:50E	0-5	5	27	4.5	1	12	9	45	4	22	66	33	17	238	48	713
	5-15	4	19	4.4	1	9	6	42	5	15	57	26	25	248	46	706

P was determined through resin extraction and spectrophotometry UV; OM was determined by sodium dichromate 4N in sulphuric acid - Walkley Black and colorimetry; K was determined through resin extraction and photometry; Ca and Mg were determined by resin extraction and spectrophotometry of atomic absorption.

SB=sum of bases; T=cation exchange capacity; V= saturation of bases.

4.2.2 Experimental design

The trials were established in December 2013 (SP), January 2014 (TO), and January 2015 (MT). In MT, 80% of the *Eucalyptus* seedlings died after planting and were replanted in October 2015. The same experimental design was replicated in all the sites (Figures 16, 17, and 18). The treatments were as follow:

- 100E - Pure *Eucalyptus* stand
- 100E + N - Pure *Eucalyptus* stand with N fertilization
- 100A - Pure *A. mangium* stand
- 50A:50E - Mixture at a 1:1 ratio between *Eucalyptus* and *A. mangium*

In 50A:50E, seedlings were planted in two rows of eucalypts alternating with two rows of acacias. Total tree densities were similar in all treatments in each site; however, they varied from 833 trees ha⁻¹ in Colinas-TO to 1111 trees ha⁻¹ in Itatinga-SP and Sinop-MT. Seedlings of *Acacia mangium* were inoculated with *Rhizobium* strains (supplied by EMBRAPA Agrobiologia) due to their high capacity to fix atmospheric N and for their high nodulation rate in nurseries.

In Itatinga-SP, 2 Mg ha⁻¹ of dolomitic limestone, 100 kg ha⁻¹ of P₂O₅, 30 kg ha⁻¹ of K₂O, 30 kg ha⁻¹ of FTE and 4.5 kg ha⁻¹ of B were applied at planting. For 100E+N treatment, 10 kg ha⁻¹ of N was additionally applied at planting. After 6 months, 120 kg ha⁻¹ of K₂O were applied in all the treatments and 90 kg ha⁻¹ of N in 100E+N.

In Colinas-TO, 400 kg ha⁻¹ of CaO, 160 kg ha⁻¹ of MgO, 44 kg ha⁻¹ of P₂O₅, 23.5 kg ha⁻¹ of K₂O, 20 kg ha⁻¹ of FTE were applied at planting. For 100E+N, 12 kg ha⁻¹ of N was additionally applied at planting. After 4 months, 26.7 kg ha⁻¹ of K₂O and 1.3 kg ha⁻¹ of B were applied in all the treatments and 16 kg ha⁻¹ N was applied in 100E+N. After 12 months, 54.4 kg ha⁻¹ of K₂O and 1.6 kg ha⁻¹ of B were applied in all the treatments.

In Sinop-MT, 50 kg ha⁻¹ P₂O₅, 15 kg ha⁻¹ K₂O and 100 kg ha⁻¹ of FTE were applied at planting. For 100E+N treatment, 15 kg ha⁻¹ of N was additionally applied at planting. Dolomitic limestone was not applied. After 45 days of planting, 50 kg ha⁻¹ P₂O₅, 15 kg ha⁻¹ K₂O and 54 kg ha⁻¹ of FTE was applied in all treatments. The main characteristics of the experimental sites are given in Table 9.

Figure 16. Itatinga-SP experimental site. a) 100E; b) 100A; and c) 50A:50E.

Figure 17. Colinas-TO experimental site. a) 100E; b) 100A; and c) 50A:50E.

Figure 18. Sinop-MT experimental site. a) 100E; b) 100A; and c) 50A:50E.

Table 9. Main characteristics of the experimental sites.

Site	Species	Origin/ Clone	Planting date	Spacing (m x m)	Stand Density (trees ha ⁻¹)	Blocks	Total plot size (trees)	Inner plot size (trees)
Itatinga SP	<i>E. grandis</i>	IPEF	December 2013	3 x 3	1111	3	12 x 12	8 x 8
	<i>A. mangium</i>	Vietnam						
Colinas TO	<i>E. grandis</i> x <i>E. urophylla</i>	VT04	January 2014	4 x 3	833	4	10 x 10	6 x 6
	<i>A. mangium</i>	Vietnam						
	<i>E. urophylla</i> x <i>E. grandis</i>	I144						
Sinop MT	<i>E. grandis</i>	Vietnam	January 2015	3 x 3	1111	4	12 x 12	8 x 8
	<i>A. mangium</i>							

4.2.3 Tree mortality and stand growth

The percentage of tree mortality was calculated at each inventory date in each experimental site. Tree total height (H) and circumference at breast height (1.30 m) (CBH) were measured for all trees in the inner plots (2 border lines removed) at 12, 24, 38, and 52 months after planting in Itatinga-SP; at 12, 36, and 54 months after planting in Colinas-TO; and at 15, 26, 36, and 52 months after planting in Sinop-MT. For acacias with multi-stem, all stems with a diameter at breast height > 2 cm were measured and an “equivalent circumference” for each stem was calculated from the sum of the basal areas of each stem of the tree.

4.2.4 Aboveground biomass

Aboveground biomass were estimated at 52 months after planting in Itatinga-SP and Sinop-MT, and at 54 months after planting in Colinas-TO. Eight trees of each species (i.e. 16 trees in 50A:50E) were sampled in each treatment. Eight trees per treatment were chosen to be distributed over the range of tree basal areas of inner trees (Nouvellon et al., 2012). The trees were separated into leaves, living branches, stem wood and stem bark and the masses were measured in the field. Leaves, branches, bark and stem sub-samples were weighed before and after drying to measure the water content and convert field measurements from fresh to dry weights.

The stand stem volume was obtained by Smalian method. The stem of each tree was divided into cross-sections each 1 m, the diameters and lengths of each section of the trunk were measured and the Smalian equation (Eq. 5) was used to calculate the volume of each section. With the results obtained it was possible to calculate the wood volume per hectare (m³)/treatment.

$$V = \sum((a1 + a2) / 2) * C \quad (\text{Eq. 5})$$

Where:

V = volume in meter cubic (m³)

a1 = area of the smaller diameter of the trunk of the cross section

a2 = area of the larger diameter of the trunk of the cross section

C = length of trunk of the cross section

Biomass equations were established for all species and trees compartments for each experimental site: Itatinga-SP (Table 10), Colinas-TO (Table 11) and Sinop-MT (Table 12). The treatment-specific allometric relationships, which relate tree CBH and/or H with component biomass, were applied to the CBH and/or H of the trees in the inner plots. All biomass values reported in this study refer to dry mass (see section 3.2.4.2).

Table 10. Biomass models for each treatment and species in Itatinga-SP. Average individual values are given (+/- Standard error).

Compartment	Treatment	Model	R ²	RMSE	Average value (kg tree ⁻¹)
Leaves	100E	1.16E-05 x DBH ^{3.21E+00}	0.96	0.81	3.56±0.08
	100E+N	1.16E-05 x DBH ^{3.21E+00}	0.96	0.81	3.60±0.14
	100A	1.78E-03 x DBH ^{2.12E+00}	0.75	2.85	6.26±0.20
	50A:50E - <i>Eucalyptus</i>	4.10E-06 x DBH ^{3.49E+00}	0.98	0.39	5.13±0.20
	50A:50E - <i>A. mangium</i>	6.23E-04 x DBH ^{2.36E+00}	0.82	0.82	4.14±0.15
Branches	100E	7.58E-07 x DBH ^{3.9278}	0.95	0.82	4.01±0.11
	100E+N	7.58E-07 x DBH ^{3.9278}	0.95	0.82	4.06±0.17
	100A	7.15E-05 x DBH ^{2.9890}	0.58	9.94	7.72±0.30
	50A:50E - <i>Eucalyptus</i>	5.82E-06 x DBH ^{3.5660}	0.92	2.97	8.17±0.24
	50A:50E - <i>A. mangium</i>	1.40E-03 x DBH ^{2.2083}	0.66	1.29	5.25±0.17
Stem	100E	0.0029 x DBH ^{2.6012}	0.99	4.69	78.96±1.39
	100E+N	0.0029 x DBH ^{2.6012}	0.99	4.69	79.37±2.73
	100A	0.0031 x DBH ^{2.5491}	0.91	16.83	58.59±2.05
	50A:50E - <i>Eucalyptus</i>	0.0323 x DBH ^{1.9989}	0.98	4.98	96.49±2.89
	50A:50E - <i>A. mangium</i>	0.0114 x DBH ^{2.1826}	0.91	4.38	39.19±1.21
Bark	100E	0.0009 x DBH ^{2.3875}	0.98	1.70	10.80±0.17
	100E+N	0.0009 x DBH ^{2.3875}	0.98	1.70	10.85±0.36
	100A	0.00104 x DBH ^{2.4386}	0.92	2.90	12.73±0.43
	50A:50E - <i>Eucalyptus</i>	0.02692 x DBH ^{1.5976}	0.89	1.78	15.93±0.44
	50A:50E - <i>A. mangium</i>	0.00105 x DBH ^{2.3910}	0.67	3.13	7.96±0.29

Table 11. Biomass models for each treatment and species in Colinas-TO.

Compartment	Treatment	Model	R ²	RMSE	Average value (kg tree ⁻¹)
Leaves	100E	3.526e-05 x DBH ^{3.035144}	0.73	0.49	2.57±0.10
	100E+N	5.49e-05 x DBH ^{2.931149}	0.93	0.33	2.77±0.14
	100A	9.68e-04 x DBH x H ^{1.195204}	0.57	2.34	1.44±0.04
	50A:50E - <i>Eucalyptus</i>	3.68e-04 x DBH ^{2.512980}	0.89	0.31	2.37±0.42
	50A:50E - <i>A. mangium</i>	3.78e-03 x DBH x H ^{1.405103}	0.76	2.07	3.96±0.25
Branches	100E	4.97e-07 x DBH ^{4.316939}	0.87	0.59	4.17±0.23
	100E+N	8.73e-06 x DBH ^{3.524508}	0.91	0.90	3.99±0.25
	100A	2.25e-03 x DBH x H ^{1.316281}	0.50	3.36	2.57±0.07
	50A:50E - <i>Eucalyptus</i>	6.70e-05 x DBH ^{3.069826}	0.95	0.39	3.09±0.66
	50A:50E - <i>A. mangium</i>	2.17e-03 x DBH x H ^{1.293104}	0.78	2.13	3.04±0.20
Stem	100E	0.00045 x DBH ^{3.1480548}	0.80	5.54	50.56±1.96
	100E+N	0.02201 x DBH ^{2.0850236}	0.94	4.52	48.43±1.66
	100A	7.04e-02 x DBH x H ^{1.0608544}	0.41	20.33	41.22±0.97
	50A:50E - <i>Eucalyptus</i>	1.70e-03 x DBH x H ^{1.5491766}	0.98	1.11	32.23±4.95
	50A:50E - <i>A. mangium</i>	2.31e-02 x DBH x H ^{1.2334228}	0.72	18.67	34.27±6.89
Bark	100E	0.00133 x DBH ^{2.3307221}	0.80	0.85	7.20±0.20
	100E+N	0.00215 x DBH ^{2.1578078}	0.90	0.74	6.21±0.22
	100A	1.69e-02 x DBH x H ^{1.0095298}	0.47	3.10	9.21±0.20
	50A:50E - <i>Eucalyptus</i>	2.39e-04 x DBH x H ^{1.5683976}	0.94	0.42	4.77±0.74
	50A:50E - <i>A. mangium</i>	8.16e-03 x DBH x H ^{1.1233723}	0.58	3.40	7.09±0.43

Table 12. Biomass models for each treatment and species in Sinop-MT.

Compartment	Treatment	Model	R ²	RMSE	Average value (kg tree ⁻¹)
Leaves	100E	5.71e-06 x DBH ^{3.364695}	0.82	1.16	3.08±0.53
	100E+N	2.33e-06 x DBH ^{3.661546}	0.87	0.97	3.57±0.23
	100A	4.52e-03 x DBH ^{1.809941}	0.61	2.13	5.34±0.06
	50A:50E - <i>Eucalyptus</i>	3.40e-06 x DBH ^{3.626186}	0.76	0.73	5.37±1.67
	50A:50E - <i>A. mangium</i>	6.05e-03 x DBH ^{1.883001}	0.76	4.54	11.55±0.93
Branches	100E	1.44e-06 x DBH ^{3.812061}	0.65	3.45	4.76±0.84
	100E+N	1.93e-0406 x DBH ^{2.601754}	0.84	0.73	4.36±0.25
	100A	2.62e-0406 x DBH ^{2.596345}	0.63	3.99	6.96±0.10
	50A:50E - <i>Eucalyptus</i>	1.28e-0206 x DBH ^{1.470200}	0.14	2.09	3.75±0.53
	50A:50E - <i>A. mangium</i>	3.03e-0306 x DBH ^{2.138495}	0.73	8.39	16.36±1.45
Stem	100E	3.86e-03 x DBH ^{2.485762}	0.97	7.98	61.08±9.67
	100E+N	2.45e-06 x DBH ^{4.385519}	0.44	18.29	68.30±4.75
	100A	6.19e-03 x DBH ^{2.386058}	0.95	10.99	71.26±0.94
	50A:50E - <i>Eucalyptus</i>	4.96e-03 x DBH ^{2.458094}	0.98	2.04	71.81±16.37
	50A:50E - <i>A. mangium</i>	4.14e-03 x DBH ^{2.453784}	0.94	16.44	80.51±7.91
Bark	100E	3.65e-03 x DBH ^{2.075406}	0.88	2.45	11.25±1.65
	100E+N	1.40e-05 x DBH ^{3.530738}	0.35	3.97	12.72±0.80
	100A	1.33e-02 x DBH ^{1.788599}	0.93	2.29	14.50±0.17
	50A:50E - <i>Eucalyptus</i>	1.51e-01 x DBH ^{1.172002}	0.92	0.69	13.78±1.55
	50A:50E - <i>A. mangium</i>	2.17e-02 x DBH ^{1.667633}	0.92	2.89	17.30±1.27

4.2.5 Soil samples

The upper soil layer was sampled in all sites at the end of the study period, at 52, 54, and 52 months after planting in Itatinga-SP, Colinas-TO, and Sinop-MT, respectively. The samples were collected according to the method of Voronoï square to represent soil heterogeneity of each plot (Voigtlaender et al., 2012). In pure plantations (100A, 100E and 100E+N), soil cores were collected in each plot at 9 positions. In mixed-species plantations (50A:50E), 18 positions were collected per plot, 9 near *Eucalyptus* trees and 9 near *Acacia* trees. Each position was located near a different tree. Soil samples were collected using an auger-type. At each sampling position, cores were collected at 0–5 cm and 5–15 cm. The samples from each plot were bulked in a single sample by plot and species. Subsequently, they were dried at 45°C to constant weight, roots were removed, the samples were passed through a 2-mm sieve, and soil analyses were then performed.

4.2.6 Water balance and historical mean monthly rainfall (10 years)

The water balance over the study period was obtained for each month in each site by the method proposed by Thorntwaite and Mather (1955), from January 2014 to April 2018 in Itatinga-SP, January 2014 to July 2018 in Colinas-TO and January 2015 to June 2019 in Sinop-MT. To analyze the impact of the low rainfall events in Brazil in 2014 and 2015 on tree mortality and tree growth, the mean monthly rainfall 10 years before planting of each site was compared with mean monthly rainfall from October 2013 to June 2016 for Itatinga-SP and

Colinas-To and from October 2014 to June 2017 for Sinop-MT.

4.2.7 Statistical analysis

The two-way ANOVA was used to analyze differences between treatments in tree rate mortality in each study site and in tree growth and biomass of acacia (100A and 50A:50E) in Itatinga-SP and Colinas-TO and in tree growth and biomass of eucalypt (100E and 50A:50E) in Itatinga-SP and Sinop-MT ($P < 0.05$). The homogeneity of variances was assessed using the Levene test. The Bonferroni's multiple range test was used to compare the treatments when there were significant differences between treatments. Statistical analyses were carried out using R 3.5.2 (R Core Team 2018).

4.3 Results

4.3.1 Tree mortality rates

Tree mortality rates changed dramatically among sites, treatments and species. At the end of the study period *Eucalyptus* mortality rate in 100E and 100E+N was 4 and 7 % in Itatinga-SP (52 months of age), 3 and 1 % in Colinas-TO (54 months of age), and 41 and 27 % in Sinop-MT (52 months of age). *Eucalyptus* mortality rate in 50A:50E was 3 %, 1 % and 56 % in Itatinga-SP, Colinas-TO and Sinop-MT, respectively (Figure 19). The percentage of *Acacia mangium* mortality in 100A and 50A:50E was 17 and 10 % in Itatinga-SP, 49 and 58 % in Colinas-TO, and 11 and 23 % in Sinop-MT (Figure 19). In Colinas at 54 months after planting, the mortality rate of *Acacia* trees in 100A and in 50A:50E was significantly higher than of *Eucalyptus* trees in 100E, 100E+N and 50A:50E. In contrast, in Sinop at 15 months after planting, the mortality of *Eucalyptus* trees in 100E+N was significantly higher than of *Acacia* trees in 100A. At 26 and 52 months after planting, the mortality of *Eucalyptus* in 50A:50E was significantly higher than of *Acacia* in 100A.

Figure 19. Time course of tree mortality rate for each treatment and species in each experimental site: Itatinga-SP (up to 52 months), Colinas-TO (up to 54 months), and Sinop-MT (up to 52 months). Different letters indicate significant differences between treatments at each age for each site ($p < 0.05$).

4.3.2 Tree growth

There was no significant difference between 100E and 100E+N regardless of the variable. We therefore decided to use only 100E for further analysis. Due to the higher *Eucalyptus* mortality in Sinop-MT and *Acacia* mortality in Colinas-TO, tree growth and tree biomass of *Eucalyptus* were compared between Itatinga-SP and Colinas-TO, and tree growth and tree biomass of *Acacia* were compared between Itatinga-SP and Sinop-MT.

The mean tree height and mean tree diameter of *Eucalyptus* in 100E and in 50A:50E were significantly higher in Itatinga (52 months of age) than in Colinas (54 months of age). The mean tree height values were 22.9 and 17.7 m in 100E, and 22.5 and 13.6 m in 50A:50E for Itatinga and Colinas, respectively. The mean diameter values were 15.4 and 12.6 cm in 100E, and 17 and 10.2 in 50A:50E for Itatinga and Colinas, respectively (Figure 20). In Colinas, the mean tree height and mean tree diameter were significantly higher in 100E than in 50A:50E, while in Itatinga the mean tree diameter was significantly higher in 50A:50E than in 100E (Figure 20).

Figure 20. Mean tree height and mean tree diameter of 100E treatment in Colinas-TO (52 months) and Itatinga-SP (52 months). Different letters indicate significant differences between sites ($p < 0.05$): A, B for differences between treatments (100E and *Eucalyptus* in 50A:50E), and a, b for differences between sites (Colinas and Itatinga).

At 52 months after planting, the mean height of *Acacia* trees did not vary between sites and between treatments for each site (Figure 21). The corresponding values of tree height were 13.1 and 13.7 m (100A), and 13.8 and 13.0 m (50A:50E) in Itatinga and Sinop, respectively. The mean diameter of *Acacia* trees was significantly higher in Sinop than in Itatinga for both 100A and 50A:50E treatments. In Sinop, there was no significant difference between treatments, while in Itatinga, the mean diameter of *Acacia* trees was significantly higher in 100A than in 50A:50E (Figure 21). The corresponding values of mean diameter of

Acacia trees were 13.4 and 15.6 cm (100A), and 12.3 and 17 cm (50A:50E) in Itatinga and Sinop, respectively.

Figure 21. Mean tree height and mean tree diameter of 100A treatment in Sinop-MT (54 months) and Itatinga-SP (52 months). Different letters indicate significant differences between sites ($p < 0.05$): A, B for differences between treatments (100A and *Acacia* in 50A:50E), and a, b for differences between sites (Sinop and Itatinga).

4.3.3 Stand biomass

Eucalyptus leaves, branches, stem wood and stem bark biomass in 100E and 50A:50E were significantly higher in Itatinga than in Colinas. In Colinas, all compartments of *Eucalyptus* stand biomass were significantly higher in 100E than in 50A:50E. In Itatinga leaves, stem wood and stem bark of *Eucalyptus* stand biomass were also significantly higher in 100E than in 50A:50E (Figure 22). *Eucalyptus* stem wood production in 100E and in 50A:50E were on average 52% and 75% higher in SP than in TO.

Figure 22. Biomass by compartment of *Eucalyptus*: leaves, branches, stem and bark of 100E and 50A:50E treatments in Colinas-TO (54 months) and Itatinga-SP (52 months). Different letters indicate significant differences between sites for each compartment ($p < 0.05$): A, B for differences between treatments (100E and *Eucalyptus* in 50A:50E), and a, b for differences between sites (Colinas and Itatinga).

Acacia leaves and branches biomass in 100A were significantly higher in Itatinga-SP than in Sinop-MT. However, biomass of stem wood and stem bark was significantly higher in MT than in SP. In 50A:50E treatment, all compartments of *Acacia* stand, biomass was significantly higher in Sinop than in Itatinga. In Sinop, stem wood and stem bark biomass of *Acacia* trees was significantly higher in 100A than in 50A:50E. In Itatinga, all compartments of *Acacia* stand, biomass was significantly higher in 100A than in 50A:50E (Figure 23). *Acacia* stem wood production in 100A and in 50A:50E was on average 18% and 51% higher in MT than in SP.

Figure 23. Biomass by each compartment of *Acacia*: leaves, branches, stem and bark of 100A and 50A:50E treatments in Sinop-MT (52 months) and Itatinga-SP (52 months). Different letters indicate significant differences between sites for each compartment ($p < 0.05$): A, B for differences between treatments (100A and *Acacia* in 50A:50E), and a, b for differences between sites (Sinop and Itatinga).

Eucalyptus stand stem volume in 100E and 50A:50E were significantly higher in Itatinga than in Colinas. In both, Colinas and Itatinga, the stand stem volume of *Eucalyptus* were significantly higher in 100E than in 50A:50E (Table 13). *Acacia* stand stem volume in 100A was significantly higher in Sinop-MT than in Itatinga-SP. In 50A:50E treatment there was no significant differences between sites. In Sinop and in Itatinga, stand stem volume of *Acacia* were significantly higher in 100A than in 50A:50E (Table 13).

Table 13. Stand stem volume for each treatment and species in each site. Different letters indicate significant differences ($P < 0.05$): A, B for differences between treatments (100E and *Eucalyptus* in 50A:50E, and 100A and *Acacia* in 50A:50E), and a, b for difference differences between sites (Colinas and Itatinga, Sinop and Itatinga).

Treatment	Volume ($\text{m}^3 \text{ha}^{-1}$)		
	Itatinga-SP	Colinas-TO	Sinop-MT
100E	230.5 $\pm$ 3.8 aA	94.9 $\pm$ 4.2 bA	-
100A	180.1 $\pm$ 6.2bA	-	216.2 $\pm$ 4.0aA
50A:50E <i>Eucalyptus</i>	129.2 $\pm$ 3.9 aB	25.4 $\pm$ 4.1 bB	-
50A:50E <i>Acacia</i>	66 $\pm$ 2.6aB	-	81.8 $\pm$ 15.4aB

4.3.4 Water balance and historical mean monthly rainfall

In Itatinga-SP, there was no water deficit during the study period. Water excess was 799 mm yr^{-1} distributed throughout months, except for April and July (Figure 24a). In Colinas-TO, water deficit was 337.8 mm yr^{-1} and occurred between May and October. Water excess was 459.9 mm yr^{-1} (Figure 24b) distributed between January and April. In Sinop-MT, water deficit was 486 mm yr^{-1} varying from May to September. Water excess was 743.2 mm yr^{-1} and occurred in all other months except for October (Figure 24c).

The average rainfall 10 years before planting (2004-2013) in the Itatinga region (according to INMET data) was an average 50% lower (729 mm) than the first year of planting (2014) (Table 5). During the months of October, November, and December 2014 the rainfall was only 175 mm while the historical mean was 547 mm (68 % below the historical mean) (Figure 25).

The average rainfall 10 years before planting (2004-2013) in the Colinas region (according to INMET data) was 36% higher (645 mm) than the second year of planting (2015). During the months of October, November, and December 2015 the rainfall was only 142.4 mm while the historical mean was 571.6 mm (75% below the historical mean). Most of the rainfall in 2015 was much lower than the historical average (Figure 25).

The average rainfall 10 years before planting (2005-2014) in the Sinop region (according to INMET data) was 27% higher (583 mm) than the first year of planting (2015). From October to December 2015, the rainfall was 488.7 mm, while the historical mean was 827 mm (40% below the historical mean). All rainy season in 2015 was much lower than the historical average (Figure 25).

Figure 24. Mean water balance for each month during the study period in Itatinga-SP (a), Colinas-To (b), and Sinop-MT (c). Parts in dark represent water deficit periods.

Figure 25. Mean monthly rainfall (mm) 10 years before the installation of each experimental site (white bars) and monthly rainfall (mm) in each site in the first two years after planting (black bars). The data were obtained from INMET. For Itatinga, data from Avaré and Itatinga weather stations were used. For Colinas, data from Araguaiana weather station were used. For Sinop, data from Matupá weather station were used.

4.4 Discussion

4.4.1 Hotter droughts enhance tree mortality

At 54 months of age, about 54% of *Acacia* trees died in Colinas-TO with a higher proportion of mortality in mixed plantations (58%) than in pure plots (49%) whilst *Eucalyptus* mortality was only 3% and 1% in 100E and 50A:50E, respectively. These results are in agreement with our first hypothesis. Studies have shown that forests are sensitive to extreme climatic events and hotter droughts could increase tree mortality and reduce stand productivity (Gonçalves et al., 2017). In the Colinas region, one year after plantation, the mean annual rainfall was 36% lower (645 mm) than the historical mean annual rainfall over the 10 years before planting. The climate type is Aw with dry winters, characterized by medium to high effects of water stress, mainly due to uneven distribution of rainfall among the seasons as well as higher temperatures (Alvares et al., 2013; Gonçalves et al., 2013). During the study period, the water deficit in the Colinas region occurred during 6 months of the year. An important parameter for water retention is soil texture (Klein and Klein, 2015). In Colinas, sand composes more than 90% of the soil. Soils with high sand contents are characterized by low to very low water retention capacity. Soils are very deep in Colinas-TO (EMBRAPA, 2020), which could be important to store large amounts of water during the wet season (Christina et al., 2017). However, drainage is very fast in Colinas (500 mm h⁻¹) (USA Soil Conservation Service, 1983), which could have prevented *Acacia* from benefiting from the water resource at depth during the dry season. Conversely, fast growth of *Eucalyptus* roots after planting possibly provided access for this species to large quantities of water stored in deep soil layers in the first stand stages (Christina et al., 2017), limiting water deficiency during the dry season. Moreover, the faster exploration and higher biomass of fine root of *Eucalyptus* than *Acacia* in mixed-species plantations (Germon et al., 2018; Bordon et al., 2020) could have amplified water *Acacia* shortage and led to higher *Acacia* mortality in 50A:50E than in 100A. Therefore, although the region of Colinas presents high temperatures and nutrient-poor soils, which are potentially favorable to *Acacia* growth (Bouillet et al., 2013; Santos et al., 2016), the long water deficit throughout the year, the very sandy soil texture combined to the hotter drought in 2015, and in the mixed-species stands, the high competition of *Eucalyptus* for water may have led to very high mortality rate of *Acacia*.

At 52 months of age, about 41% of the *Eucalyptus* trees died in Sinop-MT with the highest mortality rates observed in pure plots without N fertilizer (41%) and in mixed-species plantations (56%) whilst *Acacia* mortality was 11% and 23% in 100A and 50A:50E, respectively. These results are in agreement with our second hypothesis. The climate in Sinop

(i.e. high temperature and high air humidity) is favorable for *Acacia* development (Bouillet et al., 2013), which probably increased the competition over *Eucalyptus* trees. In addition, the clone used in Sinop was a hybrid of *E. urophylla* x *E. grandis* that is not fully adapted to hot and humid climates (Hardiyanto and Tridasa, 2000). In Amazonia, this hybrid was less productive than in South-Eastern Brazil (Demolinari et al., 2007; Behling et al., 2011), while the climatic conditions of Amazonia region are favorable for *A. mangium* growth (de Souza et al., 2004).

Studies in tropical rainforests of Borneo on the effect of El Nino in 1997-1998 (Nieuwstadt and Sheil, 2005) and in the Amazon basin in 2005 (Phillips et al., 2009) showed that drought could lead to tree mortality, even in a region with normally no water limitations (Allen et al., 2010). In the Sinop region, in the first three months after planting (January, February and March 2015), mean monthly rainfall was 36%, 24%, and 24% lower than the historical mean monthly rainfall over the 10 years before planting, which could explain the mortality of 80% of the *Eucalyptus* seedlings after planting. In October 2015, when the *Eucalyptus* seedlings were replanted, the mean monthly rainfall of October, November, and December was on average 42% lower than the historical mean rainfall. In addition, in October 2015 *Acacia* was already 9 months of age possibly leading to significant water competition against *Eucalyptus*.

Another important factor that could explain mortality of *Eucalyptus* trees was the high presence of weeds in pure plots of *Eucalyptus* trees. Although pre-plant herbicide was applied to all the plots of the experiment area, plots were covered with weeds at 50 months of age on average 60.7% and 67% of the 100E and 100E+N (Appendix B). Plots 100A and 50A:50E were covered on average with 6.4% and 9% weeds (Appendix B). The presence of weeds mainly in the first years of *E. grandis* x *E. urophylla* clones plantation had a negative effect, reducing tree height, stem diameter, leaf area, and dry mass (Pereira et al., 2013; Bacha et al., 2016). *Eucalyptus* is highly sensitive to weed competition at early stages of growth (Gonçalves et al., 2017), unlike *Acacia*, for which its slower decomposition rate of litterfall (Santos et al., 2018) and its allelopathic potential effect (Conceição, 2015) can act as a barrier against weeds. In summary, the *Eucalyptus* clone used in Sinop was not the most adapted to the climatic conditions and weeds and *Acacia* competition in mixed-species plantations, which led to high *Eucalyptus* mortality, while *Acacia* presented higher survival rate and greater tree development.

4.4.2 Tree growth and biomass production

The highest tree growth and biomass production of *Eucalyptus* in Itatinga was consistent with our third hypothesis. Bouillet et al. (2013) also found higher tree growth and stem biomass in pure plantations of *Eucalyptus grandis* in Itatinga than in Congo, characterized by higher temperatures and air humidity. Hakamada et al. (2013) conducted a study in Colinas and showed that clones of *E. grandis* x *E. urophylla* had mean annual increment $\leq 15 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ at 3.5 years of age. *E. grandis* is a native species from Queensland and New South Wales in Australia, adapted to areas with moderate mean annual temperature (20°C), and with rainfall between 1000 and 1750 mm year^{-1} (Florence, 1996), similar to the climate conditions of Itatinga. *Eucalyptus* genetic material that are more tolerant to dry condition invest more in physiological and morphological mechanisms, such as stomatal control and root system development, which make it less productive (Whitehead and Beadle, 2004). These mechanisms favor water saving in drought periods; however, they can negatively affect tree growth in periods with no water restriction (Gonçalves et al., 2017). In addition, a study performed on different clones of *Eucalyptus* across Brazil showed that an increase of 1°C in temperature led to a drop by 2.5 Mg ha^{-1} in the productivity of *Eucalyptus*, which was significantly stronger than precipitations (Binkley et al., 2017).

The highest tree growth and biomass production of *Acacia* was observed in Sinop. Santos et al. (2016) found 40.5 Mg ha^{-1} of aboveground biomass production in 100A from 30 to 60 months after planting in Seropédica-RJ. The Sinop region presents an average annual rainfall and temperature of 1370 mm and 24°C , with soils composed by 86% of sand, 7.5% of silt, and 6.8% of clay. In our study, the aboveground biomass of 100A was 108.9 Mg ha^{-1} at 54 months of age. *Acacia* trees also exhibited higher growth and stem wood production in Congo than in Itatinga (Bouillet et al., 2013). Native from northeastern Australia, Papua New Guinea, and eastern Indonesia, *Acacia mangium* is adapted to warm climates with high air moisture (Krisnawati et al., 2011). These climatic characteristics are similar to those found in Sinop. In conclusion, the pattern found in our study showed that *Eucalyptus* was more adapted to the ecological conditions of Itatinga-SP while *Acacia* was adapted to the ecological conditions of Sinop-MT.

4.4.3 Perspectives

Climate change could affect positively tree growth due to better water use efficiency and longer growing seasons, or negatively by reducing tree growth and increasing tree

mortality (Lucht et al., 2006; Scholze et al., 2006; Lloyd and Bunn, 2007; Allen et al., 2010). In our study, the incidence of extreme climatic events, such as hotter droughts, could be a risk for forest plantations in Brazil, particularly in the new regions of expansion, such as the states of Mato Grosso and Tocantins (Gonçalves et al. 2017).

It was not possible to verify if the introduction of *Acacia* into *Eucalyptus* plantations could benefit the system due to the high mortality of *Acacia* and *Eucalyptus* in Colinas and Sinop. *Acacia* trees under extreme climatic conditions cannot survive. It is necessary to choose suitable material for the region and apply procedures for weeds control to limit water deficiency. In addition, one possible way to increase resistance to water deficit would be the use of lower stand density and reduction of K fertilization to limit plant transpiration and water stress during dry events (Gonçalves et al., 2017; Christina et al., 2018).

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APPENDICES

Appendix A. Allometric equations were applied for each treatment, species, and ages to predict leaves, branches, stem wood, and bark. For all compartments, the equation used was $Y=b_0DBH^{b_1}$.

Biomass models for each species per treatment at 38 months of age.

Compartment	Treatment	Species	b0	b1	R ²	RMSE
Leaves	1	<i>A. mangium</i>	6.80E-05	2.9584	0.78	1.757
	2	<i>E. grandis</i>	5.67E-06	3.5258	0.93	1.303
	3	<i>E. grandis</i>	4.04E-04	2.4279	0.95	0.303
	4	<i>A. mangium</i>	6.80E-05	2.9584	0.78	1.757
	6	<i>A. mangium</i>	2.71E-05	3.2566	0.90	0.818
	6	<i>E. grandis</i>	5.16E-05	2.9778	0.89	0.816
	7	<i>E. grandis</i>	2.35E-05	3.1244	0.98	0.593
	9	<i>A. mangium</i>	7.24E-04	2.3029	0.93	0.336
	9	<i>E. grandis</i>	2.61E-04	2.5408	0.95	0.493
Branches	1	<i>A. mangium</i>	1.45E-06	4.0403	0.82	3.123
	2	<i>E. grandis</i>	1.73E-04	2.8188	0.87	3.046
	3	<i>E. grandis</i>	2.51E-04	2.6406	0.93	0.556
	4	<i>A. mangium</i>	1.45E-06	4.0403	0.82	3.123
	6	<i>A. mangium</i>	9.50E-04	2.4228	0.74	1.706
	6	<i>E. grandis</i>	1.62E-05	3.4100	0.87	1.923
	7	<i>E. grandis</i>	3.47E-05	3.182	0.95	1.817
	9	<i>A. mangium</i>	4.95E-04	2.609	0.81	1.475
	9	<i>E. grandis</i>	1.67E-04	2.7383	0.91	1.053
Stem	1	<i>A. mangium</i>	0.0107	2.1670	0.81	17.440
	2	<i>E. grandis</i>	0.0077	2.2742	0.96	5.162
	3	<i>E. grandis</i>	0.0156	2.1084	0.96	3.158
	4	<i>A. mangium</i>	0.0004	2.9590	0.95	7.676
	6	<i>A. mangium</i>	0.0019	2.5672	0.92	3.435
	6	<i>E. grandis</i>	0.0270	1.9702	0.92	5.597
	7	<i>E. grandis</i>	0.0050	2.4128	0.98	6.086
	9	<i>A. mangium</i>	0.0148	1.9939	0.88	2.062
	9	<i>E. grandis</i>	0.0119	2.1598	0.96	4.115
Bark	1	<i>A. mangium</i>	0.0144	1.7094	0.89	2.619
	2	<i>E. grandis</i>	0.0080	1.7699	0.98	0.504
	3	<i>E. grandis</i>	0.0320	1.4371	0.77	1.197
	4	<i>A. mangium</i>	0.0013	2.3054	0.85	2.182
	6	<i>A. mangium</i>	0.0016	2.1578	0.77	1.039
	6	<i>E. grandis</i>	0.0063	1.8413	0.94	0.522
	7	<i>E. grandis</i>	0.0012	2.238	0.99	0.462
	9	<i>A. mangium</i>	0.0038	1.9575	0.93	0.487
	9	<i>E. grandis</i>	0.0011	2.2980	0.97	0.524

Biomass models for each species per treatment at 45 months of age.

Compartment	Treatment	Species	b0	b1	R ²	RMSE
Leaves	1	<i>A. mangium</i>	1.11E-03	2.2246	0.94	2.058
	2	<i>E. grandis</i>	4.83E-04	2.3812	0.71	1.824
	3	<i>E. grandis</i>	1.39E-05	3.2429	0.97	0.378
	4	<i>A. mangium</i>	4.74E-04	2.4119	0.76	2.127
	6	<i>A. mangium</i>	2.19E-03	1.9370	0.82	0.676
	6	<i>E. grandis</i>	2.86E-05	3.0421	0.85	0.653
	7	<i>E. grandis</i>	2.64E-05	3.0376	0.96	0.553
	9	<i>A. mangium</i>	2.19E-03	1.9370	0.82	0.676
	9	<i>E. grandis</i>	5.03E-05	2.9117	0.94	0.681
Branches	1	<i>A. mangium</i>	1.33E-03	2.1813	0.54	4.370
	2	<i>E. grandis</i>	8.24E-06	3.4806	0.98	0.724
	3	<i>E. grandis</i>	8.24E-06	3.4806	0.98	0.724
	4	<i>A. mangium</i>	1.33E-03	2.1813	0.54	4.370
	6	<i>A. mangium</i>	1.75E-02	1.5084	0.59	1.267
	6	<i>E. grandis</i>	1.24E-05	3.4201	0.93	0.851
	7	<i>E. grandis</i>	5.70E-06	3.5476	0.98	0.411
	9	<i>A. mangium</i>	1.75E-02	1.5084	0.59	1.267
	9	<i>E. grandis</i>	1.17E-05	3.3970	0.96	1.435

Biomass models for each species per treatment at 52 months of age.

Compartment	Treatment	Species	b0	b1	R ²	RMSE
Leaves	1	<i>A. mangium</i>	1.78E-03	2.12E+00	0.75	2.856
	2	<i>E. grandis</i>	2.37E-05	3.06E+00	0.92	1.692
	3	<i>E. grandis</i>	1.16E-05	3.21E+00	0.96	0.815
	4	<i>A. mangium</i>	1.78E-03	2.12E+00	0.75	2.856
	6	<i>A. mangium</i>	6.23E-04	2.36E+00	0.82	0.826
	6	<i>E. grandis</i>	4.10E-06	3.49E+00	0.98	0.399
	7	<i>E. grandis</i>	5.69E-06	3.4226	0.97	0.569
	9	<i>A. mangium</i>	1.17E-04	2.8302	0.87	0.703
	9	<i>E. grandis</i>	5.02E-05	2.9096	0.98	0.665
Branches	1	<i>A. mangium</i>	7.15E-05	2.9890	0.58	9.940
	2	<i>E. grandis</i>	2.01E-05	3.2267	0.67	7.535
	3	<i>E. grandis</i>	7.58E-07	3.9278	0.95	0.828
	4	<i>A. mangium</i>	7.15E-05	2.9890	0.58	9.940
	6	<i>A. mangium</i>	1.40E-03	2.2083	0.66	1.293
	6	<i>E. grandis</i>	5.82E-06	3.5660	0.92	2.975
	7	<i>E. grandis</i>	1.04E-06	3.8879	0.90	1.119
	9	<i>A. mangium</i>	1.40E-03	2.2083	0.66	1.293
	9	<i>E. grandis</i>	5.82E-06	3.5660	0.92	2.975
Stem	1	<i>A. mangium</i>	0.0031	2.5491	0.91	16.838
	2	<i>E. grandis</i>	0.0032	2.5518	0.97	13.742
	3	<i>E. grandis</i>	0.0029	2.6012	0.99	4.694
	4	<i>A. mangium</i>	0.0031	2.5491	0.91	16.838
	6	<i>A. mangium</i>	0.0114	2.1826	0.91	4.382
	6	<i>E. grandis</i>	0.0323	1.9989	0.98	4.985
	7	<i>E. grandis</i>	0.0066	2.41E+00	0.98	5.654
	9	<i>A. mangium</i>	0.0114	2.1826	0.91	4.382
	9	<i>E. grandis</i>	0.02199	2.0598	0.96	8.330
Bark	1	<i>A. mangium</i>	0.0010	2.4386	0.92	2.901
	2	<i>E. grandis</i>	0.0150	1.7176	0.89	2.533
	3	<i>E. grandis</i>	0.0009	2.3875	0.98	1.701
	4	<i>A. mangium</i>	0.00104	2.4386	0.92	2.901
	6	<i>A. mangium</i>	0.00105	2.3910	0.677	3.133
	6	<i>E. grandis</i>	0.02692	1.5976	0.89	1.788
	7	<i>E. grandis</i>	0.00031	2.7129	0.95	1.658
	9	<i>A. mangium</i>	0.00105	2.3910	0.67	3.133
	9	<i>E. grandis</i>	0.0066	1.8890	0.94	1.170

Appendix B. The line intercept technique was used to analyze the presence of weeds in Sinop-MT (Brower and Zar, 1984). It was used a 29.7 m cord and made an X diagonally connecting the ends of each plot. In every 0.10 m was verified the presence or absence of weed. Measurements were made in 100E+N, 100E, 100A, and 50A50E treatments at 50 months after planting.

SUPPLEMENTARY MATERIAL

Figure S1. Allometric relationships established at 52 months of age for *A. mangium* (T1:100A 3mx6m, T4:100A 3mx3m, T6:50A:50E 3mx3m and T9: 50A:50E 2mx3m) and *E. grandis* (T2:100E 3mx6m, T3:100E 3mx3m, T6, T7:100E 2mx3m and T9) which relate tree density breast height (DBH) with component biomasses: stem, bark, branches and leaves. Different letters indicate significant differences ($P < 0.05$).

Figure S2. Mean biomass of aboveground compartments of *Acacia mangium* and *Eucalyptus grandis* in each treatment at 52 months of age. Different letters indicate differences between treatments ($p < 0.05$) in the total tree.

Table S1. Mean aboveground biomass per hectare at 52 months of age.

Design	Spacing	Density (tree ha ⁻¹)	Species	Biomass (Mg ha ⁻¹)
100A	3m x 6m	555	A	86.98
100E	3m x 6m	555	E	89.24
100E	3m x 3m	1111	E	108.15
100A	3m x 3m	1111	A	94.76
33A:67E	3m x 3m	741	E	88.19
		370	A	19.28
		1111	A+E	107.47
50A:50E	3m x 3m	555	E	69.77
		556	A	31.38
		1111	A+E	101.16
100E	2m x 3m	1666	E	123.75
33A:67E	2m x 3m	1100	E	86.64
		566	A	21.65
		1666	A+E	108.29
50A:50E	2m x 3m	833	E	70.46
		833	A	35.77
		1666	A+E	106.23
100E+N	3m x 3m	1111	E	108.74

Table S2. The output data obtained from CAN_EYE and destructive measurements (LAI), and the LAI adjusted ($v5_{adjusted}$) for each treatment on each age. Where: v6 and v5=Plant Area Index (PAI) of version 5 and version 6 of CAN_EYE software, p57= PAI with a gap fraction at 57.5°, fCover=vegetation cover fraction, clumping=measure of foliage grouping relative to a random distribution of leaves in space, ALAv6 and ALAv5=Average Leaf inclination Angle of version 5 and version 6 of CAN_EYE software, FAPARmod=Photo-Active Absorbed Radiation Fraction modelled and FAPARmean= Photo-Active Absorbed Radiation Fraction measured. $v5_{adjusted}=1.7397+0.7309*v5$.

Age (months)	Treatment	v6	v5	p57	millier	fCover	clumping	ALAv6	ALAv5	FAPARmod	FAPARmea	LAI	$v5_{adjusted}$
38	1	5.52	5.19	5.52	3.69	88	23.56	54	48	0.90	0.92	4.73	5.53
38	2	3.5	3.72	3.5	2.68	59.8	3.14	62	64	0.79	0.81	3.71	4.45
38	3	2.53	2.58	2.53	1.98	62.3	2.21	54	54	0.71	0.75	3.62	3.62
38	4	6.1	5.32	6.1	4.51	81.4	12.43	66	62	0.98	0.92	5.2	5.62
38	5	4.06	3.69	4.06	2.6	82.3	9	50	42	0.83	0.86	4.74	4.43
38	6	4.67	4.48	4.67	2.75	75.9	11.83	62	64	0.83	0.84	5.06	5.01
38	7	3.36	3.52	3.37	2.91	66.2	3.59	62	64	0.83	0.84	4.56	4.31
38	8	4.2	4.32	4.2	3.05	81.7	8.1	58	60	0.88	0.86	5.45	4.90
38	9	5.03	4.54	5.03	3.09	79.3	12.91	62	58	0.91	0.87	5.72	5.06
38	10	2.5	2.77	2.5	2.09	63.6	3.16	52	58	0.78	0.75	3.47	3.76
45	1	4.68	4.48	4.68	3.19	72.2	18.44	62	60	0.88	0.86	4.15	5.01
45	2	2.4	2.38	2.4	1.69	38.7	2.64	68	68	0.66	0.65	2.97	3.47
45	3	1.49	1.6	1.49	1.16	40.2	2.75	56	60	0.58	0.54	3.10	2.91
45	4	4.34	4.11	4.34	2.96	61.6	11.12	70	68	0.81	0.82	4.96	4.74
45	5	2.58	2.41	2.58	1.52	55.8	5.97	62	60	0.67	0.66	3.44	3.50
45	6	3.99	3.72	3.99	2.33	56	8.48	70	68	0.82	0.76	3.63	4.45
45	7	2.81	2.9	2.81	2.21	47.2	3.95	70	70	0.76	0.73	3.22	3.85
45	8	3.41	3.33	3.41	2.28	62.1	6.28	66	64	0.84	0.75	4.46	4.17
45	9	3.74	3.45	3.74	2.16	57.4	8.42	68	66	0.75	0.75	4.59	4.26
45	10	1.79	1.77	1.79	1.29	44.2	3.32	60	60	0.59	0.59	2.97	3.03
52	1	4.46	4.51	4.46	3.13	82.3	14.22	54	56	0.89	0.88	5.12	5.04
52	2	2.18	2.28	2.18	1.49	41.9	3.23	66	68	0.60	0.62	3.05	3.40
52	3	1.6	1.63	1.6	1.15	46.5	2.62	56	56	0.56	0.58	2.83	2.93
52	4	4.59	5.26	4.6	3.89	70.9	13.82	64	68	0.89	0.88	5.58	5.58
52	5	3.18	3.22	3.18	2.13	62.9	7.31	60	60	0.78	0.76	3.90	4.09
52	6	3.81	3.6	3.81	2.3	65	8.94	62	60	0.73	0.77	4.34	4.37
52	7	2.12	2.17	2.13	1.61	43	3.46	66	66	0.67	0.65	3.75	3.32
52	8	3.51	3.6	3.51	2.39	65.7	8.06	62	62	0.83	0.78	5.48	4.37
52	9	3.77	3.73	3.77	2.32	68.1	9.1	60	60	0.82	0.79	6.02	4.47
52	10	1.69	1.72	1.69	1.19	44.8	2.86	60	60	0.59	0.58	2.78	2.99