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DIVERSIDADE, USO E CARACTERÍSTICAS FUNCIONAIS DE ESPÉCIES ARBÓREAS EM SISTEMAS AGROFLORESTAIS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Magister Scientiae*.

Orientadora: Irene Maria Cardoso

Coorientador: Heitor Mancini Teixeira

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DIVERSIDADE, USO E CARACTERÍSTICAS FUNCIONAIS DE ESPÉCIES ARBÓREAS PRESENTE EM SISTEMAS AGROFLORESTAIS

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RESUMO

SILVA, Arthur da Cruz, M.Sc., Universidade Federal de Viçosa, outubro de 2020. **Diversidade, uso e características funcionais de espécies arbóreas em sistemas agroflorestais**. Orientadora: Irene Maria Cardoso. Coorientador: Heitor Mancini Teixeira.

A Floresta Atlântica é considerada um dos “hotspot” de biodiversidade do planeta, por apresentar alta diversidade e ao mesmo tempo estar muito ameaçada devido as atividades antrópicas. Seus remanescentes de florestas encontram-se envoltos por uma matriz agrícola em monocultivos que não contribuem para a preservação dos fragmentos florestais. Os Sistemas Agroflorestais (SAFs) biodiversos se apresentam como alternativa à essa matriz, pois podem funcionar como fonte de sementes para regenerar a paisagem, prover alimento e habitat para vida selvagem, melhorar o microclima, ao mesmo tempo produzir alimentos e gerar renda para as famílias agricultoras. Uma matriz biodiversa, como nos SAFs, pode imitar os ecossistemas naturais e contribuir para conectar e conservar os fragmentos florestais. Entretanto, nos SAFs os agricultores utilizarão espécies arbóreas com funções conhecidas. Muitas destas funções são conhecidas por eles, pois trabalham diariamente com a natureza e dependem da biodiversidade para sobreviver. Este conhecimento é fundamental para a implementação de SAFs que respeitem as caraterísticas ambientais e sociais locais. Para desempenhar as funções, as espécies arbóreas consorciadas interagem ecologicamente abaixo e acima do solo. O sistema radicular das árvores impacta as interações entre as plantas e as funções ecossistêmicas. A arquitetura radicular é uma característica importante dos sistemas radiculares e junto com as associações com microrganismos, a exemplo dos fungos micorrízicos arbusculares (FMA), influenciam a capacidade da planta de acessar água e nutrientes. A pesquisa objetivou analisar a similaridade entre a diversidade de árvores presentes em fragmentos florestais e em sistemas agroflorestais, as funções reconhecidas pelos agricultores das árvores presentes nos SAFs e as características das raízes e o potencial ecológico de árvores consorciadas nos SAFs. Avaliamos a diversidade de árvores (riqueza de espécies, índice de Shannon e Simpson) nos sistemas agroflorestais (cafezal e pastagem) nas propriedades da agricultura familiar e comparamos com áreas de floresta utilizando curva de rarefação (números de Hill); realizou-se inventário florestal nos sistemas agroflorestais com café e pastagem e entrevistas com as famílias agricultoras e identificou-se as funções das árvores através de pesquisa etnobotânica. A riqueza de espécies dos sistemas agroflorestais foi similar a floresta de sucessão intermediária (40 e 50 anos) e a diversidade baseada nos índices de Shannon e Simpson, foi similar a floresta no início da sucessão (5 e 25; 5 e 10 anos, respectivamente). De acordo com os agricultores, houve 15 diferentes funções para as árvores consorciadas nos SAFs (cafezal e pastagem). A função das árvores diferiu entre os SAFs. As cinco funções mais citadas no cafezal foi alimentação humana, cobertura do solo, sombra para plantas de café, atração de fauna e lenha. Na pastagem foi, atração de fauna, sombra para pastagem, embelezamento, estaca e lenha. A atração de insetos benéficos foi uma função indentificada apenas em SAF com café, enquanto a função sombra para a criação foi citada somente na pastagem. Houve uma alta relação linear entre a diversidade de funções das árvores e diversidade de espécies, sugerindo que o conhecimento dos agricultores sobre a função das espécies pode contribuir para a implementação de SAF`s mais diversos. Os SAFs utilizados pelas famílias agricultoras são importantes para manter a diversidade de árvores nas propriedades e a nível de paisagem, visto que apresenta riqueza de espécies similar a uma floresta no meio (40 e 50 anos) da sucessão ecológica. A articulação entre o etnoconhecimento com estudo de características ecológicas das espécies permitiu a identificação de espécies arbóreas boas para o consórcio em sistemas agroflorestais. Características funcionais das espécies *Miconia cinnamomifolia*, *Senna macranthera*, *Solanum pseudoquina*, *Zeyheria tuberculosa* foram avaliadas utilizando rhizobox contendo substrato com inóculos de fungos micorrízicos arbusculares (FMA). O delineamento do experimento foi inteiramente casualizado em fatorial de 4x2, com quatro espécies e dois tratamentos de inoculação (inoculado e não inoculado com FMA). Números de esporos, colonização radicular, arquitetura de raiz, comprimento específico de raiz, comprimento de raiz : volume de solo, peso seco de raiz, peso seco de raiz : peso seco da parte aérea, comprimento específico de raiz, altura da parte aérea e peso seco da parte aérea foram analisados utilizando ANOVA, teste t, e Análise dos Componentes Principais. No geral *S. macranthera* e *Z. tuberculosa* foram colonizadas por FMA e apresentaram estratégia conservativa de desenvolvimento. *S. pseudoquina* também foi colonizada e apresentou estratégia aquisitiva de desenvolvimento. *M. cinnamomifolia* não apresentou colonização nas raízes e apresentou estratégias aquisitivas e conservativas dependendo do parâmetro analisado. *Z. tuberculosa, S. macranthera* e *S. pseudoquina* apresentaram raiz pivotante, na qual as raízes secundárias derivam da principal; *M. cinnamomifolia* apresentou raiz fasciculada, com sistema radicular formado por diversos eixos, sem apresentar uma raiz principal. Os resultados confirmaram as informações indicadas por agricultores em outros trabalhos de que *S. macranthera* e *Z. tuberculosa* são compatíveis para o consórcio com cafezal e pastagem. *S. pseudoquina* apresentou características mais apropriadas para ser consorciada com pastagem, porém as árvores precisam ser protegidas do gado, já que os animais, segundo os agricultores, se alimentam de sua casca. O maior conhecimento das funções e das características ecológicas as espécies arbóreas podem contribuir nos processos decisórios sobre quais árvores consorciar com SAFs. O rhizobox permitiu a avaliação rápida do sistema radicular das espécies arbóreas. Estudos mais aprofundados precisam ser desenvolvidos para melhor análise das características da *M. cinnamomifolia.*

Palavras-chave: Agricultura familiar. Funções de espécies arbóreas. Árvores nativas. Características ecológicas. Etnoconhecimento.

ABSTRACT

SILVA, Arthur da Cruz, M.Sc., Universidade Federal de Viçosa, October, 2020. **Diversity use and functional traits of tree species in agroforestry systems**. Adviser: Irene Maria Cardoso. Co-adviser: Heitor Mancini Teixeira.

The Atlantic Forest is considered one of the “hotspots” of the planet's biodiversity, due to its high diverse and threaten diversity of plant and animal species. The forest reminescents are sorrounded by a monocultural agricultural matrix that does not contribute to the preservation of the forest fragments. Diversified Agroforestry Systems (SAFs) are an alternative to increase the sustainability of the agricultural matrix, as they can function as a source of seeds to regenerate the landscape, provide food and habitat for wildlife, improve the microclimate and, at the same time, produce food and generate income for local farmers. A biodiverse matrix that includes SAFs, can also contribute to connect and conserve forest fragments, as they imitate the natural ecosystems. However, in the SAFs, farmers will use species with known functions. Several of those functions are known by farmers since they work on a daily basis in interaction with nature and depend on the biodiversity to survive. Understanding farmer´s knowledge is, therefore, key to implemente systems that respect the environmental and social local characteristics. The impact of trees on ecosystem functioning is influenced by multiple interactions that occur above and belowground. Root traits of tree species, including the presence ofmicrorganisms such as arbuscular mycorrhizal fungi (FMA), will influence the ability of trees to access water and nutrient. This research aimed to analyze the similarity between the diversity of trees present in forest fragments and in agroforestry systems, the functions of intercropped trees recognized by farmers, the relationship between farmer’s knowledge and tree diversity, and the characteristics of the roots and the ecological potential of intercropped trees in the SAFs. We evaluated the diversity of trees (species richness, Shannon, and Simpson index) in agroforestry systems (coffee and pasture) in family farms and compared them to forest areas using a rarefaction curve (Hill numbers); a forest inventory was carried out in the agroforestry systems with coffee and pasture and farming families were intervwied and; we evaluated the function of trees through ethnobotanical survey. The species richness of the agroforestry systems was similar to the forest in the middle of the succession (40 and 50 years), while the diversity based on the Shannon and Simpson indexes was similar to the forest in the beginning of the succession (5 and 25; 5 and 10 years, respectively). According to the farmers, there were 15 different functions performed by intercropped trees in the SAFs (coffee and pasture). The function of the trees differed between the SAFs. The five most-cited functions in the coffee plantation were food production, soil cover, shade for coffee plants, attraction of fauna, and firewood. In the pasture, the main functions were fauna attraction, shade for the animals, aesthetic, wood for local use, and firewood. The attraction of beneficial insects was indentified only in the coffee systems and shade for cattle only in the pasture systems. There was a high linear relationship between the diversity of tree functions recognised by farmers and species diversity in the field. We suggest that SAFs used by family farmers are important to maintain the diversity of trees at farm and landscape level, since they show similar species richness compared to the forest in the middle of the ecological succession. The articulation between ethno-knowledge with the study of ecological characteristics of the species allowed the identification of tree species that have a high potential to be intercropped in agroforestry systems. Functional traits of the species *Miconia cinnamomifolia, Senna macranthera, Solanum pseudoquina, Zeyheria tuberculosa* were evaluated using rhizobox method containing substrate with inoculum of arbuscular mycorrhizal fungi (AMF). The design of the experiment was completely randomized in a 4x2 factorial, with four species and two inoculation treatments (inoculated and not inoculated with AMF). Ten root traits, spore numbers, root colonization, root architecture, specific root length, root length: soil volume, root dry, root dry mass : shoot dry mass, specific root length, shoot height, and shoot dry mass, were analyzed using ANOVA, t-test, and Principal Component Analysis. In general, *S. macranthera* and *Z. tuberculosa* were colonized by AMF and presented a conservative development strategy. *S. pseudoquina* was also colonized and presented an acquisition development strategy. *M. cinnamomifolia* did not present colonization in the roots and presented acquisitive or conservative strategies depending on the analyzed parameter. *Z. tuberculosa, S. macranthera* and *S. pseudoquina* presented tap roots, with secondary roots derived from the main root; *M. cinnamomifolia* presented a fibrous root, with the root system formed by several axes, without presenting a main root. Our results confirmed the information given by the famers in other studies that *S. macranthera* and *Z. tuberculosa* are compatible to be intercropped with coffee and pasture systems because of the root traits and growth parameters. *S. pseudoquina* showed more appropriate characteristics to be intercropped with pasture because of the root's architecture, however, the trees need to be protected, as the catle, according to the farmes, feed on the bark of the trees. More knowledge on the functions and ecological characteristics of tree species can contribute to the selection of apropriate trees to be used in agroforestry systems. The rhyzobox allowed the quick evaluation of the architecture of the radicular systems of the trees. More studies need to be done to better analyse the characteristics of *M. cinnamomifolia.*

Keywords: Family farmer. Functions of trees. Native trees. Ecological traits. Ethnoknowledge.

SUMÁRIO

INTRODUÇÃO GERAL

A Floresta Atlântica é considerada um dos “hotspot” de biodiversidade do planeta (Myers et al., 2000) e estima-se que contenha de 15 a 20 mil espécies de plantas vasculares (Myers et al., 2000; Stehmann et al., 2009), das quais, 48% são endêmicas (Stehmann et al., 2009). A Floresta Atlântica possui apenas 12,4 % da cobertura original (Fundação SOS Mata Atlântica & INPE, 2020) e mais de 80% dos fragmentos florestais são menores que 50 hectares (Ribeiro et al., 2009).

A Zona da Mata mineira, localizada no sudeste de Minas Gerais, inserida no bioma da Floresta Atlântica, também apresenta alta biodiversidade e sofreu forte desmatamento. Atualmente apenas 7% da região é coberta com vegetação florestal nativa (IEF, 2019a) e está muito fragmentada. A matriz agrícola em que os fragmentos florestais estão inseridos é predominantemente familiar (Souza et al., 2009) e os principais usos da terra são com a cultura do café e com a criação de gado, seguidos de outros cultivos, como eucalipto, feijão, milho, cana-de-açúcar e diversos produtos produzidos nos quintais da agricultura familiar (Ferrari, 1996; Cardoso et al., 2001). Estes cultivos são importantes fontes de renda e garantem segurança e soberania alimentar às famílias (Oliveira, 2015). A combinação de pastagem, café, outros cultivos e os fragmentos florestais, em unidades da agricultura familiar, formam um mosaico de habitats para a biodiversidade. Neste mosaico, o uso do solo e o manejo da matriz agrícola são determinantes para a conservação ou degradação da biodiversidade. Por um lado, a utilização de técnicas da revolução verde, como monoculturas, utilização de agrotóxicos e sementes transgênicas, diminuem a biodiversidade, por outro, a adoção de práticas agroecológicas pelas famílias aumenta e conservam a biodiversidade (Perfecto et al., 2009).

Compreender como as famílias agricultoras utilizam e o conhecimento que elas têm sobre seus agroecossistemas é fundamental para a busca de alternativas que levem a conservação da biodiversidade. O conhecimento dos agricultores sobre as funções e benefícios das espécies nos agroecossistemas nem sempre é reconhecido pela academia. O conhecimento produzido pelas famílias através das gerações pode ser acessado utilizando a etnobotânica. Este conhecimento subsidia a identificação das funções de espécies arbóreas nativas e contribui para difundir para a população o conhecimento sobre as funções e usos das espécies (Albuquerque et al., 2017). O estudo mais detalhado sobre características funcionais destas espécies arbóreas identificadas auxilia na compreensão das estratégias ecológicas das espécies. O conhecimento dessas espécies, de suas funções e estratégias contribuem, por um lado, para identificar espécies com potencial de uso agrícola, por exemplo em sistemas agroflorestais (SAFs) e os serviços ecossistêmicos ou benefícios proporcionados por elas para as famílias. Por outro lado, o uso das espécies pelas famílias contribui para a preservação das espécies e sistemas agrícolas biodiversos, com os SAFs, contribuem para a restauração das florestas nativas (Souza et al., 2010).

SAFs podem ser definidos como uma forma de cultivo múltiplo, no qual pelo menos duas espécies de plantas interagem biologicamente, pelo menos uma espécie é arbórea e pelo menos uma espécie é manejada para produção agrícola ou pecuária (Sommariba, 1992). Os SAFs contribuem para a restauração florestal porque, ao contrário dos monocultivos, as relações tróficas se assemelham aos sistemas naturais devido a maior biodiversidade, o que permite ampliar os nichos ecológicos existentes. Com isto, aumenta-se, por exemplo, a ciclagem de nutrientes e a proteção do solo através da cobertura vegetal, portanto, os agroecossistemas podem ser grandes repositores de biodiversidade, dependendo de como são manejados (Perfecto et al., 2009).

Em 1994 na Zona da Mata Mineira, foram implementados, de forma participativa, 37 SAFs com café e dois com pastagens em 25 comunidades e 11 municípios. A implementação teve como objetivo regenerar o solo (Cardoso et al., 2001), que se encontrava enfraquecidos devido a utilização não adequada das terras. As espécies implementadas nos sistemas foram escolhidas com base no conhecimento das famílias agricultoras e mais tarde, em um processo de sistematização participativa da experiência, estas famílias indicaram os critérios para a seleção das espécies a serem consorciadas com o café (Souza et al., 2010).

Dentre os critérios, os agricultores indicaram que as árvores deveriam ser compatíveis com a produção de café, o que pode ser observado a partir das folhas saudáveis sem indicação de deficiência de nutrientes e doenças. O sistema radicular das árvores foi apontado também como um critério importante. As árvores devem possuir raízes secundárias mais profundas do que as do café, para evitar competição de água e nutrientes. Os agricultores apontaram ainda que árvores caducifólias ou com arquitetura de copa mais abertas devem ser preferidas, de forma a contribuir para a entrada de luz no cafezal. A disponibilidade de sementes ou mudas, a facilidade de poda, a produção de biomassa e a diversidade da produção (alimentação para a família, animais domésticos, fauna, lenha, pequenas construções) foram ainda apontados como critérios importantes (Souza et al., 2010).

Nas pastagens, devido as características das gramíneas, os critérios de escolha das espécies não são necessariamente os mesmos que nos SAFs com café. Os agricultores apontaram que no consórcio com os pastos as árvores devem: i) possuir copa aberta para permitir a penetração da luz solar, e ii) serem perenifólias ou com folhas pequenas para não abafar o capim quando caírem (Meier, 2008; Freitas et al., 2009).

Com base nestes critérios, as famílias agricultoras apontaram 81 espécies consideradas compatíveis com o café e presentes em seus sistemas (Souza et al, 2006). Em um estudo etnobotânico, identificou-se também 85 espécies utilizadas nos SAFs com café (Fernandes et al., 2014; Siqueira, 2008), a maioria delas compatíveis com o café. Dentre as espécies identificadas, 45 correspondem às citadas no estudo de Souza et al. (2010) e, portanto, já reconhecidas pelos agricultores como compatíveis com café (Souza et al., 2010; Fernandes et al., 2014; Siqueira, 2008). Assim, considerando os dois estudos, mais de 100 espécies arbóreas foram consideradas compatíveis com o café. Em geral, as áreas da agricultura familiar manejadas com SAFs possuem grande diversidade arbóreas (Vandermeer & Perfecto, 2007), o que também foi indicado nos trabalhos de Fernandes et al., (2014) e Souza et al. (2010). Apesar do número elevado (mais de 100), muitas outras espécies compatíveis com café ou pastagem ainda podem ser identificadas, inclusive com a participação das famílias agricultoras, pois a Zona da Mata localiza-se no Bioma Mata Atlântica, um hotspot de biodiversidade (Myers et al., 2000).

Para isto, além dos critérios apontados pelos agricultores para identificar as espécies a serem consorciadas com sistemas agroflorestais (Souza et al., 2010), pode se agregar outros critérios, como os traços funcionais, que as fazem desempenhar determinadas funções no ecossistema. Os traços dependem das diferentes estratégias de investimento de energia na construção das estruturas da planta. Eles podem ser morfológicos, fisiológicos e fenológicos e ser mensuráveis ao nível foliar, radicular e da planta por completo (Pérez-Harguindeguy, 2013). Os traços funcionais indiretamente afetam o desempenho dos indivíduos, pois influenciam o crescimento, a reprodução e a sobrevivência dos mesmos (Violle et al., 2007). Os traços funcionais apontam ainda respostas das espécies ao ambiente, e os efeitos que essas espécies têm nas funções ecossistêmicos (Violle et al. 2007). O estudo dos traços funcionais das espécies permite aprofundar o entendimento do ecossistema para além da riqueza das espécies nos gradientes florestais, pois aprofunda o conhecimento sobre o funcionamento do ecossistema (Kattge et al., 2011).

A análise dos traços funcionais da raiz pode dar informações úteis para o entendimento do comportamento das espécies em SAFs. As raízes contribuem para o fornecimento de biomassa para os organismos do solo, o enriquecimento do solo com matéria orgânica, a redução da lixiviação de nutrientes, a ciclagem de nutriente e melhoram as propriedades físicas do solo (Snyder et al., 2008; Maeght et al., 2013).

Nos SAFs também há competição entre as plantas por luz, nutrientes e água. Para diminuir a competição por nutrientes e água, o sistema radicular das espécies arbóreas deve explorar uma profundidade diferente da cultura principal, como apontado pelos agricultores da Zona da Mata mineira (Souza et al., 2010). Além disto, deve-se priorizar as espécies que possuem sinergia com microrganismos, tais como micorrizas e rizóbios, para melhor ciclagem de nutrientes e a fixação de nitrogênio (Isaac & Borden, 2019; Ingleby et al., 2007; Bainard et al., 2011).

A dissertação está estruturada em introdução geral, dois capítulos e considerações finais. O capítulo 1, intitulado *Diversity and function of tree species in agroforestry systems in the Atlantic Forest biome*, objetivou analisar a similaridade entre a diversidade de árvores presentes em fragmentos florestais e em sistemas agroflorestais e e as funções das árvores consorciadas nestes sistemas reconhecidas pelos agricultgores. O capítulo 2, intitulado *Root of native Atlantic Forest trees used in agroforestry systems*, teve como objetivo analisar as característics das raízes e o potencial ecológico de árvores consorciadas em sistemas agroflorestais.

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Capítulo 1: Diversity and function of tree species in agroforestry systems in the Atlantic Forest biome

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ABSTRACT

The use of native species in agroforestry systems can be beneficial for ecosystem functioning, as native trees can serve as a source of seeds for the landscape as well as perform multiple functions, such as connect forest fragments, conserve biodiversity, provide food and habitat for wildlife, improve the microclimate, among other nature benefits. To design agroforestry systems that mimic natural forests and provide ecosystem services, farmers' knowledge is essential, because they work on a daily basis in contact with nature and have great knowledge on the local biodiversity. Therefore, the selection of multi-functional local species can benefit from farmers' knowledge. We assessed tree diversity (species richness, Shannon, and Simpson index) in agroforestry systems (coffee and pastures) in family farms and compared them with forest areas using rarefaction curve (Hill's number). We also assessed the functions of the trees using an ethnobotanical survey. The number of species in agroforestry systems was similar to the forest in the middle (40 to 50 years) of succession, whereas the diversity, based on Shannon and Simpson index, was similar to the forest in the beginning of succession (5 to 25 and 5 to 10 years, respectively). Besides, there was a high correlation between the diversity of uses, based on farmers' knowledge, and diversity of species in the field. Farmers identified 15 functions performed by trees in their coffee and pasture agroforestry systems. The tree functions differed among SAFs, whereas attraction of beneficial insects was indicated only in coffee systems and shade for cattle was only mentioned in pastures. We suggest that SAFs used by family farmers are important to maintain the diversity of trees at farm and landscape level. In addition, farmer`s knowledge on the functions of tree species is crucial for the design of hilghly diverse agroforests.

Keywords: Family farmer, native forest, ethnobotany, tree uses, tree diversification.

INTRODUCTION

In the last century, the Atlantic forest biome faced huge deforestation due to economic activities, urbanization, and agricultural intensification (Steege et al. 2015; Metzger & Sodhi 2009; FAO 2007). The remaining forest area in the biome is fragmented, and most forest fragments are smaller than 50 ha in size (Ribeiro et al. 2009). Besides, the surronding agricultural matrix is composed mainly of monocultures, such as sugar-cane, full-sun coffee, and pastures (Scarano & Ceotto 2015). Monocultures have negative consequences for the natural environment, such as the isolation of forest fragments and reduction of pollinators and dispersal agents (Tischendorf & Fahrig 2001). An agricultural matrix with a high-quality habitat to biodiversity, such as agroforestry systems, can contribute to the connectivity among forest fragments, hence improve forest functioning (Harvey et al. 2011).

Agroforestry systems (AS) are composed by at least two species, whereas one is a tree, and the other is a crop (Sommariba 1992). Therefore, agroforestry systems (AF)are an alternative to an agriculture´s matrix based on monocultures. To support the use of agroforestry systems, studies that demonstrate the similarity among agroforestry systems and forest fragments are important to understand the role of agroforestry systems in the conservation of local biodiversity. Agroforestry systems are suggested to host high levels of species richness (Bandeira et al. 2005; López-Gómez et al. 2008) and to mimic the natural forest in terms of structure and floristic composition (Altieri & Nicholls 2000; Perfecto et al., 2009). Agroforestry systems are important to maintain local and regional biodiversity (Salgado et al. 2006), as agroforestry systems can host native species that are also found in forest fragments (Souza et al. 2010). Agroforestry systems are also important to provide ecosystem services, such as pollination, biological control, and soil protection, water supply and food production (Perfecto et al., 2009). The use of trees in agriculutral systems can also provide additional income to farmers, because of the production of fruits, wood, and other products (Sanchez 1995; Young 1997). Moreover, agroforestry systems can increase the connectivity of natural forests in fragmented landscapes, acting as a buffer zone (Laurance 2004; Vandermeer & Perfecto 2007; Harvey et al. 2008; Mcginty et al. 2008).

The connection among forest fragments using agroforestrysystems help to develop ecological corridors. This connection could facilitate the migration of animals and the dispersion of seeds to forest fragments. However, if these systems are functioning as corridors, it is necessary to manage them to guarantee that they are not facilitating the propagation of exotic or generalist species leading to changes in forest structure (Schroth et al. 2004; Tucker 2000).

Farmer´s knowledge is essential to design agroforestry systems that mimic natural forests and provideecosystem services, as farmers work on a daily basis with nature and have great knowledge on the local biodiversity. The knowledge of farmers is key to design systems according to the local characteristics of the farms, land, and natural assets (Mekoya et al. 2008; Souza et al. 2010). As farmers gain experience in managing their systems, their knowledge also progressively increases (Rigal et al. 2018). Moreover, involving farmers in research about agroforestry systems is relevant because as more farmers understand the ecological processes and benefits of standing trees, more they will protect forests fragments and will keep and plant trees in their farms (Souza et al. 2010).

Research in the Atlantic forest biome demonstrated that each farmer has his/her own criteria for selecting or rejecting tree species to be intercropped in agroforestry systems (Souza et al., 2010). The selection criteria can influence vegetation richness and structure (Valencia et al. 2015). Studies have demonstrated that farmers select shade trees based on their compatibility with the cash crops (Albertin & Nair 2004; Soto-Pinto et al. 2007; Souza et al. 2010; Anglaaere et al. 2011; Cerda´n et al. 2012). However, Souza et al., (2010) showed that they also select trees based on biomass production, labor intensity, and production diversity. Therefore, the understanding of tree functions seems to be a key factor driving the selection of trees to be intercropped by farmers. Despite the advances to understand the influence of farmers' perceptions and knowledge on farm management (Lohbeck et al. 2020), there is still limited empirical evidence showing a direct relationship between farmers' knowledge of the uses of tree species and actual tree diversity in their systems.

The general objective of this study was to analyse the similarity of tree diversity present in forest fragments and agroforestry systems and to identify the functions of the intercropped trees in these systems recognized by the farmers. The specific objectives were (i) to estimatetree diversity in agroforestry systems (coffee and pasture) and forest areas in different successional stages; (ii) to analyze the floristic similarity between agroforestry systems and forest areas; (iii) to assess farmers` knowledge on the functions of trees found in agroforestry systems; and (iv) to understand the relationship between farmers' knowledge and the diversity of trees in their fields.

MATERIAL AND METHODS

Study area

The study was carried out in the rural community of Frossard, located in Divino, municipality of Zona da Mata region, southeast of the Brazilian state of Minas Gerais, in the Atlantic Forest Biome (Figure 1). The landscape of Zona da Mata, part of the Morfoclimatic Domain denominated “Sea of Hills” (Ab´Saber, 1996), is characterized by a mountainous topography, with altitudes ranging from 400 to 2700 meters above sea level. The average temperature is 18ºC and precipitation 1300 mm year^{-1}. Oxisols are the main type of soil occurring in the area, which are deep, well structured, well-drained and with low nutrient concentration (Ker, 1995). Agriculture is the major economic activity in the region, and the most important land uses are forest fragments, pasture, and sun-grown coffee, often intercropped with maize, beans, cassava, pumpkin, and other cultivated plants (Teixeira et al., 2018a; Cardoso et al., 2001).

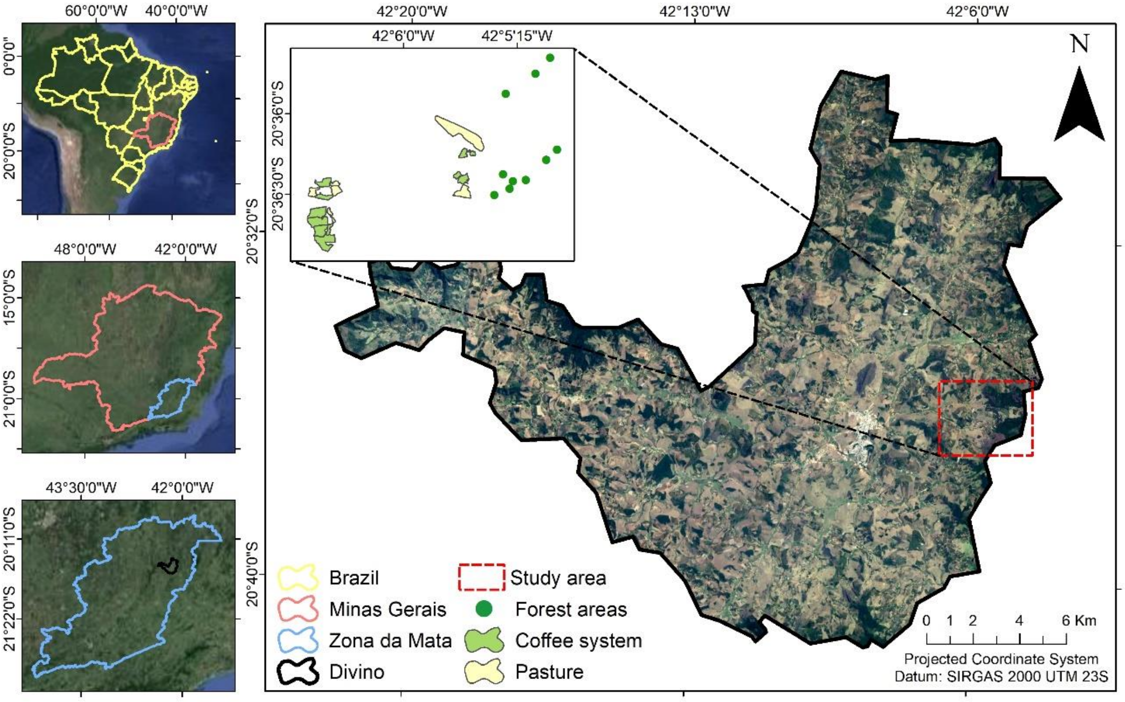

Figure 1 - Localization of the studied area (community of Frossard), Zona da Mata, of Minas Gerais, Brazil

Delimitation of studied sites

Eight coffee and six pasture systems were selected (Figure 2). The size of coffee systems ranged from 8.600 m² to 27.700 m², totalizing 13,39 ha. The size of pastures ranged from 4.300 m² to 66.100 m², totalizing 11,13 ha. Coffee and pasture systems were intercropped with native and exotic trees constituting agroforestry systems (AF).

In two farms the coffee system consisted of two separated areas (Fig. 2; coffee 1, 4), but in the analyses the areas were clustered because they were in the same landscape. Pasture 5 and 6 were from the same farm and considered individually in the analysis, because they were in different places in the landscape. Thus, we considered, for the analysis, 8 coffee systems and 6 pastures (Fig. 2).

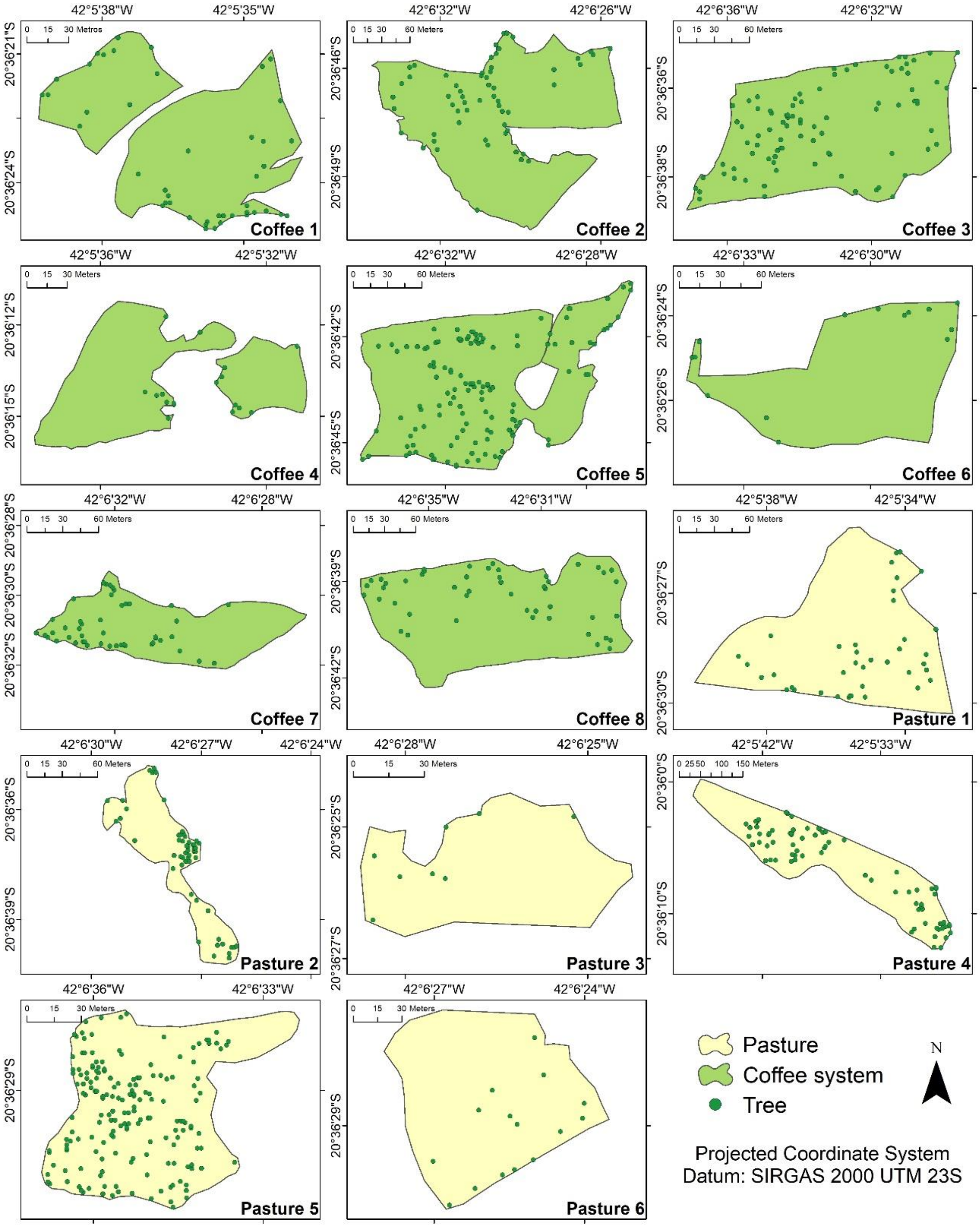

Figure 2 - Coffee systems (green) and pasture fields (yellow) studied in the community of Frossard, Zona da Mata of Minas Gerais, Brazil. Coffee area 1 and coffee area 4 are in the same farm; coffee areas 1, 3, 6, 7 are in the same farm than pastures 1, 2, 3, 5 and 6; and coffee 7 is in the same farm than pastures 5 and 6.

Forest areas in different successional stages were selected with the help of farmers to represent a gradient of secondary forest succession. In total, we selected eight secondary forest areas with ages ranging from 5 to 80 years, and two forest areas of primary forest (>100 years).

In each forest area, a plot of 900 m² (45 m x 20 m) was established (for more details see Teixeira et al., 2020). The age of each forest area was indicated by local farmers. In each plot, the soil was sampled along a central axis (45 m), uphill, in the convex part of the pedoform. In this way, the same soil type was sampled in each plot (Teixeira et al., 2020). The plots were clustered into five groups, representing ages of forest succession (F1: 5 and 10 years, F2: 15 and 25 years, F3: 40 and 50 years, F4: 60 and 80 years, F5: $\geq$100 years).

Forest inventory of agroforestry systems and forest

In the agroforestry systems and the forest plots we measured the diameter and height of all trees with a diameter at 1.3 m height (DBH) $\geq$ 5 cm. In the agroforestry systems the trees were georeferenced using the GPS Garmin Gpsmap 64s and plotted in ArcGis (Fig. 2). Most of the trees were identified at species and some at genus level. For that, botanical material was collected using a pruning pole and prepared following the usual techniques (Bridson & Forman 1999). Species were identified with the use of botanical books (Lorenzi 1992; 2000; 2009), help from specialists, and when necessary, consultation in the local herbarium of Federal University of Viçosa.

Ethnobotanical survey

The “snowball” method was used to select the families to be interviewed. This method consists of selecting an initial family, which indicates another family, and so on, until reaching a representative number of families or farms (Albuquerque & Lucena, 2004). The first family interviewed is affliated to the Union of Family Farmers and Rural Workers of Divino and a reference for the agroecological movement in the region. The family participated in several projects developed in partnership with the Federal University of Viçosa and the Center for Alternative Technologies of the Zona da Mata (CTA-ZM), a non-governmental organization that works with agroecology in the region for 32 years.

We interviewed nine families, among which, four families had both coffee systems and pastures, one family had only pasture and four families had only coffee system. A semi-structured interview (appendix 1) was conducted with each family. The first past of the interview was conducted to assess their socioeconomic characteristics, the number of household members, age, and land ownership. Following the interview, a “guided tour” method was used to assess the information about trees intercropped in coffee and pastures. The tour was done through the agroforestry systems and carried out with the main responsible for the respective land use (Albuquerque et al., 2008). During the guided tour, the second part of the semi-structured interview was conducted to assess the common name and use of each tree species and whether they were planted or grown from natural regeneration. The second part of the interview was recorded.

We submitted the research to the ethics committee of the Federal University of Viçosa, and the participants signed a Free and Informed Consent Form, stating that they agreed to share the ethnobotanical information.

BOTANICAL AND ETHNOBOTANICAL INDEXES

Cluster Analysis

We used the Jaccard similarity index (Real & Vargas, 1996) to analyse the similarity of species of trees among agroforestry systems and forest areas. This analysis consists of grouping the species that occured in the agroforestry system and forest areas. We used Agnes-algorithm to construct a hierarchy of clustering.

Diversity index

The diversity within the areas was analyzed with the rarefaction and extrapolation methods to reduce sampling of larger samples until they contain the same number of observed individuals or observations as the smallest sample (Hsieh et al., 2016). The method was based on the first three Hill's numbers (Colwell et al., 2012; Chao et al., 2014): species richness ($q = 0$), the Shannon entropy index ($q = 1$), and the inverse Simpson index ($q = 2$). The Hill's numbers differ in the weight given to the rare species, being maximum in species richness, intermediary in Shannon entropy, and low in the inverse of Simpson index (Melo, 2008).

The species richness does not consider the abundance of species and does not distinguish between dominant and rare species, because both receive the same weight. It refers to the number of species in the area. Shannon's entropy index analysis gives the species the same weight for their relative abundance (Jost & González-Oreja 2012). If there is a specie with a high level of relative abundance, and the majority is rare, the Shannon entropy will be close to zero. Shannon entropy measurement can be considered as the effective number of common species in the community (Hsieh et al. 2016). The inverse of Simpson index ($q =2$) is sensitive to the abundance of the plentiful species in the sample (Whittaker 1965) and can be interpreted as the effective number of dominant species in the assemblage (Hsieh et al. 2016).

The rarefaction test was developed based on the number of individuals present in each site and creates a relationship between diversity and the number of individuals and allows us to compare the different sites with different sizes. The rarefaction curve represents the observed value and there is a confidence interval (95%) based on the bootstrap method. This test consists of 100 repetitions based on the papers by Chao et al. (2014) and Budka et al., (2019). The extrapolation curve was twofold the number of the individuals of each curve and represent the expected value.

We also calculated the Shannon (H') index (Krebs 1989) to species diversity for each farm.

$$H' = - \sum_{i=1}^{S} (pi)\,(\log^{2} pi)$$

S = species number; pi = ni/N; ni = abundance of each species; N = abundance total of trees; H'= Shannon index.

Ethnobotanical analysis

The data on the use of trees were analyzed qualitatively, and a categorization of the uses was made based on the interview. The number of uses cited by each farmer was used to calculate a quantitative ethnobotanical Shannon (H'_e) index (Krebs 1989), for each farmer.

$$H'_e = - \sum_{i=1}^{S} (pi)\,(\log^{2} pi)$$

H'_e = Ethnobotanical Shannon index; S = number of uses; pi = ni/N; ni = number of species per use; N = total number of species.

We used a linear regression model to analyze the relationship between the diversity of uses and diversity of species in both pastures and coffee systems. The diversity of species was used as the response variable and diversity of uses as the explanatory variable. The statistical significance was assessed according to the p-values. The adjusted coefficients of determination (R^2) were used to estimate the explained variance of the model. The analysis was performed using R 3.6.1.

The cluster, linear regression and diversity analysis were performed in accordance with the parameters of R software (R Core Team 2019).

RESULTS

Tree species in agroforestry systems (coffee and pasture) and forest areas

In the coffee systems, there were more individuals, botanical families and species of trees than pastures (Table 1). There were, on average, 23 species (ranging from 9 to 40) in coffee systems and 13 species (ranging from 5 to 23) in pastures. No pattern of tree distribution was observed in the agroforestry systems. In some systems, the trees were on the edges, and in others, the trees were scattered (Fig. 2).

In the five forest areas together, we found 1,494 individual trees, on average 1660 individuals (Table 1), 166 species, and 54 families (some species and families repeated in the forest studied areas). Together, the forest areas presented higher number of individuals (Table 1), species and families of trees than the agroforestry systems.

Tabela 1 – The number of individuals, families, and species in the agroforestry systems (eight coffee systems and six pastures) and forest (F) areas.

	Coffee systems	Pastures	F1	F2	F3	F4	F5
Tree individuals	716	521	131	194	266	418	485
Tree individuals/ha	54	47	728	1078	1478	2322	2694
Families	28	26	10	19	33	41	39
Species	80	55	10	24	54	93	98

F1: forest with 5 and 10 yr; F2: forest with 15 and 25 yr ; F3: forest with 40 and 50 yr; F4: forest with 60 and 80 yr; F5: forest with $\geq$ 100 yr.

A list of all species identified in the forest and agroforestry systems are presented in the supplementary material. We could not identify 12 species, eight in the forest areas, one in the coffee systems, and three in the pastures. Some non-identified trees occur in more than one studied area (supplementary material, Table S1).

In coffee systems, nine species represented 50% of all individuals; in the pasture, six species represented 52% of all individuals; in F1 (forest with 5-10 yr) one species represented 81,78% of all individuals; in F2 (forest with 15- 25 yr) one specie represented 60,31% of all individuals; in F3 (forest with 40-50 yr), six species represented 52,63% of all individuals; in F4 (forest with 60-80 yr), ten species represented 50,96% of all individuals; in F5 (forest with $\geq$ 100 yr), one species represented 50,52% of all individuals (Table 2).

Table 2 - Tree species that represent 50 % or more of all individuals in coffee systems, pastures, and forest areas.

Species	Common name	Coffee	Pasture	Forest				
				F1	F2	F3	F4	F5
Aegiphila sellowiana Cham.	Papagaio	x						
Annona dolabripetala Raddi	Pinha/Araticum						x	
Bauhinia forficata Link	Unha -de-vaca					x		
Carica papaya L.	Mamão	x						
Casearia sylvestris Sw.	Guaçatonga		x					
Cordia trichotoma (Vell.) Arrab. ex. Steud.	Louro-pardo	x	x					
Euterpe edulis Mart.	Juçara					x	x	x
Handroanthus ochraceus (Cham.) Mattos	Ipê-amarelo		x					
Inga edulis Mart.	Ingá-de-metro	x						
Machaerium nyctitans (Vell.) Benth.	Jacarandá-bico-de-pato					x	x	
Machaerium stipitatum (DC.) Vogel	Sapuva					x		
Mangifera indica L.	Mangueira	x						
Maytenus robusta Reissek.	Cafezinho						x	
Miconia paniculata (Mart. & Schrank ex DC.) Naudin	Miconia					x		
Myrcia splendens (Sw.) DC.	Guamirim-miúdo						x	
Piptadenia gonoacantha (Mart.) J. F. Macbr.	Pau-jacaré		x	x	x	x	x	
Platymiscium pubescens Micheli.	Jacarandá	x	x					
Platypodium elegans Vogel.	Jacaranda-do-campo	x						
Prunus myrtifolia (L.) Urb.	Pessegueiro-bravo						x	
Solanum mauritianum Scop.	Capoeira-branca	x						
Styrax sp.1	-						x	
Tabernaemontana fuchsiaefolia A.D.C.	Leiteira		x					
Tetrorchidium rubrivenium Poepp.	Canemaçu						x	
Tibouchina estrellensis (Raddi) Cogn.	Quaresmeira						x	
Toona ciliata var. australis	Cedro-australiano	x						

F1: forest with 5 and 10 yr; F2: forest with 15 and 25 yr ; F3: forest with 40 and 50 yr; F4: forest with 60 and 80 yr; F5: forest with ≥ 100 yr.

Most trees intercropped in the agroforestry systems were grown from natural regeneration (Table 3). In the coffee systems, there were more planted trees than in pastures (Table 3). In the coffee systems, 61.9% of the trees were from natural regeneration, 29.8% were planted and the origin of 8.3% was unknown. In pasture sites, 90.7% were from natural regeneration, 5.6% were planted and the origin of 3.7% was unknown. In the forest areas, only one tree species was exotic, while the others were native to Atlantic forest and grown from natural regeneration.

Table 3 - Percentage of species from natural regeneration (NR), planted (P), and unknown origin (UO) in each coffee systems (Cof), and pasture (Past) and Shannon diversity of species (H') and ethnobotanical Shannon diversity (H'e) index.

Agroforestry systems	**Origin (% species)**			**H'**	**H'e**
	NR	P	UO		
Cof_1	69.23	30.77	0.00	2.50	1.79
Cof_2	57.14	42.86	0.00	2.86	2.41
Cof_3	80.49	14.63	4.88	3.37	2.26
Cof_4	37.50	56.25	6.25	2.31	1.27
Cof_5	70.00	23.33	6.67	3.17	2.02
Cof_6	70.00	10	20.00	1.94	1.73
Cof_7	67.86	28.57	3.57	2.49	1.97
Cof_8	38.46	61.54	0.00	2.78	1.45
Past_1	100.00	0	0.00	2.43	1.89
Past_2	83.33	8.33	8.33	2.65	2.27
Past_3	100.00	0	0.00	1.50	0.69
Past_4	95.65	4.35	0.00	2.36	1.67
Past_5	95.45	0	4.55	2.26	2.14
Past_6	100.00	0	0.00	1.89	1.89

In coffee systems, 68% of the planted species were exotic and 32% were native. The native species are considered of natural occurrence in the Atlantic Forest. The species from natural regeneration were 12% exotic and 88% native. The mean value of the Shannon diversity of species was 3.74, and of the ethnobotanical Shannon diversity was 2.42. In the pastures, 66.7% of the planted species are exotic and 33% are native species. The species from natural regeneration are 10% exotic and 90% native. The mean values of Shannon diversity of species, and the ethnobotanical Shannon diversity were 2.45.

Floristic similarity

In the cluster analysis (Fig. 3), four groups were formed. The agroforestry systems were grouped, showing that they have similar species composition. There is a clear secondary forest succession pattern, confirming the initial separation of the forest areas in initial, medium, and old ages. The agroforestry systems differ from the forest areas, especially from F1 and F2, the youngest secondary forest areas (Fig. 3).

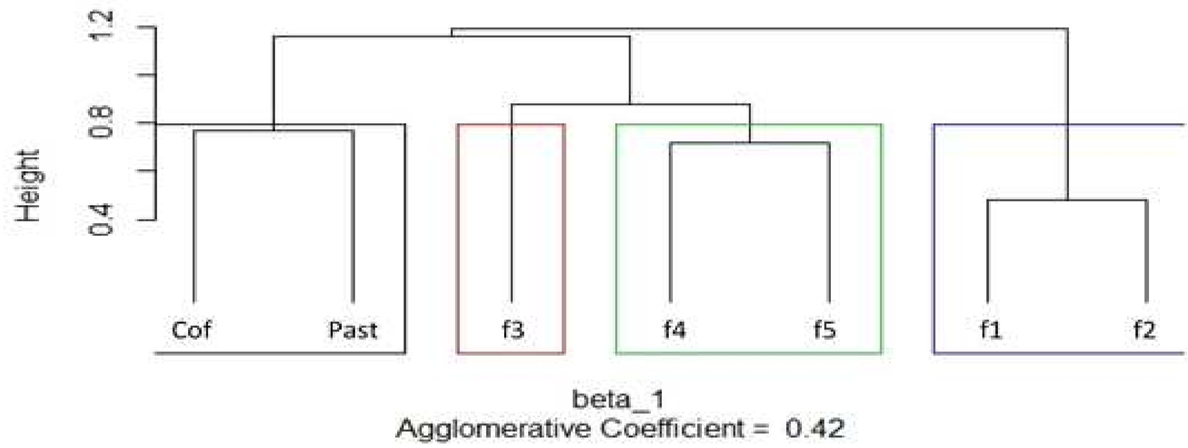

Figure 3 - Floristic similarity among forest (f) and agroforestry systems (Cof = coffee systems and Past = pastures). Numbers following the letter f represent the different ages of the forest (F1: five and ten years; F2: fifteen and twenty-five years; F3: forty and fifty years; F4: sixty and eighty years; F5: 100 and > 100 years).

We also found 130 species that occur only in the forest (126 natives, one exotic, and three unknown), 37 only in the coffee systems (18 natives, 16 exotics and three unknown), and 12 only in the pasture (eight natives and four exotic). We found 19 species that occur in coffee systems, pasture, and forest fragments, five that occur in coffee systems and forest, four that occur in pasture and forest, and 18 that occur in coffee systems and pasture.

Diversity of tree species

There were no differences in species richness between agroforestry systems (Fig 4 A1). Based on rarefaction, the tree species richness of the agroforestry systems is higher than F1, F2 (Fig. 4 A1), equal F3 (Fig. 4 A2) and lower than F4 and F5 (Fig. 4 A1). The species richness increases with the ages of the forest areas (Fig. 4 A1).

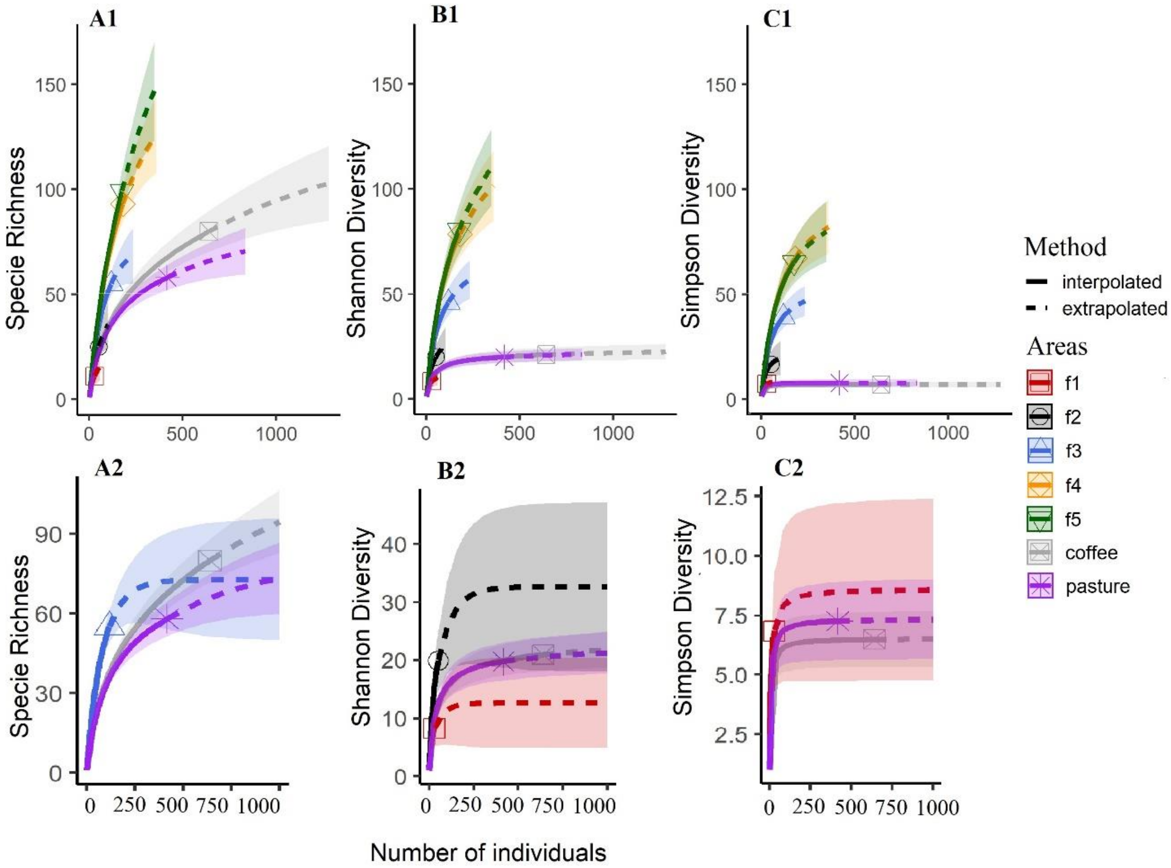

Figure 4 - Individual-based rarefaction (solid lines) and extrapolation curves (dashed lines) of tree species diversity; A1) species richness (first Hill number); B1) Shannon's entropy index (second Hill number); C1) inverse of Simpson's index (third Hill number). Figures A2 (species richness), B2 (Shannon's entropy index), and C2 (inverse of Simpson's index)compared agroforestry systems and forest areas. F1: forest of five and ten years; F2: fifteen and twenty-five years); F3: forty and fifty years; F4: sixty and eighty years; F5: 100 and > 100 years). The shade in each line represents the confidence interval with 95% confidence. X-axes give the number of individuals. All extrapolations are extended to double sample size.

The Shannon index did not differ among agroforestry systems (Fig 4 B1), and among agroforestry systems and F1, F2 (Fig. 4 B2), however, according to the cluster analysis (Fig. 3), the species composition of agroforestry systems differs from F1 and F2. The Shannon index differed among agroforestry systems and F3, F4, and F5 (Fig. 4 B1). There were fewer common species in agroforestry systems than in F3, F4, F5. In F4, and F5, the species richness and the common species were higher than in agroforestry systems.

The number of species (Fig 4 A1), the Shannon's entropy index (Fig 4 B1), the inverse of Simpson's index (Fig 4 C1) showed the same pattern of forest succession, separating then in four groups, F1, F2, F3, and F4 and F5 together.

However, the indexes showed different pattern among agroforestry systems. According to the Simpson's analysis the agroforestry systems were similar to F1 (Fig. 4 C2).

Farmers' ethnoknowledge

The average number of household members per family was four. The main householder was in average 53 years old (range 29 to 79 years). Besides, at the time of the interviews, five families were still paying for their land, which they acquired through governmental rural credit funding, while the other families already owned their farms.

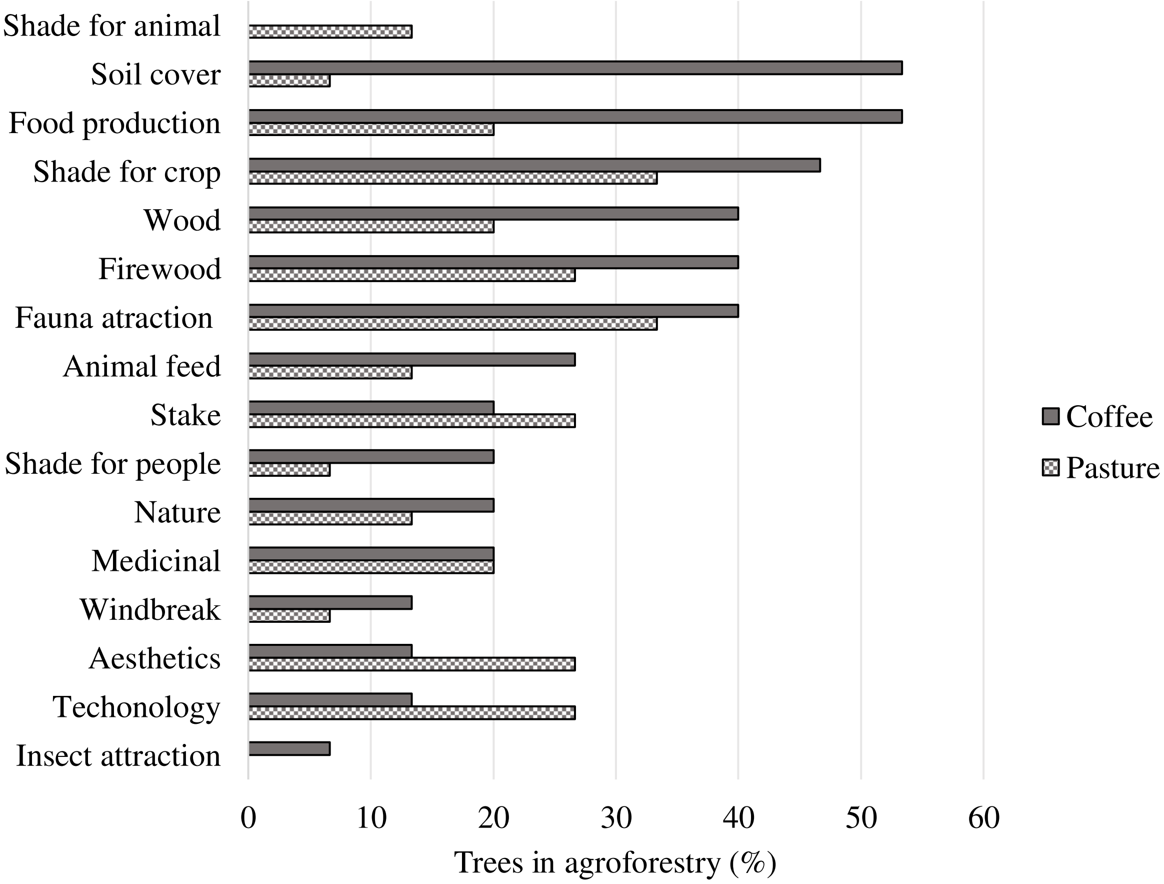

Figure 5 - Percentage of trees assigned to each use by farmers. Trees presented in the agroforestry systems without any use indicated by the farmers were not included in the calculation. Crop refered to coffee and grass; animals refered to cattle.

According to the farmers, there were 15 different uses (considered functions) for trees intercropped with coffee systems and 15 with pasture (Fig. 5). The trees' functions differed among the agroforestry systems. For instance, the attraction of beneficial insects was indicated only for coffee systems and shade for animals only for pastures. The five most-cited functions for trees in coffee systems were food production, soil cover, shade for coffee plants, fauna attraction, and firewood. In the pastures, the most cited functions were fauna attraction, shade for the grass, aesthetics, stake, and firewood (Fig. 5).

The linear regression analysis showed that there was a positive significant linear relationship between tree diversity in the agroforestry systems (coffee and pasture) and diversity of functions mentioned by farmers ($p < 0,05$, Fig. 6).

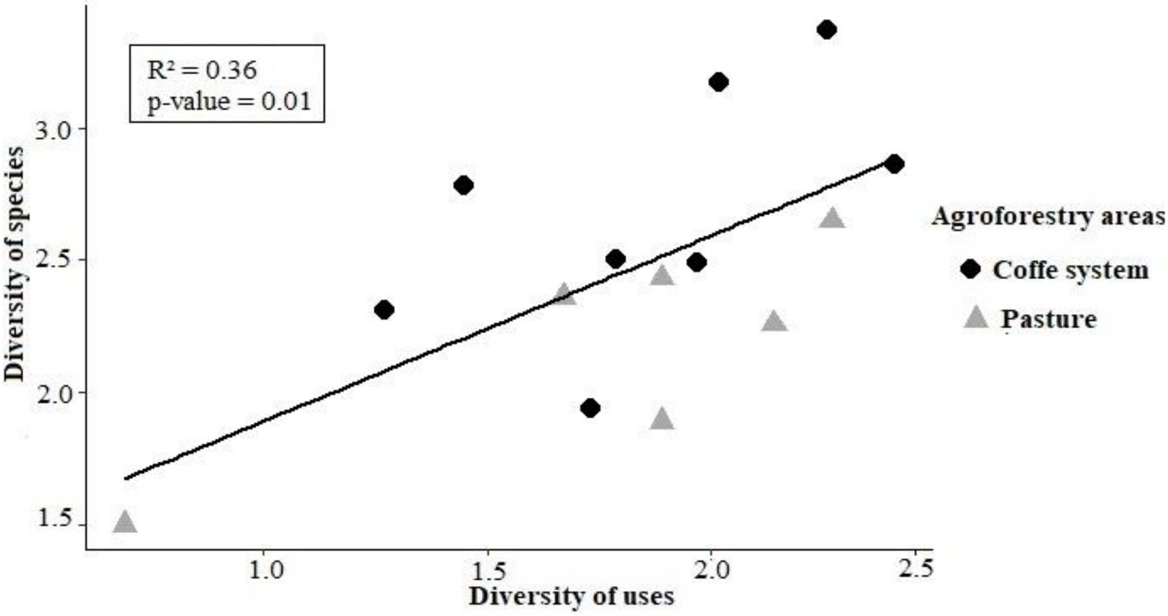

Figure 6 - Relationship between diversity of tree species in coffee and pastures and diversity of functions mentioned by farmers. Diversity of species is the response variable and diversity of functions is the effect explanatory variable.

DISCUSSION

The conservation value of agroforestry systems

Although agroforestry systems are frequently managed, they still presented a high species diversity (Table 2) and presented less eveness than the forest area F3. Our study corroborates with Bandeira et al. (2005), who showed that coffee systems can harbor a high number of wild tree species richness. Thus, at the landscape level, agroforestry systems showed the same species richness (Fig. 4 A2) as the forest in the middle stage of secondary succession (F3 with 40-50 years).

Due to the high number of species richness, the set of all farms (landscape level) have the potential to conserve the diversity (Valencia et al. 2014). However, a single farm can have limited potential to conserve tree richness (mainly due to a small area).

The number of tree species in the agroforestry systems and the species shared among the forest fragments and agroforestry systems (supplementary material, Table S1) showed the importance of these systems to promote a biodiverse agricultural matrix. A biodiverse agricultural matrix, as the agroforestry system, is important to connect forest fragments contributing to the maintenance of ecological processes, such as migration of species. Without migration among fragments, species can even get extincted (Perfect et al., 2009).

Although coffee systems and pastures were different in terms of the main crop, they showed similar trees diversity (species richness, Shannon, and Simpson index). However, when comparing the occurrence of trees in those areas, in pastures there was a larger number of species that originated from natural regeneration, possibly because coffee systems were more intensively managed than pastures, thus, farmers selected some known species that might fulfill theirs need, especially in terms of production diversification (Souza et al., 2010). Also, because in the pasture only native and rustic species survive, because of grazing (Lohbeck et al. 2020). Thus, although the pastures had fewer individuals and diversity of trees than the coffee systems, they are also an important source of biodiversity.

Most the tree species from natural regeneration present in the agroforestry system are native to the Atlantic Forest, while most of the planted tree species in the agroforestry systems were exotic. Among the exotics, most of the trees (79%) were fruit trees, which are important to the consumption of the family, to the domestic and wild animals, and to increase farmer income (Vaast et al. 2005; Siqueira 2008). The fruit tree diversity in the Atlantic forest is enormous, however, their use is little, this being a legacy of the colonization (Oliveira & Engemann 2011; Dean 1997), that introduced the exotic species. These reveal the necessity of further studies on forest fragments and tree intercropping to seek native trees that could perform the same function.

The most of exotic trees found in our inventory (Table S1, supplementary material) are not potential invaders, but farmers need to be careful because of exotic trees can negatively impact the biodiversity in native forests (Aide et al. 2000), for instance, jackfruit is shown to impact forest fragments (Mileri 2012). However, exotic fruit trees such as avocado, can also be a source of food to the native fauna of forest fragments during the dry season.

Although at the landscape level (Fig. 4 A1) agroforestry systems presented a higher number of species than the mid-successional forest fragments, there was less diversity than mid-successional forest fragments, which means that coffee and pastures systems have a considerable number of species, but few species dominate. Therefore, primary, and secondary forest fragments remain crucial and need to be protected to guarantee the conservation of biodiversityin the landscape.

The forest fragments are important to provide many ecosystem services, as water supply and pollination. Besides, forest fragments are also important to enhance biodiversity in agroforestry systems as most trees found in agroforestry systems are native and originate from the natural regeneration. These species depend onforest fragments as the main source of seeds (Lohbeck et al. 2020).

In our study, the secondary forest took approximately 60 years after agricultural abandonment to reach the same diversity (species richness, Shannon, and Simpson index) of a mature forest (>100 years). Safar et al. (2020) demonstrated that species richness in tropical forests complete recovery of the mean species richness of mature forest within the 78 to 83 years of succession. Other studies showed that 60 years old forest reached the same diversity as the mature forest (Teixeira et al. 2020; Guariguata & Ostertag 2001; Martin et al. 2013; Rozendaal et al., 2019). The difference in time to reach maturity is probably related to the previous land use (Teixeira et al. 2020) . Less intensive use leads to quick recover.

From farmers knowledge to shade tree diversification

The knowledge about the function of trees is related to tree diversity in the fields (Fig. 6) becausefarmers specifically select tree species that do not compete with the main cash crop and are capable to provide multiple ecosystems services, or benefits (Souza et al., 2010). As in our study, Fernandes et al. (2014) also found that, in the same region, the main uses of trees intercropped with coffee were food production, soil cover, and shade for the coffee. In pastures, our study corroborated with the results founded by Siqueira (2008), who also found, in the same region, that the main uses of trees were shade for the animals, fauna attraction, and firewood.

According to the main uses attributed to trees by farmers, they are important to provide food and enhance the microclimate, both important ecosystem services to maintain crop yields and crop health. Although other uses were less cited, such as respect to nature and medicinal use (fig. 5), they are also important to the environment and families, because they provide cultural and provisional ecosystem services. Moreover, agroforestry systems improve soil quality because they provide conditions to increase soil fauna under the canopy (Silva et al.

2015). They also can decrease deforestation, as they provide wood for different needs, such as firewood and timber for small constructions (Souza et al., 2010).

However, if the agroforestry coffee system is not well managed, crop productivity can decrease when compared with full sun coffee. If well managed, coffee productivity can be similar (Souza et al., 2010) in full sun coffee and agroforestry systems. Besides, agroforestry systems can provide other benefits such as reduction of the biennially of coffee production, increase of bean size and improve beverage quality because the shade delays the ripening of coffee bean (DaMata 2004; Vaast et al. 2005).

Well-maneged agroforestry systems rely on farmers knowledge. Farmers' knowledge was acquired through generations and through the interaction with nature. As a result, farmers know about the cash crop and about the ecological features of native tree species (Souza et al., 2010; Soto-pinto et al., 2007. When farmers understand the importance of biodiversity for both the environment and the cash crops, they will maintain and plant some trees, which is considered as planned diversity, to attract several associate biodiversity, responsible for many ecosystems' services (Perfecto et al., 2009).

Thereby, to preserve the biodiversity, the knowledge of the farmers is essential because if they understand and recognize the function of the biodiversity, they will have more interest to keep the trees in their fields (Teixeira et al., 2018b, Souza et al. 2010, Cardoso et al., 2001), as shown by the linear regression (Fig. 6). Farmers learn trough generations and with nature, but they also learn from scientists. Therefore, to increase scientifical knowledge about intercropping trees in agroforestry systems is also important. For instance, to understand tree root caractheristics can help in the selection of potential new native trees to be intercropped with coffee. Trees with root systems below coffee root systems can avoid competion between coffee and trees for water and nutrients (Isaac & Borden, 2019).

In the region, where the research took place, since 2008, meetings called *intercâmbio agroecológico* (agroecological meetings) are being promoted by family famers' organizations, the Federal University of Viçosa, and the Centro de Tecnologias Alternativas (CTA – ZM). These meetings occur monthly and allow farmers and scientists to share their knowledge about trees. Through these meetings, farmers, scientists, students, and technicians are increasing the use of trees, and research about trees (such as the one here presented) are being requested and carried on. One frequent request is about which tree species can be intercropped without harming coffee production.

CONCLUSION

Agroforestry systems are similar to mid-succesional forests in terms of species richness. Therefore, the use of agroforestry systems is an effective strategy to maintain tree diversity at farm and landscape level.

At landscape level, agroforestry systems connect forest fragments, contributing to the maintenance of ecological processes. At the same time, forest fragments are also important for promoting biodiversity in the agroforestry systems, because they are the main source of tree seeds, as most trees found in agroforestry systems are native and derive from the natural regenaration.

The diversity in agroforestry systems was only possible due to increased farmers' knowledge on the functions of the trees. Thus, it it remains crucial to promote the exchange of knowledge among farmers, scientists, technicians, and other actors to benefit from farmers' knowledge but also to increase their knowledge, which can result in more biodiversity in the farms. For that, it is important to promote meetings among famers and scientists and also to increase scientific knowledge about the carachteristics of trees that can be intercropped without harming the productivity of the main crop, while providing multiple ecosystem services.

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Capítulo 2: Root systems of native Atlantic Forest trees used in agroforestry systems

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ABSTRACT

Trees that are present in agroforestry systems can perform several functions that benefit farmers and society in general. Many of these functions are regulated by the below and aboveground interactions involving trees, crops, microorganisms, and the environment. For instance, root traits such as roots architecture, specific root length, as well as root colonization by mycorrhizal fungi can alter the capability of plants to access water and different pools of nutrients. There are few studies of root functional traits in agroforestry systems, and we did not find any study about the root system of *M. cinnamomifolia, S. macranthera, S. pseudoquina and Z. tuberculosa*, native species of the Atlantic Forest Biome. The common methods to excavate the soil, extract roots, cleaning and measurement in the laboratory are extremely laborious and time consuming. To study root systems is important due to the interactions between trees and crop, and is even more difficult to study in agroforestry systems, due to the multiple root interactions that occur among species. Thus, we used a rhizobox method to evaluate root traits and shoot growth parameters of the four native trees. The experiment design used was completely randomized in a 4x2 factorial, with four species and two inoculation treatments (inoculated and non-inoculated with arbuscular mycorrhizal). Data obtained was analysed using ANOVA, t-test, and Principal Component Analyses. In general, *S. macranthera* and *Z. tuberculosa* were colonized by arbuscular mycorrhiza and showed a conservative strategy of development. *S. pseudoquina* also was colonized by arbuscular mycorrhizal but presented an acquisitive strategy of development. *M. cinnamomifolia* did not show root colonization and showed both acquisitive and conservative characteristics depending on the parameter analyzed. The species *Z. Tuberculosa* and *S. Macranthera* are suitable for intercropping in coffee and pastures because they presented roots traits that will not harm the crops, as tap roots. The specie *S. pseudoquina* is suitable for intercropping in pastures because it presented roots traits that can harm the coffee, as fibrous roots, but in this case the tree must be protect as catle feed on their barks. Further studies should be developed to better analyse the characteristics of *M. cinnamomifolia.*

Keywords: Root traits, belowground interaction, rhizobox.

INTRODUCTION

Trees that are present in agroforestry systems can perform several functions that benefit farmers and society in general. (Perfecto et al., 2009). Many of these functions are regulated by the below and aboveground interactions involving trees, crops, microorganisms, and the environment. For instance, the roots system can influence the vertical distribution of soil fauna, as the fauna can harbor and feed on roots (Maeght et al., 2013). The management of the systems must be done in a such way that the interactions do not harm the production of cash crops. Below ground research on root traits, including information on root architecture, nutrient cycling, and water competition is useful to better understand the ecological interaction between the plant species (Isaac & Borden, 2019).

Trees root system can raise water from the deep and humid soil layers and redistribute it in the shallow dry layers (Peñuelas & Filella, 2003; Goldstein et al., 2008; Zapater et al., 2011). For instance, water transported by the tree root system can influence the availability of some nutrients (Snyder et al., 2008) that are absorbed via mass fluxes (Hetrick 1991). Roots architecture, an important trait of tree species, physiology, and symbiotic interactions influence the capability of plants to access water and different pools of nutrients (Maeght et al., 2013). Thus, to avoid water and nutrient competition, crop, and tree roots should explore different layers of the soil.

The amount of soil explored by trees depends on the morphology and the spatial distribution of roots and the ecological strategy of the species. Tree species can be classified as acquisitive or conservative according to their growth and repreductive strategies resource . Generally, species that grow fast and have short-lived leaves, fast photosynthetic rates, and low-wood density are classified as acquisitive species (Poorter & Bongers, 2006; Selaya & Anten, 2010; Westoby et al., 2002). In contrast, species with slow growth rates, long-lived leaves, tissues with protection against predators, and shade-tolerant show a conservative strategy (Augspurger and Kelly, 1984; Coley, 1983; Reich et al., 2003). These ecological characteristics influence the root system, since acquisitive species has a greater degree of branching and root tips (Comas & Eissenstat, 2004), and higher specific root length than conservative species (Kramer-Walter et al., 2016).

The root system is highly responsive and plastic to the soil environment. However, when plants are growing in the same biotic and abiotic conditions, the tree genetotype will coordinate the response of the roots to the environment (Das & Chaturvedi, 2008; Borden et al., 2017). According to Canadell et al. (1996), worldwide, trees have in average deeper roots (7.0 ± 1.2m) than shrubs (5.1 ± 0.8m). In the tropics, roots from deciduous trees can be deeper than 3.7 m, and in case of evergreen tree species, 20 m (Canadell et al., 1996). The analysis of crop species frequently used in agroforestry systems, such as coffee and Brachiaria spp., showed that fine roots of coffee (a tap type of root) are distributed in the first 0.20 m of soil depth (Carducci et al. 2014) and while thethe fine roots of Brachiaria spp. (a fibrous type of root) are distributed in the first 60 cm of soil depth, Huot et al. (2020). Besides, the root biomass of Brachiaria spp. decreases when it reaches one meter deep (dos Santos et al., 2007).

The contribution of deep roots for nutrient uptake in tropical soils is poorly studied (Hinsinger et al., 2011). However, according to Maeght et al. 2013, roots are important to uptake mobile nutrients that were leached to deeper layers of the soil (Isaac & Borden, 2019). Moreover, deep roots can also have synergies with microorganisms, such as arbuscular mycorrhizal fungi (AMF), that uptake immobile nutrients from deep layers of soil (Cardoso et al., 2003). The interaction with AMF is beneficial for both fungi and plants, as plants can increase their nutrient and water absorption (Silva et al., 2020), while fungi can receive a supply of Carbon from the plants (Bago et al., 2008).

More than 80% of the mycorrhizal association with terrestrial plants is formed by AMF (Sanders 2003), hence, there is an active process of nutrient acquisition by AMF that enhance a better nutrient cycling, as well as benefit the ecological interaction between trees and crops in agroforestry systems (Isaac & Borden, 2019; Ingleby et al., 2007; Bainard et al., 2011). On the one hand, AMF can increase the volume of soil accessed by tree roots, and therefore, expand the capacity of trees to absorb water and nutrients. (Isaac & Borden, 2019). Besides, AMF is suggested to access pools of nutrients that are not available to plants (Cardoso et al., 2004). On the other hand, as the AMF depends on the root systems, the root architecture will influence the AMF distribution and the volume of soil explored by the AMF (Mulia and Dupraz, 2006; Isaac et al. 2014; Cardinael et al., 2015; Kumar and Jose, 2018; Borden et al., 2019; Cardoso et al., 2003).

In agroforestry systems, root traits, plant physiology, and symbiotic interactions between roots and microorganisms will influence the capability of trees to access nutrients and water (Maeght et al., 2013). Besides, root traits caninfluence the competition of water and nutrient among trees and crops. Thus, it is important to study the root system of trees in agroforestry systems.

Senna macranthera (Collad.) H.S.Irwin & Barneby and Zeyheria tuberculosa (Vell.) Bureau ex Verl are species commonly used in agroforestry systems in the Zona da Mata Mineira (Souza et al., 2010). Duarte (2007) showed that these species increase nutrient cycling through leaf decomposition, especially of N, P, K, Cu, and Zn. These two species fulfill the criteria pointed out by farmers of Zona da Mata to select tree species to be intercropped with coffee. One important criterium pointed out by farmers is that the root system of trees should explore a different soil layer than the coffee roots (Souza et al., 2010). According to Isaac & Borden (2019), the best strategy to manage a tree-based intercropping system is to select species with complementary root distribution. *Solanum pseudoquina A. St-Hill.* and *Miconia cinnamomifolia (DC.) Naudin* are also used in agroforestry systems (Moreira & Carvalho 2018; Steenbock et al 2013). Silva et al. (unpublished) identified *S. pseudoquina* in agroforestry coffee systems where *S. macranthera and Z. tuberculosa* were also presented.

Although important, there are few studies of root functional traits in agroforestry systems (Martin & Isaac, 2015). We did not find any study about the root system of *M. cinnamomifolia, S. macranthera, S. pseudoquina and Z. tuberculosa*, native species of the Atlantic Forest Biome. It is difficult to study the root system and their interactions, as they are “hidden” in the soil (Smit et al., 2000). The common methods to excavate the soil and extract roots are extremely laborious (Van Noordwijk & Purnomosidhi, 1995; Mulatva et al., 2002). Root cleaning and measurement in the laboratory is also time consuming. To study root systems is even more difficult in agroforestry systems, due to the multiple root interactions that occur among species.

In this study, we proposed to use the rhizobox method to evaluate plant growth and root traits. This method has been used to study the rhizosphere and nutrient uptake (Youssef & Chino 1988; Wenzel et al. 2001). We used the rhizobox method to assess root traits because it allows us to easily assess the root system in vivo and to measure the root system. Moreover, this method is cheap because the rhizobox can be produced with recycled materials. We assumed that root traits of plants growing in the rhizobox are also representative of adult plants.

The general objective of this study was to assess the ecological potential of four tree species for intercropping in agroforestry systems. The specific objectives were: (i) to analyze root functional traits of the selected species, (ii) to analyze the effect of AMF in roots traits and growth parameters. The root system of *M. cinnamomifolia, S. macranthera, S. pseudoquina and Z. tuberculosa* were studied and iv) to evaluate the use of rhizobox to study root traits.

MATERIAL AND METHODS

Species description

Tree seeds from *M. cinnamomifolia, S. macranthera, S. pseudoquina*, *and Z. tuberculosa,* native species of the Atlantic Forest, were collected in a forest fragment in the Frossard community in Divino – MG. we did not find about the root system of these species in literature.

M. cinnamomifolia (common name: Jacatirão-açu) height ranges from 15 to 22 m. It is a perennial, helophyte, and pioneer species with high seed production during the year and zoochoric (Lorenzi, 2008; Borgo et al., 2011). The species has potential for logging, as the wood can be used for construction (Schuch et al. 2008) and charcoal production (Brand et al. 2013).

S. macranthera (common name: Fedegoso) height ranges from 6 to 8 m. It is semi-deciduous or deciduous during the winter, helophyte, and pioneer species, with high seed production during the year, and barochoric (Lorenzi, 2008). It is a species commonly used in agroforestry systems in the region of Zona da Mata (Souza et al., 2010). Although the species does not have a rhizobium association, it can promote N fixation (Duarte et al. 2013). *S. macranthera* is one of the species studied by Duarte (2007) with the highest amount (dag kg^{-1}) of N (3.6), P (0.19), and K (1.23) in the fresh leaves.

S. pseudoquina (common name: Quina de São Paulo) height ranges from 4 to 7 m of shoot height. It is semi-deciduous, helophyte, selective xerophyte, and pioneer species with high seed production during the year and zoochoric (Lorenzi, 1998). This species has medicinal components in its bark that present antimalarial properties and that can be used against fever and (Cosenza et al., 2013). Moreover, one agroecological farmer from Zona da Mata reported that the cattle eat the tree bark of this species, indicating that it can also be medicinal for the cattle.

Z. tuberculosa (common name: Ipê tabaco) height ranges from 15 to 23 m. It is semi-deciduous, helophyte (adapted to full sun) and pioneer or early secondary (Silva & Soares, 2002; Jesus, 1997) with an intermediate seed production during the year and anemochoric (Lorenzi, 2008). It is also a species commonly used in agroforestry systems in the region of Zona da Mata (Souza et al., 2010). *Z. tuberculosa* is among the species studied by Duarte (2007) with the highest amount (dag kg^{-1}) of, Ca (0,50), Cu (20.35), and Zn (34.52) in the fresh leaves.

Rhizobox

The rhizobox consisted of a pine wood frame 30 cm high, 15 cm wide, and 2.5 cm thick. At one side, glass was installed to see the development of the roots. The glass was covered with aluminum foil to protect the roots against sunlight. In the back, a PVC was installed to maintain the substrate. The rhizobox was placed at an angle of 65° to allow the growth of the roots in contact with the glass (Fig. 1a).

The seeds of *S. macranthera* present dormancy (Lemos Filho et al., 1997), thus, before planting, the dormancy was broken with chemical scarification with sulfuric acid for 15 minutes, and all seeds were pre-germinated in a BOD germination chamber in the Forest Seed Analysis Laboratory at Federal University of Viçosa (UFV). After germinating, the seeds were transplanted to a rhizobox (Figura 1b). The rhizoboxes were placed in a greenhouse, located at the UFV (Fig. 1C), and covered with a shading screen.

Each rhizobox was filled with 700 ml (450 g) of the substrate. The substrate used was a mixture of 60% of a commercial substrate BIOMIX (*Mudas & Plantio Orgânico* ®), 35% of a B horizon of an Oxisol, 5% of an organic substrate produced by an agroecological farmer containing charcoal, chicken eggshell, and cow manure and 70 ml (77.13 g) of viable or no viable inoculum of arbuscular mycorrhizal fungus. We added inoculum in the substrate, to increase the possibility of root colonization.

The inoculum was a mixture of fungi species obtained from the Mycorrhizal Associations Laboratory of the Federal University of Viçosa, through the on-farm method (Moreira et al. 2019; Czerniak & Stürmer 2014). There was a mean of 3.7 fungus spores per ml of soil. To sterilize, for the control treatment, the inoculum was submitted to the autoclave process with 1.2 kgf/cm³ pressure, approximately 121ºC for one hour. After 48 hours the process was repeated. We did not sterilize the substrate, first because we did not expect to have colonization with the soil-born inoculum, second because we want to know if native species could colonize with or without the inoculation.

The rhizoboxes were daily irrigated with approximately 150 ml of distilled water, corresponding to the field capacity. One fertirrigation, 45 days after planting, was applied using half of the nutrient's concentration of Clark's nutritive solution (Clark, 1975).

The experiment was a factorial 4 x 2, in a completely randomized design with four tree species and two treatments with viable (inoculated) and not viable (non-inoculated) inoculum.

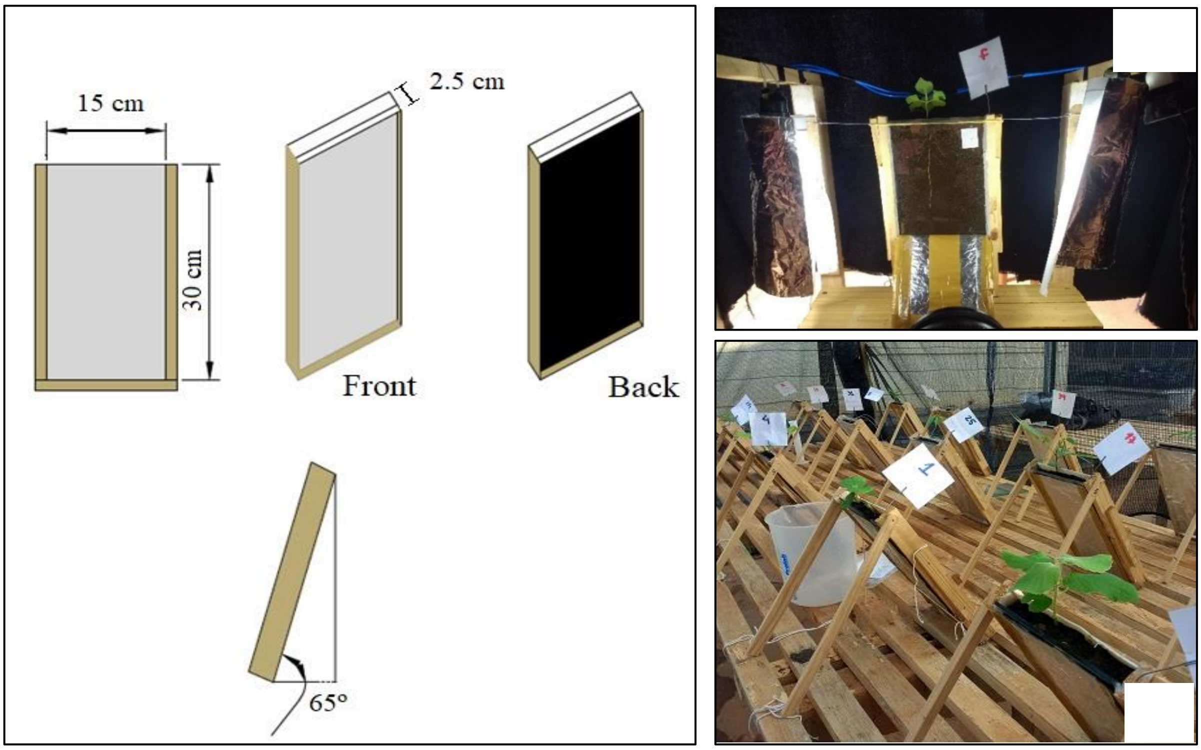

Figure 1 - Rhizobox structure (a); rhizobox with plant in the dark studio (b); rhizoboxes in the green house (c).

Root colonization of FMA (%) and Number of spores (n)

Roots of each sampling unit were cleared with 10% KOH approximately for 15 minutes or until they reach a clear color and stained with trypan blue (Phillips & Hayman, 1970) and stored in lactoglycerol to analyze the percentage of roots colonization. The AMF colonization was assessed according to Trouvelot et al. (1986) and expressed as a percentage of mycorrhization.

We collected 100 g of soil from each rhizobox to count the number of spores. Spores were extracted using the wet sieving method (Gerdemann & Nicholson, 1963), followed by centrifugation with water and 50% sucrose solution and post-wash in running water. The samplings were placed in piped petri dishes and the spores were counted.

Shoot analyzes

After 75 days of growing, almost all the tree´s roots reached the bottom of the rhizobox and the height of the seedling was measured. The height was measured using a ruler, from the soil surface to the highest leaf. The shoots were cut near the soil surface, dried in a forced air-drying oven at 60º C until constant weight to obtain the shoot biomass.

Roots analyses

After 75 days of seedling growth, the rhizobox glass cover was removed and pictures of the root systems were taken, with a professional camera, Canon EOS 550D. The photos were taken from approximately 0.5 m distance of the rhizobox, in a dark studio (Fig. 1b) to avoid reflections on the glass. The pictures were treated in photoshop and the length of the roots was measured in ImageJ (Schneider, 2012), with the SmartRoot plugin. After the photos, the experiment was dismantled, and the roots were washed and dried in a forced air-drying oven at 60º C until constant weight to obtain root biomass.

Root traits

Specific root length (cm g^{-1}), ratio of root length to soil volume (cm cm^{3-1}), and ratio of root biomass to shoot biomass (g) were calculated. Specific root length is the root length divided by the dry mass of the roots. The specific root length is a standard unit of acquisition (root length) to resource investment (mass) (Pérez-Harguindeguy 2013). The ratio of root length to soil volume (cm cm^{3-1}) indicates the intensity of soil exploration. The ratio of root biomass to shoot biomass (g) provides the investment of the species in root or shoot. The ratio of root biomass to shoot biomass (g) provides the investment of the species in root or shoot. Acquisitive species have higher specific root length and higher root length to soil volume, and conservative species have lower specific root length and lower root length to soil volume.

STATISTICAL ANALYSIS

Number of spores, root colonization, shoot and root parameters

The number of spores, root colonization, shoot height, shoot biomass, specific root length, root length: soil volume, and root biomass: shoot biomass was analyzed using ANOVA. Tukey test ($p < 0.05$) was used to compare means. For the statistical analysis, the number of spores was boxcox transformed, root colonization was arcsin ($\sqrt{x}/100$) transfomed, height values were log transformed, and specific root length was boxcox transformed, to improve the normality and homogeneity distribution of residuals.

Principal component analysis and ecological strategies

A principal component analysis (PCA) was performed for species, shoot, and roots parameters. We tested two models, one with the four species, and another without *M. cinnamomifolia*. The PCA is a multivariate analysis that reduce the dimensionality of the data to show the associations among variables. PCA was tested with and without *M. cinnamomifolia* because the species was skewing the results.

The analyses were run in R 3.6.1 (R Development Core Team, 2019).

RESULTS

Root colonization and number of spores

In the non-inoculated treatment, there was no root colonization in *Z. tuberculosa* and *M. cinammomifolia* and low root colonization in *S. pseudoquina* and *S. macranthera* (Table 1). Among inoculated, the root colonization differed within species, which *S. macranthera* showed the highest colonization, followed by *Z. tuberculosa*, and *S. pseudoquina* and *M. cinnamomifolia* had the smalles amount of root colonization (Table 1).

Within each species, the number of spores was similar in the inoculated and non-inoculated treatment. As the number of spores was similar, averages of inoculated and non-inoculated treatments were used for comparisons among species, and the average number of spores was greater in *S. pseudoquina* than in *Z. tuberculosa* (Table 2).

Table 1 - Root colonization on each tree species within inoculum treatment (inoculated and non-inoculated).

Species	Variable	Root colonization
	unit	%
Zeyheria tuberculosa	non-inoculated	0.00 (0.00) **Ba**
	inoculated	13.11 (9.64) **Ab**
Senna macranthera	non-inoculated	12.66 (10.14) **Ba**
	inoculated	79.56 (34.76) **Aa**
Solanum pseudoquina	non-inoculated	20.22 (25.51) **Aa**
	inoculated	1.33 (1.82) **Ab**
Miconia cinnamomifolia	non-inoculated	0.0 (0.00) **Aa**
	inoculated	1.11 (1.11) **Ab**

Capital letter compares the effect of inoculation within the same species and lower-case letters compares the species within each inoculation treatment. Mean follow by the same letter do not differ for t-test (>0.05).

Shoot

For the shoot height and shoot biomass, the was no difference between inoculated and non-inoculated treatments, so we averaged the results (Table 2). The species order of shoot height was *S. pseudoquina* > *S. macranthera* = *Z. tuberculosa* > *M. cinnamomifolia* (Table 1). The order of shoot biomass was *Z. tuberculosa* = *S. macranthera* > *S. pseudoquina* > *M. cinnamomifolia* (Table 2).

Root biomass and traits

Within species, the root biomass was similar between the inoculated and non-inculated treatments, so the average within each species was calculated (Table 2). The species order of root biomass was *Z. tuberculosa* = *S. macranthera* = *S. pseudoquina* > *M. cinnamomifolia* (Table 2).

Table 2 - Results of ANOVA and t-test of shoot and root measures on each tree species within inoculum treatment (inoculated and non-inoculated). The inoculation tratemnet did not show statistical differences (p>0,05).

Species	**Spores**	**Shoot heigth**	**Shoot dry mass**	**Root dry mass**	**Root biomass : Shoot biomass**
	n	cm	g	g	g
Zeyheria tuberculosa	37.20 (12.43) **b**	9.69 (1.23) **b**	1.63 (0.28) **a**	1.34 (0.26) **a**	0.82 (0.08) **b**
Senna macranthera	51.40 (10.16) **ab**	11.95 (2.76) **b**	1.57 (0.35) **a**	1.03 (0.36) **a**	0.64 (0.13) **bc**
Solanum pseudoquina	66.20 (33.54) **a**	17.50 (3.45) **a**	1.01 (0.28) 1.02 **b**	1.26 (0.36) **a**	1.29 (0.38) **a**
Miconia cinnamomifolia	65.00 (48.99) **ab**	5.49 (1.07) **c**	0.15 (0.06) **c**	0.07 (0.03) **b**	0.46 (0.11) **c**

The specific root length differed between inoculation treatment and among species (Table 3). The ratio was always higher in the non-inoculated treatment. The species order of specific root length for the non-incolulated and inoculated treatment was *M. cinnamomifolia* = *S. pseudoquina* > *S. macranthera* = *Z. tuberculosa* (Table 3).

The root length : soil volume differed between treatments, and among species. The ratio was always higher in the non-inoculated treatment. The species order for non-inoculted and inoculated treatment of root length : soil volume was *S. pseudoquina* > *S. macranthera* = *Z. tuberculosa* > *M. cinnamomifolia* (Table 3).

The ratio root biomass : shoot biomass was similar between the inoculated and non-inculated treatments, so the average within each species was calculated (Table 2). The species order of root biomass: shoot biomass was *S. pseudoquina* > *Z. tuberculosa* = *S. macranthera*= *M. cinnamomifolia.* (Table 2).

Table 3 - Results of ANOVA and t-test of and root measures on each tree species within inoculum treatment (inoculated and non-inoculated). The non-inoculated treatment showed higher means than inoculated treatment for all species (p>0,05).

	Variable	Specific root length	Root length : Soil volume
	unit	cm g-1	cm cm³-1
Zeyheria tuberculosa	non-inoculated	211.64 (41.40) **Aa**	0.43 (0.08) **Aa**
	inoculated	152.74 (41.16) **Ba**	0.27 (0.08) **Ba**
	average	182.19 (49.78) **b**	0.35 (0.12) **b**
Senna macranthera	non-inoculated	221.13 (34.15) **Aa**	0.28 (0.06) **Aa**
	inoculated	139.9 (69.94) **Ba**	0.19 (0.06) **Ba**
	average	180.51 (67.27) **b**	0.24 (0.07) **b**
Solanum pseudoquina	non-inoculated	463.77 (94.84) **Aa**	0.92 (0.16) **Aa**
	inoculated	503.88 (181.27) **Ba**	0.72 (0.16) **Ba**
	average	483.82 (138.01) **a**	0.82 (0.18) **a**
Miconia cinnamomifolia	non-inoculated	808.87 (239.13) **Aa**	0.07 (0.03) **Aa**
	inoculated	1317.27 (1691.46) **Ba**	0.11 (0.11) **Ba**
	average	1063.07 (1169.95) **a**	0.09 (0.08) **c**

Roots architecture

Z. tuberculosa, S. macranthera and *S. pseudoquina* (Figs. 2 A, B, and C) presented tap roots, i.e., the secondary roots derived from the main root; *M. cinnamomifolia* presented fibrous roots, i.e., there was no main root, and the entire root system is formed by several axes. The root system of *M. cinnamomifolia* and *S. pseudoquina* have highly branched roots with secondary roots presenting many fine roots equally distributed in the soil, including the soil surface.

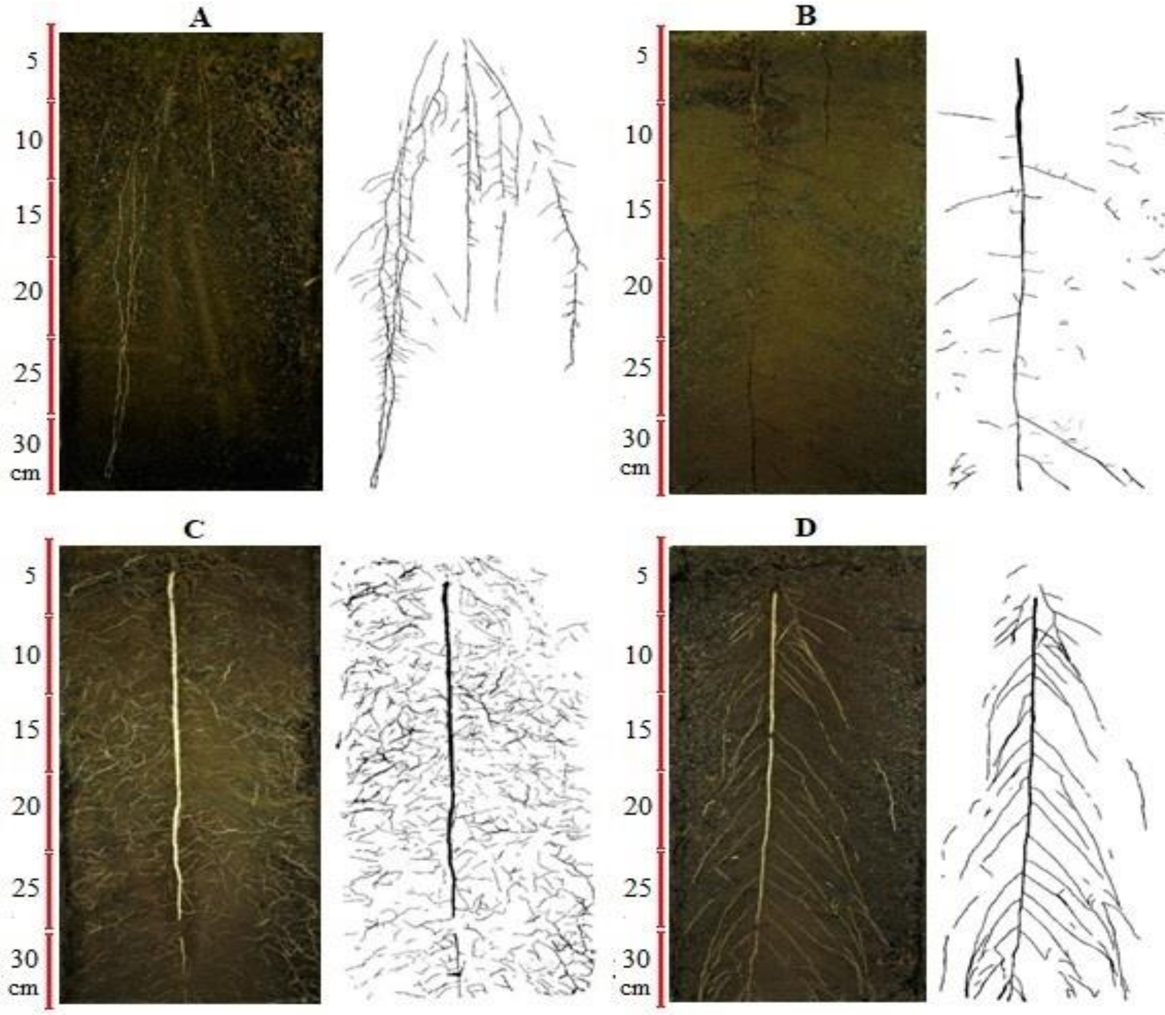

Figure 2 - Roots system, A - *Miconia cinnamomifolia;* B - *Senna macranthera;* C - *Solanum pseudoquina;* and D *Zeyheria tuberculosa.* The photos to the left show the root system in the rhizobox, and the photo to the right shows the root system treated with photoshop. The red line represents the scale (cm).

From the top to the botton of the rhizobox, the secondary roots of *M. cinnamomifolia, S. macranthera* and *Z. tuberculosa* were approximately 7 cm longer than *S. pseudoquina*. The secondary roots of *S. pseudoquina* were equally distributed in the soil in a larger amount than the other species.

Ecological strategies

The principal component analysis (PCA) with the four species (Fig. 3 A) showed that *M. cinnamomifolia* was positively associated only with specific root length. The result showed that *M. cinnamomifolia* was skewing the analyses, because its specific root length was negatively related to other acquisitive characteristics, such as shoot height and high root length : soil volume. Then, the *M. cinnamomifolia* was excluded from the analyses (Figure 3 B).

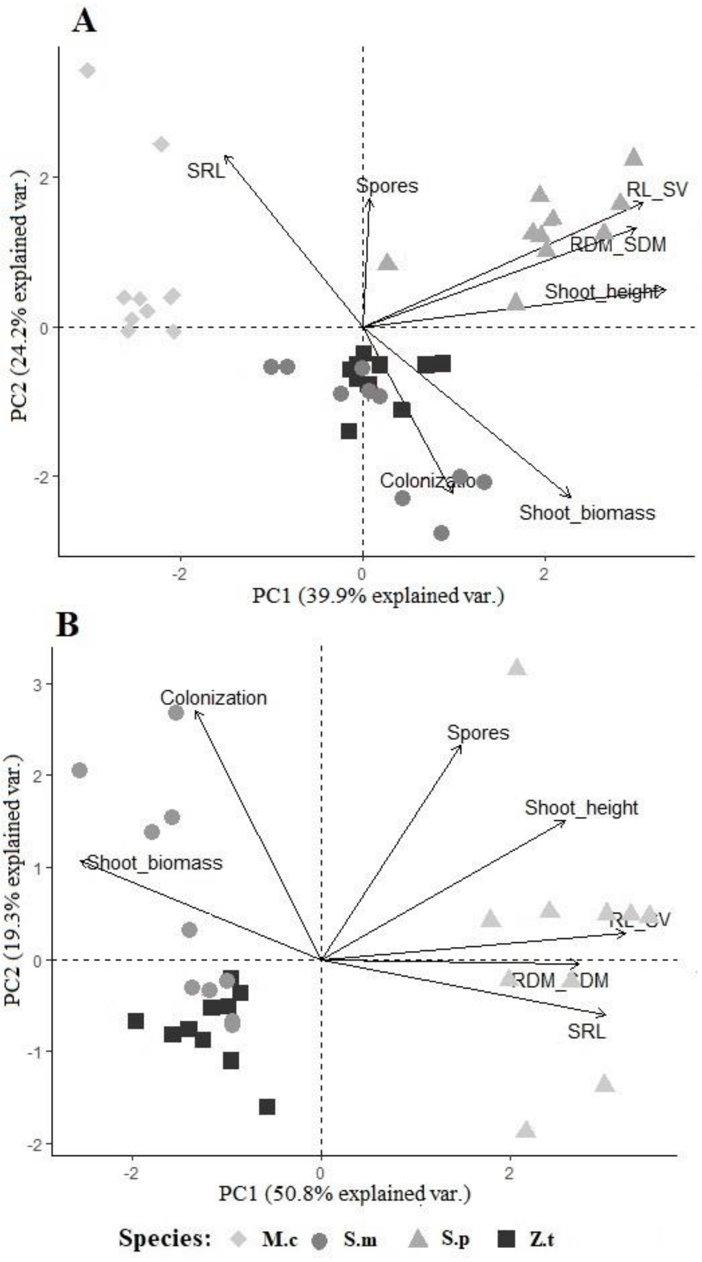

Figure 3 - Principal component analysis (PCA) of shoot and roots variables, and species. A = PCA with the four species; B = PCA without *M. cinnamomifolia*. Species: M.c = *Miconia cinnamomifolia*, S.m = *Senna macranthera*, S.p = *Solanum pseudoquina*, Z.t = *Zeyheria tuberculosa*. Colonization = Percentage of roots colonized by arbuscular mycorrhizas fungus (AMF); Spores = number of spores of AMF; RL_SV = ratio of root length : soil volume; RDM_SDM = ratio of roots dry mass : shoot dry mass; SRL = specific root length.

The PCA, without *M. cinnamomifolia* (Fig. 3 B), showed a clear pattern of acquisitive or conservative characteristics of the three species. *S. pseudoquina* was influenced positively by shoot height, as well as a high ratio on root length : soil volume, specific root length, and the ratio root biomass : shoot biomass, related to acquisitive characteristics, such as fast growth. The species invest more in roots than in shoot per soil volume than the other species, which means that the roots of these species explore more soil volume than the other three species during the time analyzed.

S. macranthera and *Z. tuberculosa* were negatively influenced by shoot height, high root length : soil volume, specific root length, and root biomass : shoot biomass and were positively influenced by shoot biomass, related to a more conservative strategy. *S. macranthera* was more influenced by root colonization than the other species. In both PCAs (Fig. 3 A and B) the colonization of the roots was inversely proportional to spores' number.

DISCUSSION

Number of spores and root colonization

The results of number of spores and root colonization confirmed that *S. macranthera* and *Z. tuberculosa* (Table 1) are species that have AMF association (Carneiro et al. 1998; Pagano & Scotti (2010)). In our study, we also found root colonization of *S. macranthera* after 75 days of of plant growth, different of Pouyú-Rojas & Siqueira (2000) who found root colonization of the species only after 90 days. Pagano & Scotti (2010) and Carneiro (1998) reported that *Z. tuberculosa* presented root colonization in greenhouse and nursery. However, as demonstrated by our results and according to Carneiro et al. (1998), the species has low root colonization. For both species, there was a difference between inoculation treatments, in the presence of inoculum the root colonization was higher, showing that both species had a positive response to non-native fungus.

No study has been found on mycorrhizal colonization of *S. pseudoquina*. The roots colonization of *S. pseudoquina* (20%) in the non-inoculated treatment indicated that the species is more sensitive to the native soil born inoculum than the mycorrhizal species present in the inoculum. In the presence of the inoculum, root colonization of *S. pseudoquina was low*, probably because the fungus species present in the inoculum inhibited the colonization by the soil born fungus.

We did not find information about the mycorrhization of *M. cinnamomifolia*. Despite the low percentage (Table 1) of root colonization, our study showed that *M. cinnamomifolia* can be associated with mycorrhizic fungi. Maybe, the short time (75 days) of the experiment was not enough to enable higher root colonization for this species. It is known that the genetic variability of the plants, the species of AMF, and the time (Klironomos, 2003), especially in perennial species (Abbott & Robson, 1981), influence the establishment of root colonization. Therefore, further studies are necessary to be sure if *M. cinnamomifolia* is associated with AMF.

The similarity in the number of spores among inoculated and non-inoculated treatments (Table 2) was expected. The spores are resistance structures, and the sterilization process (if used) does not destroy them, and the method used to count the number of spores did not distinguish viable from non-viable spores, i.e., part of the counted spores could no longer be viable (Smith & Read, 2010). Moreover, the sporulation process occurs when the AMF is under stress, i.e. drought, low nutrient availability, high light intensity (Smith & Read, 2010). The plants were not stressed in the rhizobox, and the environmental conditions such as water supply, nutrient availability, and light conditions were good for plant growth. Thus, the spores found probably were soil born.

M. cinnamomifolia and *S. pseudoquina* presented higher means of specific root length, indicating that these species have an acquisitive strategy. However, *S. pseudoquina* grew more than the other species (higher shoot height). Regardless of acquisitive strategy, each species has its specific ecological characteristic of development, such as higher growth rate and wood density was observed in *S. pseudoquina* than in *M. cinnamomifolia* (Lorenzi, 1998, 2008; Poorter and Bongers, 2006; Selaya and Anten, 2010; Westoby et al., 2002). Those characteristics indicated that *S. pseudoquina* can grow rapidly, investing less energy to build its structural tissue. Considering only this characteristic, species, such as *S. pseudoquina* can be a good option for agroforestry systems, as the specie can grow fast, providing shade faster, generating a balanced and stable microclimate (Beer et al., 1997; Lin, 2007). The small leaf size of *S. pseudoquina,* is a caracteristic especially good to intercrop the specie in pastures (Freitas et al., 2009).

The higher shoot biomass and the lower specific root length of *S. macranthera* and *Z. tuberculosa* indicate that these species present conservative strategies (Augspurger & Kelly, 1984; Coley, 1983; Reich et al., 2003), than the others. The higher shoot biomass ad the lower specific root length indicate that these species probably invest energy in storing carbon in their tissues, instead of growing quickly.

Traits of the roots

S. macranthera, S. pseudoquina, and *Z. tuberculosa* presented the same values of root biomass (Tale 1), however, they show different patterns of shoot development. While *S.*

pseudoquina invests more in shoot height, *S. macranthera* and *Z. Tuberculosa* invest more in shoot biomass, highlighting that *S. pseudoquina* has a more acquisitive strategy while *S. macranthera and Z. tuberculosa* have a more conservative strategy. *M. cinnamomifolia* presented the lower values of root biomass, shoot height, and shoot biomass, showing that the lower investment in root biomass affected the shoot height and biomass. *M. cinnamomifolia* and *S. pseudoquina* showed higher specific root length than *S. macranthera* and *Z. tuberculosa,* indicating a more acquisitive strategy. This parameter indicates the absorptive capability (root length) in relation to carbon storage (mass), that is, species with higher values of specific root length tend to have lower density of tissue and higher nutrient absorption capacity. Tree species with high nutrient absorption rates are desirable for in agroforestry systems because the root system of trees can uptake nutrients of deeper soil layers (Isaac & Borden 2019).

The species *S. pseudoquina* showed higher values of root length : soil volume and root biomass : shoot biomass than the other species(Table 1), indicating that it can explore a higher amount of soil volume than the others. However, the shallow and branchy, root system of *S. pseudoquina*, can increase the competition with coffee for water and nutrients. In pastures, the root system of grasses explores a larger volume of soil (dos Santos et al. 2007) and can support more competition for nutrients and water than coffee.

S. macranthera and *Z. tuberculosa* presented intermediate values of root length : soil volume and root biomass : shoot biomass (Table 1). These species are largely used in the agroforestry systems in Zona da Mata of Minas Gerais (Souza et al., 2010), and the values of the ratios in combination with roots architecture and root colonization values show that both species can have a good nutrient absorption, hence nutrient cycling, without harming the main crops. In contrast, *M. cinnamomifolia* presented the lowest values of root length : soil volume and root biomass : shoot biomass, that is, the species does not explore much soil volume, and at the same time it does not invest much in roots or in shoot compared with the other studied species. However, further studies with the species are needed.

Effect of AMF inoculation on seedlings development

The lack of influence of mycorrhizal colonization in the development of *S. macranthera*, *S. pseudoquina* and *Z. tuberculosa* (species that were colonized) indicated that the action of AMF external hyphae to increase the root surface area did not enhance absorption of nutrients, hence, plant growth (Sanders & Tinker, 1971). Although, the external hyphae explored more soil than the roots of *S. macranthera and Z. tuberculosa*, the development of the plants was not affected, probably due to the low volume of the soil explored by hyphae. Time used for the development of the seedling may also have influenced the results.

The larger specific root length and root length : soil volume in the non-inoculated treatment (Table 1) can be a result of an investment of *S. macranthera and Z. tuberculosa* in the fungi association instead of in the root growth, which lead to low formation of roots (Smith & Read 2010; Berta et al., 1990, 1991. The low investment in root does not mean less fitness of the species, because in field conditions, the lower specific root length and root length : soil volume can be compensated by a larger volume of soil explored by the mycorrhizal mycelium. The AMF external hyphae act to increase the root surface area, which enhances absorption of nutrients, hence, plant growth (Sanders & Tinker, 1971).

Although we found no effects of root traits on plant growth, there are other benefits, not measured in our study, that AMF colonization can provide to plants, for instance the uptake of poorly mobile nutrient ions, absorption of water, protection from soil toxins, and protection from soil pathogens (Fitter, 1991). In the long term and in field conditions, it is expected that these benefits can result in better growth of tree species.

Although *M. cinnamomifolia* root colonization in inoculated treatment was extremely low, the largest root length and root length : soil volume in the non-inoculated (Table 3) may be due to plant growth-promoting rhizobacteria present in the soil. The rhizobacteria present in inoculum may have competed with rhizobacteria of the soil, delaying the development of the roots, while in the non-inoculated treatment there was no competition and the rhizobacteria of the soil provided a better development. As the inoculum was prepared using on-farm method, there could be other microorganisms, as growth-promoting rhizobacteria. These microorganisms can colonize the surface of the root system and stimulate root growth (Barea et al., 2005; El Zemrany et al., 2007; Vacheron et al., 2013; Barazani & Friedman, 2000).

Roots architecture

As pointed out by other authors (Courtois, 2013; Nguyen & Stangoulis, 2019), the rhizobox was a good non-destructive method to analyze roots architecture, using two-dimensional (2D) high-resolution photo and image processing software.

The analysis of root architecture is important to select species to be intercropped in agroforestry systems since it influences the capability of trees to access the pool of nutrients

(Maeght et al. 2013). The roots architecture which can influence the crop, as well as the ecological functioning of the system. Our results showed that species have different characteristics of root architecture. *S. macranthera*, and *Z. tuberculosa* have the most appropriate tap root architecture to be intercropped in agroforestry coffee systems. The secondary roots of these species were not too branchy and grow downwards, thus exploring a soil layer not reached by most of the fine roots of coffee, which can occupy the first 20 cm of the soil depth (Carducci et al., 2014). These species are frequently found in agroforestry systems in Zona da Mata of Minas Gerais (Souza et al., 2010).

The fibrous root of *M. cinnamomifolia* are also present in agroforestry systems (Silva, et al. unpublished; Moreira & Carvalho, 2018; Steenbock et al., 2013), however, it is necessary to be careful in intercropping these species. These species can have better use in agroforestry pasture systems because the grass root system explores a larger layer of soil (dos Santos et al., 2007) and can support more competition for nutrients and water than coffee. However, to be used in agroforestry pasture systems, *S. pseudoquina* must be protected because the cattle can eat its' bark.

Ecological strategies

M. cinnamomifolia showed both acquisitive (large specific root length) and conservative (small shoot height) strategies. Moreover, the negative relationship betweenspecific root length and shoot height of *M. cinnamomifolia* showed that specific root length of *M. cinnamomifolia* is independent of the other acquisitive characteristics, as shoot height (Kramer-Walter et al., 2016). Kramer-Walter (2016) showed that it is not clear that specific root length coordinates with other acquisitive characteristics, such as fast growth.

When excluding *M. cinnamomifolia* from the analysis the positive relationship among specific root length, root length : soil volume and shoot height showed that *S. pseudoquina* is more acquisitive and *S. macranthera* and *Z. tuberculosa* are more conservative (Fig. 3 B). Therefore, increasing specific root length, root length: soil volume, and root biomass : shoot biomass is positively assoictaed with shoot height, but not necessarily with shoot biomass (carbon investment), because conservative species present lower specific leaf area (area of a leaf, divided by its oven-dry mass) (Pérez-Harguindeguy, 2013).

Shoot biomass indicates the capacity of the species to allocate carbon in the leaves. Species that allocate more carbon in their tissues show a conservative strategy comparing to other species that construct their tissues with a low amount of carbon (acquisitive strategy) (Kramer-Walter et al. 2016). Thus, based on shoot height and shoot biomass (Table 1), we can also indicate that *S. pseudoquina* is more acquisitive and *S. macranthera* and *Z. tuberculosa* are more conservative species.

Root architecture is also important to determine the reproductive strategy of the species (acquisitive or conservative). Besides greater specific root length, acquisitive species have greater branching and root tips than the more conservative species (Comas & Eissenstat, 2004; Kramer-Walter et al., 2016). Thereby, based on root architecture, *S. pseudoquina* (more branching and root tip) can also be considered an acqusitive species, and *S. macranthera* and *Z. tuberculosa* (less branching and root tips) can also be considered conservative species.

CONCLUSION

S. macranthera and *Z. tuberculosa* can be considered species with more conservative strategies because they have lower specific root length and root length : soil volume, higher shoot biomass, and higher mycorrhizal root colonization. *S. pseudoquina* has a more acquisitive strategy because it has higher shoot height, specific root length and root length : soil volume, and low shoot biomass. Further studies are necessary to indicate the ecological strategy of *M. cinnamomifolia,* as our results showed that the species has characteristics that can be linked to both acquisitive and conservative strategies.

S. macranthera, *Z. tuberculosa* and *S. pseudoquina* are colonized by arbuscular mycorrhiza, but *S. pseudoquina* are more sensitive to the native fungus. The low root colonization in *M. cinnamomifolia* indicates the need of further studies to confirm whether the species is associated with AMF.

Based on root traits and shoot characteristics, *S. macranthera* and *Z. tuberculosa* can be intercropped with agroforestry coffee systems, without great concerns about water and nutrients competition. Moreover, these species showed higher biomass production than *M. cinnamomifolia* and *S. pseudoquina* that can improve soil quality, and are mycorrizal, which can also benefit coffee crops. The root traits indicated that *M. cinnamomifolia* and *S. pseudoquina* can compete for water and nutrient with plants with a more superficial root system, such as coffee. Therefore, they are more recommended for agroforestry pasture systems. If intercropped with coffee, it is better to use these species in low density. However, our study was performed in a greenhouse, thus it is necessary to carry out field studies with *S.*

pseudoquina, but also with *M. cinnamomifolia* to evaluate whether these species can be intercropped in agroforestry systems. The consortium in agroforestry systems of these two species are less studied than *S. macranthera* and Z. *tuberculosa*.

The rhizobox was a good non-destructive method to analyze root architecture.

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CONSIDERAÇÕES FINAIS

Os Sistemas Agroflorestais (SAFs) biodiversos apresentam-se como alternativa às matrizes agrícolas em monocultivo, pois podem funcionar como fonte de sementes para regenerar a paisagem, prover alimento e habitat para vida selvagem, melhorar o microclima e, ao mesmo tempo, produzir alimentos e gerar renda para as famílias agricultoras. Uma matriz biodiversa como nos SAFs imita os ecossistemas naturais e pode contribuir para conectar fragmentos e promover a conservação de florestas. Entretanto, os agricultores utilizam espécies em seus sistemas se suas funções foram conhecidas. Os agricultores possuem muitos conhecimentos sobre as funções das árvores já que trabalham diariamente com a natureza e dependem da biodiversidade para sobreviver. Este conhecimento é fundamental para a implementação de sistemas de acordo com as características ambientais e sociais locais.

Para desempenhar as funções, as espécies arbóreas consorciadas interagem ecologicamente abaixo e acima do solo. O sistema radicular das árvores impacta as interações entre as plantas e as funções ecossistêmicas, sendo a arquitetura radicular e as interações com os microrganimos, a exemplo dos fungos micorrízicos arbusculares (FMA), são características importantes das espécies arbóreas por influenciar a capacidade da planta de acessar água e nutrientes.

O objetivo geral deste projeto foi avaliar a diversidade e uso de espécies arbóreas presente em sistemas agroflorestais da Zona da Mata mineira e analisar as características funcionais de raiz de quatro espécies de árvores. Inventários florestais e agroflorestais foram desenvolvidos e o conhecimento etnobotânico das espécies arbóreas utilizadas em sistemas agroflorestais foram identificados. *Senna macranthera* (nome comum: Fedegoso), *Solanum pseudoquina* (Quina-de-São Paulo) e *Zeyheria tuberculosa* (nome comum: Ipê Felpudo) estão entre as espécies encontradas em áreas florestais, cafezais e pastagens e *Miconia cinnamomifolia* (nome comum: Jacatirão) foram encontradas somente nas áreas florestais. Estas quatro espécies foram selecionadas para estudos mais aprofundados sobre o sistema radicular.

O conhecimento dos/as agricultores/as sobre as funções das espécies levou os sistemas agroflorestais a abrigar elevada diversidade de espécies arbóreas nativas da Floresta Atlântica. Houve uma relação linear entre as funções atribuídas pelos agricultores e a diversidade de árvores nos sistemas, portanto o conhecimento dos agricultores sobre as funções das espécies contribui para a manutenção das mesmas nos sistemas agroflorestas. Portanto, espera-se que o maior conhecimento das funções das árvores e por mais pessoas irá aumentar o número de espécies presentes em agroecossistemas, com os sistemas agroflorestais, o que, por sua vez, contribui para a conservação das espécies.

Com base no estudo das características funcionais de raiz e estratégia de crescimento entre as espécies estudadas, *S. macranthera* e *Z. tuberculosa* apresentaram sistema radicular com raízes secundárias que se desenvolvem em profundidades maiores. Estas espécies, entre as quatro estudadas, apresentaram os maiores valores de biomassa da parte aérea, o que indica serem espécies que possuem estratégias ecológicas mais conservadoras. Portanto, como apontado pelas famílias agricultores, essas espécies apresentam características ecológicas que são compatíveis com o cafezal, visto que as raízes não irão competir diretamente com o café por água e nutrientes.

Apesar da *S. pseudoquina* ter sido encontrada nas três áreas, o estudo das características funcionais mostrou que a espécie apresenta estratégia ecológica mais aquisitiva, com maior altura e sistema radicular altamente ramificado, inclusive nas camadas superficiais do solo, ou seja, esta espécie compete diretamente com café por água e nutrientes, mas não necessariamente com forrageiras. A espécies possui rápido desenvolvimento da parte aérea, uma característica importante para gerar um rápido sombreamento, e embora em pastagens pode gerar rapidamente maior conforto animal tem como inconveniente o fato de que o gado se alimenta de suas cascas. Com isto, *S. pseudoquina* parece ser mais compatível com pastagens do que com o café, porém ao consorciá-la com pastagens deve-se protegê-las para que o gado não mate as árvores ao alimentarem de sua casca. As características funcionais que *M. cinnamomifolia* apresentou (a exemplo de elevado comprimento específico de raiz e raiz fasciculada) indicam a espécie pode ser consorciada com pastagens, mas mais estudos precisam ser realizados para analisar sua compatibilidade ou não com o café.

O etnoconhecimento permitiu identificar as funções de espécies arbóreas que podem ser consorciadas com sistemas agroflorestais e os estudos acadêmicos permitiram o entendimento de estratégias ecológicas de cada espécie. A articulação entre o etnoconhecimento com estudo de características ecológicas das espécies contribuiem nos processos decisórios de consorciação ou não das espécies com SAFs. Portanto, precisa-se reconhecer a importância do conhecimento dos agricultores e promover ambientes de socialização deste conhecimento com outros agricultores, cientistas, técnicos e demais interessados. Precisa-se ainda pomover estudos científicos sobre as espécies para que aumente o conhecimento sobre suas funções e sobre saus características de forma a selecionar aquelas que podem ser consorciadas sem grandes prejuízos à produtividade da cultura principal.

Appendix 1 – Socioeconomic semi-structured interview

FICHA DE CADASTRO DOS ENTREVISTADOS

Data: ____/____/____

Nome do/a informante: ____

Comunidade: ____

CARACTERIZAÇÃO SOCIOECONÔMICA DOS ENTREVISTADOS

Naturalidade:____

Idade:_________; escolaridade: _________________________________

Sempre residiu na zona rural? Sim ( ) Não ( )

Em caso negativo, quanto tempo? _____________________________________

Há quanto tempo reside na comunidade/ propriedade? Terra própria

____

Dados sobre os outros residentes no domicílio

Nome	Parentesco	Idade	Sexo	Escolaridade	Ocupação

Supplementary material

Table S1 - Tree families and species names identified in coffee systems, pastures, and forest areas with the uses of species in the coffee and pasture, present in Frossard community, rural area of Divino, Minas Gerais, Atlantic Forest, Brazil. AF: Animal feed (domestic and farm animal), SA: Shade for animal, AE: Aesthetics, FA: Fauna attraction, FI: Firewood, ME: Medicinal, FP: Food production, IA: Insect attraction, NA: Nature preservation, SCR: Shade for crop, SP: Shade for people, SC: Soil cover, ST: Stake, TE: Technology, WI: Windbreak, WO: Wood. Technology includes building roof tiles from tree trunk, building brooms, and other small buildings for daily useful.

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
Anacardiaceae							
	Anacardium giganteum Hancock ex Engler	Cajueiro-do-mato	x			-	exotic
	Anacardium occidentale L.	Cajueiro		x		-	exotic
	Astronium graveolens Jacq.	Gonçalo-alves/Guaritá	x	x		-	native
	Mangifera indica L.	Mangueira	x	x		ME, FP, SC	exotic
	Spondias dulcis Parkinson	Cajá-manga	x			FP	exotic
Annonaceae							
	Annona dolabripetala Raddi	Pinha/Araticum			x	-	native
	Annona muricata L.	Graviola	x			FP	exotic
	Annona sp	Pinha	x			-	-
	Annona sp. 1	Pinha			x	-	-
	Annona squamosa L	Fruta do conde	x			FP, FA	exotic
	Annona sylvatica A.St.-Hil.	Pinha/Araticum		x	x	ME	native
	Guatteria sellowiana Schltdl.	Embira/Pindaiba-negra			x	-	native
	Guatteria sp.1	-			x	-	native
	Rollinia mucosa (Jacq.) Baill.	Biribá	x			FP	native
	Xylopia sp.1	-			x	-	native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
Apocynaceae							
	Aspidosperma pyricollum Müll.Arg.	Peroba-vermelha			x	-	native
	Himatanthus sp.1	-			x	-	native
	Malouetia sp	-	x			-	native
	Tabernaemontana fuchsiaefolia A. DC.	Leiteira	x	x		TE, WO	native
Aquifoliaceae							
	Ilex brevicuspis Reissek	Caúna-da-serra			x	-	native
Arecaceae							
	Archontophoenix cunninghamiana H Wendl & Drude	Palmeira real	x			AE	exotic
	Arecaceae	-	x			-	-
	Cocos nucifera L	Coqueiro	x			-	exotic
	Euterpe edulis Mart.	Juçara	x		x	AE	native
	Syagrus romanzoffiana (Cham.) Glassman	Jerivá/Coquim-babão	x	x		FA, FP, NA	native
Asteraceae							
	Baccharis dracunculifolia DC.	Alecrim-do-campo		x		TE	native
	Baccharis sp.1	-			x	-	native
	Gochnatia polymorpha (Less.) Cabrera	Cambará-guaçu	x	x		WI	native
	Piptocarpa sp. 1	-			x	-	native
	Piptocarpha macropoda (DC.) Baker.	Piptocarpa			x	-	native
	Vernonia sp.	-			x	-	native
	Vernonia polysphaera Baker.	Assa-peixe	x	x		ME, SCR, SC	native
Bignoniaceae							
	Handroanthus cf. albus (Cham.) Mattos				x	-	Native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Handroanthus chrysotrichus (Mart. ex DC.) Mattos	Ipê-amarelo	x	x	x	AE, FA, FI, SCR, SC, ST, WO	native
	Handroanthus ochraceus (Cham.) Mattos	Ipê-amarelo	x	x		AE, FA, SCR, WO	exotic
	Handroanthus roseo albus (Cham.) Mattos	Ipê-branco		x		AE, ME	exotic
	Handroanthus serratifolius (Vahl.) S. O. Grose	Ipê-amarelo	x	x		FA, FI, SCR, SP, WO	native
	Handroanthus sp.	Ipê	x			-	-
	Jacaranda macrantha Cham.	Caroba			x	-	native
	Sparattosperma leucanthum (Vell.) K. Schum.	Ipê-branco	x	x	x	ME, NA, ST, WO	native
	Zeyheria tuberculosa (Vell.) Bureau ex Verl.	Ipê-felpudo	x	x	x	AE, FI, SCR, ST, WI, WO	native
Boraginaceae							
	Cordia silvestris Fresen.	Louro-branco			x		native
	Cordia trichotoma (Vell.) Arráb. ex Steud.	Louro-pardo	x	x	x	FA, FI, SCR, SC, ST, WI, WO	native
Burseraceae							
	Crepidospermum atlanticum Daly	-			x	-	native
	Crepidospermum sp.1	-			x	-	native
	Crepidospermum sp.2	-			x	-	native
Cactaceae							
	Cactaceae	-		x		-	exotic
Cannabaceae							
	Trema micrantha (L.) Blume	Crindiúva	x	x	x	AF, FI, SCR, WI	native
Cardiopteridaceae							
	Citronella paniculata (Mart.) R.A.Howard	Falsa-congonheira			x	-	native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
Caricaceae							
	Carica papaya L.	Mamão	x			AF, FA, FP	exotic
	Jacaratia spinosa (Aubli) A. DC.	Jacaratiá			x	-	native
Celastraceae							
	Maytenus ilicifolia Mart. ex Reissek	Cafezinho			x	-	native
	Maytenus robusta Reissek	Cafezinho			x	-	native
	Maytenus sp.1	Cafezinho			x	-	native
	Monteverdia floribunda (Reissek) Biral.	Cafezinho			x	-	native
Clusiaceae							
	Tovomitopsis saldanhae Engl.	-			x	-	native
Cunoniaceae							
	Lamanonia ternata Vell.	Guaraperê			x	-	native
Elaeocarpaceae							
	Sloanea garckeana K. Schum.	Urucurana			x	-	native
Euphorbiaceae							
	Alchornea glandulosa Poepp.	Tanheiro			x	-	native
	Alchornea sidifolia Müll.Arg.	Taipá-guaçu			x	-	native
	Alchornea triplinervia (Spreng.) Müll.Arg.	Taipá-vermelho			x	-	native
	Croton floribundus Spreng.	Capixingui			x	-	native
	Sapium glandulosum (L.) Morong	Pau-de-leite			x	-	native
	Tetrorchidium rubrivenium Poepp.	Canemaçu			x	-	native
Fabaceae Caesalpinoideae							
	Apuleia leiocarpa (Vogel) J.F. Macbr.	Gurapiá	x	x	x	SCR, ST, WO	native
	Bauhinia forficata Link.	Unha-de-vaca		x	x	-	native
	Cassia ferruginea (Schrad) Schrad ex. DC.	Cana-fístula		x			native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Schizolobium parahybae Vell.	Guapuruvu	x			AE, WO	native
	Sclerolobium sp.	-	x			-	native
	Senna macranthera (Collad.)H.S.Irwin & Barneby	Fedegoso	x	x	x	AF, FA, FI	native
	Senna multijuga (Rich.) H.S.Irwin & Barneby	Pau-cigarra	x		x	FI, SC	native
Fabaceae Faboideae							
	Dalbergia nigra (Vell.) Benth.	Jacaranda-da-bahia	x	x	x	AE, FI	native
	Erythrina falcata Benth.	Mulungu			x	-	native
	Erythrina verna Vell.	Mulungu		x		ST, TE	native
	Platypodium elegans Vogel.	Jacaranda-do-campo	x	x	x	AE, FI, SCR, SC, WO	native
Fabaceae Mimosoideae							
	Albizia polycephala (Benth) Killip ex Record	Farinha-seca	x	x		WO	native
	Albizia sp.1	-			x	-	native
	Anadenanthera macrocarpa (Benth) Brenan	Angico-vermelho		x		-	exotic
	Anadenanthera peregrina (L.) Speg. var. peregrina	Angico-do-morro	x	x		FA, FI, NA, ST, WO	exotic
	Inga cylindrica (Vell.) Mart.	Ingá			x	-	native
	Inga edulis Mart.	Ingá-de-metro	x	x		FA, FI, ME, FP, SCR, SC	native
	Inga laurina (Sw.) Willd.	Ingá-branco	x				native
	Inga subnuda Salzm. ex Benth.	Ingá			x	-	Native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Parapiptadenia zehntneri (Harms) M.P. Lima & H.C. Lima	Angico-gurucaia			x	-	native
	Piptadenia gonoacantha (Mart) J. F. Macbr.	Pau-jacaré	x	x	x	FI, SCR, ST	native
	Piptadenia paniculata Benth.	Angico-de-espinhos			x	-	native
	Senegalia polyphylla (DC.) Britton & Rose	Monjoleiro			x	-	native
	Senegalia sp.1	-			x	-	-
	Fabaceae sp.	-	x			-	native
	Fabaceae sp.1	-	x			-	native
	Fabaceae sp.2	-	x			-	native
Fabaceae Papilonoideae							
	Ateleia glazioviana Baill.	Timbó			x	-	native
	Machaerium aculeatum Raddi.	Pau-de-angu	x	x		SCR	native
	Machaerium isadelphum (E.Mey.)Standl.	Jacaranda-bico-de-pato			x	-	native
	Machaerium nyctitans (Vell.) Benth.	Jacaranda-bico-de-pato	x	x	x		native
	Machaerium sp.	-		x			native
	Machaerium stipitatum (DC.) Vogel	Sapuva	x	x	x		native
	Machaerium villosum Vogel	Jacarandá-paulista	x	x			native
	Peltophorum dubium (Spreng.) Taub.	Canafístula		x			native
	Platycyamus regnellii Benth.	Pau-pereira			x	-	native
	Platymiscium floribundum Vogel.	Jacarandá-do-litoral			x	-	native
	Platymiscium pubescens Micheli.	Jacarandá	x	x	x		native
	Pterocarpus rohrii Vahl.	Aldrágo-miúdo			x	-	native
	Swartzia oblata Cowan	Saco-de-bode	x	x			native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Zollernia ilicifolia (Brongn.) Vogel	Pau-santo			x	-	native
Lamiaceae							
	Aegiphila sellowiana Cham.	Papagaio	x	x	x	AF, SA, FA, FI, SC, WI	native
	Vitex montevidensis Cham.	Tarumã	x				native
	Vitex polygama Cham.	Tarumã-do-cerrado	x			FA, FI, FP, SCR, SC	native
Lauraceae							
	Aiouea acarodomatifera Kosterm.	Canela			x	-	native
	Aiouea cf. saligna Meisn.	Canela			x	-	native
	Nectandra grandiflora Nees & Mart.	Canela-sebo			x	-	native
	Nectandra lanceolata Nees & Mart.	Canela-amarela			x	-	native
	Nectandra membranacea (Sw.) Griseb.	Canela-embuia	x		x	SCR, SP	native
	Nectandra oppositifolia Nees. & Mart.	Canela-amarela	x		x	AF, FI, SP, SC, WO	native
	Nectandra sp	Canela	x			-	native
	Nectandra sp. 1	Canela			x	-	native
	Ocotea sp.1	Canela			x	-	native
	Persea americana Mill.	Abacateiro	x			FA, ME, FP, TE, WI	exotic
	Persea major (Meisn.) L.E.Kopp	Abacateiro-do-mato			x	-	native
Lecythidaceae							
	Cariniana estrellensis (Raddi) Kuntze	Jequitibá-branco			x	-	native
	Lecythidaceae	-		x		-	native
Lythraceae							
	Lythraceae	-			x	-	-

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
Malvaceae							
	Bombacopsis glabra (Pasq.) A. Robyns	Castanha-do-maranhão	x			FP	native
	Ceiba speciosa (A.St.-Hil.) Ravenna	Painera			x	-	native
	Luehea candicans Mart.	Açoita-cavalo			x	-	native
	Luehea divaricata Mart. & Zucc.	Açoita-cavalo			x	-	native
	Luehea grandiflora Mart. & Zucc.	Açoita-cavalo	x		x	FI, ME, SCR, SC, ST	native
	Pseudobombax grandiflorum (Cav.) A. Robyns	Embiruçu		x	x	-	native
Melastomataceae							
	Miconia cinnamomifolia (DC.) Naudin	Jacatirão-açu			x	-	native
	Miconia latecrenata (DC.) Naudin	Pixirica			x	-	native
	Miconia paniculata (Mart. & Schrank ex DC.) Naudin	Miconia			x	-	native
	Tibouchina estrellensis (Raddi) Cogn.	Quaresmeira			x		native
Meliaceae							
	Cabralea canjerana (Vell.) Mart.	Canjerana			x	-	native
	Cedrela fissilis Vell.	Cedro			x	-	native
	Guarea guidonia (L.) Sleumer	Cedro-branco			x	-	native
	Toona ciliata var. australis	Cedro-australiano	x			SC, WO	exotic
	Trichilia elegans A. Juss.	Catiguazinho			x	-	native
	Trichilia lepidota Mart.	Baga-de-morcego			x	-	native
	Trichilia pallida Sw.	Baga-de-morcego	x	x		FA, SCR, SP, SC	native
	Trichilia sp.1	-			x		native
Monimiaceae							
	Mollinedia schottiana (Spreng.) Perkins	Capixim			x	-	native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Mollinedia sp.1	-			x	-	native
	Mollinedia widgrenii A.DC.	Capixim			x		native
Moraceae							
	Artocarpus heterophyllus Lam.	Jaqueira	x			-	exotic
	Brosimum guianense (Aubl.) Huber ex. Ducke	Leiteira-vermelha			x	-	native
	Ficus enormis (Miq.) Miq.	Figueira-de-pedra			x	-	native
	Ficus obtusiuscula (Miq.) Miq.	Figueira			x	-	native
	Maclura tinctoria (L.) D. Don ex Steud.	Taiúva			x	-	native
	Sorocea bonplandii (Baill.) W.C.Burger, Lanj. & de Boer	Araçari			x	-	native
Myristicaceae							
	Virola bicuhyba (Schott) Warb.	Virola			x	-	native
	Virola gardneri (A.DC.) Warb.	Virola			x	-	native
Myrsinaceae							
	Myrsine coriacea (Sw.) R.Br. ex Roem. & Schult.	Pororoca-branca			x	-	native
Myrtaceae							
	Campomanesia guaviroba (DC.) Kiaersk.	Guaviroba			x	-	native
	Campomanesia guazumifolia (Cambess.) O. Berg	Sete-capotes			x	-	native
	Campomanesia sp.1	-			x	-	native
	Eucalyptus sp	Eucalipto	x			ME, WO	exotic
	Eugenia oblongata O. Berg	Guamirim-rugoso			x	-	native
	Eugenia pisiformis Cambess.	Uvaia-vermelha			x	-	native
	Eugenia sp	-	x			-	native

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Eugenia sp.1	-			x	-	native
	Eugenia sp.2	-			x	-	native
	Eugenia subterminalis DC.	Cambuí-pitanga			x	-	native
	Myrcia sp. 1	-			x	-	native
	Myrcia sp. 2	-			x	-	native
	Myrcia splendens (Sw.) DC.	Guamirim-miúdo			x	-	native
	Myrciaria cauliflora (Mart.)	Jabuticaba-paulista	x			FP	native
	Myrtaceae	-	x			-	native
	Psidium cattleyanum L.	Araçá	x			-	native
	Psidium guavaja L.	Goiabeira	x	x		FI, ME, FP	native
	Psidium sp.1	-			x	-	native
	Syzygium cumini L.	Jamelão	x			FA, FP	exotic
Nyctaginaceae							
	Guapira opposita (Vell.) Reitz	Flor-de-pérola			x	-	native
Phyllanthaceae							
	Hyeronima alchorneoides Allemão	Urucurana			x	-	native
Phytolaccaceae							
	Gallesia integrifolia (Spreng.) Harms.	Pau-d'alho	x			IA	native
	Seguieria langsdorffii Moq.	Limão-bravo	x	x	x	-	native
Proteaceae							
	Roupala montana Aubl.	Carvalho-do-cerrado			x	-	native
Rhamnaceae							
	Hovenia dulcis Thunb.	Uva-do-japão	x			-	exotic
Rosaceae							
	Eriobotrya japonica (Thunb.) Lindl.	Amexinha	x			FA, ME, FP	exotic
	Prunus myrtifolia (L.) Urb.	Pessegueiro-bravo			x		native
Rubiaceae							

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Bathysa australis (A.St.-Hil.) K.Schum.	Fumão-doce			x	-	native
	Myrcia fenzliana O. Berg.	Ameixa-do-mato			x	-	native
	Psychotria carthagenensis Jacq.	Juruvarana			x	-	native
	Psychotria sp.1	-			x	-	native
	Psychotria vellosiana Benth.	Jasmin-verdadeiro			x	-	native
	Simira sampaioana (Standl.) Steyerm.	Araribá			x		native
Rutaceae							
	Citrus bigaradia L.	Limão-cravo	x			FI, ME, FP, SC, TE	exotic
	Citrus sinensis L.	Laranjeira	x			FI, ME, FP, SC	exotic
	Zanthoxylum fagara (L.) Sarg.	Mamica			x	-	native
	Zanthoxylum rhoifolium Lam.	Mamica-de-cadela	x	x		TE	native
	Zanthoxylum riedelianum Engl.	Mamica-de-porca		x	x	-	native
Salicaceae							
	Banara serrata (Vell.) Warb.	Tirivaiba			x	-	native
	Casearia decandra Jacq.	Pitumba			x	-	native
	Casearia gossypiosperma Briq.	Espeteiro			x	-	native
	Casearia guianensis (Aubl.) Urb	Fruta-de-saíra			x	-	native
	Casearia sylvestris Sw.	Guaçatonga	x	x	x	-	native
Sapindaceae							
	Allophylus edulis (A.St.-Hil., A.Juss. & Cambess.) Radlk.	Chal-chal			x	-	native
	Allophylus laevigatus (Turcz.) Radlk.	Caboatã-de-rego			x	-	native
	Allophylus racemosus Sw.	Fruta-de-pomba			x	-	native
	Cupania furfuracea Radlk.	Cubatã-folha-larga			x	-	native
	Cupania vernalis Cambess.	Arco-de-peneira			x	-	native
	Litchi chinensis Sonn	Lixia	x			FI	exotic
Sapotaceae							

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Pouteria gardneri (Mart. & Eichler ex Miq.) Baehni.	Maçaranduba-vermelha			x	-	native
	Pouteria macrophylla (Lam.) Eyma	Sapota-verde			x	-	exotic
	Pouteria sp.1	-			x		native
Siparunaceae							
	Siparuna brasiliensis (Spreng.) A. DC.	Limoeiro-bravo			x	-	native
	Siparuna guianensis Aubl.	Catinga-d'anta			x		native
Solanaceae							
	Aureliana fasciculata (Vell.) Sendtn.	Coerana			x	-	native
	Cestrum sp.1	-			x	-	native
	Solanum leucodendron Sendtn.	Jurubeba-branca			x	-	native
	Solanum mauritianum Scop.	Capoeira-branca	x	x	x	AF, SCR, SC, ST	native
	Solanum melianthum	-			x	-	native
	Solanum paniculatum L.	Jurubeba		x		FP	native
	Solanum pseudoquina A. St-Hill.	Quina-de-são paulo	x	x	x	AF, SCR	native
	Solanum rufescens Sendtn.	-			x	-	native
	Solanum sp.	-		x		-	native
Styracaceae							
	Styrax sp.1	-			x		native
Symplocaceae							
	Symplocos pubescens Klotzsch ex. Benth.	Sete-sangrias			x		native
Urticaceae							
	Cecropia glaziovii Snethl.	Embaúba-vermelha	x	x		FA	native
	Cecropia hololeuca Miq.	Embaúba-prateada	x	x	x	FA	native
	Cecropia pachystachya Trécul.	Embaúba			x	-	native
	Urera baccifera (L.) Gaudich. ex Wedd.	Urticão			x	-	native
Verbenaceae							

Family	*Species*	*Common name*	*Coffee*	*Pasture*	*Forest*	*Uses*	*Origin*
	Aloysia virgata (Ruiz & Pav.) Juss.	Cambará-de-lixa			x	-	native
Vochysiaceae							
	Vochysia cf. bifalcata Warm.	Guaricica			x	-	native
	Vochysia magnifica Warm.	Voquísia			x	-	native
	Vochysia tucanorum Mart.	Pau-de-tucano			x	-	native
No identified	Twelve individuals		x	x	x	-	-

SUPPLEMENTARY MATERIAL

Table S1 – Analysis of variance of spores

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	0.11598	5	3.3577	0.0308
Inoculation	1	0.00347	2	0.3017	0.58665
Specie*Inoculation	3	0.06608	4	1.9129	0.14734
Residual	32	0.36845	3		
Total	39	0.55399	1		
CV = 4.99 %					

Table S2 – Analysis of variance of shoot height

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	7.0114	5	72.658	0
Inoculation	1	0.0025	2	0.079	0.78069
Specie*Inoculation	3	0.1674	4	1.735	0.17955
Residual	32	1.0293	3		
Total	39	8.2107	1		
CV = 7.75 %					

Table S3 – Analysis of variance of shoot dry mass

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	14.1449	5	72.3	0
Inoculation	1	0.1809	4	2.774	0.10557
Specie*Inoculation	3	0.3247	3	1.66	0.19523
Residual	32	2.0868	2		
Total	39	16.7374	1		
CV = 23.39 %					

Table S4 – Analysis of variance of root dry mass

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	10.308	5	44.76	0
Inoculation	1	0.0624	2	0.813	0.374
Specie*Inoculation	3	0.4181	4	1.816	0.1642
Residual	32	2.4564	3		
Total	39	13.245	1		

CV = 29.99 %

Table S5 – Analysis of variance of root dry mass : shoot dry mass

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	3.7909	5	26.6283	0
Inoculation	1	0.0334	3	0.7028	0.40807
Specie*Inoculation	3	0.0656	2	0.461	0.71145
Residual	32	1.5186	4		
Total	39	5.4085	1		
CV = 27.21 %					

Table S6 – Analysis of variance of root colonization

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	3.0783	5	19.898	0.00000018
Inoculation	1	0.6001	4	11.637	0.00176834
Specie*Inoculation	3	1.7128	3	11.072	0.00003826
Residual	32	1.6502	2		
Total	39	7.0414	1		
CV = 75.88 %					

Interaction of specie in inoculation

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr.Fc**
Inoculation	1	0.6001	0.6	11.637	0.0018
Specie:Inoculated	3	4.1638	1.4	26.914	0
Specie:Non-inoculated	3	0.6274	0.2	4.0551	0.015
Residual	32	1.6502	0.1		
Total	39	7.0414	0.2		

Interaction of inoculation in specie

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr.Fc**
Specie	3	3.0783	1	19.898	0
Inoculation:Specie 1	1	0.2702	0.3	5.2388	0.0288
Inoculation:Specie 2	1	1.8247	1.8	35.383	0
Inoculation:Specie 3	1	0.2018	0.2	3.9126	0.0566
Inoculation:Specie 4	1	0.0164	0	0.3175	0.577

Residual	32	1.6502	0.1
Total	39	7.0414	0.2

Table S7 – Analysis of variance of specific root length

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	0.33359	5	35.326	0
Inoculation	1	0.01734	4	5.509	0.025276
Specie*Inoculation	3	0.01844	3	1.953	0.140971
Residual	32	0.10073	2		
Total	39	0.47009	1		
CV = 2.23 %					

Table S8 – Analysis of variance of root length : soil volume

Analysis of variance					
	GL	**SQ**	**QM**	**Fc**	**Pr>Fc**
Specie	3	2.985	5	93.19	0
Inoculation	1	0.103	4	9.686	0.00389
Specie*Inoculation	3	0.081	3	2.518	0.07564
Residual	32	0.342	2		
Total	39	3.511	1		
CV = 27.56 %					