UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PÓS-GRADUAÇÃO EM ECOLOGIA, CONSERVAÇÃO E MANEJO DA VIDA SILVESTRE

TESE DE DOUTORADO

Efeitos da facilitação por espécies nativas na restauração da Mata Atlântica

CRISTIANI SPADETO

Orientador: Prof. Geraldo Wilson Fernandes

Belo Horizonte/MG
Janeiro/2019

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VALE

Aos meus pais,

Theresa e Nemesio,

dedico.

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Sumário

Resumo

Nesta tese foi avaliado o papel facilitador de algumas espécies arbóreas (espécies facilitadoras) da Mata Atlântica e como estas espécies influenciam as mudas plantadas e a regeneração natural de ambientes em processo de restauração florestal. A tese foi dividida em dois capítulos. No capítulo I é apresentado como as espécies facilitadoras (*Senna macranthera* (DC. ex Collad.) H.S.Irwin & Barneby e *Croton floribundus* Spreng) melhoram o ambiente abaixo de suas copas e como influenciam a germinação de sementes, sobrevivência e crescimento das mudas plantadas. No capítulo II foi avaliada a estrutura filogenética e funcional, e a diversidade taxonômica, funcional e filogenética da regeneração natural. Foram feitas comparações pareadas da diversidade β para todas as facetas da diversidade que regeneraram abaixo das espécies: *Bixa arborea* Huber, *Inga laurina* (Sw.) Willd., *Joannesia princeps* Vell. e *Senna multijuga* var. *verrucosa* (Vogel) H.S. Irwin & R.C. Barneby. Também foi avaliado o efeito alelopático das folhas dessas espécies, por meio do teste de germinação de sementes. Os resultados desses estudos indicam que as espécies facilitadoras reduzem a intensidade de luz abaixo de suas copas quando comparadas a áreas abertas e melhoram a germinação de sementes e o crescimento das mudas oriundas do plantio de sementes, sugerindo que a idade das espécies facilitadas é um importante modulador das interações de facilitação. Foi possível observar também que as espécies facilitadoras não modulam a estrutura filogenética da regeneração natural, mas influenciam na diversidade filogenética e funcional na comunidade que regenera abaixo de suas copas, assim como algumas espécies podem possuir efeito alelopático. Estes resultados poderão somar aos conhecimentos já existentes sobre restauração florestal e auxiliar na tomada de decisão em relação ao conjunto de espécies a serem implantadas nos futuros projetos de restauração.

Abstract

In this thesis I evaluated the facilitating role of some arboreal tree species (facilitating species) of the Atlantic Forest and how these species influence the planted seedlings and the natural regeneration of environments in the process of forest restoration. The thesis is divided into two chapters. Chapter I presents how the facilitating species (*Senna macranthera* (DC. Ex Collad.) H.S. Irwin & Barneby and *Croton floribundus* Spreng) improve the environment under their canopy and how they influence seed germination, survival and growth of planted seedlings. Chapter II evaluates the phylogenetic and functional structure, and taxonomic, functional and phylogenetic diversity of natural regeneration. Paired comparisons of the β diversity were made for all facets of the diversity that regenerated under the species: *Bixa arborea* Huber, *Inga laurina* (Sw.) Willd., *Joannesia princeps* Vell. and *Senna multijuga* var. *verrucosa* (Vogel) H.S. Irwin & R.C. Barneby. The allelopathic effect of leaves of these species was evaluated through the seed germination test. The results of these studies indicate that the facilitating species reduce light intensity under their canopy when compared to open areas and improve seed germination and seedling growth, suggesting that the age of facilitated species is an important modulator of facilitation interactions. The facilitating species do not modulate the phylogenetic structure of natural regeneration, but they influence the phylogenetic and functional diversity in the regenerating community under their canopies, as some species may have an allelopathic effect. These results may add to the existing knowledge about forest restoration and assist decision making in relation to the set of species to be implanted in future restoration projects.

Introdução Geral

A capacidade de algumas espécies florestais estabelecerem e crescerem em ambientes degradados, assim como sua capacidade para facilitar outras espécies é a chave para determinar os processos da sucessão ecológica (Walker & Del Moral, 2003). A facilitação ocorre quando as espécies melhoram as condições ambientais, modificando, por exemplo, as taxas de luminosidade (Padilla & Pugnaire, 2006; Lima & Gandolfi, 2009), umidade, temperatura e nutrientes do solo (Padilla & Pugnaire, 2006; Liu et al., 2014) e a distribuição da serapilheira (George & Bazzaz, 1999). As mudanças geradas no ambiente sob a copa das facilitadoras favorecem a germinação de sementes, sobrevivência e o crescimento de plântulas abaixo de suas copas e, deste modo, determinar a distribuição espacial e a abundância de outras espécies (George & Bazzaz, 1999; Gandolfi, 2003).

Interações facilitativas ocorrem amplamente em todo o mundo (Valiente-Banuet & Verdú, 2007; 2013), no entanto, esse tipo de interação positiva pode aumentar em importância e intensidade, onde há elevado estresse abiótico (Hacker & Gaines, 1997; Michalet et al., 2006; He et al., 2012). Contudo, a facilitação passou a ser observada cada vez mais em ambientes úmidos (e.g., Liu et al., 2013; Souza et al., 2014; Avendanõ-Yáñez et al., 2014; Spadeto et al., 2017), sugerindo que a sua prevalência pode ser relativamente mais elevada em ambientes menos estressantes do que se pensava anteriormente (Bonanomi et al., 2010; Avendanõ-Yáñez et al., 2014). Em uma área de floresta tropical, Avendanõ-Yáñez et al. (2014) observaram, por exemplo, que a sobrevivência das mudas é maior abaixo das copas quando comparado com as de áreas abertas. Spadeto et al. (2017) observaram que a abundância de indivíduos e riqueza de espécies da regeneração natural pode ser maior sob a copa que em áreas sem cobertura vegetal em uma área de floresta Atlântica no sudeste brasileiro.

As interações entre espécies podem ser guiadas por fatores evolutivos (Valiente-Banuet & Verdú, 2007), ecológicos e morfológicos (Bonanomi et al., 2010) ou acontecer de maneira aleatória, onde espécies mais abundantes interagem mais frequentemente entre si e com mais espécies que as espécies raras (Vázquez et al., 2007). Interações positivas tendem a ocorrer entre espécies filogeneticamente distintas, o que levaria a dispersão filogenética da comunidade e assim ao aumento da diversidade filogenética (Navarro-Cano et al., 2016). Esta premissa tem suporte nos estudos realizados por Valiente-Banuet et al. (2007), Verdú et al. (2009), Soliveres et al. (2012) e Valiente-Banuet & Verdú (2013). Comunidades com maior diversidade filogenética são compostas por espécies com largo espectro de traços funcionais, as quais forneceriam mais funções ao ecossistema (Navarro-Cano et al., 2016) e provavelmente mais estabilidade. Para melhor entender os processos que influenciam a distribuição e interação das espécies, a partição da diversidade tem sido frequentemente utilizada nas facetas: taxonômica, funcional e filogenética (de Belo et al., 2009; 2010; Jankowski et al., 2009). A diversidade, em todas as facetas, pode ser particionada em alfa (diversidade dentro das comunidades locais), beta (diversidade entre comunidades locais) e gama (diversidade regional) (Pavoine et al., 2004; Ricotta, 2005).

A morfologia das espécies facilitadoras também deve ser avaliada. As espécies precisam apresentar traços adequados, como o tamanho da copa suficiente para atuarem como facilitadoras (Liu et al., 2014). A probabilidade de interação aumenta com a área projetada da copa da espécie facilitadora, pois o sombreamento gerado pela copa das árvores facilita o recrutamento e estabelecimento de mudas florestais (Callaway, 1995) e reduzem os impactos das gotas de chuva, diminuindo assim o risco de erosão no solo (Aerts et al., 2007). As interações também aumentam sob a espécie facilitadora, elas servem, por exemplo, de poleiros para pássaros e outros organismos dispersores (Aukema & Martinez del Rio, 2002; Duarte et al., 2006; Zwiener et al., 2014).

Devido a estas qualidades, a facilitação tem sido aplicada na restauração ecológica (Gomez-Aparicio et al., 2004; Avendaño-Yáñez et al., 2014). Nestes ambientes, as espécies facilitadoras são recomendadas para melhorar as taxas de sobrevivência e crescimento das plântulas (veja Padilla & Pugnaire, 2006), recuperar as propriedades e as funções do ecossistema e conduzir o processo sucessional nas áreas degradadas (e.g., Ren et al., 2008). O papel das espécies facilitadoras é fundamental desde a fase inicial da restauração, embora sua relevância deva diminuir com o avanço do processo de sucessão natural. Espécies de início de sucessão utilizadas nos plantios precisam sobreviver e crescer em áreas ensolaradas e criar ambientes propícios para o desenvolvimento de espécies tardias (Avendaño-Yáñez et al., 2014). Diferentes espécies pioneiras criam padrões específicos sob suas copas (Paterno et al., 2016) podendo desempenhar um papel facilitador para o estabelecimento de espécies intermediárias e tardias (Gómez-Aparicio et al., 2004; Sánchez-Velásquez et al., 2004; Padilla & Pugnaire, 2006).

Utilizar espécies facilitadoras como estratégia para restaurar áreas degradadas representa um grande avanço para recuperar a diversidade de espécies, principalmente quando objetiva-se restaurar áreas muito biodiversas como as encontradas na Mata Atlântica, bioma considerado um dos principais *hotspots* de biodiversidade mundial (Myers, 2000). No entanto, cada vez mais, vem sofrendo com o desmatamento e fragmentação (Ribeiro et al., 2009; Juvanhol et al., 2011). Devido à atual situação que se encontra o Bioma Mata Atlântica, seu futuro está condicionado a estratégias eficientes de conservação, restauração e gestão dos recursos naturais (Lima et al., 2015). No estado do Espírito Santo, políticas públicas têm estimulado que a restauração tenha como um dos objetivos a conexão dos fragmentos florestais e tais estratégias devem ser ampliadas para toda a Mata Atlântica (Ribeiro et al., 2009).

Na região sudoeste do estado do Espírito Santo, assim como no restante do bioma Mata Atlântica, pode-se verificar a existência de uma rede de fragmentos de diversas qualidades ambientais. Nesta região, foi observado, por exemplo, que os fragmentos florestais entre as áreas do Parque Estadual de Pedra Azul e do Parque Estadual do Forno Grande possuem tamanhos menores que 5 ha (Juvanhol et al., 2011). Associado a este fato, esta região tem potencial para o desenvolvimento de atividades econômicas, especialmente as relacionadas com a agricultura, mineração e turismo. No entanto, já foi descrita como área de extrema importância biológica e determinada como área prioritária para conservação pelo Ministério do Meio Ambiente (Brasil, 2002). Na região norte do estado do Espírito Santo, também ocorre a presença de fragmentos florestais isolados (Almeida et al., 2010), circundados por pastagens e/ou áreas agrícolas. Em meio a este cenário fragmentado destaca-se a Reserva Natural Vale com um expressivo remanescente florestal e importantes projetos de pesquisa na área de restauração florestal. Nestas duas regiões do estado do Espírito Santo eu investiguei o desempenho de espécies facilitadoras em áreas em processo de restauração florestal, com o objetivo de melhor elucidar como a facilitação contribui para aumentar a diversidade em áreas em processo de restauração.

Esta tese está dividida em dois capítulos, apresentados em formato de artigos. O capítulo I segue na formatação da revista Acta Botanica Brasilica, enquanto o capítulo II foi formatado e submetido para a revista Austral Ecology.

Estruturação da Tese em capítulos/artigos:

Capítulo I – Establishment and growth of two tree species under *Senna macranthera* and *Croton floribundus*

Capítulo II – Phylogenetic and functional diversity under Atlantic rain forest trees mediated by facilitation and alellopathy dictate community organization

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Capítulo I

Establishment and growth of two tree species under *Senna macranthera* and *Croton floribundus**

*Artigo formatado para a revista Acta Botanica Brasilica.

Establishment and growth of tree species under *Senna macranthera* and *Croton floribundus*

Abstract

The need for economically efficient and practical techniques and approaches for restoring degraded environments is urgent in restoration ecology. Restoration can occur by using seedlings or direct sowing under species that has facilitation potential. Here, we investigate how the Brazilian Atlantic rain forest species *Senna macranthera* (Fabaceae) and *Croton floribundus* (Euphorbiaceae) contribute to the germination and development of seeds and seedlings of *Cariniana estrellensis* (Lecythidaceae) and *Melanoxylon brauna* (Fabaceae- Caesalpiniaceae). Height and coverage of the canopies, attributes of the soil and light intensity under the canopies of *S. macranthera* and *C. floribundus* and in open areas were recorded. Canopy height of *S. macranthera* and *C. floribundus* varied only in the first evaluation period. Light intensity was the only abiotic variable that differs between the facilitator species and the open areas. Germination and development of seedlings of *C. estrellensis* originating from seeds were significantly greater under the facilitator species compared to seedlings planted in the open areas. No statistically significant difference was observed in the survival and development of planted seedlings when compared to open area. The ontogeny of facilitated species is an important modulator of facilitative interactions, with the progression of ontogeny entailing changes from positive interactions to neutral or negative interactions.

Keywords: degraded areas; ecological restoration; facilitation; nurse plants; ontogeny.

Introduction

The need for economically efficient and practical techniques and approaches for restoring degraded environments is becoming more urgent (Gould *et al*. 2013). The introduction of species in degraded areas can be done in two ways: by direct seeding or by planting seedlings. Direct seeding is the least expensive technique, however, it has several limitations including great variability in species performance (Souza & Engel 2018). Thus, direct seeding should be used in association with facilitation, which is when the desired species are planted under the canopies of already established plants (Gómez-Aparicio *et al*. 2004; Liu *et al*. 2013; 2014).

In facilitation, established canopies create a complex microenvironment that can vary according to species and architecture (Mejía-Domínguez *et al*. 2011; Liu *et al*. 2014). In general, facilitation results in changes in the luminosity intensity that reaches the soil, consequently altering the humidity, temperature, nutrient availability and microbial community of the soil (Padilla & Pugnaire 2006; Lima & Gandolfi 2009; Mejía-Domínguez *et al*. 2011; Hortal *et al*. 2013; Liu *et al*. 2014). These modifications can limit or favor seed germination and seedling survival and growth (George & Bazzaz 1999; Gandolfi 2003). It has been shown, for example, that greater availability of nutrients and water, and reduced stress from severe light, are beneficial for the establishment and growth of seedlings under facilitatory species (Liu *et al*. 2014). In addition, some facilitatory species, such as shrubs of *Baccharis*, increase the biodiversity of native plants through ecological facilitation and hinder the development of invasive species through competition (R Perea unpubl. res.).

Few works have evaluated facilitation as a tool for the recovery of degraded environments in the tropics (Liu *et al*. 2013; 2014; Avendaño-Yáñez *et al*. 2014). In addition, the studies that have been performed only evaluated survival and growth of seedlings below the canopies of facilitatory shrubs and trees. According to Avendaño-Yáñez *et al*. (2014), planting primary successional species facilitates the establishment of intermediate and late successional trees. In the Brazilian Atlantic Forest, however, in addition to a scarcity of studies, there is also a great diversity of species (Myers *et al*. 2000) and a large number of forest fragments (Ribeiro *et al*. 2009; Juvanhol *et al*. 2011). Efforts to restore this biome have aimed at reconnecting forest fragments because they are extremely important for the movement of animals between the Atlantic Forest in northern and southern Brazil (Ribeiro *et al*. 2009).

Native species, such as *Senna macranthera* (DC. ex Collad.) H.S.Irwin & Barneby (Fabaceae) and *Croton floribundus* Spreng (Euphorbiaceae), have been used in the reforestation of areas of Atlantic Forest (Lorenzi 1992; Abdo & Paula 2006). They have also been described as facilitators for natural regeneration in environments undergoing restoration (for more details see Melo 2015; Spadeto *et al*. 2017). These species were planted in Parque Estadual da Pedra Azul, located in the state of Espírito Santo, Brazil, to restore an area previously dominated by a fern of the genus *Pteridium*. With the objective of investigating how the two native species (*S. macranthera* and *C. floribundus*) contribute to the germination and development of seeds and seedlings of later successional species, namely *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae) and *Melanoxylon brauna* Schott (Fabaceae- Caesalpiniaceae), and what microenvironmental changes they bring to previously degraded areas, we tested the following hypotheses: (i) lower soil temperature and luminosity and higher soil moisture and nutrients can be expected under facilitatory species compared to adjacent open areas; (ii) seeds of *C. estrellensis* and *M. brauna* can be expected to have higher germination rates and seedling development when they are located under the facilitatory species *S. multijuga* and *C. floribundus*; (iii) increased percentages of survival, height development, and stem diameter at ground level can be expected for the species *C. estrellensis* and *M. brauna* when planted under *S. multijuga* and *C. floribundus* than when planted in adjacent open areas.

Material and methods

Study area and studied species

The study was conducted in Parque Estadual da Pedra Azul (Pedra Azul State Park) in the mountainous region of the state of Espírito Santo, Southeast Brazil (20° 23’ 36”; 20° 25’ 56” S and 40° 55’ 23”; 41° 01’ 40” W). The elevation in the region varies from 1,200 to 1,910m and the dominant vegetation is Atlantic rain forest, with the main phytophysiognomies being Montane and High-Montane Dense Ombrophilous Forest (IBGE 2012). According to the Köppen classification, the climate is Cfb (Alvares *et al*. 2013), with mild summers and evenly distributed rains without a dry season. The mean annual precipitation is 1,263 mm and the mean annual temperature is 18.2 °C (Alvares *et al*. 2013).

The area undergoing restoration was previously dominated by a fern of the genus *Pteridium*; a highly competitive species capable of stopping the process of succession for long periods of time (Marrs *et al*. 2000; Royo & Carson 2006). Prior to planting, the area was mowed and prepared to receive seedlings of several native rain forest species, including *S. macranthera* and *C. floribundus*. One limitation of our data set is the lack of details regarding planting, such as the initial composition of the species. The seedlings were planted at the mean spacing of 2.5 × 2.5 m. A total of 880 seedlings were planted in an area of 5,500 m^2. The experiment began one year and six months after the planting of these two species, which are hereafter referred to as ‘facilitators’.

Senna macranthera is a heliophytic, autochoric and semideciduous pioneer species. It occurs naturally from the state of Ceará to the state of São Paulo, with its core distribution in the mountains of Minas Gerais, and in the Atlantic rain forest of Espírito Santo and Rio de Janeiro states (Irwin & Barneby 1982; Lorenzi 1992). It is adapted to grow in different soil types and for fast growth, so it is considered suitable for revegetation programs in degraded areas and riparian forests (Lorenzi 1992). In addition, *S. macranthera* has been reported in association with arbuscular mycorrhizae both inside seasonal forest and in the nursery (Zangaro *et al*. 2002). Mycorrhizae provide improvements to the soil, such as limiting the loss of free nutrients, improving structure and increasing availability of minerals in the more superficial layers (Zangaro *et al*. 2002), which can make the soil more suitable for the subsequent establishment and growth of seedlings of species of later successional stages. The potential for species of the genus *Senna* to serve a facilitatory role in natural regeneration has been documented in areas undergoing restoration, where species of *Senna* regenerated more individuals and greater species richness under their canopies than did other genera and open areas (controls) (Spadeto *et al*. 2017).

Croton floribundus is an autochoric (Lorenzi 1992), deciduous pioneer with very rapid growth and a short life cycle. It is very abundant in secondary formations, in the repopulation of clearings and at forest edges. It is used in mixed reforestation since it quickly covers the soil for species of more advanced stages of succession (Abdo & Paula 2006). This species is widely distributed in diverse environments (Silva *et al*. 2010), and has a high survival rate (100%) with healthy and vigorous seedlings in soil with low nutrient content (Augusto *et al*. 2003). The species possesses characteristics of a facilitatory species. For example, in areas undergoing restoration, lower rates of

Brachiaria biomass, greater density of individuals and higher richness of regenerating species were found under its canopy when compared to open areas (Melo 2015).

The species *C. estrellensis* and *M. brauna* were selected because they are native to Espírito Santo, classified as late successional species (Portela 2012; Garcia *et al*. 2011) and hold potential for forest restoration projects (Lorenzi 1992). Their seeds do not have dormancy, so after processing they can be used for planting. *Melanoxylon brauna* is listed in the Red List of Threatened Species (IUCN 2015). These species will henceforth be referred to as the ‘facilitated’.

Experimental design

Seeds of *C. estrellensis* and seedlings of *C. estrellensis* and *M. brauna* were introduced into three treatments: open area in full sun (control treatment); area under *S. macranthera*; and area under *C. floribundus* canopies.

The experiment involved a randomized block design, with six block, each block with three treatments (two facilitator treatments and one open area treatment = control). Each plot of the facilitator treatments (*S. macranthera* or *C. floribundus*) was established with four plants for a total of 24 individuals of *S. macranthera* and 24 individuals of *C. floribundus*. The open areas were exposed to full sun and located in the vicinity of the areas occupied by the facilitators, but without any canopy interference from any species.

Seeds of *C. estrellensis* were supplied by the Nova Floresta nursery in September 2016, and planted in the same month. Fifteen seeds of *C. estrellensis* were planted under the canopy of each facilitator individual (24 individuals of each facilitator species; total n= 48) at a distance of approximately 0.4 m from their trunks. The seeds were placed 10 cm apart and their location marked with a wooden stick. The same number of seeds

(360) was sown in open areas (control treatment) with the same distance between them. Thus, a total of 720 seeds of *C. estrellensis* were used.

The seedlings of *C. estrellensis* and *M. brauna* used in planting were provided by the Florestal Primavera nursery, with six-month-old. Planting was carried out in September 2016 with one seedlings of each facilitated species being planted underneath the canopy of facilitators such that they were distributed in a north-south direction at a distance of approximately 0.5 m from the trunk. The same number of seedlings was planted in open areas (24); hence serving as the control treatment. Thus, a total of 72 seedlings per species were used, 24 seedlings in each treatment. Seedlings that died within 15 days after planting were replaced. The plants were irrigated during the first week.

Attributes of facilitators, environmental variables and chemical properties of the soil

Canopy height and coverage of the facilitators was measured the day after planting the facilitated seedlings (first evaluation) and 10 months after planting (second evaluation). The height (from the ground to the top of the canopy) and coverage (canopy diameter towards north-south cardinal points) were measured using a tape measure.

Physical and chemical attributes of the soil (moisture, temperature and fertility), and light intensity, were evaluated to test the abiotic difference between the open areas (control) and areas under the facilitators treatments. Soil temperature and humidity were measured using a GS3 portable sensor (Decagon Devices, Pullman, WA, USA) in layers of 0-10 and 10-20 cm of depth after opening a small trench for sensor access. Three readings were obtained from the central position of each layer in each trench on the same day during the morning.

Soil fertility analyses were performed on samples collected 50-cm from the trunk of each facilitator, and in the open areas, at a depth of 0-20 cm. Three soil samples were collected per plot, for which the following were determined: concentration of macronutrients (P – phosphorus, K – potassium, Na – sodium, Ca – calcium, Mg – magnesium, Al – aluminium) and pH in water (Embrapa 1997; Tedesco *et al*. 1995).

Light intensity was evaluated on a partly cloud day until 10:00 h in the morning to avoid large fluctuations in the readings due to the movement of clouds. The evaluations were performed using an ITLD 270 Instrutemp portable digital luxmeter. Four measurements were recorded under each facilitator and in the open areas during each temporal replicate. Assessments of light intensity and soil temperature and moisture were performed in the spring and fall, respectively.

Plantlet emergence, survival, and development for facilitated species

The number of emerged seedlings was recorded at three, four, five, seven, nine, 16 and 24 months post-sowing, in December 2016, January, February, April and June 2017, and January and September 2018, respectively. Stem height and diameter at ground level were measured for surviving seedlings at the last evaluation using a measuring tape and a caliper. Values for height and mean diameter were calculated for each replicate. The number of seedlings that survived, and stem height and diameter at ground level of the planted seedlings, were evaluated at planting and after nine and 24 months.

Monthly growth rate in terms of height and stem diameter at ground level was estimated for the planted seedlings using the following equation (Sánchez-Velásquez *et al*. 2011; Avendaño-Yáñez *et al*. 2014):

$$\mathrm{T_c} = \frac{\ln \mathrm{C_2} - \ln \mathrm{C_1}}{\mathrm{t}}$$

where T_c is growth rate, ln is the natural logarithm, C_2 is final height (or diameter at ground level), C_1 is initial height (or diameter at ground level), and t is time interval (nine or 24 months).

Statistical analysis

Mixed linear models (MLMs) were assembled to test whether the attributes of the facilitators, the environment and the soil varied according to the facilitators (*S. macranthera* and *C. floribundus*) and open areas. The variables height and coverage of facilitators; humidity and temperature of the soil; luminosity; and soil P, K, Na, Ca, Mg, Al H and pH, were used as response variables while treatment (facilitators and open areas) as the explanatory variable. Block was used as random variable in all models.

To understand the effect of facilitation by the different facilitator species and the effect of open area on the facilitated species (explanatory variables) we established two MLMs, one using height and the other using the diameter of plantlet originating from germination as response variables. In order to understand the effect of facilitation by the different facilitator species and the effect of open area on the facilitated species (explanatory variables), but using seed germination, survival, and growth rate of height and diameter at ground level of planted seedlings, we used generalized linear mixed models (GLMMs), with Poisson distribution. For the seed germination variable, the model had interaction between the facilitators and the time after sowing. Both types of models (MLM and GLMM) were built using the “lme4” package (Bates *et al*. 2013) and the functions “lmer” and “glmer”, respectively. All analyses were performed with R software (R Development Core Team 2016).

Results

Canopy height was the only attribute of facilitators to vary and only for the first evaluation period, with *S. macranthera* having greater height than *C. floribundus* ($P < 0.05$). Intensity of luminosity differed between facilitators and the open areas ($P < 0.05$), but not between *C. floribundus* and *S. macranthera* ($P > 0,05$). There were also no differences in soil moisture and temperature under the canopies between the facilitators ($P > 0,05$) as well as in comparison with open areas ($P > 0,05$) (Table 1).

Table 1. Attributes of facilitators, environmental variables and chemical properties of the soil (mean and standard deviation) in open areas and below the facilitators

Variables	Open area	*C. floribundus*	*S. macranthera*
Facilitator height at first evaluation (m)	**-**	**2.32±0.57 a**	**2.90±0.69 b**
Facilitator height at second evaluation (m)	-	3.87±0.94 a	4.10±0.95 a
Facilitator coverage first evaluation (m)	-	2.08±0.66 a	2.15±0.61 a
Facilitator coverage second evaluation (m)	-	3.00±0.68 a	2.93±0.95 a
Soil humidity in layers of 0-10 cm of depth ($m^3 m^{-3}$)	0.18±0.038 a	0.19±0.05 a	0.21±0.03 a
Soil humidity in layers of 10-20 cm of depth ($m^3 m^{-3}$)	0.22±0.01 a	0.22±0.02 a	0.23±0.01 a
Soil temperature in layers of 0-10 cm of	25.4±1.96 a	25.35±1.33 a	25.51±1.32 a

depth (°C)			
Soil temperature in layers of 10-20 cm of depth (°C)	25.39±1.97 a	25.37±1.32 a	25.54±1.34 a
Light intensity (Lux)	**1346±266 a**	**708±277 b**	**693±262 b**
pH	5.10±0.42 a	5.19±0.37 a	5.14±0.44 a
P	**2.68±1.33 b**	**3.93±3.58 a**	**2.68±1.09 b**
K	**49.61±23.73 b**	**70.72±37.06 a**	**51.77±26.42 b**
Ca	**1.34±0.69 a**	**1.11±0.65 b**	**0.94±0.53 b**
Na	3.16±3.72 a	3.55±7.79 a	1.33±2.14 a
Mg	**0.62±0.31 a**	**0.59±0.26 a**	**0.50±0.23 b**
Al	**1.03±0.66 a**	**0.81±0.64 b**	**1.04±0.81 a**

pH (water-ground relation 1:2,5); P, phosphorus (mg/dm^3); K, potassium (mg/dm^3); Na, sodium (mg/dm^3); Ca, calcium ($cmol_c/dm^3$); Mg, magnesium ($cmol_c/dm^3$); Al, aluminum ($cmol_c/dm^3$); Means followed by the same letter in the line do not differ significantly from each other by the Tukey test, 5% probability.

Many soil chemical characteristics were affected by the presence of the canopy of the facilitators (Table 1). For example, higher P and K consentrations were found below *C. floribundus* than below *S. macranthera* and in open areas ($P < 0.05$). The Al was lower under *C. floribundus* and higher in the open areas and below *S. macranthera* ($P < 0.05$). Higher values of Ca were found for open areas when compared to those of the facilitators ($P < 0.05$). In contrast, Mg was higher in the open areas and under *C. floribundus* than below *S. macranthera* ($P < 0.05$), but did not statistically differ between the facilitators ($P > 0,05$) (Table 1).

The interaction between facilitators and time after sowing was significant ($P < 0.05$) (Fig. 1). The emergence of *C. estrellensis* plantlet was greater under facilitators than in the open areas ($P < 0.05$) (Fig. 1). The number of emerged plantlet was higher four and five months post-planting ($P < 0.05$), while in the subsequent months fewer seedlings were observed, further reducing after 16 and 24 months post-sowing (*P* < 0.05) (Fig. 1).

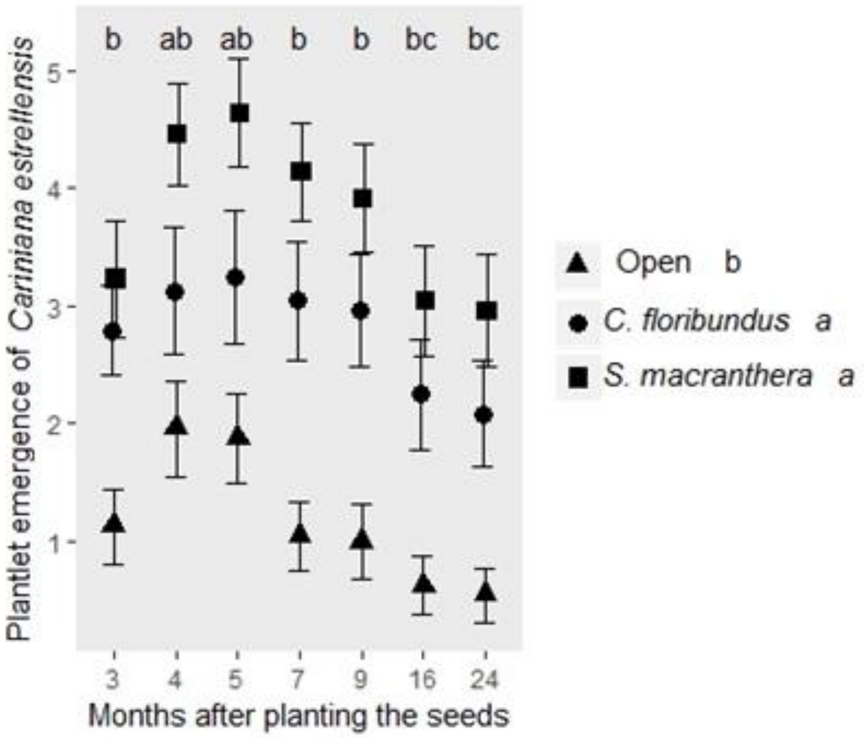

Fig. 1. Plantlet emergence of *Cariniana estrellensis* in open areas and under *Croton floribundus* and *Senna macranthera*. Values (mean and standard deviation) during the 24 months after planting the seeds. Different letters within time and within treatments differ significantly (*P* < 0.05).

The presence of facilitator correlated with the height of *C. estrellensis* plantlet. Plantlet height was higher under canopy of *S. macranthera* (*P* < 0.05) than in the open areas. However, height of *C. estrellensis* did not differ between *C. floribundus* and *S. macranthera* (*P* > 0,05) (Fig. 2A).

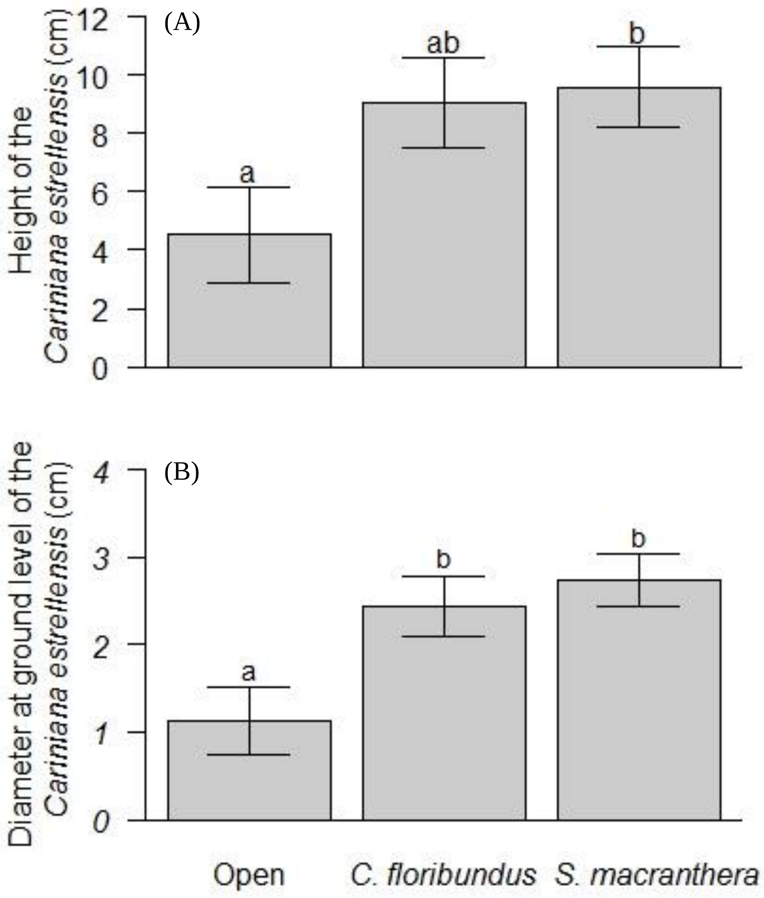

Fig. 2. Height (A) and diameter at ground level (B) of the *Cariniana estrellensis* seedlings in open area and under *Croton floribundus* and *Senna macranthera*. Vertical lines correspond to the standard deviation. Different letters differ significantly between treatments ($P < 0.05$).

Likewise the diameter at ground level of *C. estrellensis* plantlet was higher under the facilitators than in the open areas (P > 0,05), but did not differ between the two facilitator species (P > 0,05) (Fig. 2B). No statistically significant differences were found for survival (P > 0,05), and height growth rate (P > 0,05), and diameter at ground level growth rate (P > 0,05) for the planted seedlings of *C. estrellensis* and *M. brauna*, under the facilitators than in the open areas, in all evaluated periods (nine and 24 months) post-planting.

Discussion

Canopy cover did not differ between the facilitators *C. floribundus* and *S. macranthera*, while the height differed between *C. floribundus* and *S. macranthera* only in the first evaluation. Canopy architecture has been shown to be directly associated with microenvironmental changes in the understorey (Meíja-Domínguez *et al*. 2014). For example, the soil under plants with larger canopies has better water retention capacity (Liu *et al*. 2014), and larger canopies can alleviate the effects of high temperatures (Ren *et al*. 2008) and reduce the risk of soil erosion (Aerts *et al*. 2007). In addition, different canopy morphologies (such as differences in branch density and canopy height) can generate various specific niche spaces and thus harbor different species (Scrob *et al*. 2017).

Due to similar canopy height and coverage, soil moisture and temperature did not differ under the facilitators. There were also no differences in soil moisture and temperature under the canopies between the facilitators as well as in comparison with open areas. Higher humidity and lower temperature were expected in areas below canopies compared to open areas, as has been observed in other studies (Liu *et al*. 2013; Zhang & Zhao 2015). Water movement in the soil tends to occur in the direction of highest to lowest water potential, and thus be approximated between adjacent layers, both vertically and horizontally, especially in the first hours of the day (Libardi 2005). This could potentially explain the similar values of soil moisture regardless of vegetation cover in the rain forest.

The light intensity was higher in the open areas when compared to beneath facilitator species, suggesting that the canopies of these species partially impede the passage of sunlight. On average, the level of shading under the facilitators allow only 52% of the incident light to pass. However, the shading created by the canopies did not lead to changes in soil moisture and temperature, as described in previous studies (Mejía-Domínguez *et al*. 2011; Galindo *et al*. 2017; Padovan *et al*. 2018).

Some soil chemical attributes differed between the facilitators and between the facilitators and open areas. High levels of P were found in areas under *C. floribundus* compared to the open areas. Varela *et al*. (2017) obtained a similar result when evaluating the levels of P under native shrubs and in the open areas in the Monte desert in Argentina. The levels of K were also significantly higher under *C. floribundus* than in the other treatment. Jaramillo-Botero *et al*. (2008) demonstrated that *C. floribundus* has high levels of K, Ca and Mg in its leaves, with a high capacity of contributing Ca and K to the soil, compared to the values exhibited by other native species of the Atlantic rain forest. According to these authors, this species has a high capacity to contribute with nutrients through the litter, besides an accelerated growth rate and a high capacity for nutrient absorption and accumulation in its tissues. It is possible that these characteristics favored the contribution of K to the soil, which cycles rapidly (Torres & Pereira 2008). In contrast, lower levels of Al were observed under *C. floribundus*, when compared to the open areas and under *S. macranthera*. Higher concentration of Al in the soil is directly associated with pH levels less than or equal to 5.5 (Čiamporová 2002). In the present study, the pH values of all treatments were close to 5.1, showing that the soil is acidic.

In addition to the evaluated attributes, other factors may explain the mechanism of facilitation and can influence the establishment and development of species under canopies. For example, pioneer tree species may be better facilitators than later succession trees, which may better explain the interaction among plants than canopy height and diameter (Fagundes *et al*. 2018). In addition, it is also important to consider that in areas undergoing restoration/succession, the microclimatic changes generated by tree species depend on the species, age and structural characteristics of each individual (Bizuti *et al*. 2016). It has been observed, for example, that up to three years after planting, air and soil temperature and air humidity do not vary under the canopies of the species *Luehea grandiflora* Mart., *Schinus terebinthifolius* Raddi, *Solanum erianthum* D. Don, and *Croton urucurana* Baill (Bizuti *et al*. 2016). These species have functions similar to the species evaluated in the present study, such as rapid growth and broad canopies that cover and shade the soil (Bizuti *et al*. 2016).

A novelty of this work is that the presence of some planted species in areas undergoing restoration may partially explain facilitation in tropical forests, with facilitators positively influencing the germination and development of plantlet of *C. estrellensis* originating from seeds. As a technique to recover species diversity in degraded areas, planting seeds is an important tool, especially if the seeds are associated with species that allow their germination and development. In addition, planting seeds is less costly for the implementation of restoration projects (Souza & Engel 2018).

Plantlet of *C. estrellensis* originating from seeds developed better under facilitators, with no significant difference in survival and development for the seedlings of the facilitated species planted under facilitators and in the open areas. Other studies have observed similar trends, with greater intensity of facilitation during the phases of germination and establishment, and then reduced or neutralized facilitation intensity in later stages of development (Álvarez‑Yépiz *et al*. 2014; Paterno *et al*. 2016). Interactions in the facilitation system can be terminated when shade-intolerant facilitated species are involved (Soliveres *et al*. 2013). However, the species used in planting (*C. estrellensis* and *M. brauna*) are classified as late successional species

(Portela 2012; Garcia *et al*. 2011), and thus require shaded environments during the juvenile phase. Shading for species of late succession is important mainly during the phases of germination and initial development. Seedlings of shade-tolerant species absorb more light than they can use, resulting in photoinhibition or even plant death (Kitao *et al*. 2000). The performance of *C. estrellensis* seedlings, for example, has been previously assessed in pastures, edges, clearings, and forests (Sansevero *et al*. 2006). The seedlings exhibited higher mortality rates in pastures, but no differences among the other areas, which have a certain degree of shading (Sansevero *et al*. 2006).

Changes from positive interactions to neutral or negative interactions can occur when there is niche overlap between facilitator and facilitated plants (Paterno *et al*. 2016). Individuals that were previously dependent on the resources and conditions provided by nurses, become less dependent with development (Paterno *et al*. 2016). This independence is due to transformations that facilitated individuals undergo during ontogeny (Miriti 2006). This is possibly the main cause of the positive influences of *C. floribundus* and *S. macranthera* on the germination and initial development of *C. estrellensis* plantlet and the lack of benefit to the seedlings of *C. estrellensis* and *M. brauna*.

Actions of ecological restoration directly impact the germination and growth of shade-tolerant species and provide an initial idea of facilitative interaction in tropical environments. Additional studies similar to the present one are needed for the Atlantic rain forest to build a stronger theory on the role of facilitation in this highly degraded biome in Brazil. Broadening the list of facilitator species of different phylogenies is required to allow a deeper understanding of this interaction in the wild and restored areas, primarily now when such information is urgently needed (see Bustamante *et al*. 2018). Furthermore, the planting of *C. estrellensis* seeds or other species with similar characteristics can become an easy and inexpensive technique for enriching areas that are undergoing the process of restoration.

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Capítulo II

Phylogenetic and functional diversity under Atlantic rain forest trees mediated by facilitation and alellopathy dictate community organization*

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Phylogenetic and functional diversity under Atlantic rain forest trees mediated by facilitation and alellopathy dictate community organization

Abstract

Mechanisms, such as the phylogenetic relationships among plants, their diversity and their production of chemical inhibitors of germination, help to infer the main drivers of the construction and assembly of plant communities. Functional attributes (height, diameter, successional group and dispersion syndrome) were measured for all individuals or species that regenerated in open areas and below the tree species *Bixa arborea* Huber, *Inga laurina* (Sw.) Willd., *Joannesia princeps* Vell. and *Senna multijuga* var. *verrucosa* (Vogel) H.S. Irwin & R.C. Barneby. Phylogenetic and functional structures were calculated with the net relatedness index (NRI) and the nearest taxon index (NTI). Functional and phylogenetic diversities for the regenerating community in each area were calculated and pairwise comparisons made for taxonomic (TD), functional (FD) and phylogenetic (PD) components of β diversity. Tests of tree species allelopathy derived from leaf extract were performed with seeds of *Lactuca sativa* L. (Asteraceae). The effects of diversity and of extracts on seed germination were analyzed using generalized linear models. The phylogenetic structure of the regenerating communities did not differ from random, since phylogenetic and functional diversities differed significantly only for the communities that regenerated under *Joannesia* and under *Joannesia* and *Senna*, respectively, of open areas. Only *Senna* and *Inga* differed from random in relation to β TD and β FD. Seeds irrigated with the extracts of *Inga*, *Joannesia* and *Senna* inhibited germination in an intermediate way, whereas seeds irrigated with *Bixa* extract had only 1% germination. Communities at the beginning of succession have a random structure, however, *Joannesia* and *Senna* exhibited a facilitation mechanism. The communities under *Senna* and *Inga* were dissimilar to those found in open areas, suggesting possible environmental changes in these areas. In contrast, the germination test for the species of *Bixa* demonstrated allelopathic potential.

Key words: beta diversity; diversity partitioning; functional diversity; phylogenetic diversity; restoration

INTRODUCTION

Knowledge of the phylogenetic relationships among plants in a given community (whether clustered or overdispersed) helps to infer the main drivers of their construction and assembly (Weiher and Keddy, 1995; Webb et al., 2002). The search for the processes involved in the assembly of communities in different ecosystems of the world has grown (e.g., Saito et al., 2016; Carrión et al., 2017; García-Camacho et al., 2017) and special attention has been given to communities during the process of succession of restored forests (e.g., Verdú et al., 2009; Chang et al., 2015; Schweizer et al., 2015). However, the interpretation of structure depends on the phylogenetic signal in the evolution of traits (Blomberg and Garland, 2002; Wiens et al., 2010) and whether the observed structure differs from that expected by null models (Webb, 2000; Saito et al., 2016). In communities with conserved phylogenetic signals, clustered phylogenetic patterns (coexisting species are more related) are shaped by environmental filters (Webb et al., 2002; Cavender-Bares et al., 2004). On the other hand, communities with overdispersed phylogenetic patterns (coexisting species are distantly related) are formed by competition (Webb et al., 2002; Cavender-Bares et al., 2004) or facilitation (Valiente-Banuet and Verdú, 2007).

Plant diversity also provides clues as to how communities are structured. For many years diversity was assessed only with a focus on species (Gentry, 1988). However, current diversity analyses are based on phylogenetic diversity (PD) and functional diversity (FD), and generate more robust results, which can be used to better understand current patterns of biodiversity (de Bello et al., 2010; Mouquet et al., 2012). Communities with greater phylogenetic diversity are composed of species with a broad spectrum of functional traits, which provide more functions for the ecosystem (Navarro-Cano et al., 2016). For example, in areas of restoration from mining, the most efficient nurse plants had characteristics related to efficient metabolism and the root system, as well as the nutrient ratio of leaves (Navarro-Cano et al., 2017). The emergence of partitioning of diversity made it possible to better understand the processes that influence the distribution of species among taxonomic, functional and phylogenetic facets (de Belo et al., 2009, 2010; Jankowski et al., 2009).

Diversity can be portioned into alpha (diversity within local communities), beta (β; diversity among local communities) and gamma (regional diversity) (Pavoine et al., 2004; Ricotta, 2005). The measurement of beta diversity among sample units can reveal variability in composition (Anderson et al., 2006). This measure can also be performed for phylogenetic and functional diversity, in which cases variation in phylogenetic and functional composition, respectively, can be found.

Another mechanism that may influence the structuring of plant communities is allelopathy, since chemical compounds produced by some plants may interfere with adjacent vegetation (Rice, 1984; Arroyo et al., 2018). The presence of harmful allelopathic compounds in the environment can inhibit or delay seed germination (Fernandez et al., 2013) and inhibit seedling growth (Linhart et al., 2015; Arroyo et al., 2018). Studies of allelopathy have been carried out mainly with controlled temperatures and/or luminosity (Stowe, 1979; Dai et al., 2017; Hashoum et al., 2017) and with species with fast, uniform, and sensitive germination, such as *Lactuca sativa* (Maraschin-Silva and Aqüila, 2006; Tigre et al., 2012).

We are unaware of any studies that have investigated community assembly rules by simultaneously evaluating the relationships of species with a phylogenetic, functional and ecophysiological (allelopathy) approach and multifaceted β diversity (taxonomic, phylogenetic and functional). Thus, for the first time, we investigate how species planted in areas in the process of restoration influence the assembly and diversity of the regenerating communities under their canopies. This knowledge increases the chances of optimizing the selection of species for planting in future restoration projects (Chang et al., 2015), particularly in environments as diverse as the Atlantic Forest of Brazil. A previous study at the same site showed that some species are facilitators, such as: *Bixa arborea* Huber (Bixaceae), *Joannesia princeps* Vell. (Euphorbiaceae), *Inga laurina* (Sw.) Willd. (Fabaceae) and *Senna multijuga* var. *verrucosa* (Vogel) H.S. Irwin & R.C. Barneby (Fabaceae) (Spadeto et al., 2017), hereafter only *Bixa*, *Joannesia*, *Inga* and *Senna.* Higher species richness and abundance of individuals were found under *Joannesia* and *Senna*, while lower abundance of individuals was found below *Bixa* (Spadeto et al., 2017). In this context, we tested the following hypotheses: (i) The structure of the plant community recruited below tree species are overdispersed, since according to Valiente-Banuet and Verdú (2007), facilitation can act as a assembly rule favoring phylogenetic dispersion because facilitators tend to recruit unrelated species; (ii) Greater functional and phylogenetic diversity is found in communities below tree species than in open areas given that some species behave as facilitators, and thus form communities of distantly related species, which increases phylogenetic (Valiente-Banuet and Verdú, 2007) and functional (Gazol and Camarero, 2016) diversity; (iii) β diversity (for all facets: taxonomic diversity, FD, PD) between tree species and open areas is greater than expected by random. We expected communities of dissimilar plants to establish themselves under tree species and in open areas. We also expected β diversity among the communities of each tree species to be greater than expected at random because, according to Padilla and Pugnaire (2006), each species can provide different conditions and facilitate the establishment of different communities; (iv) Lower germination of *L. sativa* seeds irrigated with *Bixa* leaf extract since a lower abundance of regenerants has been previously observed under this species (see Spadeto et al., 2017).

METHODS

Study area, study species and vegetation sampling

The study was conducted in a restoration area of Reserva Natural Vale (RNV) (9°06' to 19°8' S; 39°45' to 40°19' W), located in the state of Espírito Santo, Southeast Brazil. The region is within the domain of the Atlantic Forest, one of the world's biodiversity hotspots, specifically the physiognomy of the lowland dense ombrophilous forest (IBGE, 2012). The rainy season occurs between the months of September and April and the annual averages for temperature and rainfall are 22 °C and 1,200 mm, respectively (Rolim et al., 2005). A total of 170 native species were planted in the area distributed in two spaces (2×2 and 3×3 m). For further details see Spadeto et al. (2017).

Eighteen individuals of each species were randomly selected eight years after planting: *Bixa*, *Joannesia*, *Inga* and *Senna*. These species were chosen because of their rapid growth in the area, because they produce large amounts of seeds or fruits attractive to the fauna and because they have been used in several restoration projects in Southeast Brazil (e.g., Botelho et al., 1996; Rolim et al., 2007; Ferreira et al., 2009;

Massad et al., 2011; Pedroza et al., 2011). These factors, associated with the greater richness and abundance of individuals under *Joannesia* and *Senna* (Spadeto et al., 2017), led us to investigate more deeply the mechanisms that aid in the assembly and diversity of the community below these species.

A set of 18 open areas close to the marked species, but without being influenced by the canopy, were also established to serve as controls. Plots with a radius of three meters were established in these areas and under the study species to evaluate natural regeneration. The plots marked under the study tree species were centered around individuals selected from *Bixa*, *Joannesia*, *Inga* or *Senna*.

All shrubs and trees that regenerated within each plot and were taller than 30 cm and a diameter at breast height of less than 5 cm were counted and identified. A total of 916 individuals of 96 species were recorded. The composition of the planting was described by Spadeto et al. (2017).

Measures of functional attributes

All species of regenerating shrubs and trees were used to measure functional attributes. The attributes of height, diameter of the branch at soil level, successional group and dispersion syndrome were recorded for all regenerating individuals. Analysis of these attributes for regenerating communities in areas in the process of restoration is an important strategy for monitoring such areas (Belloto et al., 2010).

The attributes height and diameter were measured *in situ*. We subsequently classified species according to their successional group as pioneer, early secondary or late secondary species (Gandolfi et al., 1995), and their dispersion syndrome as zoochoric, anemochoric or autochoric.

Categorical variables were used for calculating FD, with quantitative variables (height and diameter) being categorized into four classes of similar size (i.e., composed of 24 species in each class).

Phylogenetic analysis

The 96 species sampled in all plots were used to construct a phylogenetic tree (Appendix 1). All species were initially added to the phylogenetic tree using the software Phylomatic (Webb and Donoghue, 2005). The program generated a tree where the relationships of the species sampled followed APG IV (APG, 2016). Then, with the aid of Mesquite software (Maddison and Maddison, 2009) we corrected and improved tree resolution based on several recent molecular phylogenetic analyses. Finally, we used the *bladj* algorithm implemented in Phylocom (Webb et al., 2008) to position the undated nodes evenly between dated nodes (Appendix 1).

Phylogenetic and functional structure

After the tree was constructed, the mean distance between pairs (mean pairwise distance - MPD) and the mean distance of the nearest taxon (mean nearest taxon distance - MNTD) were calculated to evaluate the mean phylogenetic distance between all pairwise combinations of species and the mean phylogenetic distance of the nearest relative of all species, respectively. In addition, MPD and MNTD were used to calculate the net relatedness index (NRI) and the nearest taxon index (NTI). These metrics capture distinct aspects of the phylogenetic structure of communities. While NRI reflects the structure of the phylogenetic tree as a whole, particularly the basal nodes, NTI is related to the pattern shown in the terminal part of the tree (Webb, 2000; Webb et al., 2002). Both NRI and NTI are standardized measures (standardized effect size) and are calculated with the following formula: [$NRI = -(MPD_{obs.} - MPD_{rand.})/MPD\sigma_{rand.}$], where $MPD_{obs.}$ is the mean of MPD observed in the local community; $MPD_{rand.}$ is the mean of MPD obtained by randomization in null communities; and $MPD\sigma_{rand.}$ is the standard deviation of $MPD_{rand.}$ (Webb et al., 2002). To calculate NTI it is only necessary to replace MPD with MNTD. Negative values of NRI or NTI indicate communities with overdispersed phylogenetic structure (i.e., co-occurring species in the sample are phylogenetically less closely related than expected by chance), whereas positive values indicate communities with clustered phylogenetic structure (i.e., co-occurring species in the sample are phylogenetically more closely related than expected by chance). Values close to zero indicate communities with random phylogenetic structure (i.e., the phylogenetic relationship of the co-occurring species in the sample does not differ from that expected by chance). Values of NRI and NTI can also be used to calculate functional distance (Webb et al., 2008; Cianciaruso et al., 2012).

Information on the presence and absence of species in the communities was used in all analyses. The regional species bank was delimited as the sum of all the species occurring in all sampled plots (below the crowns of the evaluated tree species and in the open areas of the control plots).

The null model used was generated with the independent permutation algorithm (independent swap; Gotelli and Entsminger, 2003), which maintains constant richness and frequency in the plots, while randomizing only patterns of species co-occurrence.

All analyses were performed with the package “picante” (Kembel et al., 2010) in R software (R Development Core Team, 2016). We used the one-sample *t*-test (Kembel and Hubbel, 2006) to test whether phylogenetic or functional structure of each species (*Bixa*, *Joannesia*, *Inga* and *Senna*) differed from the null explanation (mean NRI or NTI = zero).

Functional and phylogenetic diversity and diversity partitioning

Taxonomic diversity of the recruited species was measured by species richness. In order to obtain FD and PD diversities, the dissimilarity matrix of the species were calculated using the “Gower approach” of the “trova” function (see Lepš et al., 2006; de Bello et al., 2010) in R software (R Development Core Team, 2016). The matrix used in the calculation of FD was constructed with the attributes: height class, diameter class, successional group and dispersion syndrome. The Rao index was calculated with the dissimilarity matrix to estimate FD and PD considering the occurrence of the species in each sample plot.

We constructed generalized linear models (GLMs) to test the hypothesis about functional and phylogenetic diversities by comparing species and comparing each species with open area. The models with significant differences were submitted to contrast analysis (package RT4Bio; Reis Jr. et al., 2013) to identify the difference between species, and species and open area ($P < 0.05$).

To test the hypothesis for β diversity, we used the “Rao” function (de Bello et al. 2010) to calculate TD, FD and PD and to make pairwise comparisons of diversity (TD, FD, PD) of the species (that is, the β diversity between pairs of samples). In this case, each sample was a treatment with an arboreal species, along with the open area treatment. Null models were constructed to test whether the observed values of β TD, β FD and β PD were different from those expected by chance. To do this, we used the “permatfull” function of the “vegan” package (Oksanen et al., 2018) to construct 999 matrices of random occurrence, respecting two rules: the number of species per treatment was always the same as observed and the number of treatments in which a species occurred was always the same as observed (randomization while maintaining the marginal sums of the matrix). We considered values to differ if they were outside the upper and lower range of 2.5% of the calculated values for the 999 random matrices. All analyses were performed in R software (R Development Core Team, 2016).

For pairwise comparisons of TD that were higher than expected by chance, similarity percentage analysis (SIMPER) was used to quantify the relative contribution of each regenerating species. We used Bray-Curtis similarity index in the SIMPER analysis, considering abundance. The analysis was performed using PAST 2.16 software (Hammer et al., 2001).

Allelopathy test

Approximately 100 g of leaves of each species (*Bixa, Inga, Joannesia* and *Senna*) was collected in RNV to produce aqueous extract, which was used in the irrigation of seeds of *L. sativa* L. (Asteraceae) (Maraschin-Silva and Aqüila, 2006). *L. sativa* was used in this experiment because its germination is fast and uniform, and because it is a sensitive species capable of providing evidence of allelopathic effects (Maraschin-Silva and Aqüila, 2006; Tigre et al., 2012). To produce the extract, the leaves were dried in a forced air circulation oven at 40 °C and then mechanically crushed. Distilled water was added in the ratio of 1:10 (p/v) and maintained for 48 hours, after which the solution was filtered. The test of only one aqueous extract concentration (20%) was performed with the objective of approaching the realistic concentration of the natural leachate (Hashoum et al., 2017). Distilled water was used to irrigate the seeds in the control treatment.

The bioassays were mounted on sterile Petri dishes lined with two layers of filter paper moistened with approximately 3 ml of extract or distilled water (control treatment). Twenty-five seeds were seeded in each plate and each treatment had four replicates. The bioassays were kept in a B.O.D. humid germination chamber at a constant temperature of 25 °C and a photoperiod of 12 h. The number of germinated seeds was checked daily. Germinated seeds were considered those that produced a primary root of at least 2 mm in length.

The experiment was set up in a completely randomized design with five treatments (control and leaf extracts of *Bixa*, *Joannesia*, *Inga* and *Senna*) × four replicates. We used GLMs to analyze the effect of different extracts on seed germination, with the models with significant differences being submitted to contrast analysis (package RT4Bio; Reis Jr. et al., 2013) to identify the extracts or control responsible for the differences ($P < 0.05$). These analyses were performed in R software (R Development Core Team, 2016).

RESULTS

The phylogenetic structure of the regenerating community was not significantly different from random (Fig. 1a). A similar pattern was observed for FD (Fig. 1b).

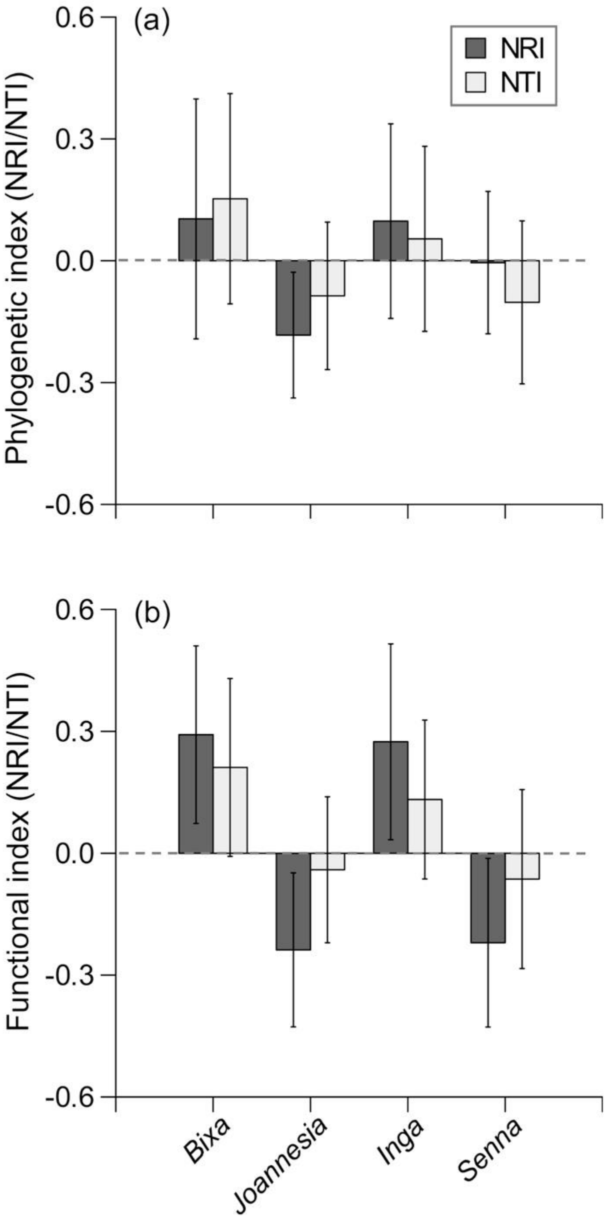

Fig. 1. Average phylogenetic patterns (a) and average functional patterns (b) using the net relatedness index (NRI; gray bar) and the nearest taxon index (NTI; black columns) in the communities under *Bixa*, *Joannesia, Inga* and *Senna*. The vertical lines represent standard deviation.

Phylogenetic diversity was higher in the communities regenerated below *Joannesia* ($P < 0.05$) (Fig. 2a). On the other hand, there were no significant differences for PD of natural regeneration between the other tree species and open areas. A significant difference was found for FD in the communities under *Joannesia* and *Senna* ($P < 0.001$), which had higher FD. Communities that regenerated in open areas and below other tree species did not differ statistically ($P > 0.05$) (Fig. 2b).

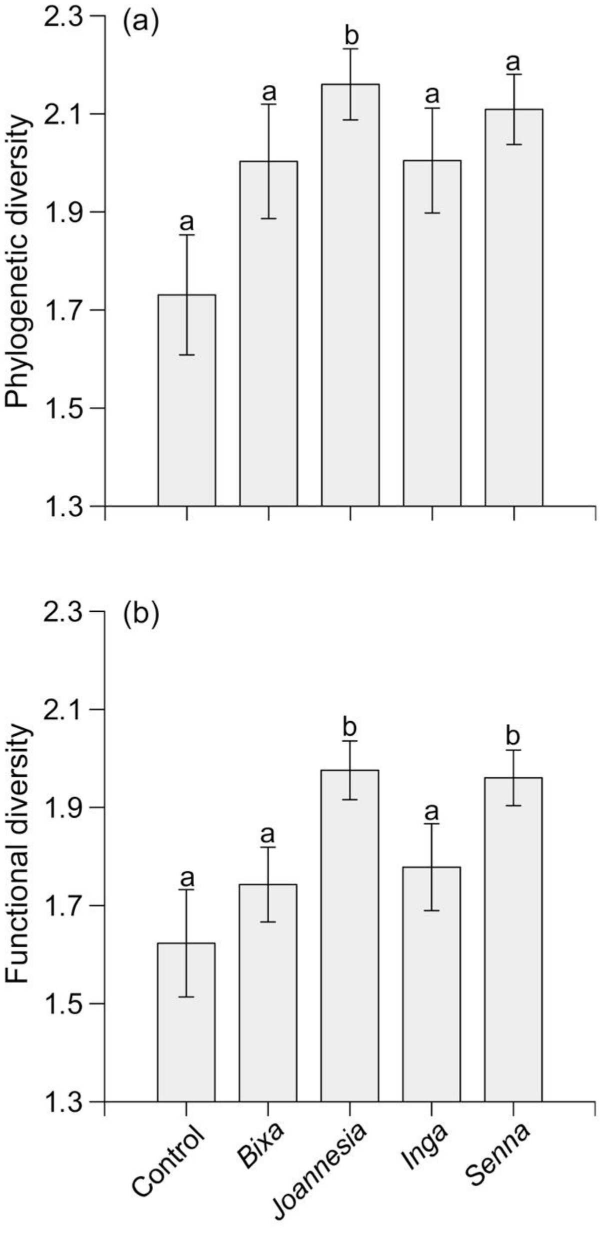

Fig. 2. Average phylogenetic diversity (a) and average functional diversity (b) in the communities in open areas (Control) and under *Bixa*, *Joannesia*, *Inga* and *Senna*. The vertical lines represent standard deviation. Different letters above the bars denote significant difference between treatments ($P < 0.05$).

Only the regenerating community under the species *Senna* differed from that expected at random for β TD (Table 1). The communities that regenerated under other species did not differ from the null model and formed a community similar to those found in open areas. The species that contributed most to the dissimilarity between *Senna* and open areas were: *Byrsonima sericea* DC., *Byrsonima stipulacea* A.Juss., *Acacia mangium* Willd., *Protium heptaphyllum* (Aubl.) Marchand subsp. *heptaphyllum*, *Anacardium occidentale* L., *Vernonanthura phosphorica* (Vell.) H.Rob., *Inga laurina* (Sw.) Willd., *Goniorrhachis marginata* Taub., *Byrsonima crassifolia* (L.) Kunth, *Terminalia kuhlmannii* Alwan & Stace, *Acacia auriculiformis* Benth., *Ceiba pubiflora* (A.St.-Hil.) K.Schum. Similarly, β FD of the regenerating communities below the tree species did not differ from the open areas except for the regenerated community under *Inga* (Table 1). This species causes the functional composition of species that regenerate under its canopy to be different from those that regenerate in open areas. The β FD of the regenerating community below *Senna* and *Joannesia*, and *Senna* and *Inga* were dissimilar. The phylogenetic composition (β PD) of the natural regeneration under the tree species did not differ from the open areas (Table 1).

Table 1. Pairwise comparisons of β diversity. Cells with the abbreviation TD or FD are significantly different than expected by random chance ($P < 0.05$). TD: taxonomic diversity and FD: functional diversity

	Control	*Bixa*	*Joannesia*	*Inga*	*Senna*
Bixa					
Joannesia					
Inga	FD				
Senna	TD		FD	FD	

The extracts of the tree species had significant effects on the germination rates of *L. sativa* ($P < 0.001$). In the control treatment 94% of the seeds germinated while only 1% of the seeds irrigated with the leaf extract of *Bixa* germinated. The seeds irrigated with extract of *Inga*, *Joannesia* and *Senna* had intermediate germination percentages (22, 22 and 30% of seeds, respectively), and did not differ significantly among themselves (Fig. 3).

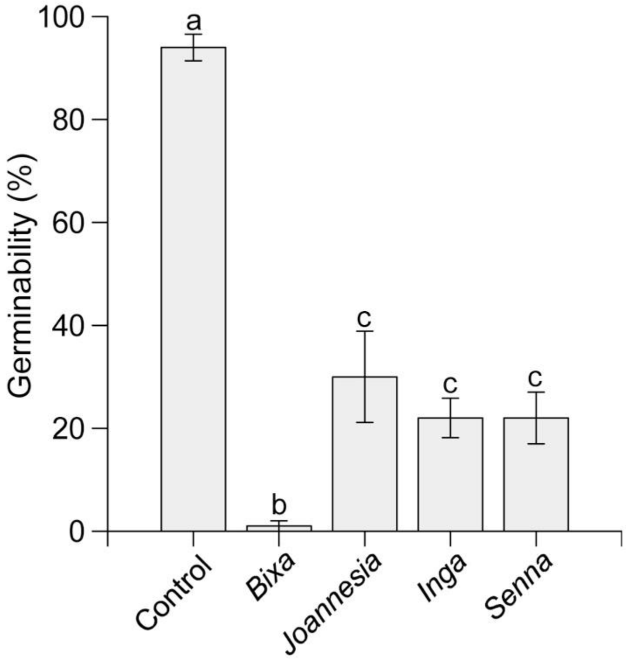

Fig. 3. Effect of the leaf extracts of *Bixa*, *Inga*, *Joannesia* and *Senna* on seed germination of *L. sativa*. The vertical lines represent standard error. Different letters above the bars denote significant difference between treatments ($P < 0.05$).

DISCUSSION

Contrary to what we predicted, the structure of the communities below the tree species was not governed by biotic interactions, both in phylogenetic and functional analyses. The mechanism of facilitation was expected to structure the communities, as previously observed in other studies (Carrión et al., 2017; Valiente-Banuet and Verdú, 2007; Verdú et al., 2009).

Communities in the early stages of succession, such as the community evaluated in this study, may have random structure for several reasons. Some authors have suggested that the phylogenetic pattern for tropical forests in the early years of succession is driven by stochastic factors, moving to clustered or overdispersed over the years (Letcher, 2010; Chang et al., 2015; Schweizer et al., 2015). However, other studies have indicated that stochastic factors are more important than deterministic factors in environments with higher productivity and higher species richness (Chase, 2010; Wang et al., 2010). Soliveres et al. (2012) postulated that climatic conditions along with biotic interactions affect the phylogenetic pattern, with one potentially overriding the other, thus leading to random structure. Similarly, Swenson and Equist (2009) found that a random phylogenetic pattern is not due to the lack of a functional mechanism, but instead is due to the simultaneous influence of abiotic filters and limiting similarity. They tested the dispersion of six functional plant traits, four of which were aggregated (leaf area, specific leaf area, leaf succulence and wood density) and two dispersed (height and seed mass).

Despite finding random structure for all communities under tree species, PD was higher below *Joannesia* and FD was higher under *Joannesia* and *Senna*, suggesting a mechanism of facilitation. In other words, the communities that regenerate under *Joannesia* and *Senna* are formed by species that cover a wide spectrum of functional characteristics, thus providing more ecosystem functions (Navarro-Cano et al., 2016). When species act as facilitators they can promote the establishment of other distantly related species, considering that in order to coexist they must differ in their regeneration niche. This mechanism organizes the community and enables an increase in PD and FD (Valiente-Banuet and Verdú, 2007; Navarro-Cano et al., 2016).

Restoration practices can be more efficient if phylogenetic information is considered (Montoya et al., 2012; Verdú et al., 2012). Using species with attributes of facilitators in restoration can benefit not only the recruitment of non-planted species, but also favor the dynamics of communities and ecosystem processes (Navarro-Cano et al., 2016; see also Le Stradic et al., 2014). For example, Navarro-Cano et al. (2016) observed that communities that regenerated below *Gypsophila struthium* Loefl. seven years after planting were phylogenetically diverse.

The comparison between tree species and open areas revealed that only the communities under the species *Senna* and *Inga* had β TD and β FD values, respectively, higher than expected by chance. It is important to note that this analysis allows us to infer that the β FD under *Senna* and *Inga* was higher than expected at random. Studied species modified the TD and FD of the communities formed below them, with the presence of species and functional attributes differing from those found in open areas. These characteristics are fundamental to ensure a successful restoration. The species that contributed the most to the dissimilarity between *Senna* and open areas were early and late secondary species, such as *B. stipulacea* and *P. heptaphyllum*. These two species were the second and fourth species, respectively, which contributed more to the dissimilarity between the *Senna* and open areas, and were found in greater abundance under *Senna*. However, other species, such as *A. occidentale*, a pioneer, which was the fifth most contributing species to the dissimilarity between the two areas, had greater abundance in open areas. The composition of species under facilitating plants can differ from that in open areas, as observed by Flores and Jurado (2003) and Schöb et al. (2013). Dissimilar communities between facilitating plants and in open areas are due to the environmental changes that occur under nurses. Such changes are highlighted by those involving: luminosity and temperature (Padilla and Pugnaire, 2006; Lima and Gandolfi, 2009), availability of water and nutrients (Koorem and Moora, 2010; Liu et al., 2014; Ramírez et al., 2015; Mihoč et al., 2016; Pueyo et al., 2016), protection against herbivores (Gómez-Aparicio et al., 2004) and reduction of competition with herbaceous species (Avendaño-Yáñez et al., 2014). Reduced stresses under facilitators creates an environment conducive to the germination and development of species that could not colonize open areas.

Species that comprise the upper stratum of a plant community form a cover that affects the diversity and structure of the lower stratum (Foré et al., 1997; Souza et al., 2015). In the present work, the species *Senna* and *Inga*, and *Joannesia* and *Inga*, seemed to form different microenvironmental conditions, because the communities under these species were functionally dissimilar. In these cases, the communities regenerating below these tree species are comprised of species that provide different ecosystem functions. Paterno et al. (2016) previously described the importance of high diversity in restored areas. In restored environments, beneficiary species make different associations with nurses, so the greater the diversity of species used in planting, the greater the different sets of beneficiary species, which increases species richness and β diversity (Paterno et al., 2016).

Another mechanism that could be acting in the structuring of the communities under the tree species of the studied restoration area is allelopathy, particularly below *Bixa*. Aqueous leaf extract of this species showed an inhibitory effect on the germination of *L. sativa* seeds, with only 1% of the seeds irrigated with this extract germinating. Although there is no record in the literature of possible allelopathic effects of *Bixa*, the present study, along with the reported low richness and abundance of individuals regenerating under *Bixa* (Spadeto et al., 2017), suggest an allelopathic effect. The community that regenerated below *Bixa* resembled the community formed in open areas, with respect to species richness and abundance. The allelopathic effect of a species can be evaluated by the relative density of plants regenerating below its crown and by seed germination tests in laboratories. The Mediterranean plant *Artemisia herba-alba* Asso was described as potentially having allelopathic effects because of the low relative density of plants that regenerated under its crown and the results of seed germination tests in laboratories. A later study carried out in natural areas (Arroyo et al., 2018) corroborated this hypothesis with the first signs of allelopathy and demonstrated the reduction of vitality of plants neighboring *A. herba-alba* (Arroyo et al., 2018).

Extracts of the other tree species resulted in intermediate germination. Previous work described the allelopathic potential of *Joannesia princeps* on the germination of cultivated species (Capobiango et al., 2008). In contrast, no allelopathic effect was recorded on the germination of seeds of *L. sativa* when they were irrigated with an extract produced with leaves of *Inga laurina* (Gonçalves et al., 2016).

All extracts reduced the germination of *L. sativa* seeds when compared to the germination of seeds irrigated with water alone. However, caution is needed when inferring allelopathic mechanisms, since they are complex and affect plant growth, soil microorganisms and resource availability (Viard-Crétat et al., 2012). In this sense, more studies should be performed with these species, especially *Bixa arborea*, and preferably in the natural environment.

We found evidence that facilitation structured the communities that regenerated under the studied tree species but only when we analyzed FD and PD and performed pairwise comparison of β diversity. We also found an evidence that the communities were influenced by the mechanism of allelopathy. In areas such as that of the present study, planted species directly influence the organization of communities that regenerate below their canopies. It is therefore important to choose species that can contribute to improving diversity, and thus favor the success of restoration projects.

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Appendix 1

Here we present details of divergence ages among nodes phylogenetic relationships upon which we built the phylogenetic tree with 96 species from Atlantic forest, Linhares - ES, southeastern Brazil.

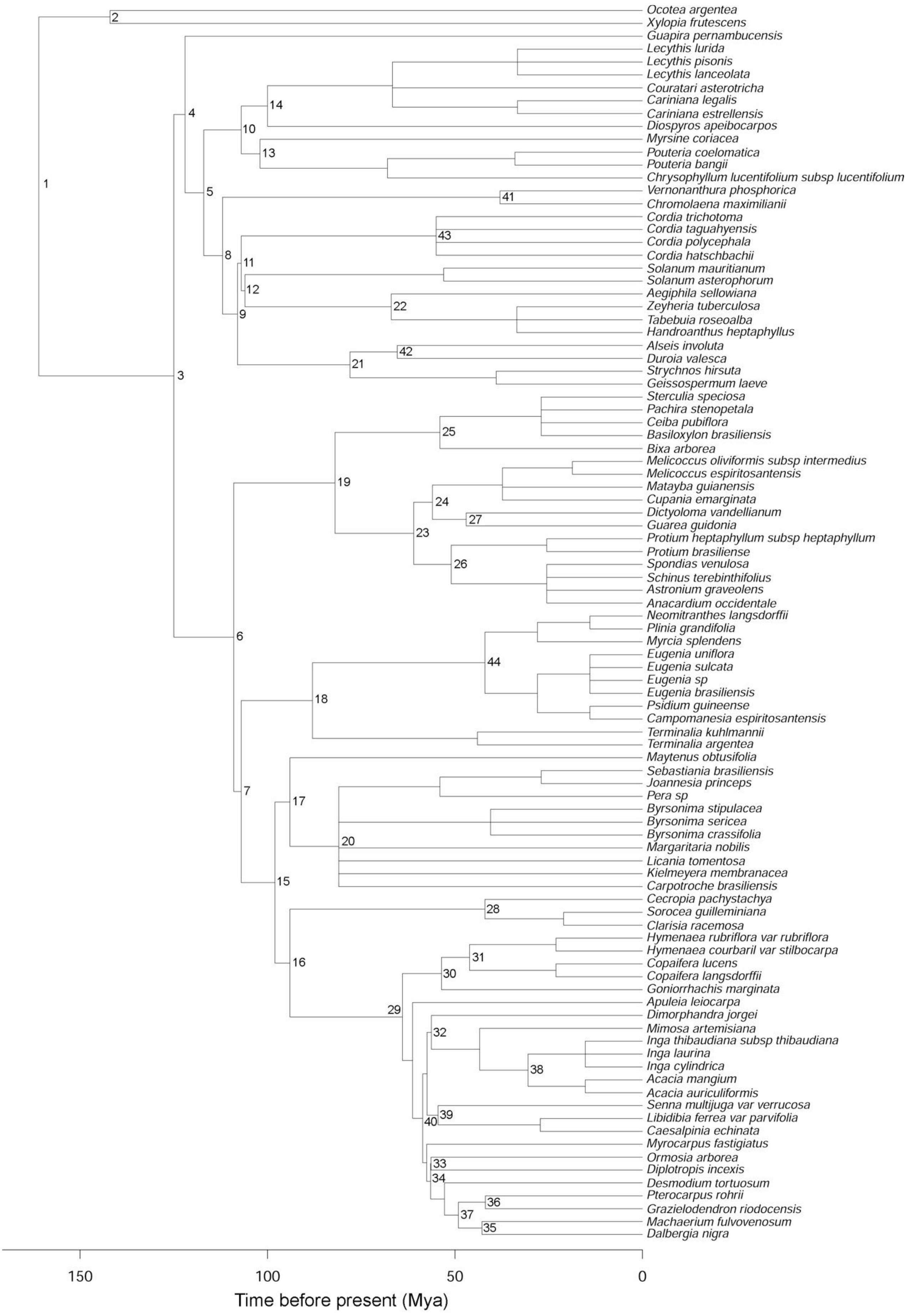
The initial topology of the phylogenetic tree of the 96 sampled species was obtained using the Phylomatic software (Webb and Donoghue, 2005) with the database of September 2015. Then we resolved polytomies within Euphorbiaceae (Wurdack et al., 2005), Myrtaceae (Lucas et al., 2007) and Fabaceae (Lavin et al., 2005; Bruneau et al., 2008). Then we estimated ages of 44 nodes based on several recent studies listed in Table S1. The ages were estimated to obtain more precise estimates of branch lengths. After this step, we positioned the non-dated nodes evenly between dated nodes using *bladj* algorithm of Phylocom software (Webb et al., 2008). Dated nodes and the phylogenetic tree are shown in Figure S1.

Table S1. Estimated age (in million years before present) of numbered nodes shown in the Figure S1.

node	estimated age	reference
1	161	Wikström et al. (2001)
2	142	Wikström et al. (2001)
3	125	Wikström et al. (2001), Forest & Chase (2009a)
4	122	Wikström et al. (2001), Forest & Chase (2009a)
5	117	Wikström et al. (2001)
6	109	Forest & Chase (2009b)
7	107	Forest & Chase (2009b)
8	112	Wikström et al. (2001)
9	108	Bremer (2009)
10	107	Bremer (2009)
11	107	Bremer (2009)

12	106	Bremer (2009)
13	102	Bremer (2009)
14	100	Bremer (2009)
15	98	Forest & Chase (2009b)
16	94	Forest & Chase (2009b)
17	94	Forest & Chase (2009b)
18	88	Forest & Chase (2009b)
19	82	Wikström et al. (2001)
20	81	Forest & Chase (2009b)
21	78	Bremer (2009)
22	67	Bremer (2009)
23	61	Wikström et al. (2001)
24	56	Wikström et al. (2001)
25	54	Wikström et al. (2001)
26	51	Wikström et al. (2001)
27	47	Wikström et al. (2001)
28	42	Forest & Chase (2009b)
29	64	Bruneau et al. (2008)
30	53.6	Bruneau et al. (2008)
31	46.1	Bruneau et al. (2008)
32	56.3	Bruneau et al. (2008)
33	56.4	Lavin et al. (2005)
34	56.5	Lavin et al. (2005)
35	42.8	Lavin et al. (2005)
36	41.9	Lavin et al. (2005)
37	49.1	Lavin et al. (2005)
38	30.5	Lavin et al. (2005)
39	54.5	Lavin et al. (2005)
40	58.6	Lavin et al. (2005)
41	38	Kim et al. (2005)
42	65.4	Bremer & Erikssom (2009)
43	55	Luebert et al. (2011)
44	42	Thornhill et al. (2012)

Fig. 1. Phylogenetic tree with the 96 species from Atlantic forest, Linhares - ES, southeastern Brazil. Dated nodes are numbered from 1 to 44.



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Considerações finais

A importância da interação de facilitação em áreas com estresse ambiental já foi reportada por diversos pesquisadores. Nestes ambientes as espécies facilitadoras melhoram as condições microambientais abaixo de suas copas e desse modo aumentam a diversidade de espécies oriundas da regeneração natural, a sobrevivência e o crescimento de mudas, plantadas.

Em florestas topicais, como a Mata Atlântica, as pesquisas no campo de facilitação são mais recentes e já foi observado, por exemplo, maior diversidade funcional e filogenética, e maior taxa de germinação de sementes e crescimento de plântulas sob a copa de algumas espécies arbóreas quando comparada a áreas abertas. Nas florestas tropicais nem sempre é possível observar mudanças em todas as variáveis ambientais avaliadas, mas a redução das elevadas taxas de luminosidade já foram descritas. A diferença de luminosidade entre áreas abertas e áreas sob as facilitadoras pode acarretar em melhorias na germinação de sementes e crescimento das plântulas.

Os atributos das facilitadoras, as transformações ambientais abaixo de suas copas e como elas favorecem as interações entre plantas sugerem a importância na escolha das espécies a serem implantadas nos projetos de restauração florestal. Estas informações (atributos, mudanças abióticas e interação com outras espécies) devem ser conhecidas anteriormente ao plantio, assim como, se as espécies a serem utilizadas no plantio produzem compostos que inibem ou retardam a germinação e o crescimento de outras espécies abaixo de suas copas. Caso estes cuidados sejam tomados, mais eficiente será o processo de sucessão natural nestas áreas e maior número de espécies poderá regenerar nestes ambientes.