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Green manure cover effects on restoration success in Southeast Atlantic Forest biome

Efeitos da cobertura de adubação verde no sucesso da restauração no bioma Mata Atlântica do Sudeste

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Abstract

Active restoration techniques, such as direct sowing, is an important strategy to benefit forest structure formation and to inhibit the exotic grasses invasion, which is an impediment to many restoration projects. The use of green manure in sowing can improve soil cover conditions and inhibit grasses. Thus, in this study we aimed to investigate the role of green manure canopy on invasive grass species' cover and on growing of native species seedlings in direct sowing sites in the Southeast Atlantic Forest biome. We hypothesized that green manure would suppress the cover of exotic grasses and would benefit native species' seedling growth. The study monitored six plots of 25 m x 4 m in a restored area (by direct sowing in pits) belonging to the Atlantic Forest biome, located in Cruzeiro municipality, in February 2021, June 2021 and January 2022. Measurements of individuals were the circumference at breast height and the plant height. We also inventoried per pit: canopy diameter, canopy cover and invasive grass cover. We found that a higher green manure canopy cover (regarding plant size and canopy) was responsible for decreasing invasive grass species cover, namely *Brachiaria* species. In addition, some species had higher canopy cover that suppressed invasive grasses more efficiently. We did not find a positive effect of green manure on native species seedling growth. However, the change from dry season to wet season in canopy cover, together with invasive grasses presence, negatively affected the growth of native species seedlings. These results provide important guidelines and indicate the role of the direct sowing technique for decreasing exotic grasses invasion and the establishment of native species.

Keywords: Permanent preservation area; Ecological restoration; Direct seeding; Native species.

Resumo

Técnicas ativas de restauração, como a semeadura direta, são uma importante estratégia para beneficiar a formação da estrutura florestal e inibir a invasão de gramíneas exóticas, que é um impedimento para muitos projetos de restauração. O uso da adubação verde na semeadura pode melhorar as condições de cobertura do solo e inibir as gramíneas. Assim, neste estudo o objetivo foi investigar o papel da adubação verde na cobertura de gramíneas invasoras e no crescimento de plântulas de espécies nativas em locais de semeadura direta no bioma Mata Atlântica do Sudeste. A hipótese é que a adubação verde suprima a cobertura de gramíneas exóticas e beneficie o crescimento de plântulas de espécies nativas. O estudo monitorou seis parcelas de 25 m x 4 m em uma área restaurada (por semeadura direta) no bioma Mata Atlântica, localizada no município de Cruzeiro, nos meses de fevereiro e junho de 2021 e janeiro de 2022. Foram medidas a circunferência em altura do peito e a altura da planta em todos os indivíduos. Também foram inventariados o diâmetro do dossel, a cobertura do dossel e cobertura de gramínea invasora por cova. Os resultados mostraram que a maior cobertura do dossel de adubo verde (em relação ao tamanho da planta e da cobertura de copa) foi responsável pela diminuição da cobertura

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de espécies de gramíneas invasoras, nomeada espécies de *Brachiaria*. Além disso, algumas espécies apresentaram maior cobertura de dossel que suprimiu as gramíneas invasoras de forma mais eficiente. O estudo verificou que a adubação verde não beneficiou o crescimento de plântulas de espécies nativas. Foi observado que a mudança de estação seca para estação chuvosa na cobertura do dossel, juntamente com a presença de gramíneas invasoras, afetou negativamente o crescimento de mudas de espécies nativas. Esses resultados fornecem diretrizes importantes e indicam o papel da técnica de semeadura direta, especialmente relacionada à diminuição da invasão de gramíneas exóticas e estabelecimento de espécies nativas.

Palavras-chave: Área de preservação permanente; Restauração ecológica; Semeadura direta; Espécies nativas.

INTRODUCTION

The Atlantic Forest biome, a biodiversity hotspot (Myers et al., 2000), has only 13% of its native vegetation cover remaining in Brazil (Fundação SOS Mata Atlântica, 2017) due to intense deforestation and human disturbance that occurred mostly in the first half of the 19th century (Dean, 1996). Nature reserves protect only 9% of the native forest left (Ribeiro et al., 2009). Lately, demographic and market shifts resulted in land abandonment on portions of cattle ranches and farms that are less suitable for agriculture, which in turn have regenerated back to forest (Silva & Vieira, 2017). At the same time, the Atlantic Forest biome has become an important global conservation and restoration focus (Brancalion et al., 2019b), with efforts of nongovernmental organizations to recover native Brazilian ecosystems (World Resources Institute, 2018) and local restoration initiatives.

Ecological restoration is “the process, active or passive, of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed” (Society for Ecological Restoration, 2004). Active and passive restoration are two important strategies to aid the recovery of large areas of deforested and degraded tropical lands (Morrison & Lindell, 2010). Revisions to the National Forest Code (Lei 12.651/Brasil, 2012) increased legal requirements (using a rural environmental registration policy) for forest recovery and conservation in areas with forest deficits like the Atlantic Forest biome (Soares-Filho et al., 2014), especially in riparian areas, hilltops, slopes, high elevations and certain types of ecosystems (the so called Permanent Preservation Areas: PPA or APP, Portuguese acronym), which have the environmental function of preserving water resources, landscape, geological stability and biodiversity, facilitating gene flows of fauna and flora, protecting soil and ensure the well-being of human populations (Brasil, 2012).

Among active restoration methods, total planting of seedlings in total area has been mostly used in Atlantic Forest restoration (Brancalion et al., 2019a), while direct sowing is usually being implemented in Cerrado areas (Sampaio et al., 2019). Direct sowing (popularly called “muvuca de sementes”) relies on a high density mixture of seeds of major functional/ecological groups sown in well prepared soil, pits, or lines (Campos-Filho et al., 2013; Rodrigues et al., 2019). The main ecological advantage of direct sowing sites is that they present higher plant density in the early stages, which could benefit forest structure formation (Antoniazzi et al., 2021) and to inhibit exotic grass and herbs invasion that spread quickly, which is a problem to many restoration projects (Mantoani & Torezan, 2016). Challenges are related to seed limitation and seedling mortality and establishment of native species in open areas (Silva et al., 2015).

Using green manure species in direct sowing can improve restoration sites promoting soil chemical, physical and biological recuperation (Sultani et al., 2007; Viani et al., 2015), besides consistently diminishing restoration costs during the maintenance phase due to the reduction of fertilization and individual protection (Vásquez-Castro et al., 2020). However, its effects on cover of invasive grasses and on growing of native species seedlings at early stages in direct seeding sites are less clear.

Direct sowing techniques might not be adaptable to all restoration possibilities. Biotic conditions associated to functional traits of seeds, predation and competition with alien species, and abiotic ones related to climate are constraints to be considered in direct sowing

(Silva & Vieira, 2017). There have been recent discussions on the role of direct sowing on Atlantic Forest restoration initiatives (Cole et al., 2011; Meli et al., 2017; Souza et al., 2021). However, the only way to understand that is through assessment and monitoring (Rodrigues et al., 2009) of "muvuca" restoration projects in these ecosystems. In the Southeast Atlantic Forest, concerns are related to effects of green manure (density and cover) on focal native species. Thus, in this study we aimed to investigate the role of green manure on invasive grass species cover and on growing of native species seedlings in direct sowing sites in the Southeast Atlantic Forest biome. We hypothesized that green manure would suppress the cover of exotic grasses and it would benefit native species seedling growth. These results provide important information for restoration success, related to decrease in exotic grass invasion and establishment of native species, in the Atlantic Forest biome (Rodrigues et al., 2015).

MATERIAL AND METHODS

This study was conducted on Permanent Preservation Area in two cattle ranches, located between the coordinates 22°31'59.1"S and 44°55'46.4"W to 22°32'20.0"S and 44°55'40.3"W, in Cruzeiro municipality, which has 304,572.00 km^2 and 82,571 inhabitants (Instituto Brasileiro de Geografia e Estatística, 2020), Southeast Atlantic Forest biome, São Paulo state, Brazil (Figure 1). The region has a strong hilly relief (Empresa Brasileira de Pesquisa Agropecuária, 1979), close to Serra da Mantiqueira mountains, and the soil is red-yellow latosol (allic or dystrophic, clayey texture: Brasil, 1960). The climate is classified as dry-winter subtropical (Köppen Cwa, reviewed by Alvares et al., 2013), with an annual mean temperature of 21.2°C, an average annual precipitation of 2169 mm, and a dry season between April and September (Climate-Data.org, 2021). The ranches are within the Atlantic Forest biome (transition between evergreen and deciduous forest). The region has two large national protected areas (Área de Proteção Ambiental da Serra da Mantiqueira and Área de Proteção Ambiental da Bacia Hidrográfica do Rio Paraíba do Sul). From the hills and mountains of the landscape drain many streams and rivers that run into the Paraíba do Sul River, providing water to the two biggest Brazilian cities (São Paulo and Rio de Janeiro).

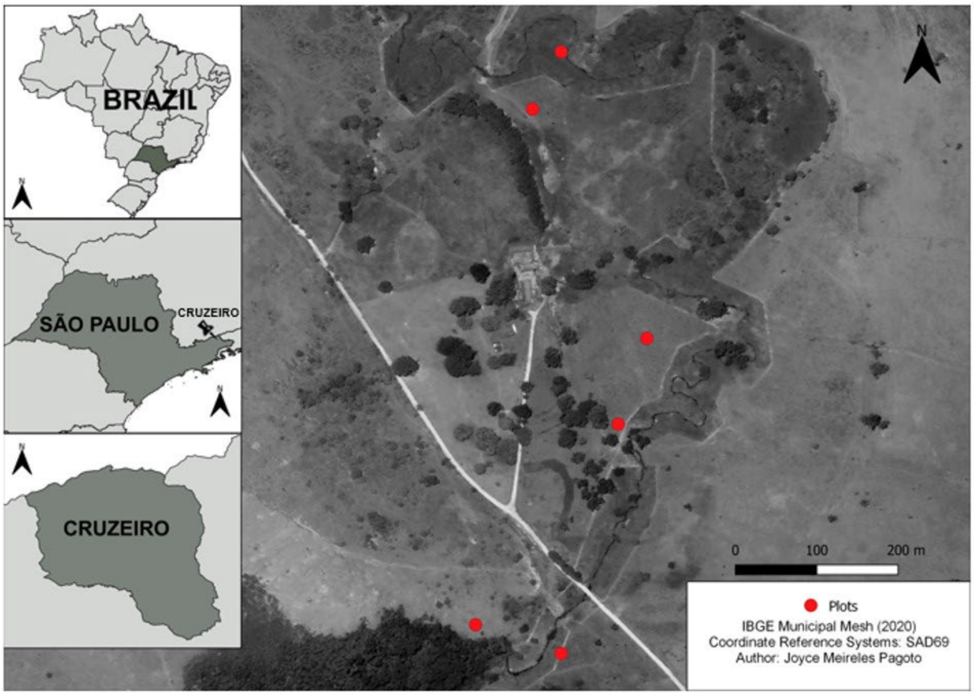

Figure 1. Satellite image of the study site, inside Cruzeiro municipality, São Paulo state, Brazil, showing plot locations.

Among them, Água Limpa River, where the cattle ranches are located, is a priority region for conservation and restoration of the Paraíba do Sul River basin (Associação Pró-Gestão das Águas da Bacia Hidrográfica do Rio Paraíba do Sul, 2017). Upstream Água Limpa River is well conserved with many forest areas, but downstream it is a degraded and silted river, almost without riparian forests (Fundo Estadual de Recursos Hídricos, 2019). Restoration sites in both ranches were pastures for cattle and buffalo before planting.

Thus, in 2019, a restoration project was approved by Fehidro (Fundo Estadual de Recursos Hídricos, number 022/2020), a São Paulo State Water Resources Fund, which aims to finance programs and actions in the area of water resources, in order to promote improvement and protection of water bodies and their hydrographic basins. The project comprised a total planting of seedlings in 22.76 ha, agroforestry systems in 0.67 ha and 10.25 ha of direct sowing. We inventoried only direct sowing areas. Sowing used 50 kg of green manure and 50 kg of native species seeds, in pits spaced 2 m x 2 m. Each pit received an average of 35 seeds of green manure species and 10-15 native species seeds. Sowing was performed in May 2020, after soil preparation, which consisted of chemical mowing, followed by 60 cm weeding, application of pesticides for ants, fertilization, soil liming, 30 cm deep ditching and hydrogel application. Exotic grass control used 300 ml of herbicide (glyphosate) in a 20 liter-pump up sprayer (3 to 4.2 liters of herbicides were applied per hectare). Ant control was carried out 30 days before planting using 5kg per hectare of powder and granulated formicide bait, along ant paths and nests. Fertilization doses were 100 g of 8-28-16 (N-P-K) per pit and 400 g of limestone per pit, due to soil degradation conditions. Before planting, seeds were stored in non-ideal conditions, which made them lose vigor.

In February 2021 we randomly established six 25 m × 4 m plots, according to the monitoring protocol of the State Resolution SMA 32 (São Paulo, 2014). All planted individuals per pit inside the plots were tagged, identified, and measured. For plant family classification, we used the Angiosperm Phylogeny Group IV and the Brazil Flora List (Jardim Botânico do Rio de Janeiro, 2010). Inventories were carried out in February 2021 (survey 1 (I1), wet season), June 2021 (I2, dry season), and January 2022 (I3, wet season) to understand differences between seasons. Measurements of individuals were the circumference at breast height (only in I3: CBH3) and the plant height (in I2 and I3, thus H2 and H3). We also inventoried per pit: canopy diameter (CD: I2 and I3, thus CD2 and CD3), canopy cover (CC: I2 and I3, thus C2 and C3) and invasive grass cover (IC: I2 and I3, thus IC2 and IC3). Canopy cover and cover of invasive grasses was quantified using the Canopeo App for phones (Patrignani & Ochsner, 2015).

We fitted generalized linear mixed-models (GLMMs), using a Poisson distribution, to assess the effects of canopy (namely canopy cover and canopy diameter) and plant size (CBH and height) on cover of invasive grasses. In addition, we performed GLMMs to verify the effects of canopy (namely canopy cover and canopy diameter) on growing of native species seedlings. Analyses were performed in R version 3.6.3 (R Core Team, 2019) using lme4 package.

RESULTS AND DISCUSSION

We sampled 167 individuals in the direct sowing sites, belonging to 16 species and 9 families (Table 1). Most individuals were Fabaceae species, used as green manure, including some native species (Table 1). In addition, 145 seedlings of native species were recorded in the whole study period (Supplementary Material 1), of which 99 could not be identified due to size and classification limitations and 25 individuals were *Dahlstedtia pinnata* (Benth.) Malme. (Fabaceae), a shrub native to the Atlantic Forest biome. Despite this high number of seedlings, due to several losses, only 21 could be sampled two times (in I2 and I3).

Table 1. Composition of direct sowing sites, regarding family, species, N (number of individuals) and whether green manure or native in the Southeast Atlantic Forest biome, Brazil.

Family	Species	N	Green manure	Native species
Anacardiaceae	*Schinus terebinthifolia* Raddi	2		x
Bignoniaceae	*Cybistax antisyphilitica* (Mart.) Mart.	1		x
	Jacaranda brasiliana (Lam.) Pers.	7		x
Fabaceae	*Cajanus cajan* (L.) Huth	16	x	
	Canavalia ensiformis (L.) DC.	1	x	
	Crotalaria juncea L.	13	x	
	Erythrina velutina Willd.	1		x
	Schizolobium parahyba (Vell.) Blake	4		x
	Senna alata (L.) Roxb.	69	x	x
Euphorbiaceae	*Mabea fistulifera* Mart.	3		x
	Sapium glandulosum (L.) Morong	1		x
Malvaceae	*Hibiscus* L.	1	x	
Myrtaceae	*Eugenia uniflora* L.	1		x
Polygonaceae	*Ruprechtia laxiflora* Meisn.	10	x	
Solanaceae	*Solanum mauritianum* Scop.	29	x	x
Urticaceae	*Cecropia pachystachya* Trécul	4		x
	Unidentifed	4		

Plant size of green manure species negatively influenced the cover of invasive grass in the last survey (DBH: z=-20.16, p<0.001; and H: z=-8.87, p<0.001). Canopy diameter of green manure did not affect grass cover in the second survey, in the dry season (z=1.11, p=0.266), but it did in the last survey in the wet season (z=-7.68, p<0.001). Canopy cover of green manure, on the other hand, negatively influenced invasive grass cover on both inventories (I2: z=-9.55, p<0.001 and I3: z=-24.42, p<0.001; Figure 2). When species were considered we found an influence of canopy cover on grass cover in the third inventory (CC3: z=-26.34, p<0.001 and species: z=9.90, p<0.001; Figure 3), but not on the second (CC2: z=-8.72, p<0.001 and species: z=0.35, p=0.725). *Senna alata* and *Solanum mauritianum* (both shrub species native of the Brazilian flora) had the biggest canopy covers and affected negatively invasive grass cover (Figure 3).

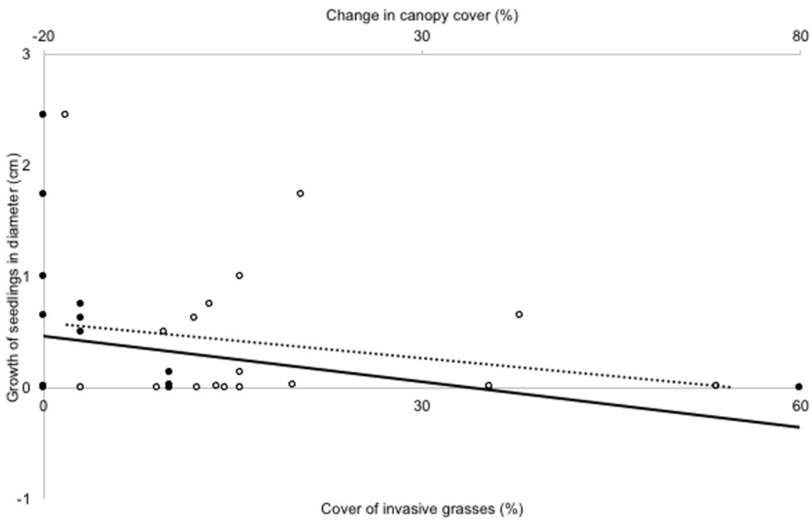

Figure 2. Relationship between canopy of green manure species and cover of invasive grasses in direct seeding areas in the Southeast Atlantic Forest biome, Brazil.

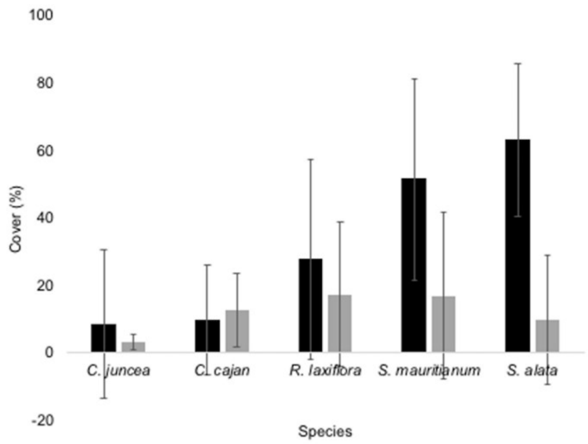

Figure 3. Cover of canopy (black) and of invasive grasses (gray) according to the most abundant species in direct seeding areas in the Southeast Atlantic Forest biome, Brazil.

Regarding the effects of canopy on growing of native species seedlings, we found that canopy diameter on the last inventory did not affect growing of seedlings in height (z=-0.40, p=0.692) and diameter (z=-0.37, p=0.713). Canopy cover also had no effect on the growth of seedlings in height (z=-0.30, p=0.765) and diameter (z=0.12, p=0.903) on the final sample.

We also analyzed the effects of canopy diameter (CD3-CD2) and canopy cover (CC3-CC2) changes on growing of native species seedlings and no effects were observed: for height (CD change: z=-0.40, p=0.693 and CC change: z=-0.38, p=0.705) and diameter of seedlings (CD change z=-1.31, p=0.191 and CC change: z=-1.13, p=0.257), respectively. When we added invasive grass cover to the models, our results showed that growing of native species seedlings (in diameter, but not in height) was negatively affected by cover of green manure species (z=-2.09, p=0.037) and also of invasive grass species (z=-2.07, p=0.038; Figure 4).

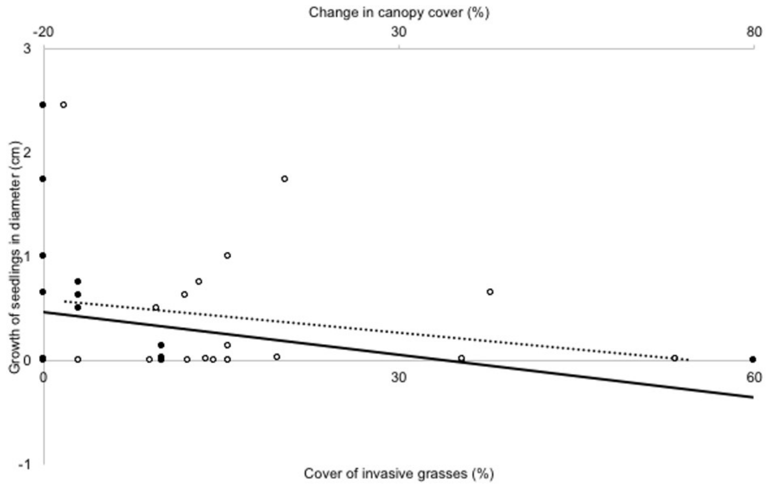

Figure 4. Effects of cover of invasive grasses (black dots and line) and change in canopy cover of green manure species (white and dotted line) on the growth in diameter of native species seedlings, in the Southeast Atlantic Forest biome, Brazil.

Weed control is being recommended in several restoration projects, resulting in higher growth and survival of rainforest planted trees (Campoe et al., 2010; Brancalion et al., 2019a; Fiore et al., 2019). In this study, we showed that the green manure canopy cover (regarding plant size and canopy) was responsible for decreasing invasive grasses cover. Thus, the fast growing of Fabaceae species (such as *Cajanus cajan* and *Senna alata*), which can be native or not, shrubs or trees, contributed to soil cover, shadow and weed control, as other studies have shown (Pellizzaro et al., 2017; Fiore et al., 2019) and might promote soil chemical, physical and biological recuperation (Sultani et al., 2007). After more than a year of sowing (in June 2021 and January 2022 inventories), several green manure species with short life cycle, have been replaced in the system and helped to decrease the cover of *Brachiaria* sp., extensively used as pasture grass in the region. Some of these species, especially *Senna alata* and *Solanum mauritianum*, both fast growing native species of the Brazilian flora, were still there in the last inventory.

Despite that, we did not find an effect of green manure canopy cover and diameter on growing of seedlings during the study period. As we are dealing with a technique by which seeds lose vigor fast, direct sowing requires a short period of seed storage. Thus, we argue that the number of studied seedlings was limiting. In addition, some disturbances happened in 2021 (such as cattle invasion, floods and frost) that made lots of seedlings die along inventories (from 145 only 21 had data for two sample dates). Thus, this result of no relations between variables should be taken with caution.

We found that the change in green manure cover from one season to another (from dry to wet), making canopies resprout, with invasive grass species presence, negatively affected growth of the remaining native species seedlings. These seedlings were mostly pioneer species that needed open environments to grow (Rodrigues et al., 2015) and that might explain that result. Again, researches should be expanded to other direct sowing sites in the Atlantic Forest biome, but it might indicate the need of pruning to diminish cover of green manure species and benefit seedlings growth, after at least a year of planting and in the wet season.

In the study region there has been an intense debate on direct sowing application of the technique in Atlantic Forest biome. Despite other well-known active restoration techniques, such as total planting of seedlings (Brancalion et al., 2019b), direct sowing studies in the biome are still scarce.

CONCLUSIONS

We hypothesized that green manure would suppress the cover of exotic grasses, which we found. In addition, some species had higher canopy cover that suppressed invasive grass species more efficiently. We also expected that green manure would benefit native species seedlings growing, but we did not find associations between them. However, the change from dry season to wet season in canopy cover, together with invasive grasses presence, negatively affected the growing of seedlings of native species. These results provide important guidelines and indicate the role of direct seeding for decreasing exotic grass invasion and establishment of native species.

Direct sowing studies in the Atlantic Forest biome are still scarce and this study brings important and novel contributions to filling this gap, especially in Permanent Preservation Areas that may accomplish the mandatory Environmental Regularization Program (PRA) of rural land in São Paulo state from 2022.

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SUPPLEMENTARY MATERIAL

Supplementary material accompanies this paper.

Supplementary Material 1. Green manure individuals, canopy diameter, canopy cover and cover of invasive grasses that had seedlings of native species under them and the number of these individuals, in direct seeding sites the Southeast Atlantic Forest biome, Brazil.

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