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Natural regeneration of woody and herbaceous species in ecological restoration areas in the Atlantic Forest

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ABSTRACT: Natural regeneration of forests is one of the most important tools for inferring about the behavior of forest restoration processes. In this context, the objective of this study was to evaluate the natural regeneration in an area of Dense Rainforest of the Lowlands after 8 years of implementation of the restoration project. The area consisted of six treatments (factorial 3 × 2), with three levels of species richness, two spacings and three repetitions, in randomized blocks. In each treatment and in the mature forest three plots of 5 × 10 m were demarcated, identifying all woody and herbaceous individuals. Floristic diversity (H') and equability (J') were calculated, in addition to the Distended Correspondence Analysis (DCA). The floristic diversity and equability were lower in areas undergoing restoration (H' = 1.82 nats indiv^{-1} and J' = 0.51). The DCA resulted in the formation of distinct groups, which differ from the mature forest of the area under restoration. In all treatments, a large number of herbaceous individuals were registered (3,963), which may have negatively influenced the establishment of woody individuals (351). The statistics revealed that the shared treatments of many woody and herbaceous species, reinforcing that planting with greater richness of species is no guarantee of success in establishing natural regeneration with high diversity.

Key words: reference ecosystem; secondary succession; tabuleiro forest

Regeneração natural de espécies lenhosas e herbáceas em áreas de restauração ecológica na Floresta Atlântica

RESUMO: A regeneração natural das florestas é uma das mais importantes ferramentas para inferir sobre o comportamento dos processos de restauração florestal. Neste contexto, o objetivo deste estudo foi avaliar a regeneração natural em uma área de Floresta Ombrófila Densa das Terras Baixas após 8 anos da implantação do projeto de restauração. A área foi composta por seis tratamentos (fatorial 3 × 2), sendo três níveis de riqueza de espécies e dois espaçamentos) e três repetições, em blocos casualizados. Em cada tratamento e na floresta madura foram demarcadas três parcelas de 5 × 10 m, identificando todos os indivíduos lenhosos e herbáceos. Foi calculada a diversidade florística (H') e a equabilidade (J'), além da Análise de Correspondência Distendida (DCA). A diversidade florística e equabilidade foram baixas nas áreas em processo de restauração (H' = 1,82 nats indiv^{-1} e J' = 0,51). A DCA resultou na formações de grupos distintos, que diferem a floresta madura da área em processo de restauração. Em todos os tratamentos foi registrado grande número de indivíduos herbáceos (3.963), o que pode ter influenciado negativamente no estabelecimento dos indivíduos lenhosos (351). As análises revelaram que os tratamentos compartilham muitas espécies arbóreas e herbáceas, reforçando que o plantio com maior riqueza não é garantia de sucesso do estabelecimento da regereração natural com alta diversidade.

Palavras-chave: ecossistema de referência; sucessão secundária; floresta de tabuleiro

Introduction

The Atlantic Forest is considered a hotspot for conservation priority, being one of the richest areas on the planet in biodiversity. However, this richness is threatened due to a long history of uncontrolled exploitation of natural resources, as a result of different types of land use (Joly, 2018). Thus, several restoration projects are implemented in order to recover the biodiversity of this biome (Brancalion et al., 2010).

The restoration of an ecosystem aims to reestablish ecological processes of biodiversity conservation and environmental services, as well as promoting succession as close to nature as possible (Reis et al., 2007; Brancalion et al., 2010). These artificial restoration processes are challenging, since nucleation techniques are developed at different succession rates (Reis et al., 2007).

Natural regeneration plays an important role in community development in forest restoration areas, thereby contributing to the floristic recomposition of the affected area (Chazdon & Guariguata, 2016). Promoting natural regeneration in restoration methodologies is an alternative to reforestation, since it is a low-cost procedure. In addition, the regeneration stratum contributes to maintaining already established forest environments, as with the canopy senescence there is a gradual replacement by individuals from this stratum (Brancalion et al., 2012).

Thus, it is important to consider the matrix in which the area under restoration is inserted (Martins et al., 2012), due to the possibility of the influence of nearby forest remnants on the increase of wealth and diversity (Chazdon et al., 2009). In addition, the majority of these are preserved forests or with minimal anthropic intervention (Numata et al., 2017).

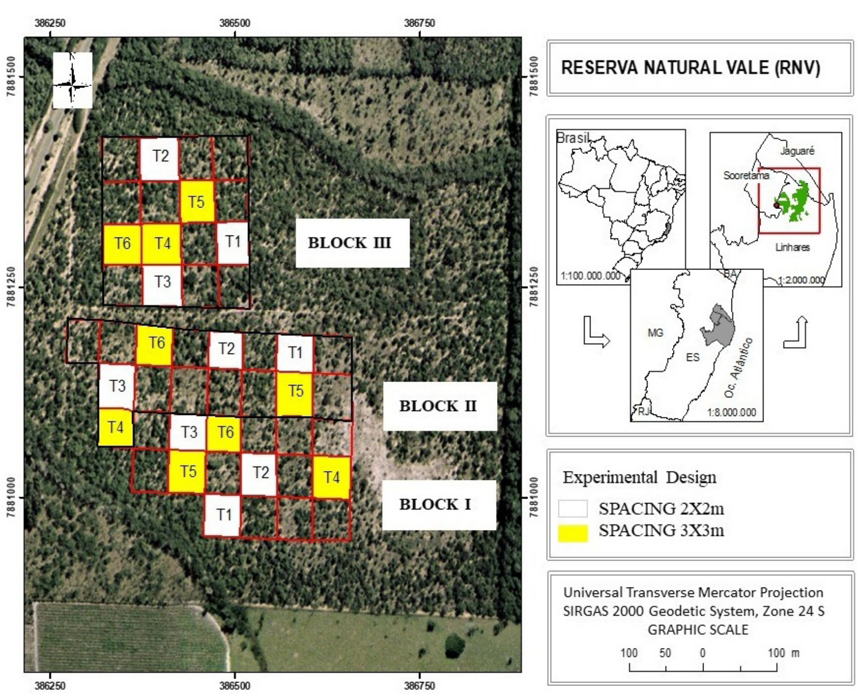
Natural regeneration contributes to verifying the future forest development and defining its successional stage, whereas in areas undergoing restoration, the evaluation of regeneration allows the analysis of whether the previously proposed objectives are being achieved (Schorn & Galvão, 2006; Miranda Neto et al., 2012). Therefore, it can be assessed if there is a need for intervention, in order to accelerate the restoration and succession process of the area (Martins & Kunz, 2007). Thus, long-term monitoring and evaluation of areas under restoration are important processes to be considered (Brancalion et al., 2012).

Due to the use of natural regeneration assessment for understanding community patterns (Trentin et al., 2018) and knowing the difficulties involved in restoration processes, the aim of this work was to assess the natural regeneration of a remnant of the Atlantic Forest after 8 years of installing a restoration project, in order to contribute to the understanding of restoration of degraded areas. For this purpose, the following hypotheses were tested: (i) the greatest floristic similarity can be observed among treatments of the area under restoration, (ii) the greatest floristic dissimilarity will be observed between treatments of the area under restoration and the mature forest, (iii) the percentage of grasses will be inversely proportional to the number of arboreal individuals sampled in the area under restoration.

Materials and Methods

Study area

The study was conducted in Reserva Natural Vale (RNV) (18° 08’ 25’’ S and 40° 04’ 21’’ W) located in the municipalities of Sooretama, Linhares and Jaguaré, in northern Espírito Santo State, Brazil (Figure 1). The study area is part of a forest



Source: Juvanhol (2014).

Figure 1. Location of the study area and the Reserva Natural Vale in the state of Espírito Santo. T1: Treatment 1 (29 species; spacing of 2 × 2 m); T2: Treatment 2 (58 species; spacing of 2 × 2 m); T3: Treatment 3 (114 species; spacing of 2 × 2 m); T4: Treatment 4 (29 species; spacing of 3 × 3 m); T5: Treatment 5 (56 species; spacing of de 3 × 3 m) e T6: Treatment 6 (114 species; spacing of 3 × 3 m)

restoration project implemented in 2005 by RNV. The climate of the region is classified as Aw type, according to the Köppen classification, as tropical hot and humid, with well defined seasons (rainy summer and dry winter). The average annual minimum and maximum temperatures are 19.6 °C and 32.0 °C respectively, while the average temperature is 23.4 °C. The average annual precipitation is 1.193 mm.year^{-1} (Viana et al., 2011).

The reserve has a forest remnant of approximately 22,000 ha, composed of a varied vegetation cover, with native grassland, Muçununga and Tabuleiro forests, where the Dense Ombrophylous Forest of the Lowlands stands out (IBGE, 2012). Besides its vast extent under the Atlantic Forest domain, the remnant forms an important ecological corridor together with the Sooretama Biological Reserve.

The forest restoration project was installed in a 4,5 ha area of the Reserva Natural Vale. Prior to the installation of the project in this area, the land use was for planting *Eucalyptus* sp. The wood extraction left the area abandoned for approximately 25 years, being later occupied by *Urochloa* sp. During this period a process of natural succession was initiated with the emergence of some regenerating trees, which were maintained in the project. The seedlings were planted in January 2005.

Data collection

Experimental design was conducted in randomized blocks design, in a 3 × 2 factorial arrangement, three levels of species richness (29, 58 and 114) and two of spacing (2 m × 2 m and 3 m × 3 m), with three repetitions, totaling 18 plots with dimensions of 50 m x 50 m each (2,500 m^2). Table 1 describes the different treatments used.

In order to assess the natural regeneration stratum in the different treatments, three 5 × 10 m (50 m^2) subplots were installed in the centre of each plot. A mature forest located 200 m from the experimental site was considered as a reference ecosystem, where three subplots with the same dimensions were installed.

The height and diameter of all regenerating shrub-arboreal and woody lianas individuals greater than or equal to 30 cm up ground were measured, as well as the diameter at breast height less than 5 cm. The herbaceous individuals were only quantified and all botanical material from the regenerating individuals was collected.

Table 1. Description of the treatments in terms of richness and spacing used in the forest restoration project in Reserva Natural Vale, ES.

Treatments	Species richness	Spacing
T1	29	2 × 2 m
T2	58	2 × 2 m
T3	114	2 × 2 m
T4	29	3 × 3 m
T5	58	3 × 3 m
T6	114	3 × 3 m

Data analysis

The identification of the species was carried out using specialized bibliography, by comparison and, whenever necessary, with the aid of specialists. The Angiosperm Phylogeny Group - APG IV (APG IV, 2016) system was used for classifying the botanical families of the species, while the appropriate scientific nomenclature was verified by means of the list of Flora do Brasil 2020 (Flora do Brasil 2020 under construction, 2020). The species were classified according to their life habit as tree, shrub, herbaceous, liana (woody or herbaceous), grass and palm.

For herbaceous plants in which individuals could not be separated, the Braun-Blanquet (1932) scale of coverage and abundance was adopted in this study. The percentage of grass cover in the soil was estimated visually within the demarcated plots, and in the field record only the scale corresponding to the coverage of these individuals was noted.

The Shannon-Weaver diversity index (H'), the Pielou equation (J') and the absolute density (adapted from Durigan's proposal, 2003) have been estimated for the entire regenerating community and individually for woody and herbaceous species. All analyses were performed through the PAST 3.26 software (Hammer et al., 2001).

Floristic similarity relationships between the areas were investigated by means of a Distended Correspondence Analysis (DCA), building an abundance matrix of treatment species and mature forest, considering all the woody species that had occured. A Cluster Analysis was performed employing the Mortisita-Horn index (Felfili et al., 2011). Subsequently, a Similarity Analysis permutation test (ANOSIM) was conducted using the Bray-Curtis dissimilarity matrix. The analyses were implemented in PAST software version 3.26 (Hammer et al., 2001).

Pearson correlation analysis was applied to each plot to assess the correlation between the presence of grasses and regenerating individuals, using the variables of grasses percentage and number of regenerants (trees, shrubs and woody vines). The correlation was calculated in electronic worksheets and tested by means of t-statistics at 5% significance level, using Microsoft Excel® 2013.

The species were classified according to the succession group (pioneer, early secondary and late secondary) and to the dispersion syndrome (zoochoric, anemochoric and autochoric).

Results and Discussion

In total, considering the area under restoration and the mature forest, 4,561 individuals belonging to 159 species and 52 families were sampled. Among these, 4,314 individuals from 89 species belong to the area in the process of restoration, while 247 individuals from 77 species were sampled in mature forest (Table 2).

Among the families with the highest values for species richness in the area under restoration were Fabaceae (33 spp.), Myrtaceae (20) and Asteraceae (7), of which species

Table 2. List with the most abundant species of natural regeneration sampled in the six treatments (T) and in the mature forest (MF), regarding life habit (LH), succession group (SG) and dispersion syndrome (DS), Reserva Natural Vale, ES.

Family/Species	LH	SG	DS	T1	T2	T3	T4	T5	T6	MF
Acacia holosericea G.Don	Tree	NC	NC						1	
Acacia auriculiformis Benth.	Tree	PI	Zoo.	6	2			3	1	
Acacia mangium Willd.	Tree	PI	Zoo.	3	3	11	1	6	2	
Acanthospermum australe (Loefl.) Kuntze	Herb.	PI	NC		1		13	10	6	
Actinostemon concolor (Spreng.) Müll.Arg.	Tree	LS	Zoo.							2
Aegiphila integrifolia (Jacq.) Moldenke	Tree	PI	Zoo.	1	1	1	3	1		
Aeschynomene sp.	Herb.	PI	NC				5			
Allagoptera caudescens (Mart.) Kuntze	Tree	NC	NC							2
Andira anthelmia (Vell.) J.F.Macbr.*	Tree	ES	Zoo.						1	
Andira fraxinifolia Benth.*	Tree	ES	Zoo.					1		
Annona tabuleirae H. Rainer	Shru.	LS	Zoo.							2
Asteraceae sp1	Herb.	NC	NC	1		47	8	79		
Asteraceae sp2	Herb.	NC	NC	2	2					
Asteraceae sp3	Herb.	NC	NC	10	1					
Astronium concinnum Schott*	Tree	ES	Ane.		1					
Borreria ocymifolia (Roem. & Schult.) Bacigalupo & E.L.Cabral	Herb.	LS	NC			200				
Borreria verticillata (L.) G.Mey.	Herb.	PI	NC	360	121	221	150	205	613	
Bowdichia virgilioides Kunth*	Tree	ES	Ane.					1		
Byrsonima crassifolia (L.) Kunth.	Tree	ES	Zoo.	6			2	1	1	
Byrsonima sericea DC.*	Tree	PI	Zoo.	39	14	18	23	16	10	
Calathea linharesana H. Kenn.	Herb.	LS	NC							40
Campomanesia espiritosantensis Landrum*	Tree	LS	Zoo.						1	
Casearia aculeata Jacq.	Tree	ES	Zoo.							2
Chamaecrista ensiformis (Vell.) H.S.Irwin & Barneby*	Tree	ES	Aut.						2	
Cordia hatschbachii J.S.Mill.	Shru.	LS	NC	1		1				
Cordia taguahyensis Vell.	Shru.	LS	Ane.		3					6
Croton sp.	Herb.	PI	NC	15	15	9	4	8	1	
Cryptanthus beuckeri E.Morren	Herb.	LS	NC							2
Cupania emarginata Cambess.*	Tree	ES	Zoo.			1				
Cyrtocymura scorpioides (Lam.) H.Rob.	Herb.	PI	NC	11		15	13	4		
Desmodium axillare (Sw.) DC.	Herb.	LS	NC						16	
Dictyoloma vandellianum A.Juss.	Tree	PI	Ane.				25			
Digitaria sp.	Gras.	PI	NC	X	X	X	X	X	X	
Dorstenia milaneziana Carauta, C.Valente & Sucre	Herb.	LS	NC							10
Elephantopus mollis Kunth	Herb.	PI	NC	1	2	106			12	
Eugenia bahiensis DC.	Tree	LS	Zoo.							4
Eugenia brasiliensis Lam.*	Tree	LS	Zoo.		1			1		1
Eugenia cf. *pisiformis* Cambess.	Tree	LS	Zoo.							12
Eugenia excelsa O.Berg	Tree	ES	Zoo.							2
Eugenia platyphylla O.Berg	Tree	LS	Zoo.							15
Eugenia plicatocostata O.Berg	Tree	LS	Zoo.				1			1
Eugenia prasina O.Berg	Tree	LS	Zoo.							8
Eugenia sp.	Tree	LS	Zoo.	2			1	1		
Eugenia sulcata Spring ex Mart.*	Tree	LS	Zoo.					1		
Geissospermum laeve (Vell.) Miers *	Tree	LS	Zoo.						1	
Goeppertia vaginata (Petersen) Borchs. & S.Suárez	Herb.	NC	NC							3
Goniorrhachis marginata Taub.*	Tree	LS	Aut.			1				
Guapira pernambucensis (Casar.) Lundl.*	Shru.	PI	NC	1	1					
Guarea guidonia (L.) Sleumer*	Tree	ES	Zoo.	1		1				
Handroanthus aff. c*hrysotrichus* (Mart. ex DC.) Mattos	Tree	ES	Ane.							3
Handroanthus heptaphyllus (Vell.) Mattos*	Tree	ES	Ane.				1			
Hyptis brevipes Poit.	Herb.	PI	NC				270		34	
Inga laurina (Sw.) Willd.*	Tree	ES	Zoo.	4	1	1	5	2	1	
Lagenocarpus verticillatus (Spreng.) T.Koyama & Maguire	Gras.	PI	NC	X	X	X	X	X	X	
Lasiacis ligulata Hitchc. & Chase	Gras.	LS	NC	X				X		
Lecythis lanceolata Poir.*	Tree	ES	Zoo.		1					
Lecythis lurida (Miers) S.A.Mori	Tree	LS	Zoo.		1			1		3
Leucaena sp.	Tree	NC	NC					2		

Continues on the next page.

Continuation of Table 2.

Family/Species	LH	SG	DS	T1	T2	T3	T4	T5	T6	MF
Margaritaria nobilis L. f.*	Tree	PI	Zoo.						1	
Megathyrsus maximus (Jacq.) B.K.Simon & S.W.L.Jacobs	Gras.	PI	NC		X	X		X		
Melicoccus espiritosantensis Acev.-Rodr.*	Tree	ES	Zoo.				1			
Melicoccus oliviformis subsp. *intermedius* (Radlk.) Acev.-Rodr.*	Tree	ES	NC	1	1			1		
Mesosphaerum suaveolens (L.) Kuntze.	Herb.	PI	NC		7	590	68		120	
Micropholis gardneriana (A.DC.) Pierre	Tree	LS	Zoo.							2
Micropholis crassipedicellata (Mart. & Eichler ex Miq.) Pierre	Tree	LS	Zoo.							4
Myrcia splendens (Sw.) DC.*	Tree	LS	Zoo.				2			
Oeceoclades maculata (Lindl.) Lindl.	Herb.	LS	NC	2			10	1	1	
Oxypetalum banksii Schult. subsp. *Banksii*	HL	ES	NC	5	4	3	2	4	4	
Paubrasilia echinata (Lam.) Gagnon, H.C.Lima & G.P.Lewis*	Tree	ES	Aut.						1	
Phyllanthus niruri L.	Herb.	PI	NC	3	6		6	11		
Plinia renatiana G.M.Barroso & Peixoto	Tree	LS	Zoo.							3
Pombalia calceolaria (L.) Paula-Souza	Herb.	PI	NC		8	11	1	9	12	
Porophyllum ruderale (Jacq.) Cass.	Herb.	PI	NC	86	70	39	33	131	14	
Pouteria coelomatica Rizzini*	Tree	LS	Zoo.	1	1	1				
Protium heptaphyllum (Aubl.) Marchand subsp. *heptaphyllum**	Tree	ES	Zoo.	7	13	2	1	4	1	
Pseudopiptadenia contorta (DC.) G.P.Lewis & M.P.Lima	Tree	ES	Ano.							4
Psidium guineense Sw.*	Tree	ES	Zoo.		2		2		1	
Psidium longipetiolatum D.Legrand*	Tree	LS	Zoo.	1						
Renvoizea trinii (Kunth) Zuloaga & Morrone	Gras.	PI	NC	X	X					
Riodocea pulcherrima Delprete*	Tree	ES	NC						1	
Schinus terebinthifolia Raddi	Tree	ES	Zoo.		2				1	
Sebastiania brasiliensis Spreng.	Tree	LS	Zoo.							17
Senegalia langsdorffii (Benth.) Seigler & Ebinger	Shru.	NC	NC							3
Senna multijuga var. *verrucosa* (Vogel) H.S. Irwin & Barneby*	Tree	PI	Aut./Ane.			1		1	1	
Sida linifolia Juss. ex Cav.	Herb.	PI	NC			2				
Solanum sooretamum Carvalho	Tree	ES	Zoo.	1						2
Sorocea hilarii Gaudich.	Shru.	LS	Zoo.							2
Stephanopodium sp.	Tree	LS	Zoo.							4
Strychnos cf. *hirsuta* Spruce ex Benth.	Shru.	LS	NC							2
Tabernaemontana salzmannii A.DC.	Tree	LS	Zoo.							2
Terminalia mameluco Pickel*	Tree	ES	Ane.	1				1		
Tontelea sp.	Tree	LS	NC							4
Toulicia patentinervis Radlk.*	Tree	LS	Ane.			1				
Trichilia pseudostipularis (A. Juss.) C. DC.	Tree	LS	Zoo.							6
Trichilia pallens C.DC.	Tree	LS	Zoo.							3
Turnera ulmifolia L.	Herb.	PI	NC	1		11	3	7		
Urochloa sp.	Gras.	PI	NC	X	X	X	X	X	X	
Varronia polycephala Lam.	Shru.	LS	Zoo.	1					1	
Vernonanthura polyanthes (Sprengel) Vega & Dematteis	Tree	PI	Ane.		1		1	1	1	
Xylopia frutescens Aubl.	Tree	ES	Zoo.	1	1		1			
Youngia japonica (L.) DC.	Herb.	PI	NC	2	18	18			38	
Zeyheria tuberculosa (Vell.) Bureau ex Verl.*	Tree	ES	Ane.	1		2	11		3	
Zornia latifolia Sm.	Herb.	LS	NC		2		1			

Tree= tree; Shru.= shrub; Herb.= herbaceous; WL= woody liana; HL= herbaceous liana; Gras.= grasses; Palm= palm; PI= pioneer; ES= early secondary; LS= late secondary; Zoo.= zoochoric; Ane.= anemochoric ; Aut.= autochoric; X= indicates the presence of the species; NC= uncharacterized.
*Species used for planting using regenerants in the area under forest restoration process.

belonging to the Asteraceae family are predominantly herbaceous. In other areas under restoration process, a high number of species and individuals from these families were also found (Trentin et al., 2018), thus corroborating our results. Some exotic species such as *Acacia auriculiformis* Benth. (26 individuals) and *Acacia mangium* Willd. (12), were found in all treatments. Exotic species may attribute risks to the successional development of the area, as most of the time they compete with native species (Souza et al., 2017).

The treatments for the woody community comprised 351 individuals and 68 species, while the mature forest included 190 individuals and 84 species. In the mature forest several species of woody lianas were found presenting a high number of species (15) and individuals (27), whereas in the area under restoration only 3 species and 7 individuals were accounted. Regarding the herbaceous community, 4,020 individuals were found distributed in 32 species. From these, the highest concentration occurred in the restoration area with 3,963 individuals (Table 3).

Table 3. General parameters of natural regeneration in treatments (T) and in mature forest (MF), Reserva Natural Vale, ES.

General community	T1	T2	T3	T4	T5	T6	MF
N. individuals*	582	315	1.317	675	519	906	247
Density (ind ha^{-1})	38.800	21.000	87.800	45.000	34.600	60.400	13.200
N. species	34	38	27	33	32	34	77
N. families	27	24	19	22	19	17	32
Diversity (H')*	1.57	2.25	1.8	2.07	1.92	1.34	3.84
Equability (J)*	0.43	0.61	0.54	0.58	0.54	0.38	0.85
Woody community							
N. individuals	81	57	44	86	48	35	190
Density (ind ha^{-1})	5.400	3.800	2.933	5.733	3.200	2.333	12.667
N. species	20	25	14	18	19	22	71
N. families	16	16	9	11	10	12	29
Diversity (H')	2.07	2.62	1.91	2.15	2.48	2.72	3.99
Equability (J)	0.68	0.81	0.72	0.73	0.41	0.88	0.9
Herbaceous community							
N. individuals *	501	258	1.273	589	471	871	57
Density (ind ha^{-1})	33.400	17.200	84.867	39.267	31.400	58.067	533
N. species	14	13	13	15	13	12	6
N. families	11	11	10	13	11	10	5
Diversity (H')*	1.02	1.59	1.65	1.62	1.53	1.12	0.97
Equability (J)*	0.36	0.6	0.62	0.57	0.58	0.45	0.54

* The number of grass individuals is not included.

The high number of herbaceous individuals in the area under forest restoration process may be related to the ancient soil use in the region and the presence of discontinuous canopies (Franco et al., 2012), caused by mortality of the planting species, as well as the arrival of propagules from the seed rain from this group of species. The studied area is surrounded by pasture and other restoration projects, which include several herbaceous species which can be dispersed by natural agents. Other factors that contribute to the increase of herbaceous species in natural regeneration are the life cycle of these species, the high productivity and the facility of dispersing their seeds (Curtinhas et al., 2010).

Species such as *Borreria verticillata* (L.) G.Mey. was confirmed in all treatments, totaling 1,670 individuals. The high infestation was also found in fragments of Dense Ombrophylous Forest of the Lowlands by Soares Junior et al. (2008). This species can be indicated for recovery of degraded areas, mainly by its high adaptation in mining areas (Jesus et al., 2016). On the other hand, the high infestation of herbaceous species may negatively influence natural regeneration, leading to resistance in the succession process (Rodrigues et al., 2010; Silva et al., 2018).

Low occurrence of woody species and individuals sampled in natural regeneration for all treatments may be associated with competition with herbaceous species (Rodrigues et al., 2010). In addition, there is low seed rains due to the absence of a propagation source and lack or limitation of dispersants (Chiamolera et al., 2011), which may negatively influence successional processes.

Concerning the richness variable, a low average number of woody species was observed in the natural regeneration in all treatments of the area under forest restoration process (Table 3). The species richness in plant communities may be associated with forest age (Liebsch et al., 2007), as observed in studies by Aide et al. (2000), which noted a gradual increase of woody species richness along the successional process in areas with anthropic interference. Brancalion et al. (2010), reported that implementing less species richness and diversity in a restoration area may lead to unsuccessful outcomes, forming environments unable to maintain high biodiversity.

The diversity and equability indices were higher for the woody community when compared to those calculated for the general and herbaceous community (Table 3). These low values may be associated with the large number of herbaceous individuals in some species (Durigan, 2003), as observed e.g, in the species *Porophyllum ruderale* (Jacq.) Cass. (373 individuals), *Mesosphaerum suaveolens* (L.) Kuntze (785) and *Borreria verticillata* (L.) G.Mey.

Considering the reference ecosystem, the diversity found for the woody regenerating layer was 3.99 nats $indiv^{-1}$. Nevertheless, in the secondary forest fragment located in the Reserva Natural Vale the diversity values of natural regeneration were higher, increasing from 4.35 to 5.00 over a ten-year period (Souza et al., 2002).

Diversity values of the woody community were low for all treatments, when compared to the reference ecosystem. During the natural succession process it is expected that floristic diversity values are higher in environments where there is a source of species propagation of advanced successional stage (Britez, 2007), however, this pattern was not observed.

Contrary to the observed in the area under restoration process, in the mature forest more species and woody individuals than herbaceous were sampled. Mature tropical forests form a continuous canopy, with scarce light reaching the soil (Guilherme, 2000), which inhibits the germination of a large number of herbaceous species. This increase in understorey shading may reduce the colonization of pioneer species, and enhance the chance of establishing late secondary species (Oliveira et al., 2019).

Moreover, the natural tropical forests, such as those located in the north of Espírito Santo, are composed of a great richness and diversity of plant species (Paula & Soares, 2011). Therefore, Melo et al. (2006) and Brancalion et al. (2010), have reported that recruitment of species from different ecological groups by means of seed rain is related to the presence of a permanent and diversified canopy.

The high percentages of grasses are associated with the presence of high number of herbaceous plants. In the area under restoration, 55% of the sampled area had more than 15% coverage by grasses, and in some the percentage was approximately 90%, which may also have interfered in the germination of woody species. However, no significant correlation was found ($r = -0.36$ and $p > 0.05$), thus suggesting that there was no significant association between the percentage of grasses and the number of arboreal individuals in the sampling area (Figure 2).

A higher proportion of late secondary arboreal species was registered in the mature forest (63.23%), whereas in the area under restoration the initial secondary species were more numerous (41.93%) (Figure 3). Initial secondary species need intermediate light for their growth, therefore they preferentially occur in less shaded areas. The presence of the discontinuous canopy in the area under restoration process allows the entry of light, which may favor the establishment of species from this group.

Furthermore, a considerable number of shadow-tolerant species (37.09%) were observed in the area under restoration (Figure 3), suggesting that over time a breakthrough in the successional process occurs. Late secondary species are establishing themselves in the area due to favourable shading conditions created by light tolerant planting species, but which have not fully covered the area.

The analysis of DCA, using the abundance of woody species as a parameter, resulted in the formation of distinct groups, despite the proximity between the restoration project and the mature forest. The ordination generated distinct groups, showing the high floristic dissimilarity between the treatments and the mature forest (Figure 4).

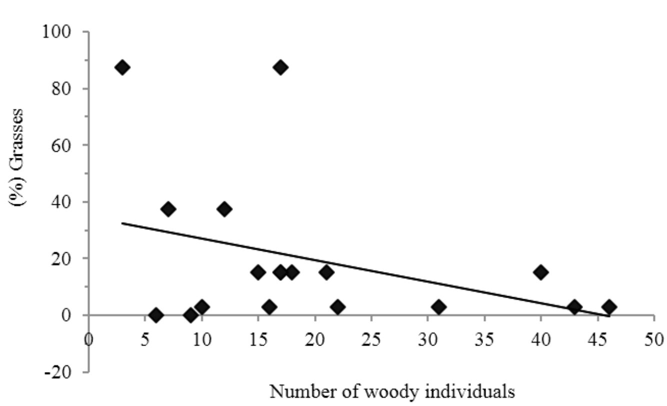


Figure 2. Diagram of dispersion between the number of woody individuals and the percentage of grasses, Reserva Natural Vale, ES.

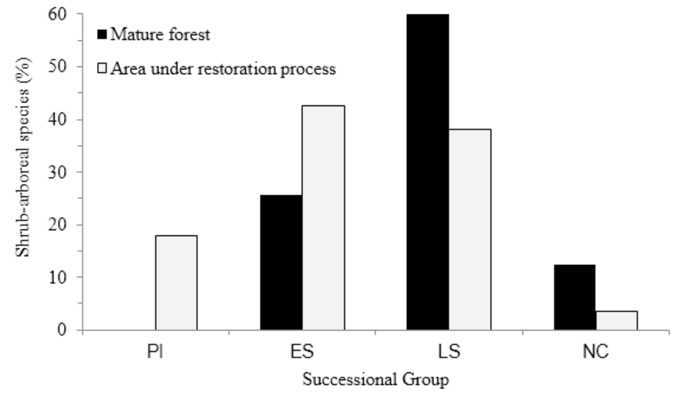
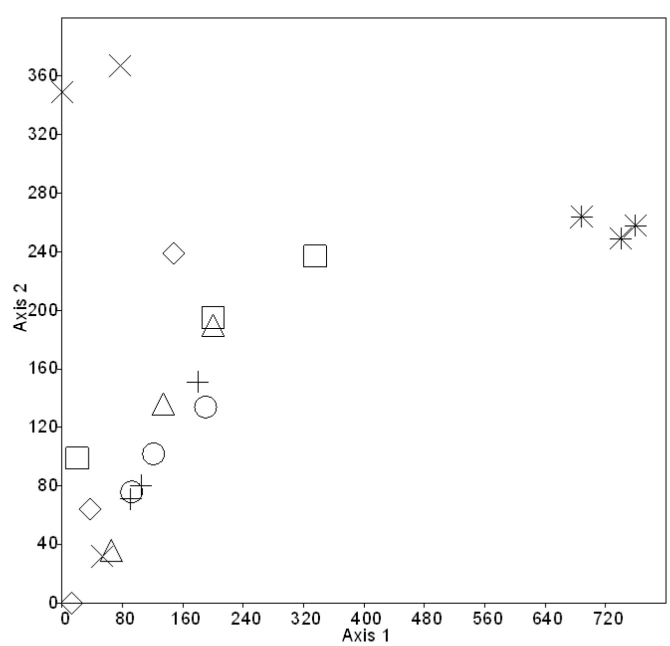


Figure 3. Percentage of pioneer species (PI), early secondary (ES), late secondary (LS) and uncharacterized (NC) species in relation to the total number of shrub-arboreal species sampled in natural regeneration, in the area under restoration and in mature forest, Reserva Natural Vale, ES.

Our results showed that even the treatments with the highest richness (T3 and T6) did not result in an increase in the number of individuals and diversity of areas under restoration, with the average number of species being 5.67 and 8, respectively. In contrast, Durigan et al. (2010), suggest that high diversity is not a guarantee of success in the restoration, since other factors such as filters or barriers can influence the stability of the restored ecosystem.



The treatments are represented by symbols (O = Treatment 1, □ = Treatment 2, Δ = Treatment 3, × = Treatment 4, + = Treatment 5, ◊ = Treatment 6, * = Treatment 7).

Figure 4. Ordination diagram of the species and plots of the woody community resulting from the Distended Correspondence Analysis (DCA), for different sample sites in the area under restoration and in mature forest, Reserva Natural Vale, ES.

Mature forest plots were grouped and another group was formed by treatments (Figure 4). It can be observed that the plots of treatment 4 are more distant, due mainly to the low sharing between species and the other plots, besides the abundance of individuals belonging to the species *Dictyoloma vandellianum* A.Juss, which obtained greater relevance and exclusivity in this treatment. It should be pointed out that the mentioned species was not used in the project planting.

Flora from the natural regeneration of the area under restoration process is composed of different species compared to mature forest, as confirmed by the One-Way ANOSIM test, which presented the values (r = 0.323 and p = 0.001). Even though there is no floristic similarity between the areas, mature forests are important sources of propagules for the natural regeneration (Britez, 2007; Peña-Domene et al., 2013).

Floristic dissimilarity was also observed by Melo & Durigan (2007), when assessing areas in the process of restoration with 7, 9 and 13 years and a mature forest. The authors reported that a long time is required to equalize the species composition of restored areas with natural forests.

Among the species used for planting, 34 were present in the stratum of natural regeneration of the area, however, several of these species were sampled in few treatments and in a small number of individuals. This finding was not in accordance with Daronco et al. (2013), whose results demonstrated that planted species have a minor influence on the floristic composition of the community under restoration.

When analyzing the dispersion syndrome of the regenerating species in both areas, an increased number of zoochoric species was registered (Figure 5).

The difference in dispersion strategies may vary according to the type of vegetation and its successional stage (Chazdon et al., 2007), however, this observed syndrome is a pattern in tropical forests (Venzke et al., 2014). Species of the restoration area are characteristics of areas undergoing an early to intermediate succession process, evidencing the successional stage, in which they were more representative in terms of abundance. On the other hand, late species were more abundant in mature forests.

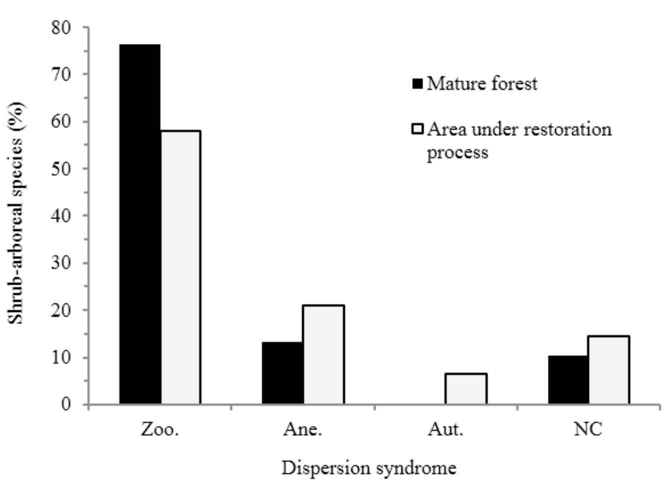


Figure 5. Percentage of zoochoric (Zoo.), anemochoric (Ane.), autochoric (Aut.) and uncharacterized (NC) species in relation to the total number of shrub-arboreal species sampled in natural regeneration, in the area under restoration and in the mature forest, Reserva Natural Vale, ES.

Conclusions

During the evaluation of the natural regeneration after 8 years of project installation, it was possible to identify that the restored area presents difficulties to advance in the succession process. The composition of the natural regeneration of the entire area under restoration, regardless of the richness used for planting, has a high similarity, and with many woody species being shared among the treatments. Moreover, the area under restoration process shares few species with the mature forest, thereby making the dissimilar environments.

It was not possible to observe a correlation between the percentage of grasses and the number of arboreal individuals in the restored areas, suggesting that other processes have a negative influence on the regeneration of woody species in these environments. Thus, it is reinforced by the obtained information that even areas of planting with major species richness are not a guarantee of success in the establishment of natural regeneration with high diversity.

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