Floresta e Ambiente 2019; 26(Spec No 1): e20180411
https://doi.org/10.1590/2179-8087.041118
ISSN 2179-8087 (online)

Original Article

Conservation of Nature

Litter as an Ecological Indicator of Forest Restoration Processes in a Dense Ombrophylous Lowland Forest

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ABSTRACT

The objective of the present study was to evaluate the influence of spacing, species richness, and sampling time on accumulated litter in forest restoration areas in the state of Espírito Santo, Brazil. The experiment was conducted under a randomized complete block design, in a subdivided plot scheme. Six treatments were evaluated, consisting of a combination of spacing levels and species richness. Accumulated litter samples were weighed and chemically analyzed to obtain nutrient concentrations. The variables analyzed in the restoration process, in general, did not influence litter biomass and nutrient concentrations. The time elapsed between restoration implantation and data sampling for the present study may have been insufficient for the occurrence of differences in variables evaluated. Despite the small amount of accumulated litter, it is of great importance for soil physical protection and fertility improvement, generating conditions for the stabilization of new forest species in the restoration area.

Keywords: restoration/reclamation/rehabilitation areas, afforestation and reforestation, forest soil, forest nutrition, ecology of forest ecosystems.

1. INTRODUCTION

Forest restoration is an important strategy in the reconstruction of forest ecosystems influenced by anthropogenic activities. This process involves interventions to restore the functional processes of degraded ecosystems to restore the natural successional process, based on the edaphoclimatic conditions of the site as close as possible to the original system. One of the precepts of the restoration process is to have previous knowledge of the area being restored, where different interventions should be applied for each situation. This information also enables adopting more economically viable practices, since it is based on the restoration potential of the area itself. One of the methods most frequently adopted in the restoration of forest environments is the planting of native species in the region to be restored (Rodrigues et al., 2009).

The planting of forest species produces a catalytic effect, causing changes in environmental conditions, and thus, generating favorable conditions for the germination and development of species (Brooker et al., 2008). One of the main restoration challenges is the selection of an ideal combination of species, spacing, density, and distribution of seedlings in the field to achieve soil shading and protection by the canopy cover and control weed competition. Thus, restoration model is a constantly changing process, which is assisted by information regarding the physical and biological environment of the region, before and after its implantation (Rodrigues et al., 2009). Considering the fragmentation of the Atlantic Forest in the state of Espírito Santo and the importance of forest remnants of the Northern tableland of the state, it is fundamental to prepare strategies for the maintenance and expansion (mainly by forest restoration) of these natural resources.

Litter is the organic waste layer that accumulates on the soil surface of forest ecosystems. It consists of leaves, twigs, branches, stems, fruits, flowers, seeds, and unidentified plant parts, as well as animal remains and fecal material. Litter plays an important role in forest ecosystems due to its participation in nutrient cycling (soil-plant-soil), soil protection against erosion, leaching and compaction, nutrient for soil fauna, and soil temperature and moisture regulation (Martinelli et al., 2017). The contribution of litter is influenced by biological, edaphic, and climatic factors, and may be lower in cold regions, such as temperate forests, and higher in tropical and subtropical forests (Vogel et al., 2007; Godinho et al., 2013a). The composition of species, forest cover intensity, successional stage, sampling season, forest type, soil and climatic conditions, silvicultural management, canopy proportion, decomposition rate, fire, insect attack, litter removal, and crops occurring in the forest or settlement also influence litter accumulation (Caldeira et al., 2008; Godinho et al., 2014).

Studies where litter serves as an ecological restoration indicator allow the gathering of information that assists in the choice of forest species, spacing, and richness for the formation of fragments (Caldeira et al., 2008; Sperandio et al., 2012; Villa et al., 2016). In this sense, studies on restoration areas are essential, especially those that allow understanding the direct relationships between vegetation and environment, such as those related to nutrient cycling. Data generated by these studies are fundamental, since they support new proposals for restoration and guide decision-making, especially in heavily anthropized and degraded regions.

In view of the above, the purpose of the present study was to fill some of existing gaps regarding factors that influence the accumulation of litter and nutrients in forest restoration areas. Combinations of different spacing levels and species richness are expected to promote differences in nutrient cycling, micro and macronutrient concentrations or plant growth. To verify this hypothesis, the present study aimed to evaluate the influence of spacing levels, species richness, and sampling season of accumulated litter in restoration areas in a region of Dense Ombrophilous Lowland Forest in the Vale Nature Reserve, state of Espírito Santo, Brazil.

2. MATERIAL AND METHODS

2.1. Characteristics of the study area

The present study was carried out at Vale Nature Reserve, which is located in the municipality of Linhares, state of Espírito Santo, Brazil. The native vegetation of the Vale Nature Reserve is classified as Dense Ombrophylous Lowland Forest (IBGE, 2012). The region has hot and humid climate, with rainy season during summer and dry season during winter, which corresponds to Aw type based on the Köppen classification system. Mean annual air temperature is 23.5 °C, with means of 25.9 and 20.9 °C during March and August, respectively. Mean annual precipitation is 1294 mm, with means of 121 and 58 mm during March and August, respectively (Alvares et al., 2013).

The relief of the experimental area is considered plain (0–3% slope). Based on terminology reported by Caldeira et al. (2017), the soil is classified as dystrocohesive yellow Argisol with moderate A horizon, presence of B textural, well drained with texture varying from sandy to medium in horizon A, and medium to clay in horizon B (EMBRAPA, 2013). The soil chemical attributes, which have little spatial variation, collected in March 2013, at different depths are shown in Table 1.

Until the 1980s, the restoration area under study was planted with *Eucalyptus* sp. After cutting, the area remained fallow for 25 years. In December 2004, the restoration project began, with mowing and chemical weeding of the grass forage. During the same year, soil preparation was completed by manual hollowing (0.30 × 0.30 × 0.30 m) with fertilization using a 200-g superphosphate simple pit. The planting of seedlings began in January 2005 and, at the end of the year, dead seedlings were replanted. Periodic project maintenance was performed, until the fifth year via chemical weeding and ant control.

2.2. Description of treatments

In the experimental model, six treatments (primary factor) were evaluated in a randomized complete block design with three replicates, totaling eighteen plots of 50 × 50 m each, in a subdivided plot scheme. The six treatments consisted of a combination of spacing levels (3 × 3 m and 2 × 2 m) and species richness (29, 58, and 114 species per treatment). Treatments were analyzed over two seasons (March [wet season] and August [dry season] 2013). Descriptions of treatments are shown in Table 2.

Table 1. Chemical soil attributes at four depths in a forest restoration area in Linhares, Espírito Santo (ES), Brazil.

Depths	N	P	K	pH	Al	Ca	Mg	H + Al	SB	t	T	V	m	SOM	OC
(cm)	$g\ kg^{-1}$	$mg\ dm^{-3}$		H_2O	$cmol_c\ dm^{-3}$							%		$g\ kg^{-1}$	
Diversity 1 - species richness - 29 species per treatment															
0-5	1.9	0.9	37.3	5.2	0.3	1.3	0.4	3.2	1.9	2.2	5.1	37.2	13.6	27.8	16.1
5-10	1.4	1.3	26.4	5.1	0.4	0.9	0.3	2.8	1.3	1.7	4.1	31.6	22.1	15.8	13.3
10-20	1.1	0.7	20.0	4.8	0.5	0.6	0.2	2.5	0.9	1.4	3.4	25.9	38.1	16.4	9.5
20-40	1.1	0.7	12.0	4.7	0.8	0.4	0.1	3.8	0.6	1.4	4.4	14.3	57.3	13.9	8.0
Diversity 2 - species richness - 58 species per treatment															
0-5	1.7	1.4	28.3	5.1	0.3	1.1	0.4	3.4	1.5	1.9	4.9	32.2	17.7	25.3	14.7
5-10	1.6	1.4	24.3	5.1	0.5	0.9	0.3	2.9	1.3	1.8	4.3	31.2	25.9	23.0	13.3
10-20	1.3	1.1	15.7	4.8	0.6	0.5	0.2	2.9	0.8	1.4	3.6	20.2	46.5	17.6	10.2
20-40	0.1	0.8	12.7	4.8	0.7	0.5	0.2	3.4	0.7	1.4	4.2	17.2	49.7	12.5	7.2
Diversity 3 - species richness - 114 species per treatment															
0-5	1.9	1.3	34.3	5.0	0.4	1.2	0.4	3.4	1.7	2.1	5.2	33.7	19.4	25.8	15.0
5-10	1.9	1.1	27.7	4.8	0.5	0.9	0.3	3.4	1.3	1.7	4.7	27.1	27.1	23.5	13.6
10-20	1.4	0.8	26.3	4.8	0.6	0.6	0.2	3.1	0.7	1.5	4.0	22.9	39.5	16.7	9.7
20-40	1.1	0.6	21.3	4.8	0.6	0.7	0.2	3.7	1.0	1.6	4.7	21.7	38.6	12.1	7.0

Note: N (nitrogen), P (phosphorus), K (potassium), Al (aluminum), Ca (calcium), Mg (magnesium), H + Al (potential acidity), SB (sum of bases), t (effective cation exchange capacity), T (potential cation exchange capacity), V (base saturation), m (aluminum saturation), SOM (soil organic matter), OC (organic carbon). Source: Caldeira et al. (2017).

Table 2. Description of treatments applied in the forest restoration area at Linhares, ES, Brazil.

Treatment	Spacing (m)	Density ($tree\ ha^{-1}$)	Number of species
1	2 × 2	2500	29
2	2 × 2	2500	58
3	2 × 2	2500	114
4	3 × 3	1111	29
5	3 × 3	1111	58
6	3 × 3	1111	114

Species were selected from a list of 170 forest species belonging to 41 families that are native to the Dense Ombrophilous Lowland Forest. In each treatment, species were distributed in the same proportion as the ecophysiological groups (i.e., 25% pioneers, 25% initial secondary, 25% late secondary, and 25% climax).

2.3. Sampling and processing of accumulated litter

Sampling of accumulated litter was carried out in March and August 2013. In each plot, litter was collected from the forest ground. Eight samples of accumulated litter, one at each end and four in the center of the plot, were collected with the aid of a wooden implement (0.25 m × 0.25 m). Accumulated litter samples were separated into two fractions: a) leaves + miscellaneous – consisting of senescent leaves accumulated on the ground together with reproductive plant materials, bark, animal remains or their feces, and material not identified as fragmented material or of unknown origin; and b) branches – consisting of branches with diameters smaller than 2 cm.

After separation, samples were placed in brown paper bags to dry in a circulation oven and air renewal at 65 °C until reaching constant mass. They were then individually weighed on a precision scale for quantification of accumulated biomass. For chemical analysis, the material was ground in a Wiley mill, passed through a 1.0 mm sieve and stored in a glass vial. Concentrations of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Fe, Cu, Mn, and Zn) in accumulated litter were analyzed according to methodology proposed by EMBRAPA (2009). The nutrient stock of accumulated litter was obtained from dry biomass measurements and concentrations of nutrients estimated by equation (1) (Cuevas & Medina, 1986):

$$NS = [NC] * DLB \quad (1),$$

Where NS = nutrient stock (kg ha^{-1}), NC = litter nutrient concentration (g kg^{-1} or mg kg^{-1}), and DLB = dry litter biomass (Mg ha^{-1}).

2.4. Data analysis

Data from biomass and micro and macronutrient concentrations in accumulated litter were submitted to Shapiro-Wilk residue normality test and Cochran variance homogeneity test. When data did not have normal distribution and/or homogeneity of variance, they were transformed in different ways, e.g., via logarithmic function, square root of data, inverse square root of data, or square exponential. To verify if the effects of restoration methods were significant, analysis of variance and F-tests were performed and, where significant differences occurred, the Scott-Knott test ($p<0.05$) was performed to group factors that not differed significantly from each another.

3. RESULTS

3.1. Accumulated litter biomass

Total litter biomass, as well as biomass of fractions (leaves+miscellaneous and branches) did not have significant interaction ($p>0.05$) between treatments and sampling seasons. Analyzing each factor individually, no significant difference was verified among treatments, ($p>0.05$). However, for the sampling seasons, significant difference ($p<0.05$) was observed for both fractions and accumulated total litter, with the highest values found in August, which was the period of lower precipitation and air temperature (Table 3).

3.2. Nutrient concentration in accumulated litter

For the leaves+miscellaneous fraction, in relation to treatments significant difference was verified only for Fe concentrations ($p<0.05$). For the two sampling seasons, with the exception of N, macronutrient concentrations in accumulated litter were significantly different ($p<0.05$), being higher during August, which was the period of lower precipitation. However, micronutrient concentrations were uniform throughout the year and did not vary between seasons ($p>0.05$) (Table 4).

Unlike the leaves + miscellaneous fraction, the only nutrient that had significant difference in concentration among evaluation treatments for branches fraction was Ca. In the branches fraction, similarly to leaves + miscellaneous fraction, the concentrations of most macronutrients (except for N and Mg) had differences from one season to another, with higher values during August when precipitation was lower. For micronutrients; however, there was uniformity in concentrations between treatments and throughout the year (Table 5).

Table 3. Biomass of leaves + miscellaneous fraction, branches fraction, and total accumulated litter in different treatments and seasons in a forest restoration area in Linhares, ES, Brazil.

Treat.[1]	March/2013			August/2013			Average of two seasons		
	L + M	Bran	Total	L + M	Bran	Total	L + M	Bran	Total
	Mg ha^{-1}								
T1	2.71	0.82	3.53	3.38	1.79	5.17	3.05[ns2]	1.31[ns]	4.35[ns]
T2	2.80	0.97	3.76	3.25	1.82	5.07	3.02	1.39	4.42
T3	3.38	0.88	4.26	4.57	1.39	5.96	3.97	1.14	5.11
T4	2.57	0.76	3.33	3.20	1.30	4.49	2.88	1.03	3.91
T5	2.80	0.78	3.57	3.27	1.75	5.02	3.03	1.26	4.30
T6	3.04	0.59	3.63	4.13	1.31	5.45	3.59	0.95	4.54
Average	2.88b[3]	0.80b	3.68b	3.63a	1.56a	5.19a	3.26	1.18	4.44

[1]Treatments: T1 = richness of 29 species in 2 × 2 m spacing; T2 = richness of 58 species in 2 × 2 m spacing; T3 = richness of 114 species in 2 × 2 m spacing; T4 = richness of 29 species in 3 × 3 m spacing; T5 = richness of 58 species in 3 × 3 m spacing; T6 = richness of 114 species in 3 × 3 m spacing. [2]NS = Not significant: the means of each treatment, from two sampling seasons, did not differ at 5% probability level ($p<0.05$) by the Scott-Knott test. [3]The mean values for fractions and litter total, between sampling times, considering all treatments, followed by different letters, differ from each other at 5% probability level ($p<0.05$) by the Scott-Knott test.

Table 4. Mean values of nutrient concentrations in leaves + miscellaneous fraction from accumulated litter in different treatments and seasons in a forest restoration area in Linhares, ES, Brazil.

Treat.[1]	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu
	g kg^{-1}						mg kg^{-1}			
T1	12.66[ns3]	0.33[ns]	1.35[ns]	12.97[ns]	2.36[ns]	1.27[ns]	19.3[ns]	601.6a[4]	152.5[ns]	8.3[ns]
T2	12.48	0.38	1.52	13.24	2.43	1.21	17.8	545.4a	119.1	8.0
T3	12.07	0.31	1.87	11.92	2.32	1.15	17.7	299.0b	110.9	7.6
T4	13.24	0.36	1.80	12.27	2.31	1.24	20.0	332.0b	117.5	7.3
T5	12.66	0.32	1.85	13.96	2.44	1.26	21.7	357.2b	134.7	8.2
T6	12.77	0.34	1.24	14.80	2.36	1.28	22.6	414.4b	135.0	8.5
CV(%)[2]	10.14	21.81	17.07	11.15	15.87	11.10	25.65	30.61	23.17	22.36
Season[5]										
March	12.42[ns]	0.29b	1.28b	12.08b	2.17b	1.06b	18.0[ns]	411.4[ns]	120.5 [ns]	8.0[ns]
August	12.87	0.39a	1.94a	14.31a	2.57a	1.41a	21.6	438.5	136.1	8.0
CV(%)	12.65	0.34	1.61	13.19	2.37	1.24	19.18	424.96	128.33	8.04

[1]Treatments: T1 = richness of 29 species in 2 × 2 m spacing; T2 = richness of 58 species in 2 × 2 m spacing; T3 = richness of 114 species in 2 × 2 m spacing; T4 = richness of 29 species in 3 × 3 m spacing; T5 = richness of 58 species in 3 × 3 m spacing; T6 = richness of 114 species in 3 × 3 m spacing. [2]Coefficient of variation in %. [3]NS = Not significant: averages in the same column for treatments or sampling season, do not differ by Scott-Knott's test ($p<0.05$). [4]Means followed by different letters, in the same column for treatments or sampling season, differ by Scott-Knott's test ($p<0.05$). [5]Year 2013.

Table 5. Mean values of nutrient concentrations in the branches fraction from accumulated litter on soil in different treatments and seasons in a forest restoration area in Linhares, ES, Brazil.

Treat.[1]	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu
	g kg^{-1}						mg kg^{-1}			
T1	10.27[ns3]	0.25[ns]	1.25[ns]	15.31a[4]	2.31[ns]	0.71[ns]	33.4[ns]	342.0[ns]	123.2[ns]	8.5[ns]
T2	8.87	0.27	1.92	16.18a	2.33	0.69	37.7	236.1	126.9	8.4
T3	8.87	0.24	1.20	12.80b	1.97	0.62	29.2	218.0	95.0	7.5
T4	9.74	0.25	1.45	13.30b	2.15	0.69	30.0	194.8	75.8	7.0
T5	8.46	0.22	1.47	13.73b	1.91	0.56	34.5	193.3	96.2	7.1
T6	9.10	0.25	1.08	14.24b	2.11	0.67	36.9	239.1	89.0	7.9
CV(%)[2]	18.97	16.12	13.81	10.49	13.82	20.19	16.39	19.33	3.21	16.86
Season[5]										
March	9.55[ns]	0.22b	1.12b	13.24b	2.05[ns]	0.55b	31.6[ns]	239.4[ns]	101.8[ns]	7.9[ns]
August	8.89	0.27a	1.67a	15.27a	2.20	0.76a	35.7	235.0	100.2	7.6
CV(%)	12.22	16.88	15.80	12.69	18.38	14.31	21.32	10.85	2.75	16.21

[1]Treatments: T1 = richness of 29 species in 2 × 2 m spacing; T2 = richness of 58 species in 2 × 2 m spacing; T3 = richness of 114 species in 2 × 2 m spacing; T4 = richness of 29 species in 3 × 3 m spacing; T5 = richness of 58 species in 3 × 3 m spacing; T6 = richness of 114 species in 3 × 3 m spacing. [2]Coefficient of Variation in %. [3]NS = Not Significant: averages in the same column for the treatments or sampling season, do not differ by Scott-Knott's test ($p<0.05$). [4]Means followed by different letters, in the same column for treatments or sampling season, differ by Scott-Knott's test ($p<0.05$). [5]Year 2013.

3.3. Nutrient stock in accumulated litter

Nutrient stock in total accumulated litter is shown in Table 6. The mean of total accumulated nutrients in all treatments and seasons was 138.96 kg ha^{-1}, of which 136.63 kg ha^{-1}was related to macronutrients and 2.37 kg ha^{-1} to micronutrients, at ratio of approximately 58:1.

In the leaves + miscellaneous fraction, a total of 104.93 kg ha^{-1} of nutrients were accumulated, with 103.05 kg ha^{-1} of macronutrients and 1.88 kg ha^{-1} of micronutrients, considering the average of all treatments and seasons (Table 7). Macronutrients that obtained the highest and lowest stocks were Ca and P, respectively, which followed the same pattern as concentrations. The accumulation order was Ca>N>Mg>K>S>P. Regarding micronutrients, the elements with the highest and lowest stocks were Fe and Cu, respectively. The micronutrient accumulation order was Fe>Mn>Zn>Cu.

The branches fraction accumulated a total of 33.25 kg ha^{-1} of nutrients, with 32.81 kg ha^{-1} of macronutrients and 0.44 kg ha^{-1} of micronutrients, when considering the average of all treatments and seasons (Table 8). The macronutrient accumulation order was Ca>N>Mg>K>S>P and the micronutrient order was Fe>Mn>Zn>Cu, which were the same as that observed for the leaves + miscellaneous fraction.

4. DISCUSSION

4.1. Accumulated litter biomass

Although treatments were composed of different amount of species, the proportion of successional groups was the same for all treatments. The amount of litter on the soil surface can vary depending on the composition of species, successional stage, and intensity of forest cover (Caldeira et al., 2008; Grugiki et al., 2017). Therefore, the use of the same proportion of successional groups in the present study might have caused a similar response in the analyzed treatments. In addition, the age of the restoration area was only

Table 6. Nutrient stock, in kg ha^{-1}, of total accumulated litter in different treatments and seasons in a forest restoration area in Linhares, ES, Brazil.

Nutrient	Season	Treatments[1]					
		T1	T2	T3	T4	T5	T6
		kg ha^{-1}					
N	March/2013	45.10	41.15	45.58	39.93	44.70	44.04
	August/2013	58.34	58.23	71.90	56.90	53.33	64.81
P	March/2013	0.99	1.22	0.95	0.97	0.93	1.01
	August/2013	1.79	1.85	2.14	1.61	1.65	1.99
K	March/2013	3.92	5.54	5.50	6.15	3.70	3.08
	August/2013	7.80	9.82	12.85	7.27	11.64	8.60
Ca	March/2013	43.20	45.85	47.43	40.95	46.52	52.49
	August/2013	78.15	82.40	78.43	60.45	72.17	81.92
Mg	March/2013	8.05	8.48	8.50	7.54	7.43	7.83
	August/2013	12.76	13.04	14.78	9.77	12.28	13.72
S	March/2013	3.65	3.10	3.67	3.20	3.55	3.83
	August/2013	6.11	6.30	7.19	5.55	5.45	6.94
Zn	March/2013	0.07	0.07	0.07	0.07	0.09	0.08
	August/2013	0.14	0.14	0.14	0.10	0.13	0.15
Fe	March/2013	2.26	1.69	0.93	0.83	1.33	1.16
	August/2013	2.28	2.26	2.04	1.36	1.39	2.22
Mn	March/2013	0.47	0.43	0.43	0.38	0.42	0.45
	August/2013	0.76	0.67	0.66	0.49	0.61	0.71
Cu	March/2013	0.03	0.03	0.03	0.02	0.03	0.03
	August/2013	0.05	0.04	0.05	0.03	0.04	0.05

[1]Treatments: T1 = richness of 29 species in 2 × 2 m spacing; T2 = richness of 58 species in 2 × 2 m spacing; T3 = richness of 114 species in 2 × 2 m spacing; T4 = richness of 29 species in 3 × 3 m spacing; T5 = richness of 58 species in 3 × 3 m spacing; T6 = richness of 114 species in 3 × 3 m spacing.

Table 7. Nutrient stock, in kg ha^{-1}, of the leaves + miscellaneous fraction on the soil in the different treatments and seasons in a forest restoration area in Linhares, ES, Brazil.

Nutrient	Season	Treatments[1]					
		T1	T2	T3	T4	T5	T6
		kg ha^{-1}					
N	March/2013	36.22	32.95	37.23	32.36	37.97	38.50
	August/2013	41.31	42.50	60.19	44.63	38.81	53.11
P	March/2013	0.80	0.97	0.76	0.81	0.78	0.89
	August/2013	1.27	1.33	1.77	1.27	1.21	1.60
K	March/2013	3.18	3.69	4.68	5.02	3.03	2.61
	August/2013	4.97	5.77	10.79	5.56	8.15	6.84
Ca	March/2013	31.81	31.49	36.04	32.07	35.49	44.84
	August/2013	48.17	49.49	60.37	41.06	48.65	61.40
Mg	March/2013	6.22	6.10	6.75	5.94	5.95	6.77
	August/2013	8.30	8.75	11.95	7.12	9.04	10.52
S	March/2013	3.11	2.54	3.24	2.78	3.15	3.54
	August/2013	4.64	4.89	6.13	4.48	4.43	5.84
Zn	March/2013	0.047	0.043	0.050	0.047	0.063	0.067
	August/2013	0.070	0.067	0.097	0.067	0.070	0.100
Fe	March/2013	1.94	1.42	0.77	0.70	1.17	1.07
	August/2013	1.74	1.92	1.69	1.11	1.09	1.83
Mn	March/2013	0.36	0.31	0.35	0.32	0.35	0.41
	August/2013	0.55	0.42	0.55	0.40	0.45	0.58
Cu	March/2013	0.023	0.023	0.027	0.017	0.023	0.027
	August/2013	0.030	0.027	0.037	0.023	0.027	0.037

[1]Treatments: T1 = richness of 29 species in 2 × 2 m spacing; T2 = richness of 58 species in 2 × 2 m spacing; T3 = richness of 114 species in 2 × 2 m spacing; T4 = richness of 29 species in 3 × 3 m spacing; T5 = richness of 58 species in 3 × 3 m spacing; T6 = richness of 114 species in 3 × 3 m spacing.

Table 8. Nutrient stock, in kg ha^{-1}, of the branches fraction in the different treatments and seasons in a forest restoration area in Linhares, ES, Brazil.

Nutrient	Season	Treatments[1]					
		T1	T2	T3	T4	T5	T6
N	March/2013	8.89	8.20	8.35	7.57	6.73	5.53
	August/2013	17.03	15.73	11.71	12.27	14.53	11.70
P	March/2013	0.19	0.26	0.20	0.16	0.15	0.12
	August/2013	0.52	0.51	0.37	0.34	0.44	0.38
K	March/2013	0.73	1.86	0.82	1.12	0.67	0.47
	August/2013	2.83	4.05	2.06	1.71	3.48	1.76
Ca	March/2013	11.39	14.36	11.39	8.88	11.03	7.65
	August/2013	29.98	32.91	18.06	19.39	23.52	20.51
Mg	March/2013	1.83	2.38	1.75	1.60	1.48	1.06
	August/2013	4.46	4.29	2.83	2.65	3.24	3.19
S	March/2013	0.54	0.56	0.44	0.43	0.40	0.29
	August/2013	1.48	1.41	1.07	1.07	1.03	1.11
Zn	March/2013	0.02	0.03	0.02	0.02	0.02	0.01
	August/2013	0.06	0.07	0.04	0.04	0.06	0.05
Fe	March/2013	0.32	0.27	0.16	0.13	0.17	0.10
	August/2013	0.54	0.34	0.35	0.24	0.30	0.38
Mn	March/2013	0.11	0.13	0.08	0.06	0.08	0.05
	August/2013	0.22	0.25	0.12	0.09	0.16	0.13
Cu	March/2013	0.00	0.00	0.00	0.00	0.00	0.00
	August/2013	0.01	0.01	0.01	0.00	0.01	0.01

[1]Treatments: T1 = richness of 29 species in 2 × 2 m spacing; T2 = richness of 58 species in 2 × 2 m spacing; T3 = richness of 114 species in 2 × 2 m spacing; T4 = richness of 29 species in 3 × 3 m spacing; T5 = richness of 58 species in 3 × 3 m spacing; T6 = richness of 114 species in 3 × 3 m spacing.

nine years, which is considered relatively young and the canopy did not close in some sections of the experimental area. Thus, the existing clearings allowed the entry of solar rays and favored both the action of rain on the vegetal material and the soil wetting and drying cycles, which accelerate the litter decomposition process (Rodrigues et al., 2010) and, consequently, unify biomass accumulation in the area.

When analyzing the sampling seasons, there was lower air temperature and rainfall in August, which coincided with the period of greatest total litter accumulation and leaves + miscellaneous and branches fractions, as observed by Pinto et al. (2009) and Godinho et al. (2014) (Table 3). According to these authors, litter decomposition is influenced by seasonal variations, mainly temperature and humidity, which is increased in warmer months and reduced in colder months, thus causing the increase in litter accumulation during the coldest period that, in the region under study, also had the lowest rainfall.

Another important issue to explain the greater biomass accumulations of fractions and total litter is the presence of deciduous species in the area, which lose their leaves during periods of lower precipitation. However, according to Godinho et al. (2014) and Pinto et al. (2009), seasonal variation in litter accumulation occurred due to water stress during the period of lower precipitation.

The average amount of total accumulated litter found in the present study (4.44 Mg ha^{-1}) is below values found in other studies performed in recovery or initial succession stage areas in Dense Ombrophilous Forests, where values ranged from 4.5 Mg ha^{-1} to 9.1 Mg ha^{-1} (Caldeira et al., 2008; Klippel et al., 2016). In the study by Caldeira et al. (2008), the authors verified litter accumulation of 4.5, 5.0, and 5.3 Mg ha^{-1} in a Dense Ombrophylous Submountain Forest, in Blumenau, Santa Catarina, during the initial, intermediate, and advanced recovery stages, respectively, indicating a tendency of increasing litter accumulation with increasing recovery age.

The leaves + miscellaneous fraction was the main component of litter accumulated on the soil (73%). From the nutrient cycling point of view, leaves represent the fastest and most nutrient-rich pathway, which constitutes a strategy of trees in the use of nutrients for their growth (Caldeira et al., 2008; Pinto et al., 2009; Godinho et al., 2014).

The production of litter varies according to the successional stage of the forest ecosystem, i.e., with forest age. In a review by Martinelli et al. (2017), the authors summarized data from 105 estimates of fine litterfall production from 45 sites in the Atlantic Forest domain, including two types of forests, evergreen and seasonal, and two successional stages, secondary and old growth. The average litterfall was 8.0 ± 2.5 Mg ha^{-1}. They emphasized that litterfall was significantly higher in seasonal forests than in evergreen forests and in old growth versus secondary forests. Leaves were the major litterfall component, contributing with 68% of the total. The second most important component was branches, contributing with 22%, followed by reproductive organs (flowers and fruits) with 6%.

4.2. Nutrient concentration in accumulated litter

In general, nutrient concentration in the leaves + miscellaneous fraction was higher than in the branches fraction. Leaves are composed of a physiologically more active tissue, where most living cells are found, which tend to accumulate higher amounts of nutrients due to transpiration and photosynthesis processes (Godinho et al., 2013a).

Ca and N were the macronutrients with the highest concentrations in fractions for the studied seasons. The different nutrient concentrations in accumulated litter are related to their respective mobility within the plant. Ca redistribution does not occur readily in plant tissues because it plays a structural role, being part of the cell wall and mainly being present in lignified structures (Godinho et al., 2014). Consequently, with foliar aging and abscission, Ca stocks tend to increase in litter. Despite the high N mobility in plants, N deposition and accumulation in the litter layer can be associated to dominance of the Fabaceae family, which are N_2 fixing species (Cunha Neto et al., 2013; Diniz et al., 2015).

In the branches fraction, Ca concentrations were higher in T1 and T2 treatments (Table 5). Considering other treatments of the same richness ([T1 and T4] and [T2 and T5]), the influence that spacing levels had on Ca concentrations was observed, with concentrations higher in more densely spaced treatments. A different result was obtained by Kolm & Poggiani (2003), who studied the influence of progressive thinning on nutrient cycling in *Eucalyptus* plantations and concluded that nutrient concentrations in litter were higher in treatments submitted to thinning and higher spacing.

For both leaves + miscellaneous and branches fractions, Ca concentrations were higher during the period of lower precipitation (Table 4 and 5). According to Sanches et al. (2009), water deficiency leads to an increase in the concentration of some nutrients in plant tissues, including Ca and N. In the present study, N concentrations; however, did not show any difference between seasons or between treatments within different fractions.

P was the macronutrient with the lowest concentration in both fractions, in all treatments and in both seasons. This fact is related to its low concentration in the soil (Godinho et al., 2013b), which is sometimes conditioned by P adsorption in soil. There were no significant differences in P concentrations between treatments for any of the two fractions under study. However, higher P concentrations were observed during the period of lower rainfall, which refers to a period of lower productivity of forests, when there is less demand for P, lower biochemical cycling and, consequently, this element is lost with the senescence of vegetal tissues.

K concentrations in leaves+miscellaneous and branches fractions were also low, which is related to the low biogeochemical cycling rates. Higher mobility nutrients in plant tissue, such as K, have more expressive cycling via retranslocation inside the plant (Godinho et al., 2014), which causes senescent tissues to have low concentrations. Differences in K concentrations between seasons were recorded for the leaves+miscellaneous and branches fractions, and were higher during the lower precipitation period. This variability in K concentrations present in litter observed between seasons evaluated is related to rainfall variation. This is explained by the high K susceptibility to leaching via washing of leaves and litter because K does not contribute to organic compounds, rather, K occurs in the soluble or adsorbed form in the cell fluid (Godinho et al., 2013a). The cycling of K in the plant-soil-plant relationship is faster than that of other nutrients because it is a monovalent cation (Godinho et al., 2014).

Similarly to K, Mg and S had low concentrations in leaves + miscellaneous and branches fractions. Although they were considered moderately washable or removable, there were minor variations. Mg concentrations were reduced in leaves, which is due to chlorophyll oxidation that occurs when leaves are in senescence and when they begin to decompose on the soil (Godinho et al., 2014). The concentrations of this macronutrient had seasonality only in the leaves + miscellaneous fraction, with lower value during the period of higher precipitation.

Regarding micronutrients, Fe was the element showing the highest concentrations, both in the leaves + miscellaneous and the branches fraction. This can be justified by the low Fe mobility in plant tissues. In the leaves + miscellaneous fraction, Fe concentrations had similar behavior to Ca between treatments, with T1 and T2 having higher concentrations compared to the other treatments. Similarly, T1 and T4 treatments that had the same richness as T2 and T5 showed influence of spacing on Fe concentration. The other micronutrients (Zn, Mn, and Cu) showed no differences in concentrations between treatments and seasons in both fractions.

4.3. Nutrient stock in accumulated litter

In general, nutrient stock reflects the accumulation/deposition tendency of litter biomass (Pinto et al., 2009; Godinho et al., 2014), as it is obtained by multiplying nutrient concentrations by accumulated biomass. According to Godinho et al. (2013a), nutrient stock assumes a greater proportionality ratio with litter biomass than with nutrient concentrations of litter biomass. Thus, considering the uniformity of accumulated biomass between treatments, it was decided not to perform statistical analyses for nutrient stock (Table 6).

Ca was the macronutrient that had the highest stock accumulated in litter (60.83 kg ha^{-1}), followed by N (52.00 kg ha^{-1}), due to the fact that these elements were present in the highest concentrations (Table 6). Based on results of a study by Vitousek (1984), Ca accumulation was high in the majority of tropical forests. Like Ca, N accumulation is commonly high in tropical forests due to to the presence of materials enriched with this element, such as those from the Fabaceae family.

In addition, there is frequent increase in N stock in the material already accumulated in the soil due to the addition by atmospheric precipitation, to the activity of N-fixing microorganisms from the atmosphere while using the source of litter carbohydrate, to the concentration of organic compounds produced or released by the decomposing microorganisms or by contamination through the fall of materials of animal or vegetal origin (Vuono et al., 1989). As a strategy for commercial or restoration plantations, leguminous species can be added for being able to contribute to biological N fixation (Rodrigues et al., 2009).

As for the other macronutrients, 10.45 kg ha^{-1} of Mg, 7.17 kg ha^{-1} of K, 4.88 kg ha^{-1} of S, and 1.42 kg ha^{-1} of P were accumulated in the litter, when considering the average among all treatments and seasons. In most cases, P is a limiting element in ecosystems (Selle, 2007) due to its adsorption in the soil, which explains its low concentrations in litter. Micronutrients that had the highest and lowest stock were Fe (1.64 kg ha^{-1}) and Cu (0.036 kg ha^{-1}), respectively, which were also influenced by their concentration values. The high Fe stock can be justified by the fact that this nutrient is the most absorbed by plants due to its higher concentration in the soil solution, as well as its low mobility in plant tissues (Caldeira et al., 2008). Cu is highlighted due to its low concentration and stock, which is in agreement to Caldeira et al. (2008), who found only 0.060 kg ha^{-1} of accumulated Cu in the litter of a Dense Ombrophylous Submontane Forest in Blumenau, Santa Catarina (Table 6).

Based on the results above, it is possible to verify that litterfall is especially important in forests of humid tropics, where almost 70% of soils have low nutrient reserves (Martinelli et al., 2017). Vegetal waste quickly decomposes in tropical areas (Powers et al., 2009), but nutrient loss is prevented by the presence of a thick mat of roots on the soil surface (Godinho et al., 2013b).

5. CONCLUSIONS

Different spacing levels, species richness, and sampling season analyzed in the restoration process, in general, did not influence biomass and nutrient concentrations of accumulated litter. The time elapsed between restoration implantation and data sampling for the present study may have been insufficient for the occurrence of differences in the variables evaluated. The biomass of different fractions (leaves + miscellaneous and branches) and total accumulated litter, as well as nutrient concentrations, were higher during the period of lower temperatures and precipitation. Despite the small amount of accumulated litter, it is of great importance for soil physical protection and fertility improvement, generating conditions for the stabilization of new forest species in the restoration area.

ACKNOWLEDGEMENTS

The authors thank the organizers of the VII Latin American Forestry Congress and the editor of the journal.

SUBMISSION STATUS

Received: 26 oct., 2018
Accepted: 4 dec., 2018

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FINANCIAL SUPPORT

The authors thank the support provided by the Vale Nature Reserve for hosting, study area concession, and fieldwork support.

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