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Original Article

Conservation of Nature

Soil Microbial Biomass as an Edge Effect Indicator in Semi-Deciduous Seasonal Forest Fragments

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ABSTRACT

This work had the objective to evaluate the activity and microbial biomass of the soil as an indicator of edge effect in Semi-Deciduous Seasonal Forest fragments. Three fragments of different sizes (small, medium and large) located in the Southwest region of Bahia state were evaluated, in which three sampling ranges were defined in each, and soil samples were collected. The carbon and nitrogen contents of the microbial biomass were determined by the fumigation-extraction method and soil respiration. The microbial biomass carbon (MBC), metabolic carbon quotient (qCO₂) and the microbial C:N and MBC:C ratios were discriminating indicators of the edge effect on the soil quality of the studied forest fragments. The integrated analysis of soil microbiological attributes is adequate to evaluate the edge effect in forest fragments. The size of the fragments and their isolation influence the MBC contents, showing greater vulnerability of the smaller fragments in relation to the actions of the external environment.

Keywords: fragmentation, microbial activity, forest soils.

1. INTRODUCTION AND OBJECTIVES

Humankind has used forest resources for their development since the earliest times, which over the years has caused the original forest cover to change. As a consequence of this process, there is a reduction in native vegetation areas and the formation of forest fragments. In Brazil, the Atlantic Forest is the biome most affected by fragmentation. Its remnants have been reduced to about 13% of its original area and are mostly divided into fragments with an area of less than 100 hectares (Silva et al., 2016).

Within the Atlantic Forest biome, one of the phytophysiognomies most affected by the fragmentation process is the Semi-deciduous Seasonal Forest, which occurs in the transition zone with the Caatinga biome (Bahia state and Northeast of Minas Gerais) and in the South of the country (Iesb, 2007). However, even though its territory has been reduced, this formation is still little studied.

Forest fragmentation causes the division of a population into two or more non-interconnected subpopulations (Laurance et al., 2003). This results in an increase in the amount of edges and abrupt edges which are subject to higher temperature, luminosity, wind speed and low relative humidity due to higher exposure (Holanda et al., 2010). As a result of this influence, several ecosystem functions, including biological and hydrological cycles, are also altered.

In this way, the edge effect in the forest ecosystem can be reproduced in different ways, such as changes in biodiversity and species interactions (Laurance et al., 2011), plant composition and richness (Riguete et al., 2013), litter production and accumulation (Vidal et al., 2007), and soil attributes (Freitas et al., 2008; Santos et al., 2011). Such changes can be used to define edge effect indicators capable of reflecting the conservation status of forest fragments.

Any transformation in the ecosystem can directly alter soil characteristics such as density and porosity (Martinkoski et al., 2017), organic matter content, biological activity (Freitas et al., 2008), and, consequently, fertility. Thus, soil quality assessment may aid in interpreting the magnitude of the effects of natural or anthropogenic interferences on the ecosystem.

Soil quality is usually measured through its chemical, physical and biological attributes, which

reflect soil condition and ecosystem sustainability (Araújo & Monteiro, 2007). The biological attributes of the soil are considered sensitive indicators to the anthropic disturbances and modifications in the soil, allowing their use in studies related to the quality and maintenance of ecosystems (Pôrto et al., 2009).

Among soil biological attributes, microbial biomass (MB) represents the labile fraction of soil organic matter (OM) and has a dynamic nature, being easily affected by biotic and abiotic factors (Gama-Rodrigues & Gama-Rodrigues, 2008). For this reason, although it represents a small proportion of the organic carbon in the soil, MB is usually more responsive to initial changes in OM levels than the soil organic C content (Anderson & Domsch, 1993; Gama-Rodrigues & Gama-Rodrigues, 2008). Thus, evaluating microbial biomass can help to understand the behavior of soil microorganisms, for example indicating the decomposition rate of organic matter and the release of carbon and nutrients into the soil (Padilha et al., 2014).

There are still few studies in Brazil related to the evaluation of the edge effect on soil quality in general, and in particular on the microbiological attributes of the soil. In this context, studies related to this theme may significantly contribute to understand the real consequences of fragmentation on the soil dynamics, in addition to providing subsidies for elaboration of more efficient conservation techniques.

This work had the objective to evaluate the activity and microbial biomass of the soil as an indicator of edge effect in Semi-deciduous Seasonal Forest fragments.

2. MATERIALS AND METHODS

2.1. Characterization of the study area

The study area is located in the city of Vitória da Conquista (BA), in the Planalto da Conquista, at altitudes varying from 857 to 1,000 m. The municipality is inserted in a transitional stretch between the Caatinga and Atlantic Forest biomes and its predominant vegetation is Montana Semi-deciduous Seasonal Forest, regionally known as *cipó* forest. The climate of the region is tropical altitude (Cwb, according to the Köppen classification), with an average temperature of 25 °C and annual precipitation of 850 mm. The Conquista Plateau Forest is a relatively low forest, with trees that have an average height between 10 and 15 meters, about 50% deciduous and with a species predominance of the *Parapiptadenia* and *Anadenanthera* genera, normally associated to the *Cavanillesia, Tabebuia* and *Cedrela* genera (IBGE, 2012).

2.2. Forest fragment selection

Three fragments of semi-deciduous seasonal forest were selected, a small one (7.3 ha), one of medium size (45 ha) and one of large size (142 ha), all with visually similar forest structure (given the stratification, tree sizes, and life forms present) and with similar successional and historical stages of disturbance. The selected fragments also had the same environmental situation, which was predominantly agricultural, and soils with clayey texture, classified as Dystrophic Red-Yellow Latosol. In addition, the fragments presented different characteristics in relation to the landscape: the small fragment (fragment 1) and the middle fragment (fragment 2) were totally isolated from other forest patches by agricultural areas, while the large fragment (fragment 3) was connected through a forest corridor to two smaller fragments, which were not evaluated in this study.

Fragment 1 is located in the vicinity of the BR-116 highway (14° 56' 40" S and 40° 53' 50" W). Fragment 2 is located in the Agricultural Field of the State University of Southwest Bahia (Uesb), Campus of Vitória da Conquista (14° 52' 46" S and 40° 47' 34" W). Fragment 3 is located near the BA-263 highway, between the municipalities of Vitória da Conquista and Barra do Choça (14° 52' 53" S and 40° 41' 33" W).

The Trakemaker Pro software was used in order to characterize the condition of the fragments in the landscape (Table 1), incorporating images from Google Earth from the last quarter of 2014, from which the total area of each fragment (ha) was obtained; nuclear area (ha) – fragment area excluding edges of 50 m (Murcia, 1995); distance from each fragment to other forest fragments greater than 50 ha (ISO) and proximity to forest fragments (PROX) – obtained by the formula PROX = S (A/D²), where A corresponds to the area of the neighboring fragment (m²) and D the distance edge to edge between the main fragment and the neighboring fragment, considering a maximum radius of 800 m from the edge, as adopted by Vidal et al. (2007).

2.3. Delimitation of sampling ranges

Three sampling ranges were defined in each of the forest fragments: (1) edge – positioned at the edge of the forest, 0-10 m from the edge; (2) transition – intermediate range, located 40-50 m from the edge, representing a transition zone within the forest; (3) interior – corresponding to the fragment matrix, located in the half of the total distance between the ends of each fragment, in the sense of traversing the demarcation of the strips (180, 400, and 500 m, respectively, for fragments 1, 2 and 3).

2.4. Soil collection

Soil samples were taken in September 2015. Three plots of $10 \times 30 \text{ m} (300 \text{ m}^2)$ were installed at random in each of the strips. In each plot, 10 simple soil samples were collected to form a composite of the 0-10 cm layer.

2.5. Soil chemical analysis

The soil samples were analyzed for P and K (extractable by Mehlich⁻¹), Ca, Mg, Al (exchangeable for KCl 1 mol L⁻¹), organic C by oxidation with $K_2Cr_2O_7$ 1.25 mol L⁻¹ in acid medium, total N by the Kjeldahl method, pH (in water) and granulometry, as described

Tabl	le 1.	. Indices ref	erring to t	he spatial	configuration	of the studied	l fragments (Vitória da	ι Conquista-BA	۱).
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Eusant	Are	a (ha)	Elevation (m)	ISO (m)	PROX ⁽¹⁾ (m)	
Fragment	Total	Nuclear	Elevation (m)	150 (m)		
1	7.3	2.5	890	683.0	0.16	
2	45.0	32.0	915	661.5	2.87	
3	142.0	113	930	342.5	29.48	

In which: 1: small-sized fragment (7.3 ha); 2: medium-sized fragment (45 ha); 3: large fragment (142 ha); ISO: isolation index and PROX: proximity index.

by Embrapa (1997). Table 2 summarizes the obtained results, presenting the chemical and granulometric characterization of the soil (layer 0-10 cm) in the different strips of the three studied forest fragments.

2.6. Analysis of soil microbial biomass

For the microbial biomass evaluations, the soil samples were homogenized and sieved with 2 mm mesh, incubated for seven days in a flask containing one vial of water and another with 1 mol L^{-1} NaOH to absorb CO₂ from the soil and to eliminate the effect of sieving, since this fragments the soil organic matter, making it more available to microbial attack. After this procedure, the moisture content of the soil samples was adjusted to 40% saturation capacity.

The microbial biomass of the soil was determined by the fumigation-extraction method, adopting the approach proposed by Tate et al. (1988) for C, and the method by Joergensen & Brookes (1990) for N. Microbial activity was measured in terms of soil respiration (CO₂ released). The amount of CO₂ released was estimated over a seven-day incubation period, using jars containing two glass flasks, one with soil (50 g) and another with 10 ml of 1 mol L⁻¹ NaOH. The jars were hermetically sealed and the NaOH solution was titrated with 0.5 mol L⁻¹ HCl

after the incubation period, using 2 drops of the phenolphthalein indicator.

Next, microbiological indexes were determined based on the obtained data: C ratio of microbial biomass/organic C (MBC:C), N ratio of microbial biomass/total N (MBN:N), C ratio of microbial biomass/N of microbial biomass (C:N mic), and metabolic quotient (qCO_2) calculated by the relation between the accumulated respiration and the C of the microbial biomass (Anderson & Domsch, 1993), expressed in mg CO₂ g⁻¹ CBM day⁻¹.

2.7. Data analysis

The Student's t-test at 5% of significance was used to compare the attributes and microbiological indexes of the soil between the ranges of each forest fragment, using the SAEG'v.9.1 statistical program. In addition, microbiological and soil chemical data were submitted to principal component analysis (PCA), with the purpose of condensing the multidimensional variation of the data in a diagram, ordering the treatments in components according to their similarities. The Canoco[®] v.5.0 program was used in the PCA.

In addition, Pearson correlations were established at a 5% significance level between chemical and microbiological soil attributes, also using SAEG[®] v.9.1.

Strip	pН	Р	Al	К	Na	Ca	Mg	H+Al	Clay	Sand	Silt
		mg dm-3		cmolcdm ³					gKg⁻¹		
			Fragment 1 ⁽²⁾								
Edge	4.6	3.0	1.2	0.12	0.02	1.1	0.41	11.6	454.8	518.0	27.2
Transition	4.4	3.2	1.5	0.11	0.03	0.7	0.26	11.2	450.0	516.4	33.6
Interior	4.6	3.4	1.2	0.10	0.02	0.8	0.39	8.9	451.1	273.0	275
		Fragment 2									
Edge	5.2	3.6	0.3	0.16	0.02	2.1	0.63	6.9	229.9	740.9	29.2
Transition	5.0	4.2	0.5	0.11	0.01	1.9	0.51	6.8	211.8	762.4	25.9
Interior	5.0	5.8	0.3	0.17	0.02	2.2	0.62	7.9	155.4	833.0	11.7
		Fragment 3									
Edge	4.8	3.4	0.9	0.13	0.02	1.2	0.51	9.1	361.6	610.6	27.8
Transition	4.6	2.8	1.4	0.10	0.02	0.7	0.28	11.7	385.7	586.9	27.4
Interior	4.7	3.2	1.2	0.11	0.01	0.7	0.38	9.4	308.8	671.7	19.5

Table 2. Chemical characterization and soil granulometric composition in three Semi-deciduous Seasonal Forest fragments in the Southwest region of Bahia state, Brazil.

In which: Fragment 1: small fragment (7.3 ha); Fragment 2: medium fragment (45 ha); Fragment 3: large fragment (142 ha).

3. RESULTS AND DISCUSSION

The studied soils are of high acidity and low to medium fertility. They presented clay contents varying between 199 g kg⁻¹ (fragment 2) and 452 g kg⁻¹ (fragment 1), with intermediate values in fragment 3 (mean of 352 g kg⁻¹) (Table 2). The total N contents were quite close between the fragments (mean of 1.6 g kg⁻¹), while the mean organic C contents ranged from 21.2 g kg⁻¹ in fragment 2, to 47.3 g kg⁻¹ in fragment 3 (Table 3). Rangel and Silva (2007) studied a Red Latosol with clayey texture (layer 0-10 cm) in the Semi-deciduous Seasonal Forest in the state of Minas Gerais, observing organic C and total N levels close to those found in this work (38.9 and 1.4 g kg⁻¹, respectively).

No significant variations were observed between fragments regarding MBC contents. When analyzing each of the studied areas, a variation between the strips was only observed in fragment 3, with a higher edge value $(331.9 \ \mu g \ g^{-1})$ in relation to the interior $(115.7 \ \mu g \ g^{-1})$.

A similar trend was found by Freitas et al. (2008) in an Atlantic Forest remnant (Dense Ombrophilous Forest) in the state of Pernambuco, in which they observed higher values for MBC at the edge (662.70 µg g⁻¹) in relation to the interior of the fragment (367.57 μ g g⁻¹). These authors observed a relationship between MBC values and soil carbon availability, which was also higher at the edge $(63.49 \text{ g kg}^{-1})$ than the interior $(30.59 \text{ g kg}^{-1})$. However, organic C soil levels in the present study do not explain the variation in MBC contents, since no significant differences were observed between the strips of the evaluated fragments, suggesting that other factors would favor greater carbon immobilization by microbial biomass at the edge, such as the physicochemical composition of litter accumulated on the soil. Changes in the quantity and quality of organic residues have a direct influence on the C contents of the soil microbial biomass, since it conditions the C and N additions to the soil (De-Polli & Guerra, 2008).

The results of MBN and accumulated respiration (AR) showed no variation between the ranges of any

Table 3. Organic carbon, total nitrogen, carbon and nitrogen from microbial biomass, accumulated respiration and soil microbiological indexes in three Semi-deciduous Seasonal Forest fragments in the Southwest region of the state of Bahia, Brazil.

Strip		Ν	MBC	MBN	AR	C:N _{mic}	MBC:C	MBN:N	qCO ₂	
	g kg ⁻¹		mg g⁻¹				%		mg g ⁻¹ day ⁻¹	
	Fragment 1 ⁽¹⁾									
Edge	38.6a	2.4a	275.9a	66.5a	215.2a	4.6a	0.71a	3.6b	306.2a	
Transition	41.4a	1.7a	247.3a	86.6a	101.9a	5.2a	0.58a	3.1b	165.9ab	
Interior	38.9a	1.9 a	174.3a	57.8a	85.4a	2.4a	0.45a	6.1a	67.6b	
Mean	39.6B	2.02A	232.5A	70.3A	134.2AB	4.0A	0.58AB	4.29A	179.89A	
	Fragment 2									
Edge	21.3a	1.1a	163.7a	47.5a	120.3a	2.8a	0.76a	4.5a	129.5ab	
Transition	31.6a	1.1a	137.8a	40.9a	160.9a	3.3a	0.43a	3.7a	207.1a	
Interior	31.8a	1.6a	176.6a	69.6a	137.6a	2.6a	0.56a	5.0a	103.9b	
Mean	61.7A	1.29A	159.4A	52.7A	139.6A	2.9A	0.84A	4.40A	146.83B	
Edge	42.9a	1.4a	331.9a	56.9a	110.4a	5.5a	0.78a	4.8a	84.6b	
Transition	47.2a	1.6a	166.1ab	48.7a	94.9a	3.9ab	0.35b	3.7a	149.7b	
Interior	51.8a	1.5a	115.7b	65.4a	141.7a	1.71 b	0.23b	4.8a	257.4a	
Mean	47.3AB	1.53A	204.6A	57.0A	115.7B	3.7A	0.45B	4.44A	163.91AB	

In which: Fragment 1: small fragment (7.3 ha); Fragment 2: medium fragment (45 ha); Fragment 3: large fragment (142 ha); C: Organic carbon; N: Total Nitrogen; MBC: Microbial biomass C; MBN: Microbial biomass N; AR: Accumulated respiration; C:N_{mic}: C ratio of microbial biomass: organic C; MBN:N: N ratio of microbial biomass: total N; qCO_2 : Metabolic quotient. Means followed by the same lower case or upper case letter in the column do not differ from one another by the t-test at 5%.

of the three studied fragments (Table 3). Similar results were reported by Silva et al. (2012), who also did not observe significant differences in MBN between secondary forests in different successional stages: initial (37 mg kg⁻¹), intermediate (48 mg kg⁻¹), and advanced (35 mg kg⁻¹).

The MBN:N ratio only showed a distinction between strips in the smallest fragment, ranging from 3.1 to 6.1%, with higher value in the interior and lower in the edge and transition strip (Table 3), which suggests that the biomass in the conditions of the interior yields greater efficiency in the conversion of soil nitrogen to microbial N.

On the other hand, the MBC:C ratio only varied significantly in the larger fragment, with lower value in the interior and transition (0.23%) in relation to the edge (0.78%). This is an indication that, under edge conditions, microbial biomass would be acting on better quality organic matter, since higher values of this ratio indicate greater efficiency in the conversion of soil carbon into microbial C (Wardle, 1992). These results corroborate previously discussed results and may be related to the occurrence of a higher proportion of pioneer leguminous species at the edge, which would be contributing to depositing plant residues with higher nutritional quality with high levels of N. In this sense, Malmivaara-Lämsä et al. (2008) evaluated urban forest fragments of boreal vegetation in Finland and observed that the C:N ratio of the soil increased in the edge-interior direction of the fragments. However, in this study, the C:N ratio of the soil varied little within the fragments.

The C:N_{mic} ratio also only showed significant differences between strips in fragment 3, being smaller in the interior (1.7) in relation to the edge (5.5) (Table 3). Lower values of this ratio demonstrate greater efficiency of microbial biomass in immobilizing soil carbon and nitrogen (Wardle, 1992). Therefore, the observed results indicate that the performance of the edaphic microbiota on the border, when compared to the interior, would be hindered, possibly due to another stress condition or higher recalcitrance of the organic matter.

The absence of variation of MBC, C:N_{mic} and MBC:C indices between the strips of fragments 1 and 2 may be related to the influence of size and isolation of the fragments (Table 1), which made the transition strip and interior of these fragments more susceptible to the changes caused by the edge effect. In other words,

this would be providing a more vulnerable state of conservation throughout fragments 1 and 2, while because fragment 3 has a larger area and proximity index (Table 1), this would allow greater preservation of the characteristics of its interior, thus differing from the edge. Oliveira & Mattos (2014) point out that smaller fragments tend to suffer greater interferences from external factors, and therefore the internal dynamics end up losing strength from the influence that the surroundings of the fragment exerts.

The qCO_2 was the index that was more responsive to the environment variations, since it presented differentiation between the strips of the three studied fragments. The lowest values in fragments 1 and 2 were observed in the interior in relation to the edge and transition (Table 3). This result suggests that in the strips near the edge, the soil microbial population is in a more stressful condition, probably due to the greater influence of the external environment.

Freitas et al. (2008) observed greater microbial activity at the edge when compared to the interior of an Atlantic Forest fragment, attributing the result to the effect of stress on soil microorganisms, which would result in high nutrients cycling. In evaluating the quality of soils under different vegetation cover, Jakelaitis et al. (2008) verified lower qCO_2 values in native vegetation (0.05 µg of CO_2 µg biomass⁻¹ day⁻¹) compared to pasture areas (0.17 µg of CO_2 µg biomass⁻¹ day⁻¹) and corn cultivation (0.18 µg⁻¹ day⁻¹), noting the efficiency of using qCO_2 as an indicator of stress or functional imbalances in the environment.

However, a contrasting trend was observed in fragment 3, with higher qCO_2 values in the interior and lower in the edge and transition (Table 3). This result may be related to differences in the C:N ratio of the soil between the sampling ranges, since a significant positive correlation was verified between the qCO_2 and the C:N ratio of the soil under this fragment (p < 0.05, r = 0.73). According to Paul (2016), soil respiration (and as a consequence the metabolic quotient) are influenced by several factors such as humidity, temperature, nutrient availability and soil C:N ratio.

For the smaller fragment, the PCA presented eigenvalues of 64.4% for the first axis (horizontal, PC1) and 35.6% for the second axis (vertical, PC2). The graphical dispersion (Figure 1A) shows that there was dissimilarity between edge, transition and interior of

the fragment. The location of the interior to the right of the diagram, near PC1, is due to the MBC, AR and N values verified in this range, considering that these variables were the most important for explaining the variance of the first axis. These same variables also explain the positioning of the edge to the left of the graph, between PC1 and PC2. On the other hand, the location of the transition to the left, very close to PC2, shows that organic C, an attribute most correlated with the second axis, was the most influential characteristic for differentiation of this range.

The eigenvalues found in the PCA of the middle fragment (Figure 1B) were 55.6% (PC1) and 44.4% (PC2). The positioning of the transition to the right of the ordering diagram, next to PC1, shows that MBC, AR and MBN attributes are more correlated with the first axis, and were the characteristics of greater influence for differentiation of this range. The location to the left of the edge (upper quadrant) and inner (lower quadrant) charts, close to PC2, was determined by the variables total N and organic C.

In relation to the large fragment, eigenvalues in PCA were obtained from 54.3% for PC1 and 45.7% for PC2 (Figure 1-C). For the first axis, the most discriminant variables in evaluating the variation between the strips were organic C and AR, while for PC2 the most important variables were total N and MBN. The location of the edge to the left of the graph, close to PC1, shows that MBC was the most influential characteristic for differentiation of this range.



Figure 1. Ordering diagram produced by the analysis of main components of the soil attributes in the sample ranges of the fragments: small (A), medium (B) and large (C). C: organic carbon; N: total nitrogen; MBC: microbial biomass C; MBN: microbial biomass N; AR: accumulated respiration in relation to the border, transition and interior of the fragments.

The PCA in the three forest fragments showed low grouping of the strips, which were distributed in different quadrants, although with a slight approximation of the characteristics of the edge in relation to the transition, indicating a dissimilarity between the strips with respect to the evaluated attributes. This result suggests differentiation of the soil microbial biomass efficiency in immobilizing C and N in the three considered conditions. In working with native forest soils and agricultural and pasture areas, Silva et al. (2012) verified that the MBC, MBN and arylsulfatase enzyme contents were the higher correlated attributes with the first ordering axis, enabling differentiation of two groups; one formed by agricultural areas and another by native forest and pasture. Also using principal component analysis to interpret the relationship of chemical and microbial attributes in soils under different eucalyptus plantations, Barreto et al. (2008) observed that the organic C and total N contents were the attributes of greater correlation with the first axis, concluding that these were more discriminating indicators than microbial biomass to measure variations between plantations.

The results obtained in this study reveal that the fragment size conditions the degree of influence of the external environment, taking smaller fragments to a status of similar edge effect throughout its extension and consequently a worse state of conservation. Thus, the greater edge-interior distance in large fragments would favor maintaining the natural characteristics of the microbial biomass of the soil inside due to the lower effect of the external environment.

4. CONCLUSIONS

The MBC, qCO_2 , and the C:N_{mic}, MBC:C ratios are discriminating indicators of the edge effect on the biological quality of the soil of the studied forest fragments. The integrated analysis of different soil microbiological attributes is adequate to evaluate the edge effect in forest fragments.

The fragment size and their isolation condition the influence level of the edge effect on the biomass and microbial activity of the soil. Smaller-sized fragments are more vulnerable to the actions of the external environment in all their extension, while larger fragments present greater capacity to preserve the characteristics of their interior.

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