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Edge Effect on Soil Biochemical and Microbiological Activities in an Atlantic Forest Fragment in the State of Pernambuco, Brazil

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ABSTRACT

The Atlantic Forest is one of the most important terrestrial biomes due to its high biodiversity and species endemism. However, the degree of fragmentation produced by anthropogenic activity results in an edge effect, changing ecological processes and affecting the environment. The degree to which the edge affects soil microorganism dynamics, nutrient cycling and ecosystem stability is poorly understood. In this paper the influence of the edge effect on soil quality was evaluated. Soil samples were collected from the edge of and from within an Atlantic Forest remnant and from an adjacent sugarcane field, at Igarassu, Pernambuco State, Northeast Brazil. The physical, chemical, biochemical and microbiological properties of samples were evaluated. Significant differences ($P<0.05$) were observed for all of the variables studied, with the exception of easily extractable glomalin (< 1 mm). Of the 21 variables measured, 14 were highest in the soil from the fragment edge while inside of the fragment only the percentage of mycorrhizal colonization was higher than in the edge. Significant and positive correlations between physical, chemical, biochemical, and microbiological variables were recorded, mainly in relation to soil organic carbon. Multivariate analysis demonstrated that the soil from the edge was chemically, biochemically and microbiologically different from that observed inside the fragment and also in the sugarcane field, which were similar to one another. In general, higher microbial activity occurred at the fragment edge, indicating the effect of stress on soil microorganisms, which results in high nutrient cycling in the system.

Keywords: environmental fragmentation, Humid Forest, soil quality, sustainability

INTRODUCTION

The Atlantic Forest is the second largest humid rain forest on the American continent (Tabarelli *et al.* 2005) and is considered one of the 25 global "hotspots" of biodiversity (Meyers *et al.* 2000). Due to anthropogenic pressures such as intensive agriculture it is also considered one of the most threatened biomes of the world (Barbosa and Thomas 2002) with 7.5% of its original area currently remaining. In the State of Pernambuco, Northeast Brazil, sugar cane crops have fragmented the Atlantic Forest, producing an ecological 'edge effect' (Tabarelli *et al.* 2005). However, it is not known if the edge effect interferes with soil microbial activity, which is important for nutrient cycling and maintenance of terrestrial ecosystems.

The fragmentation of the Atlantic Forest is reducing biodiversity thus compromising sustainability of the ecosystem (Tabarelli *et al.* 2005). This increase in environmental degradation is driving the search for sensitive indicators of soil quality to be used for rapid evaluation of the environment as well as for predicting which practices can degrade the soil and its capacity for recovery. Microbiological and biochemical rather than physical and chemical indicators are recommended because they are more sensible to variations in fertility and edaphic quality (Pinzari *et al.* 1999) and indicate not only the actual condition but also the potential sustainability of the system. Examples of such indicators include general or specific enzymatic activity, soil microbial biomass, respiratory activity, byproducts of microbial origin, such as polysaccharides and glomalin-related soil proteins (GRSP), produced by arbuscular mycorrhizal fungi and important for soil aggregation and also for copper and zinc sequestration in polluted soils (Cornejo *et al*. 2008). These parameters can be used to describe soil quality, and to predict the sustainability of perturbed systems. In this work we tested the hypothesis that the edge effect alters the microbial activity in Atlantic Forest fragments left among sugar cane fields.

MATERIALS AND METHODS

Study area

The studies were performed in a remnant of Atlantic Forest classified as Dense Ombrophyla (356.93 ha; 07° 45′ 48″ S, 38° 59′ 24″ W). The fragment, which is known as "Mata dos Macacos", belongs to the Usina São José (USJ) located in the Municipality of Igarassu, State of Pernambuco. The climate is hot and humid (As' -Koppen) with a mean annual temperature and precipitation of 24.9°C and 1.689 mm, respectively (Posto Metereológico USJ, 1998-2003). Soil samples were collected from the edge and within the fragment and also from the adjacent sugar cane field. The following plant species, among others, are found at the edge of the fragment: *Annona coriacea* Mart. (Annonaceae), *Bowdichia virgilioides* Kunth. (Fabaceae), *Brosimum rubescens* Taub. (Moraceae), *Dioclea bicolor* Benth. (Fabaceae), *Poinsenttia heterophylla* (L.) Klotzsch & Garcke (Euphorbiaceae), and *Solanum baturitense* Huber (Solanaceae). In the interior of the fragment *Tovomita mangle* G. Mariz (Clusiaceae), *Mabea occidentalis* Benth. (Euphorbiaceae), *Eriotheca crenulaticalyx* A. Robyns (Bombacaceae), *Chaetocarpus myrsinites* Baill. (Euphorbiaceae), *Erythroxylum squamatum* Sw. (Erythroxylaceae), and *Anaxagorea dolichocarpa* Sprague & Sandwith (Annonaceae) were present.

Sampling

Soil samples were collected from three areas: a) edge of the fragment (up to 100 m); b) inside the fragment (400 m); c) adjacent

Received: 3 July, 2008. Accepted: 11 November, 2008.

Original Research Paper

sugar cane field (at 150 m from the edge). The collection points were in the form of a zigzag - seven points, 20 m apart, each 0-20 cm deep. The samples were kept in plastic bags and transported to the laboratory in a cooler, for analysis.

Physical and chemical soil properties

Total soil carbon was determined by oxidation with potassium dichromate in medium with sulfuric and phosphoric acids, followed by titration with ammonium iron sulfate, using diphenylamine as a pH indicator. Values were expressed in g kg^{-1} (Embrapa 1999).

***Humic (HA) and fulvic acids (FA)*:** 30 mL of 0.1 N NaOH were added to 7.5 g of soil and the solution shaken at 200 rpm for 24 h, centrifuged (2000 × *g*/10 min) and acidified with concentrated hydrochloric acid (HCl) at pH 2.0. After 24 h the supernatant (FA) was separated from the precipitate (HA). Each component (HA, FA) was gravimetrically determined after drying (105°C/24 h) and expressed in g^{-1} soil (Frighetto 2000; Tiquia 2005). The ratio HA/FA was calculated using the obtained values.

Soil humidity was gravimetrically determined after drying 2 g of soil (105°C/24 h); values were expressed as a percentage.

Soil pH was evaluated in solution of soil/distilled water (1:2.5 v/v) in a potentiometer (Analyser, pH/lon 450 M) after vigorous agitation and resting for 1 h.

Microbiological and biochemical soil properties

***Microbial respiration*:** 100 g of soil was incubated in recipients of 1000 mL with 10 mL of KOH (0.5 N) for 15 d, in the dark. The liberated CO_2 captured by the KOH solution was quantified by titration with 0.1 N HCl, using phenolphthalein (0.1% in ethanol) and methyl orange (1%) as pH indicators. The CO_2 carbon liberated by microorganism respiration was expressed in µg C-CO_2 g^{-1} of dry soil day^{-1} (Grisi 1978).

Carbon of the microbial biomass was estimated by fumigation with chloroform free of ethanol in 20 g of soil, followed by extraction of carbon with 50 mL of potassium sulfate (0.5 M) and oxidation with 2 mL of potassium dichromate (0.66 mM) in medium with 10 mL of concentrated sulfuric acid and 5 mL concentrated phosphoric acid. The carbon was quantified by titration with ammonium iron sulfate (0.033 N), using diphenylamine 1% as indicator. Values were calculated from fumigated and non fumigated soil samples, using the correction factor K_C= 0.33 and the values expressed in µg C g^{-1} dry soil (De-Polli and Guerra 1997). The qCO_2 was determined by the relation between the liberated CO_2 carbon and the soil microbial biomass carbon. The relation carbon of the microbial biomass and total carbon (C-BM/total C) was also calculated and expressed as a percentage.

***Cellulase activity*:** soil samples (5 g) were incubated (24 h/50°C) with 15 mL of carboxymethylcellulose (0.7% in sodium acetate buffer 2 M; pH 5.5) and 15 mL of acetate buffer (2 M; pH 5.5). The supernatant was filtrated and the reduction of sugars determined after reaction with potassium ferric hexacyanate, and formation of the complex called Prussian blue, measured in a spectrophotometer (690 nm). The standard curve was prepared from a monohydrated glucose solution (28 mg L^{-1}) and the values expressed in µg glucose g^{-1} dry soil 24 h^{-1} (Schinner and von Mersi 1990).

***Saccharase activity*:** 15 mL of sodium acetate buffer (2 M; pH 5.5) and 15 mL of sucrose solution (1.2% in sodium acetate 2 M; pH 5.5) were added to 5 g of soil. After incubation (3 h/50°C), the supernatant was filtered and the reduction of sugars determined as for cellulase. The standard curve constructed for cellulase was used and the values expressed in µg glucose g^{-1} dry soil 3 h^{-1} (Schinner and von Mersi 1990).

***Dehydrogenase activity*:** 5 g of soil were incubated with 5 mL of 1% TTC (2,3,5 triphenyltetrazolium chloride) in water bath (37°C) per 24 h. After that, the reaction was stopped with 10 mL methanol and the TTF (triphenylphormazan) formed by the action of dehydrogenase in the TTC reduction measured in a spectrophotometer (485 nm). The standard curve solution of TTF (1% in methanol) was used and the values of the enzymatic activity expressed in µg TTF g^{-1} dry soil (Casida *et al.* 1964).

***Hydrolysis of fluorescein diacetate activity (FDA)*:** soil samples (5 g) were incubated in an Erlenmeyer flask with 20 mL potassium phosphate buffer (66 mM; pH 7.6) and 200 µL of FDA solution (0.02 g/10 mL acetone) for 20 min. The reaction was interrupted by addition of 20 mL acetone, with measurements in a spectrophotometer (490 nm). For the standard curve increasing concentrations of FDA previously hydrolyzed by heat (100°C) were used. The enzymatic activity was expressed in µg of hydrolyzed fluorescein g^{-1} dry soil h^{-1} (Swisher and Carrol 1980).

***Polysaccharides of microbial origin*:** 30 mL of sodium hydroxide 0.5 N (NaOH) was added to 7.5 g of soil and stirred for 3 h; the pH was adjusted to 2.5 and the solution centrifuged (2500 rpm/5 min) to precipitate humic acids. After filtration 40 mL of acetone was added to 20 mL of the filtrate and the mixture left to rest for new centrifugation; the supernatant was discard by decanting. Later, 5 mL of water/acetone solution (1:1 v/v) was added to the sediment, followed by agitation and centrifugation for 10 min. After discarding the supernatant, the sediment was dried (40°C/10 min) and the polysaccharide carbon was determined by oxidation in medium with 1 mL of potassium dichromate (1 N) and 2 mL of concentrated sulphuric acid. The readings were taken using a spectrophotometer (600 nm) and the values were expressed in mg g^{-1} dry soil. The standard curve was prepared from agar solution (1 mg mL^{-1}) (Frighetto 2000).

***Density of AMF spores*:** spores of arbuscular mycorrhizal fungi (AMF) were extracted from 50 g of soil samples by wet sieving and sucrose centrifugation (Gerdemann and Nicolson 1963; Jenkins 1964) and counted using a stereomicroscope (40X).

Mycorrhizal colonization was determined by the gridline intersect method (Giovannetti and Mosse 1980), in roots cleared with KOH (10% w/v) and H_2O_2 (10% v/v) and stained with Chlorazol Black-E 0.01%, and expressed as a percentage.

***Glomalin-related soil proteins (GRSP)*:** quantified in two fractions (Wright and Upadhyaya 1998) from aggregates < 1 mm and 1-2 mm in diameter. The easily extracted fraction (EEF) was obtained from 0.25 g of soil aggregates. The glomalin fractions were quantified by the Bradford (1976) method using a spectrophotometer (595 nm) with bovine serum albumin (BSA) as standard. The data were expressed as mg of glomalin g^{-1} of aggregates after correcting the extraction volumes. Glomalin carbon (C-glomalin) was estimated from total glomalin, considering that carbon represents 43.1% of the molecule (Rillig *et al.* 2003), and expressed in mg g^{-1} aggregates. The percentage contribution of glomalin to total soil carbon was calculated based on the relation between C-glomalin and the total soil carbon in aggregates of < 1 mm and 1-2 mm.

Experimental design and data analysis

The experimental design was entirely random with 3 treatments: fragment edge, interior of the fragment and sugar cane field, with 7 replicates, totaling 21 experimental units. The data were submitted to analysis of variance and the means compared by Tukey's test ($P<0.05$). Single Pearson (r) correlation analysis was also performed between the studied variables. Multivariate analysis was performed considering all evaluated parameters and the methods of Euclidian Distance and UPGMA (un-weighted pair-group method). Statistica 6.0 (Statsoft 2002) was used for the analyses.

RESULTS AND DISCUSSION

There was an effect ($P<0.05$) of the treatments on all studied variables, with the exception of the metabolic coefficient (qCO_2), microbial carbon biomass/total carbon and easily extractable glomalin (< 1 mm) (**Table 1**).

The highest values of C were registered in the edge soil,

Table 1 Analysis of Variance for the studied parameters.

Parameters	Effect
CO_2 evolution	**
Carbon of microbial biomass (C-MB)	**
Metabolic quotient (qCO_2)	ns
C-MB/total organic C	ns
Celulase	**
Saccharase	**
Dehydrogenase	**
Hydrolysis of FDA activity	**
Microbial polysaccharides	**
Easily extractable glomalin (< 1mm)	ns
Easily extractable glomalin (< 1-2 mm)	**
Total glomalin (< 1mm)	**
Total glomalina (1-2 mm)	**
Glomalin carbon	**
Glomalin-C (< 1 mm)/soil-C	**
Glomalin-C (1-2 mm)/soil-C	**
Density of AMF spores	**
Mycorrhizal colonization	**
Total organic carbon	**
Humic acids (HA)	**
Fulvic acids (FA)	*
Relation HA/FA	**
Soil pH	**
Soil humidity	**

*(P<0.05); **(P<0.01); ns (not significant)

Table 2 Content of carbon, humidity, humic acids (HA) and fulvic acids (FA), relation HA/FA and pH in soil from the edge and inside of an Atlantic Forest fragment (Mata dos Macacos) and adjacent sugar cane field at the Municipality of Igarassu, Pernambuco State, Brazil.

Variables	Areas		
	Sugar cane	Edge	Interior
Total carbon (g kg^{-1})	10.87 c	63.49 a	30.59 b
Soil humidity (%)	5.20 b	14.30 a	6.70 b
Humic acids (g g^{-1} solo)	0.04 b	0.34 a	0.04 b
Fulvic acids (g g^{-1} solo)	0.18 a	0.13 ab	0.15 b
Relation HA/FA	0.22 b	2.61 a	3.10 b
Soil pH (H_2O) (1:2.5 v/v)	5.61 a	4.39 b	4.71 b

Means followed by the same letter in a row do not differ according to Tukey's test (P<0.05).

followed by those from inside the fragment and the sugar cane field (**Table 2**). Similar results were obtained by Dinesh *et al.* (2003), who observed higher carbon content in the soil of a Tropical Rain Forest in relation to commercial crops of *Pterocarpus dalbergioides* DC. and *Tectona grandis* Linn. In general, crop areas present lower values of soil organic matter when compared to Tropical Forest systems (Nogueira *et al.* 2006).

Soils from the sugar cane field and from the interior of the fragment had similar humidity values, which were lower than those from the edge (**Table 2**). Similarly, forest disturbed areas in Costa Rica had higher humidity than soils from preserved forests and pasture fields (Groffmann *et al.* 2001). Templer *et al.* (2005) found higher humidity in soil of an impacted forest under regeneration comparing with a preserved area.

The content of humic acids and the relation between humic acids and fulvic acids (HA/FA) were similar for the soils from the interior and from the sugar cane field but were significantly higher in the soil from the fragment edge (**Table 2**). A high HA:FA ratio has been suggested as indicator of degree of humification of the system (Tiquia 2005). Soils from Tropical Forests in Bangladesh had higher amounts of humic acid in relation to a sugar cane field (Islam and Wueil 2000). As observed in the present study the edge effect can alter the degree of humification of soil organic matter.

The pH of the forest fragment (edge and interior) was comparatively more acid than the adjacent field (**Table 2**). In Ethiopia, sugar cane fields were less acidic than forest soils (Teklay *et al.* 2006). This can be the result of liberation of organic acids and H^+ from the decomposition of organic matter, whose content is higher in the forest (**Table 2**). However, for this variable no effect was observed.

Higher values of CO_2 evolution and carbon of microbial biomass (C-MB) occurred in the soil of the fragment edge, with the lowest values obtained in the sugar cane field (**Table 3**). In general, higher values of CO_2 mineralization and C-BM occur in Tropical Forests, in comparison with cultivated areas (Templer *et al.* 2005). The values of these variables, in the forest fragment and in the sugar cane field were similar to those obtained in other tropical areas (Dinesh *et al.* 2004a). Probably the higher values of C-MB and $C\text{-}CO_2$ were due to higher availability of carbon in this area. The increase in soil organic matter improves respiration and microbial biomass (Fernandes *et al.* 2005).

In tropical areas organic matter has been considered the main factor altering the activity of the edaphic microbiota (Dinesh *et al.* 2004a), and in disturbed areas, such as the one studied, the fast growing vegetation provides organic substrates for the microbiota, favoring its activity (Groffmann *et al.* 2001). Besides, in fragmented areas there is high emission of CO_2 of plant origin (Nascimento *et al.* 2003), and in this study this was observed for the CO_2 of microbial origin (**Table 3**).

Although considered an indicator of system maturity (Nogueira *et al.* 2006) qCO_2 did not differ between areas with different degrees of impact (**Table 3**). Similarly, qCO_2 does not effectively separate differently managed agricultural areas (Pereira *et al.* 2004). Thus, the use of qCO_2 to define the equilibrium of an ecosystem has received some criticism, considering its incapability to distinguish between the effects of disturbances from those caused by "stress" (Wardley and Ghani 1995).

The ratio C-MB/total C did not differ among the studied areas (**Table 3**). In other Tropical Forests, different uses of the soil did not change the value of this ratio (Islam and Wueil 2000). The low ratio obtained in this study (~ 1%) indicates the reduced availability of organic matter for the soil microbiota (Fernandes *et al.* 2005), characterizing the stress situation of the system as referred by Böhme and Böhme (2006) for cultivated areas.

With the exception of dehydrogenase activity, other enzymes (cellulase, saccharase and hydrolysis of FDA) had higher activity in the soil from the fragment edge than from the interior and the sugar cane field (**Table 3**). This was similar to results obtained for total C. In general, soils with high content of organic matter have enzymatic activity maximized (Kaushik *et al.* 2005). Considering that the humidity content improves the activity of soil enzymes (Nogueira *et al.* 2006), higher humidity in the fragment edge soil probably contributed to higher values of enzymatic

Table 3 Evolution of CO_2, carbon of microbial biomass (C-MB), metabolic quotient, relation C-MB/total C, polysaccharides of microbial origin and activity of cellulase, saccharase, dehydrogenase and hydrolysis of fluorescein diacetate (FDA) in soil from the edge and interior of an Atlantic Forest fragment (Mata dos Macacos) and adjacent field of sugar-cane, at the Municipality of Igarassu, Pernambuco.

Variables	Areas		
	Sugar cane	Edge	Interior
$C\text{-}CO_2$ (μg C- CO_2 g^{-1} dry soil day^{-1})	1.75 c	11.34 a	6.03 b
C-MB (μg C- CO_2 g^{-1} dry soil)	117.73 c	662.70 a	367.57 b
qCO_2	0.016 a	0.015 a	0.014 a
C-MB/total C (%)	1.08 a	1.04 a	1.20 a
Cellulase (μg glucose g^{-1} dry soil 24 h^{-1})	0.61 c	0.82 a	0.34 b
Saccharase(μg glucose g^{-1} dry soil 24 h^{-1})	0.15 c	3.12 a	1.39 b
Dehydrogenase (μg TTF g^{-1} dry soil)	10.80 a	8.95 a	5.34 b
FDA (μg fluorescein g^{-1} dry soil h^{-1})	26.02 c	69.46 a	46.04 b
Polysaccharides (mg g^{-1} dry soil)	4.58 b	10.64 a	5.95 b

$C\text{-}CO_2$ = evolution of CO_2; C-MB = carbon of microbial biomass; qCO_2 = metabolic coefficient; TTF (Triphenylphormazan). Means followed by the same letter in a row do not differ according to Tukey's test (P<0.05).

Table 4 Mycorrhizal colonization, AMF sporulation and production of easily extractable and total glomalin (in aggregates <1 mm and of 1-2 mm), carbon of glomalin and relation carbon of GRSP/soil carbon in the edge and interior of an Atlantic Forest fragment (Mata dos Macacos) and adjacent sugar cane field, at the Municipality of Igarassu, Pernambuco State, Brazil.

Variables	Areas		
	Sugar cane	Edge	Interior
Mycorrhizal colonization (%)	7.76 b	2.07 c	23.48 a
AMF spores density (50 g^{-1} soil)	38.00 a	16.00 b	11.00 b
EEG (mg g^{-1} aggregates 1-2 mm)	0.62 b	1.73 a	0.86 b
TG (mg g^{-1} aggregates < 1 mm)	1.31 b	2.71 a	1.65 b
TG (mg g^{-1} aggregates 1-2 mm)	0.62 b	2.25 a	0.86 b
Glomalin-C (mg g^{-1} aggregates)	0.27 b	0.96 a	0.37 b
Glomalin-C (< 1 mm) / soil-C (%)	7.49 a	1.76 b	1.96 b
Glomalin-C (1-2 mm) / soil-C (%)	5.52 a	1.56 b	1.61 b

EEG = easily extractable glomalin; TG = total glomalin; glomalin-C = carbon from glomalin; soil-C = soil carbon. Means followed by the same letter in a row do not differ according to Tukey's test (P<0.05).

activity.

Cellulose is one of the main polymers in forest soils, and is positively related to production of cellulases; the high activity of these enzymes at the edge of the fragment (**Table 3**) suggests that a higher input of cellulosic material occurred in this area, providing substrate for enzymatic action (Dinesh *et al.* 2004b). Probably the products of enzymatic action (oligosaccharides and glucose) were important for edaphic microbiota activity and consequently for nutrient cycling in this system. Moreover, the higher content of carbon at the edge of the fragment contributed for higher activity of cellulase, once that a large amount of this enzyme may be adsorbed to soil organic matter (Sinegani *et al.* 2005).

Similarly, the participation of sucrose on nutrient cycling results from liberation of low molecular weight sugars to the microbiota (Zhang *et al.* 2005). The higher acti-vity of these hydrolases in the soil from the fragment edge indicates that soil fertility is changing (Caravaca *et al.* 2006).

The soil from the fragment edge had dehydrogenase activity similar to that of the sugar cane field (**Table 3**). The activity of this enzyme is related with soil carbon (Casida *et al.* 1964), but this was not observed in this study. In some situations it was not possible to distinguish areas with different degrees of soil impact using this enzyme as a parameter (Graham and Haynes 2005). Probably the synthetic acceptor (TTC) used in the enzymatic assay was not sensitive to the acceptance of electrons (Casida *et al.* 1964). Thus, dehydrogenase should not be considered as an indicator of changes in soil quality of Atlantic Forest fragments.

The input of substrates, due to the content of C in the edge of the fragment probably contributed to higher hydrolytic activity of FDA in this location (**Table 3**). Moreover, the enzymes (proteases, lipases and non-specific sterases) that work on cleavage of FDA can be adsorbed to the colloids of organic matter, contributing to higher enzymatic activity at the edge of the forest fragment. This activity, in the studied areas, was comparatively superior to that referred for soils of others Atlantic Forest fragments in the State of Sergipe, also in Northwest Brazil (Silva *et al.* 2004).

Higher production of polysaccharides in the soil occurred also at the edge of the fragment (**Table 3**). In the soil, such polymers participate on particles aggregation. Probably higher synthesis of these compounds indicates that the aggregation and consequent quality of the soil is improving, considering that polysaccharides are potential indicators of improvement of edaphic health (Frighetto 2000).

Plants from inside the fragment had greater mycorrhizal colonization than those from the edge and from the sugar cane field (**Table 4**). This suggests that the environment inside the fragment is more suitable for the establishment of VAM while the edge effect compromised the formation of the symbiosis. It also shows that sugar cane roots are not easily colonized as those from the forest and confirms the low mycotrophic dependence of this grass crop (Siqueira and Franco 1988). Similar results were obtained by Muthukumar *et al.* (2003): plants from a disturbed Tropical Forest presented lower AMF colonization than those from the preserved primary forest.

Conversely, more spores were obtained in the sugar cane field than in the other studied areas (**Table 4**). High sporulation can indicate that the sugar cane field is under unfavorable or stress conditions (Picone 2000). In other fragments of Atlantic Forest in Northeast Brazil the edge effect increased AMF sporulation (Maia *et al.* 2006). This was not observed in this study, considering that the density of spores did not differ between the edge and inside the Forest fragment. The number of spores recovered in the studied fragment is similar to that registered by Muthukumar *et al.* (2003) in soil of a Tropical Forest in China, but lower than that found in a Tropical Humid Forest in Southeast Brazil (Trufem 1990).

The fractions and the PRSP carbon varied depending on the study area, with higher activity of AMF in soil of the fragment edge (**Table 4**). Higher production of glomalin-related proteins at the edge can represent a mechanism to improve soil aggregation. Rillig and Steinber (2002) observed that systems with a low degree of aggregation, such as fragment edges have high deposits of PRSG. Probably the high content of humidity and carbon stimulated the production of external mycelium, responsible for synthesis of these proteins.

The observed contents of C from glomalin were similar to those registered by Borie *et al.* (2006) in soils of Chile. At the edge and inside the fragment the level of carbon from GRSP/soil carbon was < 2%, in both fractions of aggregates studied (**Table 4**). Borie *et al.* (2006) mentioned that the GRSP represented 5% of soil carbon and the values obtained in our study were comparatively lower, probably because only aggregates were used in the analysis. Besides, it is possible that the GRSP had been used as energetic substrate in microbial oxidative processes (Rillig *et al.* 2003), decreasing its contribution to total soil carbon.

As observed in other cases, CO_2 evolution was positively correlated (**Table 5**) with content of total carbon (Menyailo *et al.* 2003), soil humidity (Groffman *et al.* 2001), carbon of the microbial biomass (Dinesh *et al.* 2004a), sucrose activity (Graham and Haynes 2005) and FDA hydrolysis activity (Nasabimana *et al.* 2004). Positive relations between the microbial biomass carbon (C-MB) and enzymatic activity (**Table 5**) were registered by Monokrauso *et al.* (2006).

The content of total carbon was linearly correlated with the enzymatic activity, CO_2 evolution and C-MB (**Table 5**). Similar correlations have been found in other studies (Silva 2006), indicating that organic matter can be an energy source for microbial processes (Carrasco *et al.* 2006).

Positive correlations between the content of humic acids and enzymatic activity (**Table 5**) can be due to the fact that these acids may act as an adsorbent of molecules, as suggested by Arancon *et al.* (2004). Schinner and von Mersi (1990) also found a positive linear correlation between humic acids in the soil and activity of hydrolases.

The evolution of CO_2 was positively correlated with the fractions of glomalin (**Table 5**), indicating that the microorganisms were able to use the glucose portion of the GRSP in the oxidative processes, as suggested by Rillig *et al.* (2003).

The linear relation between the enzymatic activity and the fractions of glomalin (**Table 5**) suggests that this glycoprotein was used as substrate for enzymatic catalysis, which has been not mentioned before.

The relation between soil pH and glomalin fractions was linear negative while soil humidity was positively correlated with pH (**Table 5**), as observed in other studies (Rillig *et al.* 2003).

The production of spores was not related with mycorrhizal colonization (**Table 5**), as also observed in other Tropical Forest area (Muthukumar *et al.* 2003). On the other

Table 5 Pearson coefficient of single correlation (r) between the studied variables (P<0.05).

Var	CO_2	C-MB	TOTC	CEL	SAC	POLI	FDA	HA	FA	HA/FA	EEG (<1)	EEG (1-2)	TG (<1)	TG (1-2)	C-GLO	SD	COL	pH	SH
CO_2	-																		
CM-B	0.95	-																	
TOTC	0.96	0.99	-																
CEL	0.94	0.97	0.99	-															
SAC	0.92	0.97	0.97	0.95	-														
POLI	0.82	0.85	0.89	0.89	0.86	-													
FDA	0.88	0.91	0.92	0.92	0.92	0.81	-												
HA	0.75	0.80	0.83	0.79	0.83	0.74	0.74	-											
FA	-0.60	-0.65	-0.63	-0.60	-0.58	-0.61	-0.51	ns	-										
AH/AF	0.79	0.85	0.88	0.85	0.87	0.83	0.76	0.97	0.97	-									
EEG (<1)	ns	ns	ns	ns	ns	ns	0.44	0.52	-0.56	0.50	-								
EEG (1-2)	0.79	0.84	0.86	0.82	0.91	0.79	0.83	0.86	ns	0.88	0.55	-							
TG (<1)	0.81	0.85	0.86	0.86	0.85	0.80	0.89	0.79	ns	0.82	0.52	0.92	-						
T G (1-2)	0.76	0.75	0.80	0.76	0.84	0.82	0.73	0.83	ns	0.83	0.59	0.88	0.78	-					
GLO	-0.75	-0.79	-0.75	-0.73	-0.75	-0.63	-0.76	-0.44	ns	-0.48	ns	-0.59	-0.63	-0.53	-				
SD	-0.61	0.59	-0.56	-0.56	-0.59	ns	-0.56	ns	0.44	ns	ns	ns	ns	ns	0.65	-			
COL	ns	ns	ns	ns	ns	ns	ns	-0.57	ns	-0.56	-0.44	-0.55	-0.54	-0.55	ns	ns	-		
pH	-0.76	-0.82	-0.80	-0.81	-0.83	-0.72	-0.81	-0.53	ns	-0.58	ns	-0.65	-0.67	-0.63	0.90	0.60	ns	-	
SH	0.89	0.89	0.92	0.92	0.90	0.82	0.81	0.86	0.44	0.90	0.56	0.87	0.88	0.82	-0.55	ns	-0.53	-0.69	-

Var = variables. CO_2 = CO_2 evolution; C-MB = carbon of the microbial biomass; TOTC = total carbon; CEL = cellulase; SAC = saccharase; POLI = polysaccharides of microbial origin; FDA = hydrolysis of fluorescein diacetate activity; HA = Humic acids; FA = fulvic acids; HA/FA = relation humic and fulvic acids; EEG = easily extractable glomalin; GT = total glomalin; (<1) = aggregates < 1 mm diameter; (1-2) aggregates 1- 2 mm diameter; C-GLO = carbon of glomalin; SD = density of AMF spores; COL = mycorrhizal colonization; SH = content of soil humidity; ns = not significant

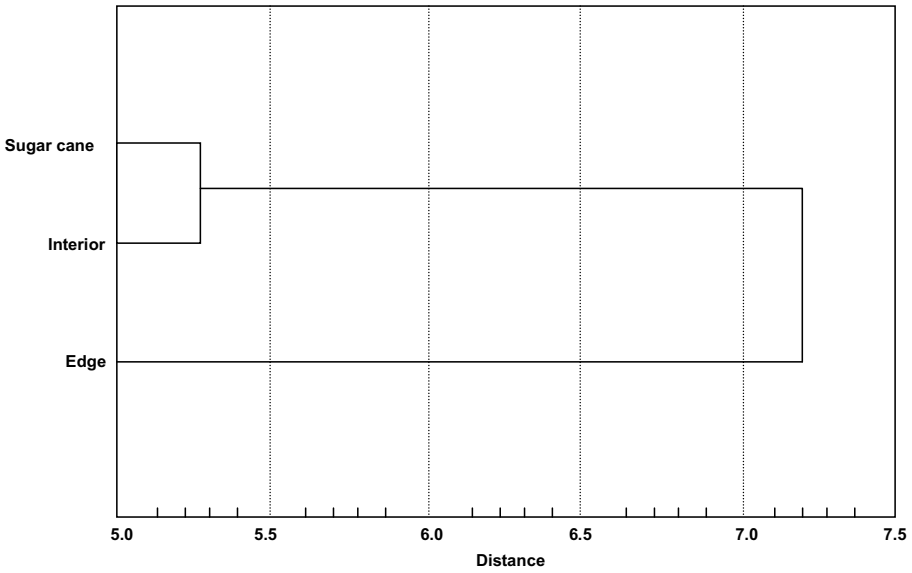

Fig. 1 Grouping analysis of similarity among the areas based in all variables: total carbon, soil humidity, pH, CO_2 evolution, microbial biomass carbon, *q* CO_2, relation C-MB/total C, cellulase, dehydrogenase, activity of hydrolysis of fluorescein diacetate, polysaccharides of microbial origin, content of humic acids (HA) and fulvic acids (FA), relation HA/FA, easily extractable glomalin and total glomalin in aggregates < 1 mm and 1-2 mm, carbon of glomalin (Glo-C), relation Glo-C/Total C, AMF spores density and mycorrhizal colonization.

hand, in a Costa Rica Tropical Forest, soil pH was positively correlated with AMF sporulation (Picone 2000).

According to the multivariate analysis, the soil from the fragment edge had chemical, biochemical, and microbiological behavior different from those observed inside the fragment and in the sugar cane field, which were similar (**Fig. 1**). This does not indicate that the soil from the sugar cane field is as stable as the soil inside the forest, but rather that the soil from this area has lower activity than that at the edge of the fragment. Templer *et al.* (2005) and Groffman *et al.* (2001) observed that for some biochemical parameters of soil quality, forest areas were similar to crop fields. The use of multivariate analysis in our study was important to distinguish environments with different microbial activity. In another context, Nogueira *et al.* (2006) used the same analysis to separate areas with different soils.

In general, higher microbial activity occurred at the fragment edge, indicating that the system presents high nutrient cycling, possibly due to the different amount of plant residues in this area (Nascimento *et al.* 2003), which were used as substrate by the soil microbiota. It also shows the hyperdynamism in fragmented areas, with edge effect (Laurence 2002) also over the soil microbiota. Thus, the initial hypothesis is confirmed, showing that besides altering plant communities (Tabarelli and Silva 2004), the edge effect also changes the edaphic microbiota activity. Further studies are needed to define whether the observed pattern is changed by season and size of the forest fragments.

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