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Changes in soil microbial biomass and activity in different Brazilian pastures

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Abstract

Microbial biomass and activity are useful indices for assessing changes in soil ecosystems. The impact of different pastures on microbial biomass and activity was studied in a long-term experiment in Northeast Brazil. For our study the pastures were divided into plots: a) *Brachiaria brizantha*; b) *Leucaena leucocephala*; c) *Cynodon dactilon*; d) *Panicum maximum*. An adjacent area with native vegetation was used as reference. Soil samples were collected in 0-10 and 10-20 cm depths. No significant differences in soil organic C (C_{org}) was found among all plots at 0-10 and 10-20 cm depth. Soil microbial C (C_{mic}) values were higher in native forest and *P. maximum* when compared to the other plots. The soil basal respiration (CO_2) values were similar among all plots evaluated. However, respiratory quotients (qCO_2) were significantly lower in native forest and *P. maximum* when compared to other plots, at 0-10 cm depth. Values of fluorescein diacetate (FDA) hydrolysis were significantly higher in native forest and *P. maximum*, while values of dehydrogenase activity were found to be significantly higher in native forest, *C. dactilon* and *P. maximum.* Soil microbial biomass and activity changed when a native forest was converted to pastures. These changes were positive with the inclusion of *P. maximum* by the high input of C sources.

Additional key words: bioindicators; qCO_2; q_{mic}; soil enzymes; soil quality.

Resumen

Cambios en la biomasa y actividad microbiana del suelo en distintos pastos brasileños

La biomasa y actividad microbiana son índices útiles para identificar cambios en los ecosistemas de suelo. Se estudió el impacto de diferentes pastos en la biomasa y actividad microbiana en un experimento a largo plazo en el nordeste de Brasil. Se dividieron los pastos en parcelas cultivadas con a) *Brachiaria brizantha*, b) *Leucaena leucocephala,* c) *Cynodon dactilon* y d) *Panicum maximum*, usando como referencia un área adyacente con la vegetación nativa. Se recolectaron muestras de suelo a profundidades de 0-10 y 10-20 cm. No se encontraron diferencias significativas en el C orgánico del suelo (C_{org}) entre las parcelas a 0-10 y 10-20 cm. Los valores del C microbiano del suelo (C_{mic}) fueron más altos en la vegetación nativa y en *P. maximum* que en el resto de las parcelas. Los valores de la respiración básica (CO_2) del suelo fueron similares en todas las parcelas evaluadas. Sin embargo, los cocientes respiratorios (qCO_2) fueron considerablemente inferiores en la vegetación nativa y en *P. maximum* que en el resto de las parcelas a la profundidad de 0-10 cm. Los valores de la diacetato de la fluoresceína (FDA) fueron significativamente más altos en la vegetación nativa y en *P. maximum,* mientras que la actividad deshidrogenasa fue significativamente más alta en la vegetación nativa, *C. dactilon* y *P. maximum*. Cuando un bosque nativo fue convertido en pastizales, la biomasa y la actividad microbiana del suelo cambiaron. Estos cambios fueron positivos con la inclusión de *P. maximum* por el elevado aporte de fuentes de C.

Palabras clave adicionales: bioindicadores; calidad de suelo; enzimas de suelo; qCO_2; q_{mic}.

Introduction

Pasture ecosystems have influence on soil environment through their potential to cycle C and N and enhancing the quality of life for humans through their provision of meat, milk and wool (Iyyemperumal *et al*., 2007). Previous studies have focused the effect of pastures on soil fertility (During and Weeda, 1973;

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Abbreviations used: C_{mic} (soil microbial biomass), C_{org} (soil organic C), DHA (dehydrogenase activity), FDA (fluorescein diacetate), qCO_2 (respiratory quotient), q_{mic} (microbial quotient), SOM (soil organic matter), TTF (trifenyl tetrazolium formazan).

Haynes and Williams, 1993). On the other hand, soil microbial biomass and activity have received less attention in pastures than in forests or grasslands. Soil biological properties are important for sustainability of pastures, once soil microorganisms start decomposition of soil organic matter and, thus, provide nutrients for plants (Kennedy and Doran, 2002). Additionally, in pastures, organic input from vegetation and animal can contribute to increased soil organic matter content and consequently cause an impact on soil biological process.

Microbial biomass and activity are the main biological indicators of soil quality and respond rapidly to changes resulting from agronomic practices (Araújo *et al.,* 2008). Soil microbial biomass, the living part of soil organic matter, functions as a transient nutrient sink and is responsible for releasing nutrient from organic matter which is used by plants (Smith and Paul, 1990). One of the basic functions of soil microbial biomass is the decomposition and transformation of organic materials, which are mostly derived from above and below-ground plant residues (Ananyeva *et al.,* 1999). Microbial biomass also acts as a small but labile reservoir of nutrients that contributes to maintaining long-term soil sustainability. Thus, the activity of soil microbial communities plays a critical role in pasture ecosystems once there is a large input of organic residue.

Soil microbial biomass and activity have been used as a sensible indicator for the assessment of the effects of soil pollutants (Araújo *et al.,* 2003; Araújo and Monteiro, 2006) and management practices (Agbenin and Goladi, 1997; Ananyeva *et al.,* 1999; Oliveira *et al.,* 2004; Araújo *et al.,* 2008). However, there are few studies focusing on the effect of practices used in pastures on soil microbial biomass and activity, mainly in tropical regions (Oliveira *et al.,* 2004; Agbenin and Adeniyi, 2005).

The hypothesis of this study was that changes in soil microbial biomass and activity should be expected when a soil with native vegetation is cropped with different pastures. In view of the above, the present study was carried out to study the changes in soil microbial biomass, respiration and enzymes activities in a long-term experiment with pasture under different managements in the Northeast region of Brazil.

Material and methods

The study was conducted as a long-term experiment with pastures of the Zootecny Department from Agriculture Science Center, Federal University of Piauí, Brazil, located in the southern American subcontinent at 05°05'21" S latitude, 42°48'07" W longitude and 74 m above sea level. The climate is tropical dry with a mean precipitation of 1,300 mm yr^{-1}. The soil type is an Orthic Acrisol (Typic Hapludult, US taxonomy).

The long-term experiment was established in 2000 and the land was divided into plots (about 1 ha each one) with different pastures: a) *Brachiaria brizantha*; b) *Leucaena leucocephala*; c) *Cynodon dactilon*; d) *Panicum maximum*. An adjacent area with native vegetation was used as reference. The characteristic of each pastures are: a) *B. brizantha* (43.3% C and C:N ratio = 2.5) without fertilization; b) *Leucanea leucocephala* (9.1% C and C:N ratio = 18.3) without fertilization; c) *C. dactilon* (45.6% C and C:N ratio = 22.4) without fertilization; d) *P. maximum* (45.9% C and C:N ratio = 28.3) with annual fertilization of 30, 75 and 30 kg ha^{-1} of P_2O_5, N and K_2O, respectively; e) native vegetation with native plant species like «caneleiro» (*Cenostigma macrophyllum*), «pau d'arco» (*Tabebuia serratifolia*), «jatobá» (*Hymenaea courbaril*), «palmeira de babaçu» (*Orbignya phalerata)*, «mofumbo» (*Combretum leprosum*), «jitó» (*Guarea kunthiana*) and «sapucaia» (*Lecythis pisonis*). The annual input of straw (air-dry) from different pastures in each plot is: a) *B. brizantha* (15 ton dry weight per ha (dw ha^{-1}); b) *L. leucocephala* (5 ton dw ha^{-1}; c) *C. dactilon* (18 ton dw ha^{-1}); d) *P. maximum* (26 ton dw ha^{-1}) applied on soil surface.

Soil sampling was carried out in March 2008. In each plot, soil samples were obtained, at 0-10 and 10-20 cm layers, at 9 points along sets of parallel lines (Dick *et al*., 1996). Soil samples were passed through a 2 mm sieve and 300 g of soil from each sample was separated, placed in plastic bags and stored in a refrigerator at 4-8°C for further evaluation of microbial biomass and activity. The remaining soil samples were air-dried. Soil samples were ground and passed through a 0.21-mm sieve to determine C_{org} by wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating (Yeomans and Bremmer, 1998).

The soil chemical analyses (Table 1) were made in the Soil Quality Laboratory located at the Federal University of Piauí. Soil pH was determined in a 1:2.5 soil/water extract. Exchangeable Al, Ca and Mg were determined using extraction with 1 M KCl. Available P and exchangeable K were extracted by Mehlich-1 and determined by colorimetry and photometry, respectively (Tedesco *et al.,* 1995).

Table 1. Soil chemical properties (0-10 and 10-20 cm) of each plot evaluated in this study

Area	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	CEC ($cmol_c$ kg^{-1})
(0-10 cm)						
Native forest	4.2[b]	5.1[c]	7.5[c]	1.35[a]	0.45[a]	5.78[a]
Brachiaria	5.4[a]	7.2[b]	10.1[b]	2.20[a]	0.75[a]	5.54[a]
Leucaena	4.8[a]	9.8[a]	12.0[a]	2.15[a]	0.70[a]	6.55[a]
Cynodon	5.0[a]	10.5[a]	11.8[a]	1.95[a]	0.75[a]	4.89[b]
Panicum	4.8[a]	11.2[a]	12.5a	2.05[a]	0.80[a]	5.35[a]
(10-20 cm)						
Native forest	3.9[b]	4.2[c]	4.4[b]	0.80[a]	0.35[a]	3.92[b]
Brachiaria	5.7[a]	7.6[b]	10.6[a]	1.50[a]	0.65[a]	3.81[b]
Leucaena	4.7[a]	8.3[b]	10.3[a]	1.40[a]	0.70[a]	5.01[a]
Cynodon	5.0[a]	9.8[a]	11.7[a]	1.35[a]	0.75[a]	3.73[b]
Panicum	4.8[a]	10.5[a]	11.3[a]	1.40[a]	0.65[a]	3.93[b]

pH = 1:2.5 soil to water. CEC: cation exchange capacity.

C_{mic} was determined according to Vance *et al.* (1987) with extraction of organic carbon (C) from fumigated and unfumigated soils by K_2SO_4. Organic C was measured using dichromate digestion and an extraction efficiency coefficient of 0.38 was used to convert the difference in soluble C between fumigated and unfumigated soil in C_{mic}.

CO_2 emission, FDA hydrolysis and DHA activity were analyzed as indicative measures of soil microbial activity. Soil respiration was determined according to Alef (1995). Soil samples (100 g) were placed in 300 mL-glass containers, moistened at 60% of the maximum water holding capacity (gravimetric method), closed with rubber stoppers and incubated for 3 d at 25°C. Glass vials holding 20 mL of 0.5 N NaOH to trap the evolved CO_2 were placed in the above containers. On the 3rd day after the incubation, the glass vial was removed and the CO_2 trapped in NaOH was then determined titrimetrically. The qCO_2 was calculated as the ratio of basal respiration to microbial biomass C. The qCO_2 results were expressed as g CO_2-C d^{-1} g^{-1} C_{mic}. Microbial quotient (q_{mic}) was calculated as the ratio of C_{mic} to C_{org} and expressed as μg C_{mic} μg C_{org}^{-1} (Anderson and Domsch, 1990). FDA hydrolysis was determined according to the method of Schnurer and Rosswall (1982) and DHA was determined using method described in Casida *et al.* (1964) and based on the spectrophotometric determination of triphenyl tetrazolium formazan (TTF) released by 5 g of soil during 24 h at 35°C.

The data were subjected to analyses of variance (ANOVA) and t-test using SPSS version 10 software to detect significant differences between the areas studied. When a significant F value was detected, the means were compared by the Tukey's test ($p < 0.05$).

Results

Soil organic C (C_{org}) content did not vary significantly among all plots, at 0-10 and 10-20 cm depth, after seven years of implantation of pastures of native vegetation (Table 2). It means that the conversion of native

Table 2. Influence of soil management on soil organic C (C_{org}), microbial biomass C (C_{mic}) and soil respiration (CO_2) at 0-10 and 10-20 cm soil depths

Plot	C_{org} (g kg^{-1})	C_{mic} (mg kg^{-1})	CO_2 (mg CO_2 kg^{-1} day^{-1})
(0-10 cm)			
Native forest	21.9[a]	85.5[a]	16.3[a]
Brachiaria	19.4[a]	54.8[b]	13.5[a]
Leucaena	24.5[a]	56.8[b]	14.3[a]
Cynodon	18.5[a]	40.6[b]	14.6[a]
Panicum	20.0[a]	90.8[a]	11.0[a]
(10-20 cm)			
Native forest	15.0[a]	48.6[a]	13.1[a]
Brachiaria	18.8[a]	43.5[a]	13.9[a]
Leucaena	14.6[a]	47.0[a]	12.1[a]
Cynodon	13.2[a]	48.8[a]	12.7[a]
Panicum	17.1[a]	50.5[a]	13.4[a]

In each column, means followed by the same letter do not differ statistically from each other at $p < 0.05$ according to the Tukey's test.

forest to pastures did not promote changes in soil organic matter (SOM) content.

Soil microbial properties differed significantly ($p < 0.05$) among the plots only at 0-10 cm depth (Table 2). In this layer, the C_{mic} values were higher in native forest and *P. maximum* (85.5 and 90.8 mg kg^{-1}, respectively) compared with other plots.

The soil basal respiration (CO_2) values were similar among all evaluated plots (Table 2). Soil respiration indicates biological activity and decomposition of organic residues. The similar results observed in all plots indicate that the soil microbial activity was not influenced by pastures.

The qCO_2 was significantly lower in native forest and *P. maximum* (0.26 and 0.10, respectively) when compared to other plots, at 0-10 cm depth (Table 3). However, qCO_2 were higher in plots under *B. brizantha, L. leucocephala* and *Cynodon dactilus* showing a small microbial biomass with high respiration and low incorporation of carbon. The q_{mic} values were higher in native forest and *P. maximum* (1.41 and 1.13 %, respectively) as compared with others plots (Table 3).

The values of FDA hydrolysis were significantly higher in native forest and *P. maximum* (30.3 and 27.0 µg FDA g^{-1}, respectively), when compared with others plots at 0-10 cm depth (Table 4). The values of DHA were significantly higher in native forest, *C. dactilum* and *P. maximum* (25.3, 21.4, 21.8 µg TTC g^{-1}, respectively, at 0-10 cm depth) (Table 4). The values of DHA were similar to that of FDA hydrolysis, except for the plot under *C. dactilum*. In this plot, the value was not consistent with the results found for soil microbial biomass.

Table 3. Influence of soil management on soil *q*CO2 and q_{mic} at 0-10 and 10-20 cm soil depths

Plot	q_{mic} (%)	qCO_2 (g CO_2 g^{-1} C_{mic})
(0-10 cm)		
Native forest	1.41[a]	0.26[b]
Brachiaria	0.96[b]	0.35[a]
Leucaena	0.83[b]	0.43[a]
Cynodon	0.90[b]	0.46[a]
Panicum	1.13[a]	0.10[b]
(10-20 cm)		
Native forest	1.03[a]	0.31[a]
Brachiaria	0.93[a]	0.32[a]
Leucaena	0.86[a]	0.31[a]
Cynodon	0.80[a]	0.27[a]
Panicum	0.98[a]	0.34[a]

In each column, means followed by the same letter do not differ statistically from each other at $p < 0.05$ according to the Tukey's test.

Table 4. Influence of soil management on fluorescein diacetate (FDA) hydrolysis and dehydrogenase activity (DHA) at 0-10 and 10-20 cm soil depths.

Plot	FDA (mg FDA kg^{-1} soil)	DHA (mg TTC kg^{-1} soil)
(0-10 cm)		
Native forest	30.3[a]	25.3[a]
Brachiaria	18.1[b]	16.4[b]
Leucaena	15.4[b]	12.8[b]
Cynodon	12.1[b]	21.4[a]
Panicum	27.0[a]	21.8[a]
(10-20 cm)		
Native forest	17.3[a]	13.6[a]
Brachiaria	14.7[a]	11.6[a]
Leucaena	16.0[a]	9.40[a]
Cynodon	19.2[a]	10.2[a]
Panicum	18.0[a]	12.0[a]

TTC: tetrazolium triphenyl chloride. In each column, means followed by the same letter do not differ statistically from each other at $p < 0.05$ according to the Tukey's test.

Discussion

In our study, the period of implantation of pastures ecosystems (seven years) did not promote significant changes in C_{org} content due, probably, the initial high C_{org} content found in these soils. Several studies of organic C content following conversion of tropical forest to pasture have shown a range of responses, including increases, decreases, or no net long-term changes in soil C (Cerri *et al.,* 1991; Tiessen *et al.,* 1992; Neill *et al.,* 1997; Agbenin and Goladi, 1997; Agbenin and Adeniyi, 2005).

The microbial properties were more sensitive for detecting differences in soil management than organic C. These findings are in agreement with previous work that reported the soil microbial biomass responded more quickly to crop management practices than organic C (Brookes, 1995; Araujo *et al.,* 2010). Similarly, seven years of winter cover cropping showed no effect on organic C levels, but microbial biomass was affected (Ndiaye *et al.,* 2000).

The higher C_{mic} content in native forest can be attributed to permanent input of plant residues that supply

available C and maintain a high microbial biomass, conforming reports by Pérez *et al.* (2004), Ndaw *et al.* (2009) and Araújo *et al.* (2010) that there were higher C_{mic} contents in areas under native vegetation compared to cultivated areas.

In our study, C_{mic} was strongly affected by pasture species composition. According to Agbenin and Adeniyi (2005) different plant species affect soil microbial biomass both by the quality as well as quantity of litter and below ground biomass that support microbial activity. The higher C_{mic} content found in soil under *P. maximum* cultivation suggests a positive effect of high content of litter added to soil by this pasture. The average annual dry matter yield of *P. maximum* is higher (about 26 tons ha^{-1}) as compared to other pastures (about 15, 5 and 18 tons ha^{-1} for *B. brizantha, L. leucocephala* and *C. dactilon,* respectively) and favored the soil microbial biomass.

In addition, the quantity and quality of root exudation by plant species may have influence on soil microbial biomass (Grayston *et al*., 1996). These exudations are dependent of plant species and environmental conditions, such as fertilization. Probably, the fertilization in plot with *P. maximum* promoted higher quantity and quality of exudation and it favored soil microbial biomass compared with unfertilized plots. Nutrients have a major impact on exudation, usually enhancing the process, particularly with regard to the supply of N, P and K (Kraffczyk *et al*., 1984). Other studies using different crops that varied in amount, rate of decomposition and quality of residue inputs showed effects on soil microbial and biochemical properties (Janzen and Lucey, 1988; Franzluebbers *et al.,* 1995; Klose *et al.,* 1999; Ekenler and Tabatabai, 2002).

The qCO_2 is the ratio of the basal respiration rate to the C_{mic}, and hence reflects the efficiency of heterotrophic microorganisms to convert organic carbon into microbial biomass (Anderson and Domsch, 1990). The results observed for native forest and *P. maximum* shows a soil microbial biomass more efficient. However, the absence of soil fertilization and lesser input of litter in pltos with *B. brizantha, L. leucocephala* and *Cynodon dactilon* contributed to a low soil microbial biomass content thus resulting in an increase of qCO_2.

Microbial quotient (q_{mic}), the ratio of C_{mic} to C_{org}, has been used as an indicator of future changes in organic matter status that will occur in response to alterations in land use (Sparling, 1997). Soil microbial biomass generally comprises only 1-4% of soil organic C (Sparling, 1992). In our study, the values of microbial quotient in native forest and *P. maximum* were within these ranges. This result is in accordance with Drury *et al*. (1991) and Iyyemperumal *et al*. (2007) that found values of q_{mic} between 1 and 2 in pastures. The higher value of q_{mic} observed in the native vegetation and in the *P. maximum* pasture may be due to the higher soil microbial biomass C content observed in these soils, suggesting a large proportion of soil organic matter occupied by microbial biomass. Additionally, the results suggest an improvement in soil microbial biomass efficiency under native forest and *P. maximum* in using available C.

Soil use and management practices modify the total amount of soil organic matter and its composition and significantly affect the enzyme levels and their activities (Dick *et al.,* 1996). Soil DHA and FDA hydrolysis activities, which are directly involved in the transformation of soil organic matter, were all increased by native forest and *P. maximum,* due mainly to high deposition of residues. Similar results were observed by Sicardi *et al*. (2004) that observed high enzymes activities under pastures than Eucalyptus. Other studies have suggested that soil enzyme activities are generally the most sensitive indicators of residue management changes on the belowground microbial community (Gregorich *et al*., 1994; Jordan *et al*., 1995).

The results also showed that FDA hydrolysis is directly proportional to the microbial growth, conform reported by Swisher and Carroll (1980). On the other hand, DHA, an intracellular activity which relates to total activity of microorganisms, cannot be strongly correlated to microbial biomass as most soil microorganisms are inactive. Therefore, this result may be due to the non contribution of the quantity of litter added to soil by this pasture, in short-term, for soil microbial biomass content, but promoted an increase in DHA. Others studies showed that organic materials, like plant litter stimulate soil DHA (Garcia *et al.,* 1998; Elfstrand *et al.,* 2007).

As final conclusion, microbial biomass and activity were more responsive than organic C to pastures establishment. Soil microbial biomass and activity changed when a native forest was converted to pastures. These changes were positive with the inclusion of *P. maximum* by the high input of C sources. The maintenance of high input of C in pasture ecosystems may promote an increase in soil microbial biomass and activity in the short- and long-term.

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