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Soil microbial biomass in an agroforestry system of Northeast Brazil

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Abstract

Agroforestry systems (AFS) are considered alternative land use options to help prevent soil degradation and improve soil microbial biomass and organic C status. However, it is unclear how different densities of babassu palm [*Attalea speciosa* (syn. *Orbignya phalerata*)], which is an important tree in Northeast Brazil, affect the soil microbial biomass. We investigated the soil microbial biomass C and activity under AFS with different densities of babassu palm associated with *Brachiaria brizantha* grass. Soil microbial biomass C (MBC), soil microbial biomass N (MBN), MBC:total organic C ratio, fluorescein diacetate hydrolysis and dehydrogenase activity showed highest values in plots with high density of babassu palm. On the other hand, the respiratory quotient (qCO_2) was significantly greater in plots without babassu palm. *Brachiaria brizantha* in monoculture may promote C losses from the soil, but AFS with high density of babassu palm may increase the potential of soils to accumulate C.

Resumen

Los sistemas agroforestales (AFS, por sus siglas en inglés) son opciones alternativas de uso de la tierra que ayudan a prevenir la degradación del suelo y mejorar la biomasa microbiana y el estado del carbono (C) orgánico. Babasú [*Attalea speciosa* (syn. *Orbignya phalerata*)] es una importante palma nativa que forma bosques en los estados Maranhão y Piauí, nordeste de Brasil; no obstante no se conoce su efecto sobre la biomasa microbiana del suelo cuando crece en diferentes densidades. En el estudio se evaluaron los efectos sobre el C y la actividad de la biomasa microbiana del suelo en AFS con 3 densidades de babasú en pasturas de *Brachiaria brizantha*. El C y el nitrógeno de la biomasa microbiana (MBC resp. MBN) del suelo, la relación de MBC:C orgánico total del suelo, la hidrólisis de diacetato de fluoresceína y la actividad de la deshidrogenasa mostraron valores más altos en las parcelas con alta densidad de babasú. Por otra parte, el cociente respiratorio (qCO2) fue significativamente mayor en las parcelas sin babasú. El pasto *B. brizantha* en monocultivo puede promover la pérdida de C del suelo, pero los AFS con alta densidad de la palma pueden aumentar el potencial de los suelos para acumular C.

Introduction

The babassu palm [*Attalea speciosa* Mart. ex Spreng. (syn. *Orbignya phalerata* Mart.)] occurs widely in Brazil, Colombia, Bolivia and Mexico and its fruits are a source of lauric oil, having both edible and industrial uses. Over 80% of babassu palms found in Brazil are from the northeast of Maranhão and Piauí states, known as Mid-North region, and produce about 200,000 tonnes of fruit annually. Babassu palm forests and primary forests are being removed and converted to improved pastures and mechanized crop production (May et al. 1985), which has contributed to land degradation in this region (Dias-Filho 2005). On the other hand, although babassu palms are seldom planted, they may be managed within a regional agroforestry system (AFS), as it is a good strategy to plant pasture in association with babassu palm (Nair 1993).

AFS is an alternative land use system to help prevent land degradation, by allowing continued use of land to

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produce crops or pastures in association with trees on a sustainable basis (Araújo et al. 2012). Such systems involve the combination of at least one woody-perennial species with a crop or pasture, which results in ecological and economic interactions between the two components (Palma et al. 2007). The AFS provides a continuous input of organic material into the soil, especially as the deep roots of the forest component (Albrecht and Kandji 2003) increase soil organic matter (SOM) stocks (Manlay et al. 2007; Fontes et al. 2010), and improve the soil microbial biomass (Udawatta et al. 2008; Yadav et al. 2010). Several authors have reported that soil microbial biomass is greater in an AFS, owing to the effects of trees and organic matter input and differences in the quality and quantity of litter and root exudates (Gómez et al. 2000; Myers et al. 2001; Mungai et al. 2005; Sørensen and Sessitsch 2007).

The main AFS involving babassu is pastures planted under native palm stands, with common palm densities of 50–100 trees/ha. Although this system covers much of the region, it is unclear how plant density of babassu palm affects soil microbial biomass. We hypothesized that soil microbial biomass is affected by the density of trees in AFS, owing to different inputs of plant litter. In order to test these hypotheses, we determined soil microbial biomass, by measuring soil microbial biomass C as the most reliable indicator, under AFS palm-pasture with different densities of babassu palm associated with a *Brachiaria brizantha* pasture in Northeast Brazil.

Materials and Methods

The study was conducted at the farm "Água Viva", Maranhão state, Northeast Brazil (05°06'25" W, 02°59'35" S). The climate is seasonally dry tropical with a mean precipitation of 1,500 mm/yr (main rainfall from November to May) and an annual mean temperature of 30 °C, with minimum and maximum monthly temperatures of 22 °C and 40 °C, respectively. According to the Brazilian Soil Survey (Embrapa-SNLCS 1986), the dominant soils are classified as Plintossols.

We evaluated 1 ha plots of babassu palm-*Brachiaria brizantha* associations with different palm densities. We labeled palm densities as: low density (LD – 80 babassu palms/ha); medium density (MD – 130 babassu palms/ha); high density (HD – 160 babassu palms/ha); and MC (*B. brizantha* in monoculture). The plots were similar in soil type and climate (see above). All plots received 300 kg urea/ha annually. The inputs of dry litter were: MC – 1.9 t/ha; LD – 4.5 t/ha; MD – 9.5 t/ha; and HD – 15 t/ha. The plant litter (dry mass) was calculated after the collection of all litter found in 1 m^2 quadrats installed at each plot (one quadrat for each plot installed at the plot center). The litter was dried at 65 ^oC until a constant weight was reached. Litter contribution per hectare was then calculated.

Soil samples were collected at 0–20 cm depth in March (rainy season) and September (dry season) of 2013. In each plot, the plant cover was carefully removed from the soil surface and soil cores (2.5 cm diameter) were taken at random. In each plot, 5 soil cores were collected from each of 4 sub-plots and pooled to form a composite sample. All samples were immediately stored in sealed plastic bags in a cooler and transported to the laboratory. The field-moist samples were sieved (2-mm mesh) and stored in sealed plastic bags at 4 °C for microbial analyses.

Subsamples of the soils were ground and passed through a 0.2-mm sieve to evaluate chemical properties. Soil pH was determined in a 1:2.5 soil:water extract. Exchangeable Ca was determined using extraction with 1 M KCl. Available P and exchangeable K were extracted using the Mehlich-I extraction method and determined by colorimetry and photometry, respectively (Tedesco et al. 1995). Total organic C (TOC) was determined by the wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating (Yeomans and Bremner 1998).

Soil microbial biomass C (MBC) and N (MBN) were determined according to Vance et al. (1987) with extraction, by K_2SO_4, of C and N from $CHCl_3$-fumigated and unfumigated soils. Extraction efficiency coefficients of 0.38 and 0.45 were used to convert the differences in C and N between fumigated and unfumigated soils in MBC and MBN, respectively. Hydrolysis of fluorescein diacetate (FDA) was determined according to the method of Schnürer and Rosswall (1982) and dehydrogenase activity (DHA) was determined using the method described in Casida et al. (1964), based on the spectrophotometric determination of triphenyl tetrazolium formazan (TTF) released by 5 g of soil during 24 h at 35 °C. The respiratory quotient (qCO_2) was calculated as the ratio of basal respiration to microbial biomass C, expressed as g CO_2-C/d/g MBC. Moreover, we calculated the ratio between MBC and TOC (qMIC), which is a common measure for carbon availability (e.g. Santos et al. 2012).

The results are expressed on the basis of oven-dry soil. Least significant difference (LSD) analysis was performed and all differences reported in the text were considered significant at $P<0.05$. Data were analyzed using multivariate ordination non-metric multidimensional scaling (NMS) with Sorensen distances. Ordination was performed using the PC-ORD v. 6.0 program.

Results

Soil chemical properties were not significantly affected by palm density (Table 1), as soil pH and Ca and K concentrations were similar in all AFS systems. However, soil P concentration showed highest values in the HD system.

Total organic carbon (TOC) concentrations were higher ($P<0.05$) in HD and MD than in LD and MC in both seasons, while soil microbial biomass C (MBC) and N (MBN) showed highest values in HD and lowest under the straight grass pasture (MC) ($P<0.05$) (Table 2).

Soil respiration did not differ ($P>0.05$) among treatments in both seasons (Table 3), while the respiratory quotient (qCO_2) was significantly ($P<0.05$) greater in MC than in treatments with babassu palms. Similar to soil microbial biomass, the qMIC was highest in HD and lowest in MC plots ($P<0.05$), as were values for FDA hydrolysis and DHA activity.

Table 1. Soil pH and Ca, P and K concentrations in agroforestry systems with different densities of babassu palm in *Brachiaria brizantha* pasture.

Treatment	Soil pH		Ca ($cmol_c$/kg)		P (mg/kg)		K (mg/kg)	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
HD[1]	6.3a[2]	6.2a	3.8a	3.6a	12.3a	12.1a	80a	75a
MD	6.0a	6.5a	3.2a	3.8a	10.3b	10.4b	91a	86a
LD	6.3a	6.1a	3.7a	3.2a	9.5b	10.4b	79a	78a
MC	5.8a	6.1a	3.9a	3.5a	9.8b	10.3b	78a	89a

[1]Babassu palm density: HD – 160 trees/ha; MD – 130 trees/ha; LD – 80 trees/ha; MC – grass monoculture (no trees).
[2]Means within columns followed by the same letter are not significantly different at $P\leq0.05$ (Tukey´s HSD test).

Table 2. Total organic C (TOC), microbial biomass C (MBC) and microbial biomass N (MBN) (± SD) in agroforestry systems with different densities of babassu palm in *Brachiaria brizantha* pasture.

Treatment	TOC (g C/kg)		MBC (mg C/kg)		MBN (mg N/kg)	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
HD[1]	8.9 ± 1.0a[2]	9.0 ± 1.1a	107 ± 13a	83 ± 15a	16.5 ± 4.3a	11.0 ± 1.3a
MD	9.6 ± 1.7a	9.3 ± 0.9a	81 ± 10b	52 ± 12b	10.9 ± 2.5b	8.7 ± 2.5b
LD	7.1 ± 0.9b	6.9 ± 0.8b	61 ± 9c	47 ± 10b	7.2 ± 1.3c	4.2 ± 0.8c
MC	5.4 ± 1.8b	5.1 ± 0.7c	32 ± 17d	18 ± 3c	3.2 ± 0.9d	1.9 ± 0.4d

[1]Babassu palm density: HD – 160 trees/ha; MD – 130 trees/ha; LD – 80 trees/ha; MC – grass monoculture (no trees).
[2]Means within columns followed by the same letter are not significantly different at $P\leq0.05$ (Tukey´s HSD test).

Table 3. Mean values (± SD) for soil respiration (RB), respiratory quotient (qCO_2), ratio between soil microbial biomass C (MBC) and total organic carbon content (TOC) (qMIC), hydrolysis of fluorescein diacetate (FDA) and dehydrogenase activity (DHA) in agroforestry systems with different densities of babassu palm in *Brachiaria brizantha* pasture.

Treatment	RB (mg C-CO_2/kg/d)		qCO_2 (g CO_2-C/d/g MBC)		qMIC (MBC:TOC)		FDA (µg FDA/g)		DHA (µg TTF/g)	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
HD[1]	58 ± 22a	49 ± 17a	0.5 ± 0.1a	0.5 ± 0.1a	1.2 ± 0.2a	1.1 ± 0.3a	24 ± 5a	15.4 ± 1.9a	3.0 ± 0.1a	1.6 ± 0.4a
MD	55 ± 13a	42 ± 15a	0.6 ± 0.2a	0.7 ± 0.2a	0.8 ± 0.2b	0.8 ± 0.2a	22 ± 6a	11.2 ± 2.1b	2.1 ± 0.4b	1.0 ± 0.3b
LD	36 ± 21a	38 ± 14a	0.7 ± 0.2a	0.8 ± 0.4a	0.9 ± 0.3b	0.8 ± 0.1a	14 ± 3b	7.6 ± 0.9c	1.7 ± 0.3b	0.7 ± 0.3b
MC	54 ± 16a	45 ± 19a	1.7 ± 0.3b	2.6 ± 0.8b	0.6 ± 0.2b	0.5 ± 0.1b	10 ± 2c	3.7 ± 0.5d	0.7 ± 0.5c	0.4 ± 0.2c

[1]Babassu palm density: HD – 160 trees/ha; MD – 130 trees/ha; LD – 80 trees/ha; MC – grass monoculture (no trees).
[2]Means within columns followed by the same letter are not significantly different at $P\leq0.05$ by Tukey´s test.

The non-metric multidimensional scaling (NMS) analysis identified the association of soil biological and chemical properties with evaluated plots (Figures 1 and 2). The MC plot was clearly separated from the other plots, whereas LD and MD were more similar. In both seasons, the first axis explained about 70% of the variation and was strongly correlated with qCO_2 values, which characterized the MC plot, and with soil microbial biomass and enzymes, which characterized the HD plot. In the MC plot, qCO_2 was higher, while the HD site was characterized by higher soil microbial biomass and enzyme activity.

Discussion

Treatments in this study were unreplicated but the large plot sizes and large numbers of soil samples taken in each plot should compensate for the lack of spatial replication. The results suggest that the density of babassu palm in AFS affects soil microbial biomass C and organic C. This may be associated with the increase of plant litter, which improves soil organic C status (Assis et al. 2010; Fracetto et al. 2010; Sousa et al. 2012; Albaladejo et al. 2013). The plots with babassu palm present high and constant litter deposition, compared with the grass in monoculture, contributing to the maintenance of soil moisture and lower soil surface temperatures and increasing the soil organic C (Stockmann et al. 2013). The increase in soil organic C is important for Northeast Brazil, owing to the low levels of organic matter in soils in this area. High organic C is important for sustainability, because organic matter has a positive influence on soil physical, chemical and biological properties.

As N fertilizer was the only chemical fertilizer applied, the values of soil pH, Ca and K content did not vary. On the other hand, available soil P content increased in the HD system, possibly due to the highest input of plant litter, which may supply P during decomposition of the organic residue.

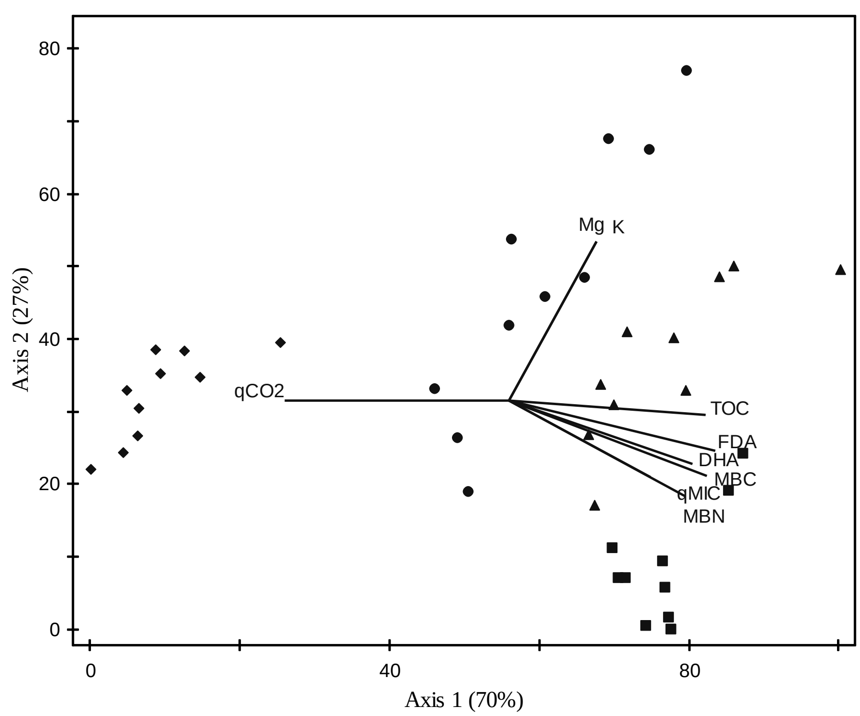

Figure 1. NMS analysis based on biological and chemical properties of soil during the dry season. Soil microbial biomass C (MBC, mg/kg); soil microbial biomass N (MBN, mg/kg); microbial respiratory quotient (qCO2, g CO_2-C/d/g soil microbial biomass C); hydrolysis of fluorescein diacetate (FDA, µg/g); dehydrogenase (DHA, µg TTF/g); and MBC:TOC (qMIC, mg/kg).
HD (■); MD (▲); LD (●); MC (◆).

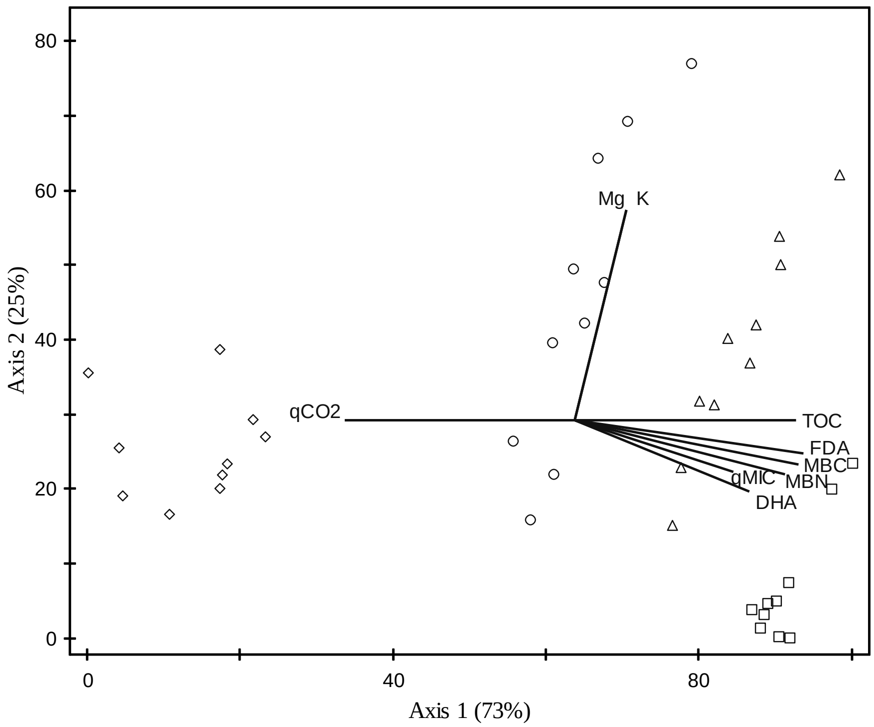

Figure 2. NMS based on biological and chemical properties of soil during the wet season. Soil microbial biomass C (MBC, mg/kg); soil microbial biomass N (MBN, mg/kg); microbial respiratory quotient (qCO2, g CO_2-C/d/g soil microbial biomass C); hydrolysis of fluorescein diacetate (FDA, µg/g); dehydrogenase (DHA, µg TTF/g); and MBC:TOC (qMIC, mg/kg).
HD (□); MD (△); LD (○); MC (◇).

Soil microbial biomass C was strongly affected by the increase in density of babassu palm, presumably by higher inputs of plant litter, that supply microbial biomass with C sources (Lopes et al. 2010). The average annual litter added in HD was much higher (15 t/ha) than in the others plots (range 1.9–9.5 t/ha) and favored the accumulation of soil microbial biomass. In addition, the quantity and quality of the rhizosphere of the plants influence soil microbial biomass (Grayston et al. 1996). Therefore, higher numbers of babassu palm may favor accumulation of soil microbial biomass through root exudation and promoting better conditions for soil microbial biomass. Other studies using different crops, that varied in amount and quality of residue inputs, showed effects on soil microbial biomass in tropical soils (Lopes et al. 2010; Araújo et al. 2013; Azar et al. 2013).

Soil respiration indicates biological activity and decomposition of organic residues (Santos et al. 2012). Our results showed similar soil respiration in all evaluated plots. Soil respiration might indicate either a disturbance of the soil or a high level of productivity in the ecosystem (Islan and Weil 2000). The respiration rate per unit of microbial biomass or respiratory quotient (qCO_2) is a variable of more straightforward interpretation (Fernandes et al. 2005). The qCO_2 reflects the efficiency of heterotrophic microorganisms to convert organic C into microbial biomass (Anderson and Domsch 1990). MC showed the lowest microbial biomass proportion (qMIC) with more efficient soil microbial communities in terms of C use than other plots.

The qMIC has been used as an indicator of changes in organic matter status and usually the values should range between 1 and 4% (Sparling 1992). The values of qMIC in the HD plot are within this range. These higher values may be due to the higher annual litter supply and the related higher soil microbial biomass C content observed in the HD plot, suggesting a large proportion of SOM being occupied by microbial biomass.

Dick et al. (1996) found that the use of different management practices, which modify SOM content, may significantly affect enzymatic activity. In our study, the increases in soil DHA and FDA, which are directly involved in the transformation of SOM, were greatest in the HD plot and lowest on the MC plot, being directly related to amounts of plant litter deposited on the soil. Previous studies reported that inputs of organic materials, like plant litter, stimulate soil DHA and FDA (Elfstrand et al. 2007; Lopes et al. 2010).

Soil microbial properties differed between seasons, and this pattern is in agreement with Silva et al. (2012) for tropical soils. Such effects of season may be mainly due to variations in soil humidity and temperature (Araújo et al. 2013). Also, it may suggest that soil microbial community structure is likely to differ between seasons and to respond differently to dry and wet conditions as reported by Araújo et al. (2013; 2014) for tropical soils from Northeast Brazil.

NMS analyses of the biological and chemical properties showed distinct patterns according to the different plots and suggest a strong relationship between the biological properties and conditions occurring in the plots. The MC plot was clustered in the NMS and characterized by a high respiratory quotient, i.e. an indicator of stress, and by low soil microbial biomass. This indicates that soil microorganisms are severely limited by low resource availability (Nunes et al. 2012; Araújo et al. 2013). On the other hand, the HD plot showed high soil microbial biomass and activity, suggesting higher availability of organic residues driven by high inputs of plant litter.

Conclusion

The results highlight that AFS has strong effects on soil organic and microbial properties. While pastures under grass monoculture may promote C losses from the soil compared with standing forest, adding babassu palms to create an AFS, especially with high density of babassu palms, may increase the potential of soils to accumulate C. Our results support the hypothesis that high palm density in pastures provides better conditions for balanced microbial diversity, reflected by higher levels of soil microbial biomass C and enzyme activity compared with grass in monoculture. Therefore, the increase in the density of babassu palm in AFS may be an important strategy to improve the function of soils considerably. As soil microbial biomass plays an important role in nutrient cycling, pasture growth should increase through higher nutrient uptake and, consequently, livestock production per hectare could be favored. However, shading effects on pasture growth need to be considered. Further research is needed to clarify these issues.

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