# **SOIL AND PLANT NUTRITION - Article**

# Activity of soil microbial biomass altered by land use in the southwestern Amazon

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**ABSTRACT:** The increasing demand for food creates environmental problems, mainly due to the removal of native vegetation cover for agriculture expansion in Brazil. These changes in land use lead to changes in the soil organic matter dynamics. Microorganisms represent the most biological and physiological diversity in soil, as well as are responsible for more than 95% of the decomposition and nutrient cycling processes. The objective in this research was to check if there is difference of patterns in activity of soil microbial biomass under varied natural vegetation, pastures in use and agricultural systems recently established. The area covered by this study corresponds to the states of Rondônia and Mato Grosso. Canonical variate analysis was used in physical, chemical and

microbiological factors in each ecoregion and land use, looking for patterns and variables that can differentiate them. The native areas showed distinct patterns in the dynamics of microbiological attributes mainly related to the amount of litter in each biome studied. For the disturbed areas, there were similar results between pastures and native areas, significantly different from the results obtained in agricultural areas, which, due to differences in management and kind of cultures analyzed, showed a great variability in the final result. The results support the recommendation for use of microbiological attributes as indicators of land use change, combined with chemical and physical factors of the soil.

Key words: microbial biomass, basal respiration, Amazon.

# INTRODUCTION

The growing demand for food, fiber and biofuel creates various environmental problems, mainly caused by the disorderly occupation of the largest agricultural border in the world, that between the Amazon Forest and the Cerrado of Central Brazil (Borlaug 2002). The flat relief facilitates the use of machines, making the occupation of this area relatively easy. In the southwestern Amazonia, the states of Rondônia (RO) and Mato Grosso (MT) are the major ones that promote deforestation for agricultural land use (INPE 2006).

The intensive land use causes negative effects on both the environment and the agricultural productivity when conservation practices are not adopted (Cerri et al. 2004; Foley et al. 2005). There are concerns regarding soil quality alterations for a long period or even in an irreversible way. Soil microbiota is an important part of biogeochemical cycles and has a key role in developing and maintaining soil structure and quality (Allen and Schlesinger 2004). The microbial biomass is the living and active part of the soil and can indicate changes in the amount of nutrients due to changes in land use (Brookes 2001). After the alteration, microbial biomass fluctuates to reach a new equilibrium (Powlson et al. 1987). In this way, the demand for rapid and reliable indicators to detect changes in soil quality has been continuously growing, mainly those based on microorganisms (Anderson 2003; Ananyeva et al. 2008).

In most soils subjected to agricultural practices, niches available are affected by the interference with soil properties. Each major change is a renewal of selection pressure favoring some microbial components and eliminating others, reorganizing the equilibrium among populations. If there is a reduction in microbial diversity, some species capable of metabolizing certain functional groups are lost, resulting in a reduction in system resilience (van Heerden et al. 2002).

This study aimed to add new information on the alterations resulting from changes in land use and their impact on soil microbial biomass. The goal was to evaluate the change in soil microbial biomass activity after human interference with the natural vegetation for land use as pasture and farming areas in the southwestern Amazon.

#### MATERIAL AND METHODS

## Study area

The study area corresponds to the states of Rondônia and Mato Grosso. The southwestern Amazon is an area of transition between the Amazon Basin and the Brazilian Central Plateau, located between 7° and 18° South latitude and between 50° and 67°West longitude, forming a watershed between the Amazon Basin to the north and the Paraná Basin to the south. This region is one of the largest agricultural frontiers in the world, covering an area of approximately 1,128,000 km² in these states. The climate varies according to latitude and can be characterized by a humid tropical regime with a short dry season. Maia et al. (2009, 2010) described the soils of the region studied as mainly represented by Oxisols, Ultisols and Entisols, with about 40, 20 and 15%, respectively.

To better select the sampling sites, the total area of the states of Rondônia and Mato Grosso was divided into 11 biogeoclimatic macroareas (Figure 1). The division was made using the Geographic Information System ArcGIS 9.0 by the overlapping of information layers related to soil, native vegetation, geology, climate and topography in order to find relatively homogeneous areas, making it possible to determine the soil properties to be carefully extrapolated to the whole ecoregion. Full description of the type of soil, climate, vegetation, topography and geology of each ecoregion can be found in Maia et al. (2009, 2010).

In each ecoregion, two municipalities were drawn to evaluate the physical, chemical and microbiological attributes of the soil due to the change in land use, totaling 22 municipalities (Figure 1). At these sites, soil samplings were conducted in representative pairs regarding the use and change of land use for determining the carbon stock. Sampling pairs are characterized by native system (reference), pasture and agricultural area.

#### Sampling

Three replicates were taken in each site at the 0 - 10 cm layer, totaling 330 samples (110 in each land use). Soil

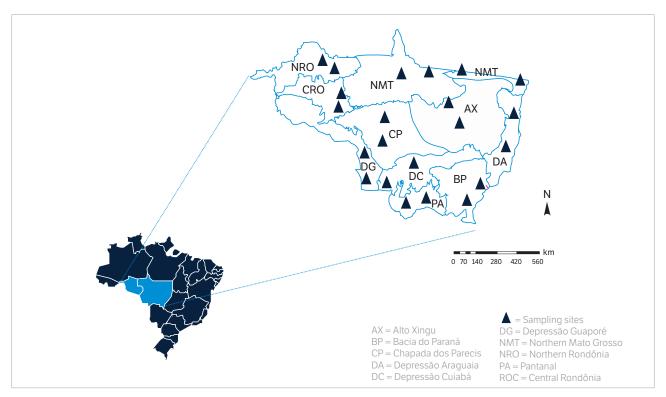


Figure 1. Distribution of the ecoregions in the study area.

samples were homogenized and sieved with the aid of a 2-mm sieve to remove non-decomposed material.

## Soil basal respiration

Soil basal respiration (BR) was determined by carbon dioxide (CO<sub>2</sub>) emission. Soil samples (5 g of dry weight) were pre-incubated in 250-mL flasks at 25 °C for three days. The tubes were then sealed, and CO<sub>2</sub> concentration was measured after 8 h of incubation by collecting 2 mL of gas using BD syringes. CO<sub>2</sub> production was determined by an infrared gas analyzer (infra-red gas analyzer — IRGA).

# Microbial biomass carbon and nitrogen

The microbial biomass carbon (MB-C) was determined by fumigation-extraction method (Vance et al. 1987). Extracts were analyzed for organic carbon content (Shimadzu - TOC 5000A), and microbial biomass was determined by the difference between the values obtained in fumigated and non-fumigated samples. To determine the microbial biomass nitrogen (MB-N), the extracts

were analyzed by the Ninhydrin method (Joergensen and Brookes 1990).

# Chemical and physical characterization of the soil

The total carbon content (C%) of the samples was determined by dry combustion in an elemental analyzer LECO CN-2000. To calculate the apparent bulk density, undisturbed soil samples were collected with the aid of a stainless steel cylinder (5 × 5 cm). The apparent bulk density was determined according to Blake and Hartge (1986). The carbon stock was calculated by multiplying its content by soil density at the 0 – 10 cm layer. The pH was determined in  $\rm H_2O$  and KCl (1.0 mol·L<sup>-1</sup>) using a soil: solution ratio of 1:2.5.

#### Statistical analysis

The results were grouped into different categories as to land use (pasture — PAST; agricultural areas — AGR; native areas — NAT). The analysis of variance (ANOVA;  $\alpha = 0.001$ ) was applied to test the differences between

land uses. Canonical variate analysis was run considering the microbiological data obtained in this study and then adding the chemical and physical data of the soil. All statistical analyses were performed using the statistical package GenStat 11 and GenStat Discovery Edition 3 (VSN International Ltd., Herts., UK).

#### **RESULTS**

MB-C ranged from 0.04 to 1.75 g C per kg soil. MB-N ranged from 0.12 to 108.4 mg N per kg soil. Microbial activity, measured as soil respiration, ranged from 0.07 to 1.82  $\mu$ g CO $_2$  per g soil·h. In general, there was a great similarity between the values of native areas and pastures, with significant differences from agricultural areas (Table 1).

The relationship between carbon immobilized in the microbial biomass and total soil organic carbon also followed the same trend, i.e. 25% higher in pasture areas. The metabolic quotient (qCO<sub>2</sub> — ratio between basal respiration and quantity of microbial biomass) was higher in native areas (1.7 times higher than in agricultural areas and 2.2 times higher than in pastures; Table 1). Soil properties also presented significant

differences between the areas analyzed, except for the total soil carbon (Table 2), with the highest values found in pasture areas.

In pasture areas, the carbon content had a highly significant correlation (p < 0.01) with the three studied microbiological attributes (basal respiration —  $r^2 = 0.29$ , MB-C and MB-N —  $r^2 = 0.43$ ), while pH KCl had a high correlation with basal respiration ( $r^2 = 0.38$ ). In agricultural areas, it was only found a highly positive correlation between basal respiration and carbon stock ( $r^2 = 0.21$ ). In turn, in native areas, MB-N showed a correlation with pH H<sub>2</sub>O ( $r^2 = 0.60$ ), pH KCl ( $r^2 = 0.63$ ) and carbon stock ( $r^2 = 0.55$ ).

The canonical analysis restricted to microbiological data demonstrated no clear distinction between pastures and native areas. An overlap of the confidence regions was observed between pasture and native areas (Figure 2). This overlap reflects the great variability in the database. On the other hand, the canonical analysis distinguished pastures and native areas from agricultural areas (Figure 2). The key attributes accounting for this separation were basal respiration and MB-C with high values of canonical vectors for the canonical axis 1 (CV1), which explains 72.84% of the observed variation (Table 3). The canonical axis 2 (CV2) explained 27.16% of the observed variation, once

**Table 1.** Microbiological attributes in the study area.

	Agricultural areas		Native areas			Pastures			
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Basal respiration (ug CO <sub>2</sub> per g soil·h)	0.37 b	0.04	21.44	0.46 a	0.04	24.89	0.50 a	0.04	38.06
MB-C (g C per kg soil)	0.53 b	0.07	41.29	0.55 b	0.07	55.50	0.69 a	0.07	25.29
MB-N (mg N per kg soil)	17.85 b	7.09	90.19	37.89 a	7.09	83.20	37.16 a	7.09	71.00
MB-C : Corg	2.98 b	0.45	49.51	3.05 b	0.45	64.65	4.08 a	0.45	33.14
$qCO_2$	1.01 b	0.49	97.14	1.71 a	0.49	169.00	0.76 b	0.49	45.09

Means followed by different letters in the same row are significantly different by Tukey's test (p < 0.05). SD = Standard deviation; CV = Coefficient of variation; MB-C = Microbial biomass carbon; MB-N = Microbial biomass nitrogen; Corg = Organic carbon; q = metabolic quocient.

**Table 2.** Physical and chemical properties in the study area.

	Agricultural areas (n = 110)			Native areas (n = 110)			Pastures (n = 110)		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Content of C (%)	1.94 a	0.67	34.90	2.11 a	0.99	46.91	1.95 a	0.79	40.81
Stock of C (Mg·ha <sup>-1</sup> )	10.86 b	3.10	28.62	11.00 b	4.07	36.97	12.54 a	4.77	38.05
Density (g·cm⁻³)	1.17 b	0.16	13.71	1.09 c	0.19	17.81	1.34 a	0.19	14.55
pH H <sub>2</sub> O	5.96 a	0.47	7.86	5.26 b	0.95	18.01	5.97 a	0.50	8.46
pH KCI	5.27 a	0.55	10.50	4.47 b	1.02	22.92	5.24 a	0.72	13.66

Means followed by different letters in the same row are significantly different by Tukey's test (p < 0.05). SD = Standard deviation; CV = Coefficient of variation.

again especially for MB-C and basal respiration, while MB-N did not contribute significantly to the separation of land uses on any of the axes (Table 3).

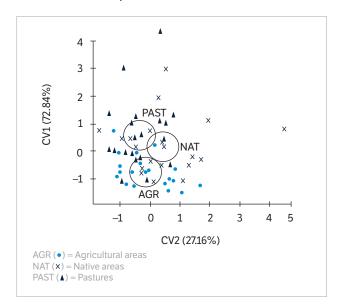


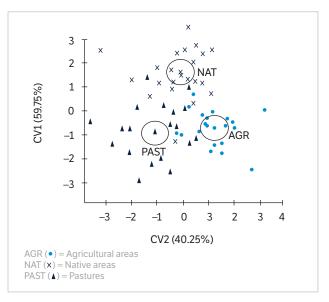
Figure 2. Canonical analysis considering only microbiological attributes.

Table 3. Canonical variate analysis of the study area..

Micro	Microbiological data			Microbiological, physical and chemical data						
Variation (%)										
CV1	72.84		CV1	59.75						
CV2	27.16		CV2	40.25						
Total	100.00		Total	100.00						
Vectors										
	CV1	CV2		CV1	CV2					
MB-C	1.39	-4.99	BR	0.4	4.43					
MB-N	0.02	0.04	C%	0.3	1.72					
BR	5.71	-1.57		2.00	-0.90					
			Stock of C	-0.05	-0.01					
			Density	6.42	6.90					
			MB-N	-0.04	0.01					
			pH H <sub>2</sub> O	1.12	0.01					
			рН КСІ	0.04	-1.05					
Canonical mean										
	CV1	CV2		CV1	CV2					
AGR	-0.74	-0.14	AGR	-0.71	1.2					
NAT	0.18	0.46	NAT	1.62	-0.10					
PAST	0.56	-0.32	PAST	-0.09	-1.10					

 $\label{eq:cv1} $\text{CV1} = \text{Canonical axis 1; CV2} = \text{Canonical axis 2; MB-C} = \text{Microbial biomass carbon; MB-N} = \text{Microbial biomass nitrogen; BR} = \text{Basal respiration; C\%} = \text{Carbon content; AGR} = \text{Agricultural areas; NAT} = \text{Native areas; PAST} = \text{Pastures.}$ 

When combining the results of physical and chemical data with the microbiological data, the separation of land uses becomes more evident. Most of the variability found was explained by CV1, which separated native and agricultural areas (Figure 3). The separation is confirmed by canonical scores of the first axis, being positive for native areas and negative for agriculture areas and pastures (Table 3). Soil density had a highly negative value related to CV1, representing the main factor for the separation verified in this axis. Other important factors in CV1 include MB-C and pH H<sub>2</sub>O (Table 3). The CV2 explained 40.25% and distinguished pastures and native areas from agricultural areas, as demonstrated by the negative scores of pasture and native areas compared to the positive score of agricultural areas. As well as in CV1, the main factor for CV2 has been soil density, but the basal respiration and total soil carbon also contributed to this separation (Table 3).



**Figure 3.** Canonical analysis considering microbiological, physical and chemical soil properties.

#### DISCUSSION

The canonical variate analysis evidenced that it was not possible to separate the three land uses considering only the microbiological data. The differentiation between pastures and native areas was only possible when the physical and chemical soil properties were included. The human interference with these land uses explains the similarities and differences observed in this study.

The similarities between pasture and native areas may be related to equivalent carbon stock, while the different behavior presented by agricultural areas may be related to soil management and the different types of crops found in the region (soybean, corn, rice, cocoa, coffee etc.). The highest values of MB-C: Corg were registered in pastures followed by native and agricultural areas (Table 1). Jakelaitis et al. (2008) also reported the same sequence. According to Balota et al. (1998), soils with high and low values of MB-C: Corg may represent, respectively, accumulation or loss of soil C. These values confirm the percentage proposed by Jenkinson and Ladd (1981), who considered that usually 1 to 4% of the total soil carbon correspond to the microbial component. This relationship indicates the soil organic matter (SOM) quality, as it allows to track disturbances promoted by ecological imbalance and changes in total SOM caused by management, reacting faster than the physical-chemical indicators (Alvarez et al. 1995). Santos et al. (2004) and Fialho et al. (2006) also presented higher values of qCO<sub>2</sub> in native areas. Mader et al. (2002) evaluated agroecosystems for 21 years and verified a high negative correlation between qCO<sub>2</sub> and microbial diversity. The low values found in pastures and agricultural areas suggest that the microbial biomass in these areas is more efficient in the energy use, characterizing more stable environments (Chaer 2001), and that these areas have also higher microbial diversity (Mader et al. 2002). Dinesh et al. (2003) assign higher values of qCO<sub>2</sub>, such as those found in the present study (1.71), to native areas due to the large amount of C content available for degradation by soil microorganisms.

The high values of microbiological attributes observed in pasture soils in relation to native areas are mainly because most pastures analyzed herein are old, between 10 and 25 years of planting. Besides the pasture age, it is also considered the overall increase of C from the photosynthesis performed by grasses (Feigl et al. 1995). Cerri et al. (2003, 2004) made simulations modeling the dynamics of SOM in pasture soils and demonstrated an increase in carbon stock in the long term (100 years) compared to native areas. Other studies show that, after several years of cultivation, the total concentration of carbon in pasture soils is comparable to those of forests (Cerri et al. 2003). The fine root biomass is a factor that can affect the response of microbiological attributes in the pasture system, with a positive correlation with the microbial biomass and with the water content of the soil (Luizão et al. 1999; Rangel and Silva 2007). The high basal respiration verified in degraded pastures may be related to the diversity of weeds, which have diversified root systems, promoting greater aeration and oxygenation of the soil and increasing the input of nutrients through the litter and exudates produced by different plant species. The highest nitrogen content of the microbial biomass in degraded pastures may indirectly indicate a change in taxonomic groups comprising the microbial biomass. Management such as pasture, where there is not soil disturbance, allows lower flow of CO, into the atmosphere due to less mineralization of SOM (Bayer et al. 2000), besides the large input of nutrients that enables, in a long term, an increase in its content (Carneiro et al. 2008). Due to similarities of microbiological attributes between native forests and pastures, the separation was only possible after the inclusion of physical and chemical factors, especially density (Table 3), which was significantly higher in pastures (Table 2). Other factors that may favor the development of microbial biomass in pastures are the grazing pressure, which results in an increase in the carbon and nitrogen of microbial biomass (Wang et al. 2006), and livestock excreta, which acts as a natural fertilizer (Saviozzi et al. 2001; Iyyemperumal et al. 2007).

Agricultural areas have significantly lower values of microbiological attributes due to anthropogenic influence in this land use. Soils under natural vegetation present a stable carbon stock due to the dynamic equilibrium of soil CO<sub>2</sub> emission and the amount of organic matter from vegetation. When the soil is cultivated, this balance is modified. Carmo et al. (2007) observed a reduction of carbon and nitrogen of microbial biomass in the state of Rondônia, caused by agricultural operations. The application of pesticides, very common in agricultural areas, has a strong impact on soil microbial biomass. Muñoz-Leoz et al. (2011) showed that the use of Tebuconazole implies a reduction in basal respiration, MB-C and metabolic diversity of soil microbial biomass. Planting also leads to losses of carbon and nitrogen of microbial biomass in relation to native areas (Reyes-Reyes et al. 2007). The separation of the agricultural areas from other land uses was possible only using the microbiological attributes due to major disturbances conducted in planting and management of crop, which significantly influences the behavior of microbial biomass (van Heerden et al. 2002). When the physical and chemical attributes are added, the separation between areas becomes more evident, due to soil density (Tables 2 and 3).

# CONCLUSION

Microbiological attributes are efficient for separation of land uses assessed in southwestern Amazon when combined with chemical and physical soil properties. When used alone, the microbiological attributes do not distinguish native areas from pastures, thus indicating a high similarity in the behavior of microbial biomass in these two areas. Soil density contributes significantly to strengthen the differences between native areas and pastures. The canonical variable analysis highlights the key attributes for the separation of land uses and ecoregions analyzed, representing a simple multivariate technique of easy application, with practical results.

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