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Soil Microbial Biomass Under Native Cerrado and Its Changes After the Pasture and Annual Crops Introduction

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1. Introduction

The Brazilian savanna (Cerrado) soils were incorporated into the agricultural production process in the 1970's. Soils were initially occupied by pastures, and later used for cropping (Ferreira et al., 1997). An area of over 12 million hectares is cultivated with annual crops under different systems of soil management (Bayer et al., 2004). The introduction of pastures and/or annual crops utilizing different management systems promoted changes in the dynamics of soil organic matter (SOM).

The SOM improves the soil structure and regulates its biological activity, as well as being directly linked with the ability to accumulate water and maintain the soil fertility. The introduction of agricultural systems in the Cerrado soils, with intensive land use, has brought direct consequences for the chemical, physical and biological properties of soil, with losses in its quality. Continued land use for the cultivation of grains, fibers and cultivated pastures can generate a rapid process of degradation, loss of organic matter, biological processes and imbalance in the flow of nutrients.

The conversion of Cerrado into pastures and croplands made by the slash-and-burn process which causes major impacts on soil fertility. In order to improve the soil conservation, maintain and increase crop productivity, a number of practices were introduced, such as the elimination of crop residues caused by burning, the adoption of conservation tillage, and the management of crop residues (Mielniczuk et al., 1983). As a consequence, SOM increased not only due to the reduction of losses caused by biological decomposition and erosion but also due to the increase of plant residues on soil surface (Bayer et al., 2000).

It is widely known that SOM improves soil structure (Feller & Beare, 1997) and regulates soil biological activity (Bayer & Mielniczuck, 1999), in addition to its role in water holding capacity and soil fertility maintenance (Dick, 1983). The dynamics of SOM is different in clayey and sandy soils and it is highly influenced by different management practices and climate conditions in each region. The stocks of SOM decrease when the soil is exposed to intensive tillage systems due to increasing losses caused by water erosion and microbial oxidation (Silva et al., 1994). However, little information is available about the effects of agricultural management practices on the dynamics of SOM in the Cerrado region. Some studies in Oxisols showed a decrease in microbial attributes in management systems compared to the native systems (Neill et al., 1995; Roscoe et al., 2000; Matsuoka et al., 2003).

Microorganisms play a key role in SOM decomposition. When their diversity or abundance is reduced, the nutrient cycling can be highly affected (Giller et al., 1998). The soil microbial community is generally influenced by variations in soil temperature, water content and aeration, rupture of aggregates, decrease in soil cover, nutrient availability, and organic substrates. These factors can be modified by soil management systems as a function of crop residue incorporation and soil disturbance intensity (Vargas & Scholles, 2000). Soil microorganisms present a high potential for use in soil quality assessments due to their abundance, biochemical and metabolic activity, providing faster responses to environmental changes (Araújo & Monteiro, 2007).

In most part of the soils under agricultural practices (tillage, fertilization, liming, incorporation of pesticides and other inputs) the communities available could be affected to changes in the physical, chemical and biological characteristics. Each change represents a profound renewal of selection pressure, favoring some components of the microbial community and eliminating others, thus experiencing the reallocation of the steady state between the populations.

The soil microbial biomass (SMB), the living fraction of soil organic matter, represents 1 to 4% of the soil organic carbon (C) (Anderson & Domsch, 1990; Sparling, 1992), 2 to 6% of the soil total nitrogen (N) (Jenkinson, 1988), and it is an N reservoir for plants. Nutrient release and immobilization depends on the microbial dynamics, the quantity and quality of plant residues, on the carbon cycling and efficiency of the soil microbial community (Beaudoin et al., 2003). Management systems influence microbial C and N concentrations and conventional tillage reduces the soil microbial biomass and microbial activity (Roscoe et al., 2000; Figueiredo et al., 2007). Due to its sensitivity to changes occurring in the soil, SMB is considered to be a good soil quality indicator (Jackson et al., 2003).

The objective of this chapter is to evaluate the changes in the soil microbial attributes due to conversion of the native Cerrado into pasture and annual crops and to determine the changes related to soil management and climate seasonality.

2. Changes in soil microbial biomass after the land-use-change in Cerrado region

Soil microbial biomass (SMB) and its activity have been suggested as appropriate indicators of changes caused by different land uses and management systems. Kaschuk & Hungria (2010) evaluated a hundreds of studies involving the microbial biomass carbon (MB-C) as one of the physical-chemical indicators of soil quality in the Brazilian cerrado region, confirming the benefits of the conservation tillage in the MB-C and increases of its concentration due to permanent organic agriculture. Thus, the application of pesticides and burning of the vegetation affected the soil microbial communities and reduced the MB-C in overgrazed pastures, but increased in pastures rotated with well-managed crops. However, these authors concluded that the direct relationship between MB-C, nutrient-cycling dynamics, microbial diversity and the soil functionality are still unclear. Further studies are needed in order to develop strategies to maximize the beneficial effects of microbial communities on soil fertility and crop productivity.

It is important to evaluate the microbial biomass nitrogen (MB-N) when nitrogen fertilization occurs. Only between 40 to 60% of the nitrogen applied as fertilizer is absorbed by plants and 20-50% is incorporated into the soil as organic nitrogen (Gama-Rodrigues et al., 2005). However, little information is available about the effects caused by successive applications of nitrogen fertilizers in the SMB, especially in soils of tropical ecosystems.

There are other studies reporting that the addition of nitrogen fertilizer on the soil surface has induced changes in the amount of SMB and its activity, not only in superficial layers but also in deeper layers (Zaman et al., 2002). Taking advantage of this evaluation Coser et al. (2007) working with an Oxisol cultivated with barley studied the effect of nitrogen fertilization (30, 60, 90 and 120 kg ha^{-1}) the microbial biomass nitrogen at different soil depths (0-5, 5-10, 10-20 and 20-30 cm). Results showed no increases in the MB-N in the soil under higher doses of nitrogen (90 and 120 kg ha^{-1}), but the MB-N decreased with depth. This clearly shows that even with addition of nitrogen an increase of SMB may not occur, but this depends increase in SMB but this depends on the type of culture evaluated.

With regard to soybean cultivation, Perez et al. (2004) quantified the microbial biomass carbon (MB-C) and the organic carbon (Corg) of cerrado soil under different systems: no tillage, disking, sub soiling and disking operations over a clayey Oxisol. Soil samples were collected at five depths (0-5, 5-10, 10-20, 20-30 and 30-40 cm) and in four periods (before soil preparation, 30 days after germination, flowering and after soybean harvest). Thus, they reported that sub soiling showed the lowest value of SOC after soybean harvest, since the values of carbon in tillage remained more stable, especially at 0-20 cm. Before planting the crop, the MB-C:Corg was higher in the Cerrado and no significant differences among the tillage systems were found. At 30 days after germination, significant differences were obtained in the values of MB-C:Corg among the types of tillage systems. The values of microbial biomass carbon under the tillage systems were more stable especially at 0-20 cm.

Matsuoka et al. (2003) show that the population of microorganisms on the rhizosphere can be increased when the soil structure has been preserved. Carneiro et al (2008) working with Cerrado soils with soybean cultivation also noted that the maintenance of organic carbon in these soils is essential to maintain its sustainability, since it provides the structure and functioning of soil microbial activity, and contribute to increase the cation exchange capacity (CEC) of these soils.

Anyway, there are many papers studying the SMB and the most important factor that diverges at the results is the type of soil cultivation associated with the type of management and the conditions of soil treatment. In general, the soils of the surface layers contain higher input of organic matter due to increasing of the SMB. The conversion of Cerrado into pasture alters the soil microbial composition.

A permanent cover of soil is an important factor for the maintenance of microorganisms, is directly reflected in the content of microbial C and N. The land use with pasture and managed over time provides high levels of microbial C and N, and that detention may be similar to that observed in the native system.

Studies evaluating soil management systems with annual crops in different soil types showed reduction of microbial C and N in these systems in relation to native Cerrado. This is the most favorable conditions for microorganisms when there is native vegetation, with species diversity and favors the preservation of soil fungal hyphae. The reduction of the most active fraction of SOM affects various functions in the soil, for example, maintaining the production of polysaccharides fundamental to the process of aggregation.

2.1 Soil microbial biomass after pasture and agriculture introduction in Cerrado region

Processes of deforestation are complex and involve many factors at different scales of time and space. Initially, the main reason for deforestation was the timber exploration and the livestock (pasture). The growing demand for grains, fiber and meat for export have caused significant changes in the agricultural scenario of the Brazilian territory. Native areas are being converted to livestock and agriculture utilizing intensive management systems (Brazil, 2009).

According to Maia (2009), the cultivated area in Mato Grosso State had increased 20 times in the period between 1970 and 2002. The increase of pastureland was 2.1 times lower in the same period. Already in Rondonia State, pastureland showed an increase of 17 times, while the agricultural area increased 10.3 times. The author related a large increase in grassland classified as degraded, but also there was a significant increase in areas with improved pasture.

The land-use-change has caused modification in the dynamics of SOM as well as vegetation influences in the activity of the microbial biomass resulting in the reduction of biomass C as studies involving deforestation have shown. When a forest is converted to monoculture or pasture there is a severe environmental impact. The natural mechanisms of nutrient recycling and soil protection are disrupted, often resulting in degradation of the area. Modifications in the microbial community can result in changes in soil quality and fertility and in the availability of carbon (C), nitrogen (N), phosphorus (P) and and others nutrients for plants.

The degradation of SOM is a property of all heterotrophic microorganisms and its rate is commonly used to indicate the soil microbial activity. Thus, it is possible to better understand the process of mineralization and verify the intensity of energy flows (Ingran et al., 2005). The microbial respiration has great potential as an indicator of soil quality in degraded areas, related to the loss of organic C from the soil-plant system to the atmosphere.

2.1.1 Study areas

This study focuses on the Southwestern Amazonian states of Rondônia (RO) and Mato Grosso (MT), a transitional region between the Amazon Basin and the highlands of the Brazilian Central Plateau. Located at latitude 7° and 18° South and longitude 50° and 67° West, the region represents the water divisor between two large basins: the Amazon on the North and the Paraná on the South. It is also one of the largest agricultural frontiers in the world, comprising an area of approximately 1,128,000 km^2.

The regional climate varies according to latitude and can be characterized as a humid tropical regime with short dry seasons. The pluviometric regime has very defined seasons: a rainy and a dry season (or low rainy season) with mean annual precipitation ranging from 1400 to 2500 mm. The types of soil in this region, as described by Mello (2007), are Oxisols, Ultisols and Entisols, comprising 40%, 20% and 15%, respectively, of the total area of both states.

The research sites were selected to cover the main bio and geo-climatic zones of the States of Rondonia and Mato Grosso (Fig. 1), following the “Guidelines for National Greenhouse Gas Inventories” issued by the Intergovernmental Panel for Climate Changes (IPCC, 2007). The delimitation of the zones was performed using the Geographic Information System ArcGis 9.0 with combined information on soils, native vegetation, geology, climate and relief. This methodology allowed to obtain relatively homogeneous areas that made it possible to perform a discerning extrapolation of the microbiological parameters for the whole region. In each one of the 11 zones, two cities were randomly chosen for data collection, totaling 22 points of research sites (Maia et al., 2009).

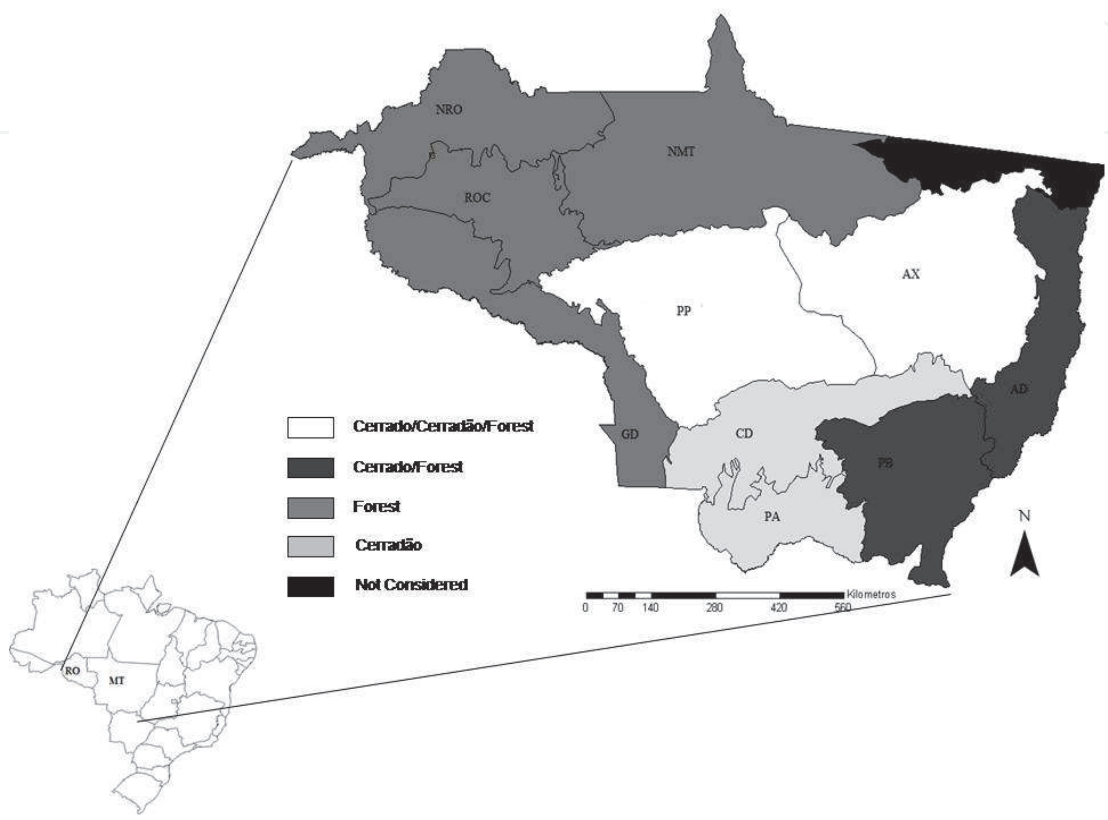

Fig. 1. Distribution of Ecoregions and native areas in study area; (AX) Alto Xingu; (PB) Parana Basin; (PP) Parecis Plateau; (AD) Araguaia Depression; (CD) Cuiabá Depression; (DG) Guaporé Depression; (NMT) North of Mato Grosso; (NRO) North of Rondônia; (PA) Pantanal; (ROC) Central Rondônia.

The follow land uses were selected for this study: native Cerrado area (CER); Agriculture (AGR) with different crops (soybean, corn, rice, etc); and Pasture area (PAS).

2.1.2 Soil sampling and analytical procedures

Sampling was carried out in June/July 2007 at 0-5 cm depth (15 in Cerrado area, 45 in agricultural area, and 55 in pasture area) in three replicates on each place, totaling 345 samples. Soil samples were harrowed and sifted through a 2 mm mesh to remove rocks and vegetation fragments.

Soil Basal Respiration (BR) was determined by CO_2 evolution. Field-moist soil samples (equivalent to 5g dw) were placed in 250 ml tubes and pre-incubated at 25°C for 3 days. The tubes were hermetically closed and he CO_2 produced from the soil after 8 hours of incubation. The samples were carried out with BD syringes (2ml) and analyzed utilizing an infra-red analyzer (IRGA LICOR-6262).

Microbial biomass C (MB-C) was determined by the chloroform fumigation–extraction method (Vance et al.,1987) with field-moist samples (equivalent to 20 g dw). The filtered soil extracts of both fumigated and no fumigated samples were analyzed for soluble organic C using a total organic C analyser (Shimadzu TOC-5000). The MB-C was estimated on the basis of the difference between the organic C extracted from the fumigated soil and that from the no fumigated soil (EC). For the quantification of nitrogen in microbial biomass (MB-N), the extracts were analyzed using the Ninhydrin Method (Jorgensen and Brookes, 1990). Ten Cerrado areas, 20 agricultural areas and 20 pasture areas were selected, in three replicates on each place in field conditions and two replicates in laboratory, totaling 300 samples.
The (qCO_2) metabolic ratio (or specific respiratory rates) was calculated based on relationship between MB-C and BR.

2.1.3 Results and discussion

The results for the basal respiration between pasture and Cerrado were similar (Table 1). These results may be related to carbon stocks equivalents, while the different behavior in the agricultural areas may be related to soil management and the different types of crops that are found in the region (soybean, corn, rice, coffee, etc.).
Studies show that after some years of cultivation, the total concentration of carbon in the pasture soil is similar to the native systems (Cerri et al., 1991; Cerri, 2003; Silva, 2004) which also influences the dynamics of SMB. This fact is due to the large amount of roots present in the pasture system which allows the increase in SOM content and available substrates for microorganisms to long-term (Carneiro et al., 2008). Maia (2009) concluded that degraded pastures in the states of Mato Grosso and Rondonia may provide an increase in the soil organic C content and consequently promote C sequestration.
Jakelaitis et al. (2008) also reported the same sequence in their studies. According to Ballota et al. (1998) soil that exhibit high and low values of MB-C:Corg may represent accumulation or loss of soil carbon, respectively. Those values are consistent with the percentage proposed by Jenkinson & Ladd (1981) who consider it normal that 1 do 4 % of soil carbon corresponds to the microbial component. This ratio is reported as an indicator of the quality of SOM (Wardle, 1994), it allows to monitor the disturbances promoted by the ecological imbalance and changes in total SOM caused by management, reacting faster than the physical and chemical indicators (Alvarez et al., 1995).
Studies in different soils and regions found higher values of qCO_2 in native areas (Xavier, 2004; Santos et al., 2004; Fialho et al., 2006). Assessing agroecosystem for 21 years, Mader et al. (2002) reported a high negative correlation between microbial diversity and qCO_2. The lower values observed in pasture (0.76) suggest that these areas have a more efficient microbial biomass energy use, featuring more stable environment (Chaer, 2001) and also have higher microbial diversity (Mader et al., 2002). Dinesh et al. (2003) assigns higher values of qCO_2 due the large amount of C content available for soil microbial degradation.
The higher values of MB-C and MB-N observed in soils under pasture (PAS) in relation to native area (CER) is due to long time of pasture implantation in these ecoregions (10 to 25 years of establishment). Luizão et al. (1999) studied pastures 2 to 13 years in the Amazon region and assign that the SMB and BR in the soil superficial layer (0-5 cm) increase until five years after the pasture establishment. After that a progressive decline occurs until the eighth year. However, De Vries et al. (2007) shows a positive correlation between SMB (fungal and bacterial) and the age of pasture.

Basal Respiration (ugCO_2 g soil h^{-1})				
	Mean	N	S.D.	C.V. (%)
AGR	0.37 b	135	0.08	21.44
PAS	0.50 a	165	0.18	38.06
CER	0.46 a	45	0.09	19.17
MB-C (g C kg $soil^{-1}$)				
	Mean	N	S.D	C.V. (%)
AGR	0.53 b	120	0.21	41.29
PAS	0.69 a	120	0.17	25.29
CER	0.53 b	60	0.29	54.86
MB-N (mg C kg $soil^{-1}$)				
	Mean	N	S.D	C.V. (%)
AGR	17.85 b	120	16.10	90.19
PAS	37.16 a	120	26.93	71.00
CER	26.56 b	60	18.74	70.55
MB-C: Corg (%)				
	Mean	N	S.D	C.V. (%)
AGR	2.98 b	100	2.40	49.51
PAS	4.08 a	100	2.50	33.14
CER	3.45 a	20	2.31	67.03
qCO_2 (g CO_2 h^{-1})				
	Mean	N	S.D	C.V. (%)
AGR	1.01 a	120	0.49	97.14
PAS	0.76 b	120	0.47	45.09
CER	1.02 a	20	0.78	76.52

Table 1. Soil microbial attributes in different areas at Mato Grosso and Rondonia states. AGR: Agriculture; PAS: Pasture areas; CER: Cerrado; S.D.: Standard Deviation; C.V.: Coefficient of Variation.

According to Luizão et al. (1999), the biomass of fine roots is a factor that may influence the response of the microbial attributes in the pasture system, having a positive correlation with the SMB and the soil water content. The higher BR observed in degraded pastures may be related to the diversity of invasive plants which have varied root systems, resulting in greater soil aeration and oxygenation (Grimaldi et al., 1992). Moreover, there is an increase in nutrient input through litter and exudates produced by different plant species.

The greatest MB-N content in degraded pastures may indicate, indirectly, a change in taxonomic groups that compose the microbial biomass (Venzke Filho, 1999). The development of nitrifying microorganisms occurs due to different physical, chemical and nutritional properties.

Other factor that promotes the development of the SMB in pasture is an intensive livestock, resulting in an increase in MB-C and MB-N (Wang et al., 2006). The livestock waste acts as a natural fertilizer and consequently causes reactions in the dynamics of soil microorganisms (Saviozzi et al., 2001; Iyyemperumal et al., 2007). The microbial biomass is sensitive to changes in soil organic carbon related to management and land-use-change. After the

alterations in the soil, the SMB undergoes fluctuations until a new equilibrium (Polwson et al., 1987).
To illustrate the dynamics of the microbial attributes within ecoregions, was performed a study in the Alto Xingu ecoregion. This ecoregion was chosen because it has great representation in the land-use-change in Southwestern Amazonia. The Alto Xingu is characterized by livestock and agriculture in the municipality of Sorriso, and the soil is classified as Oxisols with different clay contents (Belizario, 2008).
The improved pastures (IP) showed higher values of MB-C and BR in relation to degraded (DP) and typical pastures (TP). The SM-C (Figure 2) values were 0.84 (IP), 0.60 (DP) and 0.53

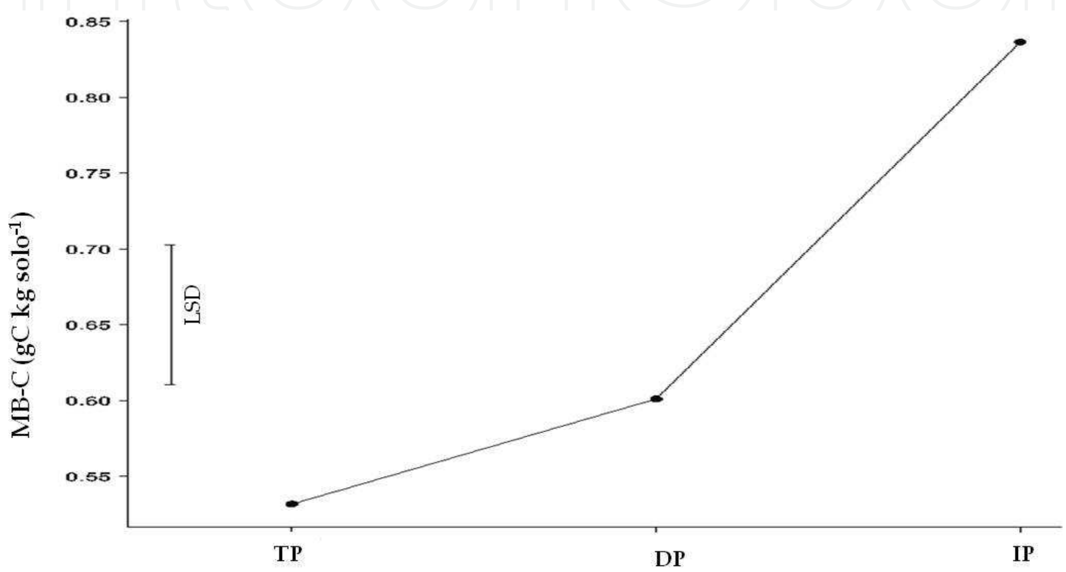

Fig. 2. Microbial biomass carbon (MB-C) in pasture areas located at Alto Xingu ecoregion. LSD, Least significant difference; TP, typical pasture; DP, degraded pasture; IP, improved pasture.

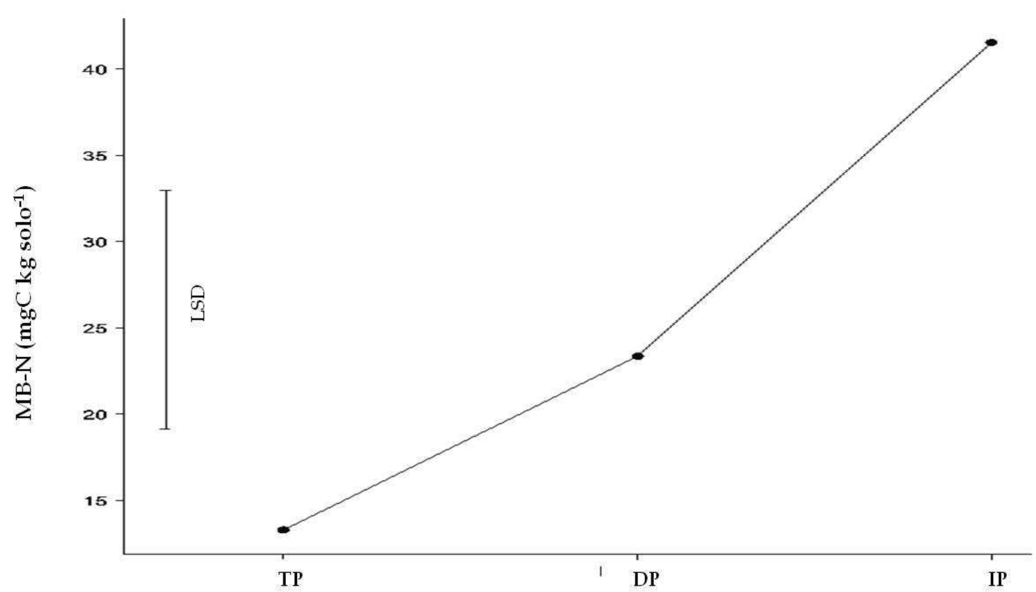

Fig. 3. Microbial biomass nitrogen (MB-N) in pasture areas located at Alto Xingu ecoregion. LSD, Least significant difference; TP, typical pasture; DP, degraded pasture; IP, improved pasture.

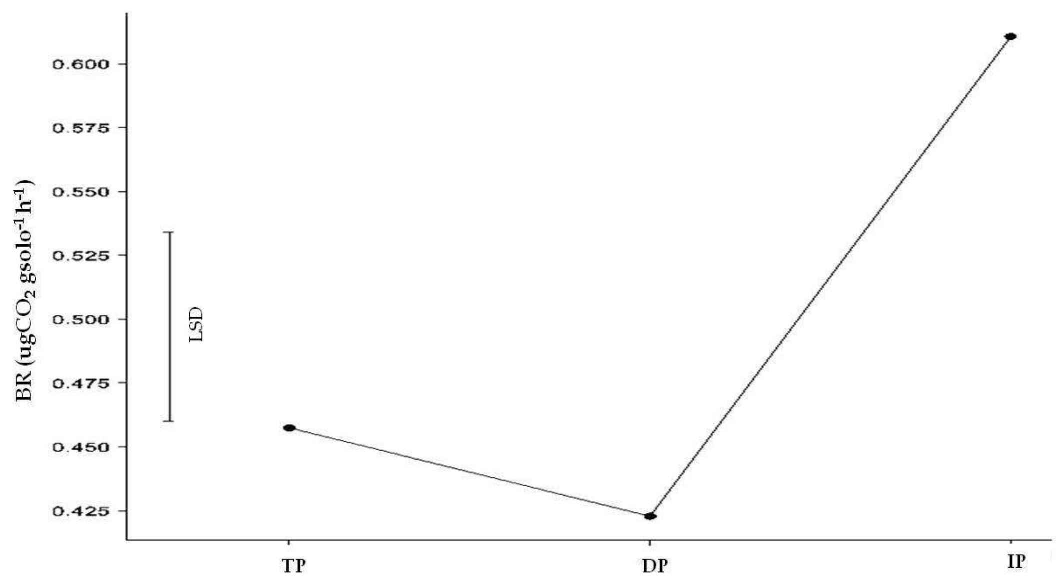

Fig. 4. Soil basal respiration (BR) in pasture areas located at Alto Xingu ecoregion. LSD, Least significant difference; TP, typical pasture; DP, degraded pasture; IP, improved pasture.

gC kg $solo^{-1}$ (TP), without statistical differences between DP and TP. The MB-N (figure 3) showed higher values to IP (41.54 mgN kg $solo^{-1}$), followed by DP (23.36 mgN kg $solo^{-1}$) and TP (13.29 mgN kg $solo^{-1}$).The BR values (Figure 4) were 0.61 (IP), 0.42 (DP) and 0.46 $ugCO_2$ $gsolo^{-1}$ h^{-1} (TP).

The highest values of soil microbial attributes founded in IP may be related to the application of fertilizers and lime in the study area. Hatch et al. (2000) demonstrated increases in basal respiration in soils after the application of fertilizers, however, no reported increases in the MB-C, different that observed in this study.

The fertilizer can initiate a process of "priming effect" on soil, promoting an increase in the active biomass (r-strategists microorganisms) which die after the exhaustion of the substrate, or become dormant due to its inability to mineralize the SOM. In contrast, the microbial biomass of slower growth (k-strategists microorganisms) remains active and increased its population due the non-degraded fractions by r-strategists microorganisms and also the substrates provided by cell lyses (Fontaine et al., 2003). The mechanisms of priming effect are not fully elucidated yet; however competition between r and k-strategists microorganisms can help to elucidate the dynamics observed in these study areas with pasture.

The increase in pH caused by the lime application promotes an impact in the composition of SMB. High pH stimulates the activity of nitrifying bacteria which combined with fertilizers cause the soil acidification, mainly in the superficial layers (Giracca, 2005).

2.2 Soil microbial biomass after the annual crops introduction in Cerrado region

The Cerrado region was incorporated for the grain production because has weather conditions favorable to cultivate annual crops (soybean, corn, sorghum, etc). Moreover, the flat topography facilitates the soil management and harvesting of grains.

The pasture and annual crops cultivation are the first land-use-change that have occurred in large proportions and quite fast in the Amazon region. The expansion of soybean cultivation

in the Southwestern of Amazon region has occurred mainly in the Rodonia and Mato Grosso states. The production of Brazilian soybean was 19 M tons in 1994 and increased to 40 M tons in 2004, and the Mato Grosso State was the largest contributor to this increase (Brasil, 2009).
It is important to report that the intensive land use reduces the quality of organic matter remaining in the soil. These changes occur, for example, in the breakdown and destruction of soil aggregates with losses to erosion, reducing the availability of nutrients to plants and the water storage capacity. These are some factors that reflect negatively on agricultural productivity, and consequently on food production and the sustainability of the soil-plant-atmosphere (Lal. 2003; Six et al., 2004; Knorr et al., 2005). However, Maia (2009) in interviews with experts noted that occurred a considerable increase in the no-tillage cultivation between 1985 and 2002, and now it is the main crop system adopted in the region.
Microorganisms play an essential role for the decomposition of SOM and its reduction in the diversity or abundance may affect nutrient cycling (Giller et al., 1998). The microbial activity is affected by soil management systems, depending how the crop residue is incorporate and the degree of soil disturbance (Vargas & Scholles, 2000). Thus, is important determine the changes in soil microbial biomass related to soil management and climate seasonality in the Cerrado region.

2.2.1 Study areas

The study was carried out at the União Farm (12º29’S, 60º00’W), a conventional farm with an area of 3,700 in Rondonia State, Brazil (Figure 5). The native vegetation of the region is classified as Cerrado, sub-group Cerradão of the dense vegetation type (Ribeiro and Walter, 1998). According to Köppen (1900) the climate is classified as Aw (humid tropical) with mean temperature of 23.1 °C and a minimum temperature of 18.0 °C during the coldest month. The region has a well defined dry season (May to September) with a monthly rainfall below 10 mm, while the mean annual rainfall is 2,170 mm. The mean altitude of the region is 600 m with undulating relief. The soil was classified as an Oxisol (Typic Hapludox) with very clayey texture (730 g clay kg^{-1} of soil).

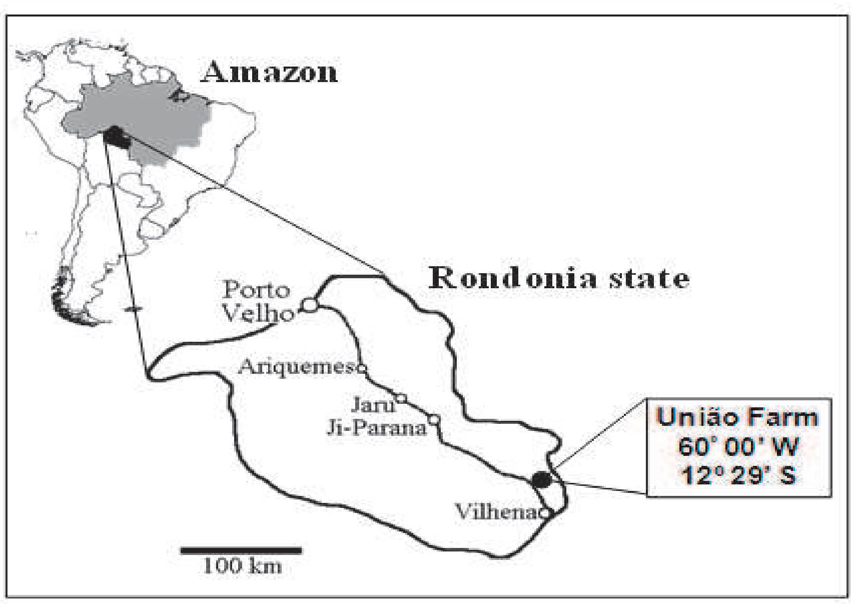

Fig. 5. Map of location of the study area in the União Farm, Rondônia State, Amazon region, Brazil.

Areas of about 500 ha of the farm were cleared yearly for cultivation between 1999 and 2004. (Figure 6). The clearing was done by tractor and blade at the end of the wet season (May/June). After a drying period of 20 days, aboveground biomass was burnt. Mechanical windrowing followed this operation and areas were subsequently cleaned by burning stumps and root residues and removing remaining material. For further soil preparation, a disc harrow was used to incorporate dolomite lime, which was applied to achieve 50 % base saturation (V) in the 0-20 cm soil layer. Next, a leveling harrow was used. These initial preparation steps had been applied to all sampled areas, except for the native Cerrado (used as control).

Fig. 6. Conversion of Cerradão to agriculture in União farm.

Every newly established area was cultivated with rice under CT. After two years of rice under CT and associated lime incorporation, leveling and cleaning of the soil surface, a NT system with soybean was introduced for one to three years. A chronosequence of six different sites was considered in this study: native Cerrado vegetation (CE), used as a reference area, a CT system cultivated with rice for 1 year (1CT) and 2 years (2CT), and a NT system cultivated with soybean for 1 (1NT), 2 (2NT) and 3 years (3NT), always preceded by a 2-year period of rice under CT alternating either with other crops or fallow land in the winter season (Table 2). This table also shows the crop cycles, annual application rates of lime, pH $CaCl_2$, available P and V in the 0-30 cm soil layer. Nitrogen fertilization rates and other nutrient additions in the study area are described in detail in Carvalho et al. (2007).
The areas were located in close proximity (less than 2 km apart from each other), with similar topography, soil and climate conditions, differing only in the time since clearing and the setting up of the sites.

2.2.2 Sampling and analytical procedures

Soil sampling was carried out in July 2004 (dry season) and in January 2005 (wet season) in six areas of approximately 1 ha (100 x 100 m) based on a completely randomized sampling design with five pseudo replicates in each area. We are considering those as pseudo replicates, since they came from the same evaluated areas.
Soil samples were taken from 5 profiles at 0-5, 5-10 and 10-20 cm depths in each site. After air-drying, the samples were sieved at 2 mm. From each sample, 10 g were ground and sieved at 0.25 mm for determination of Total Organic Carbon (TOC). The determination was carried according to Nelson & Sommers (1982) using a Carbon Analyzer – LECO® CN-2000.
As samples were collected from fixed layers, the C stock calculation needed to be adjusted for variations in bulk density (BD) after land use changes. Therefore, the methodology described

Land use	**Cultivation period**	**Main crop**	**Winter crop**	Lime	Soil Density	pH $CaCl_2$	Available P	V
				Mg ha^{-1}	g cm^{-3}		mg dm^{-3}	%
Continuous Cerrado				-	0.77	3.8	4.7	3.6
1CT	2003 – 2004	rice (CT)	fallow land	6	1.00	4.5	6.5	24.0
2CT	2002 – 2003	rice (CT)	fallow land	2	0.93	4.5	5.7	20.1
	2003 –2004	rice (CT)	fallow land	4				
1NT	2001 – 2002	rice (CT)	fallow land	2	1.11	4.7	9.4	32.5
	2002 – 2003	rice (CT)	fallow land	2				
	2003 – 2004	soybean (NT)	maize	2				
2NT	2000 – 2001	rice (CT)	fallow land	2	0.98	5.0	15.4	39.1
	2001 – 2002	rice (CT)	fallow land	2				
	2002 – 2003	soybean (NT)	sorghum	1				
	2003 – 2004	soybean (NT)	millet	1				
3NT	1999 – 2000	rice (CT)	fallow land	1	1.14	5.4	29.7	58.9
	2000 – 2001	rice (CT)	fallow land	2				
	2001 – 2002	soybean (NT)	fallow land	1				
	2002 – 2003	soybean (NT)	maize	0				
	2003 – 2004	soybean (NT)	maize	2				

Table 2. Cultivation history of the main crops (rice, soybean) and land use in the winter season in the corresponding cultivation periods under different land use practices annual lime application rates, pH $CaCl_2$, available P and base saturation in the 0-30 cm soil layer. Source: Carvalho et al. (2009). Where: 1CT and 2CT mean 1 and 2 years of rice under conventional tillage; 1NT, 2NT and 3NT mean 1, 2 and 3 years of soybean under no-tillage after a 2-year period of rice under conventional tillage; V means base saturation.

in Ellert & Bettany (1996) and Moraes et al. (1996) was used to adjust soil C stocks to an equivalent soil mass. In order to calculate C stocks in an equivalent soil mass, the depth of the considered area was adjusted, i.e., the depth of the cultivated areas containing the same soil mass as the corresponding layer (0-30 cm) in native vegetation (the reference area).

To determine soil microbial biomass and basal respiration, subsamples were carried out using a grid pattern at five points within a 100m^2 quadrant for each treatment. The soil subsamples from each treatment were bulked and thoroughly mixed in a plastic bag, and a composite sample was taken. The composite samples, in five replicates for each treatment, were transported on ice, in a cooler, to the laboratory. Field moist soils were sieved through a 2 mm screen, and immediately stored in sealed plastic bags at 4°C.

The samples used for microbial biomass and determination of soil basal respiration (BR) were adjusted to 55% of the field capacity, considered the ideal soil water content for studying microbial activity responses. The soil microbial biomass was estimated by the fumigation-extraction method proposed by Vance et al. (1987). Fumigated and non-fumigated soil samples were extracted with 0.5 M K_2SO_4 for 30 min (1:5 soil:extraction ratio), filtered, and the aliquot was analyzed. The microbial C concentration in the extracts was obtained by a SHIMADZU TOC 5000-A equipment. The microbial N was determined by the ninhydrin reactive compound quantification method (Joergensen & Brookes, 1990) using the conversion factor kEN = 0,65 (Sparling et al., 1993).

The statistical analysis of data was performed on a completely randomized sampling design, with the assumption that the areas studied had the same topographic, edaphic and climatic conditions. Six areas with five pseudo replicates were evaluated.

Data from soil C stocks under different areas were analyzed for variance (ANOVA) to determine land use effects. A Tukey test was used to test significant ($p \leq 0.05$) differences among treatments. All statistical analyses were performed using the SAS program, version 6.

2.2.3 Results and discussion

In the 0–30 cm soil layer, the C stock in CE was 50 Mg ha^{-1}, significantly smaller than the stocks in 1NT and 3NT ($p < 0.05$), in the dry season (Table 3). Corazza et al. (1999), studying a clayey Typic Hapludox under Brazilian Cerrado vegetation, measured a soil C stock in the 0-20 cm layer of 39.8 Mg ha^{-1}. Resck et al. (2000) measured in a Typic Hapludox under Brazilian Cerrado a C stock of 61 Mg C ha^{-1} in the 0-30 cm soil layer. In a Rhodic Hapludox with very clayey texture under Cerrado in Dourados (Mato Grosso do Sul State, Brazil), Salton et al. (2005) measured a soil C stock of 44.5 Mg ha^{-1} in the 0-20 cm layer. Bayer et al. (2006) reported a C stock of 54 Mg ha^{-1} in the 0-20 cm layer of a Typic Hapludox (650 g clay kg^{-1} soil) under Cerrado in Brazil. Despite the soil C contents were similar among the mentioned studies (ranging from 2.5 up to 3.1% comparable to the 2.9% of C for this research) the soil BD obtained here (weighted mean of 0.77 g cm^{-3} in the 0-30 cm soil depth) was lower than the values reported by Salton et al. (2005) and Bayer et al. (2006). Therefore, we suggest that the lower soil C stocks presented here are due to the lower BD compared to the last two studies cited above.

After the conversion of Cerrado into agricultural land, while the soil C stock in 1CT (47.6 Mg ha^{-1}) was significantly smaller than the stocks in 1NT and 3NT ($p < 0.05$), it is not statistically different from 2NT (Table 3).

In the wet season, six months after the first soil sampling, there were no significant differences among the areas in the 0-30 cm soil layer (Table 3). Average soil C stocks in

Situations	Soil C Stocks (Mg ha^{-1})	
	Dry season	Wet season
CE	50.0 ± 7.4 b	48.1 ± 2.6 a
1CT	47.6 ± 4.9 b	47.4 ± 7.2 a
2CT	55.4 ± 8.5 ab	58.5 ± 11.0 a
1NT	66.5 ± 6.5 a	65.6 ± 15.4 a
2NT	54.5 ± 5.6 ab	47.4 ± 7.9 a
3NT	67.5 ± 10.3 a	59.0 ± 18.5 a
LSD(1)	14.49	22.87
CV%(2)	13.0	21.5

Table 3. Soil C stocks (Mg ha^{-1}) in the equivalent soil mass of 30 cm depth under Cerradão in the dry (July 2004) and wet (January 2005) seasons under Cerrado (CE), conventional tillage (1CT and 2CT) and no-tillage (1NT, 2NT and 3NT) in Vilhena, Rondonia State, Brazil. Adapted from: Carvalho et al., (2009).The results are mean (n=5) ± standard deviation. Means followed by the same letter are not significantly different according to Tukey's test at 5 %. (1) Least Significant Difference. (2) Coefficient of variation

the 0-30 cm were calculated using the data of the two evaluated sampling times (dry and wet seasons presented). When the average soil C stock was considered, some significant differences were observed. The C stock in 1CT was significantly smaller ($p < 0.05$) than the stocks in 2CT, 1NT and 3NT.

In dry season, the MB-C in the 0-5 cm soil depth was higher in the CE area than the other situations (Table 4). However, only 1CT and 1NT were significantly lower ($p<0.05$). In the 5-10 cm soil layer, was again obtained higher contents of MB-C in CE area, followed by 2CT and 1NT. At 10-20 cm soil depth there were no significant differences between the situations evaluated.

The MB-C increased in January 2005. Others studies in the Amazon region (Geraldes ,1995; Frazão et al., 2010) reported modifications in SMB under different management systems and seasonal variation, with increases in the wet season.

In the rainy season the MB-C was higher in the CE situation (Table 4). At 0-5 cm, MB-C was higher in CE, followed 1NT, 1CT, 3NT, 2NT and 2CT. Cerri et al. (1985) found higher MB-C in native area in relation to cultivated areas, and this fact was linked to increased deposition of organic residues in soil and the large amount of roots with stimulate the activity of soil microorganisms, especially in the superficial layers of soil.

In both seasons studied showed the same trends of MB-C reduction with the land-use-change. Chaga (2000) studying soils in Cerrado region, did not found significant differences in MB-C values between native forest and NT system. Moreover, Hungria (1996), in study at Parana State, noted that the MB-C was 50% higher in soil under NT compared to CT.

A possible explanation for the lower amount of MB-C in NT may be related to short time of installation this system. Souza et al. (2006) founded similar results, with lower values in NT than in CT. According to D'Andrea et al. (2001) this occurs in NT areas recently implemented, where there is initially a reduction of MB-C and then after the stabilization of NT the result is an increase in soil MB-C.

Situation	Microbial biomass carbon (mg C kg soil^{-1})					
	Dry season (July 2004)			Wet season (January 2005)		
	0–5 cm	5–10 cm	10–20 cm	0–5 cm	5-10 cm	10-20 cm
CE	713 ± 147 a	644 ± 69 a	421 ± 118 ab	1262 ± 310 a	993 ± 263 a	840 ± 212 a
1CT	354 ± 118 b	342 ± 57 c	338 ± 69 ab	312 ± 146 bc	467 ± 323 bc	679 ± 217 bc
2CT	535 ± 209 ab	521 ± 135 ab	479 ± 61 a	200 ± 25 c	470 ± 103 bc	352 ± 167 bc
1NT	367 ± 87 b	367 ± 91 bc	310 ± 49 b	543 ± 141 b	657 ± 118 ab	655 ± 250 bc
2NT	465 ± 104 ab	307 ± 80 c	280 ± 116 b	228 ± 28 c	307 ± 97 c	239 ± 133 c
3NT	641 ± 183 ab	346 ± 76 c	299 ± 75 b	292 ± 110 bc	244 ± 93 c	364 ± 127 bc

Table 4. Microbial biomass carbon (MB-C) in the dry (July 2004) and wet (January 2005) seasons under Cerrado (CE), conventional tillage (1CT and 2CT) and no-tillage (1NT, 2NT and 3NT) in Vilhena, Rondonia State, Brazil. The results represent the mean (n=5) ± standard deviation. Means within each column of the same depth followed by the same letter are not significantly different by the Tukey test ($p<0.05$).

The content of MB-N was altered by changes in soil moisture (Table 5). Overall, the averages of MB-N tended to be higher in the CE situation. In dry season, the 0-5 cm layer showed increase in 3NT, followed CE and 2NT situations. The lowest MB-N contents were obtained in 1NT.

In the rainy season there was no statistical difference at 0-5 cm soil depth ($p<0.05$). It was observed the higher MB-N values in CE at 5-10 cm, and CE and 1NT at 10-20 cm soil depth.

Situation	Microbial biomass nitrogen (mg N kg soil^{-1})					
	Dry season (July 2004)			Wet season (January 2005)		
	0–5 cm	5-10 cm	10-20 cm	0-5 cm	5-10 cm	10-20 cm
CE	47 ± 16 ab	32 ± 9 a	27 ± 8 abc	36 ± 28 a	75 ± 19 a	58 ± 11 a
1CT	21 ± 4 cd	20 ± 4 bc	23 ± 12 ab	27 ± 22 a	35 ± 25 ab	43 ± 22 ab
2CT	28 ± 8 bcd	31 ± 4 ab	33 ± 4 a	22 ± 10 a	24 ± 25 b	32 ± 14 abc
1NT	15 ± 4 d	11 ± 4 c	14 ± 6 d	44 ± 20 a	42 ± 20 ab	47 ± 13 a
2NT	38 ± 5 abc	19 ± 5 bc	20 ± 4 bcd	24 ± 26 a	20 ± 10 b	18 ± 14 bc
3NT	55 ± 17 a	23 ± 8 abc	16 ± 4 cd	28 ± 15 a	22 ± 23 b	14 ± 8 c

Table 5. Microbial biomass nitrogen (MB-N) in the dry (July 2004) and wet (January 2005) seasons under Cerrado (CE), conventional tillage (1CT and 2CT) and no-tillage (1NT, 2NT and 3NT) in Vilhena, Rondonia State, Brazil. The results represent the mean (n=5) ± standard deviation. Means within each column of th same depth followed by the same letter are not significantly different by the Tukey test ($p<0.05$).

Fernandes et al. (1998) in a study at Sete Lagoas, Minas Gerais State, founded the quantity of the soil MB-N two times higher in NT than in CT.

The ratio between microbial biomass carbon and total organic carbon (MB-C:Corg) has been considered a good indicator of changes of SOM in the evaluation of soil management systems (Sá et al., 2001) and reflects the amount of C available for the growth of microorganisms.

The MB-C:Corg ratio ranged between dry and rainy seasons, confirming the results obtained by Frazão et al (2010) in a sandy soil in the Amazon region. The MB-C:Corg ratio was highest at 0-10 cm than at 10-20cm soil depth. (Figure 7). Haynes (1999) evaluated temperate soils and reported a reduction in ratio with the increase of soil depth. In dry season, considering 0-20 cm layer, the higher MB-C:Corg ratio was observed in CE (1,6%). The values ranged between 1.4 (2CT) and 0.9% (1NT) in management systems.

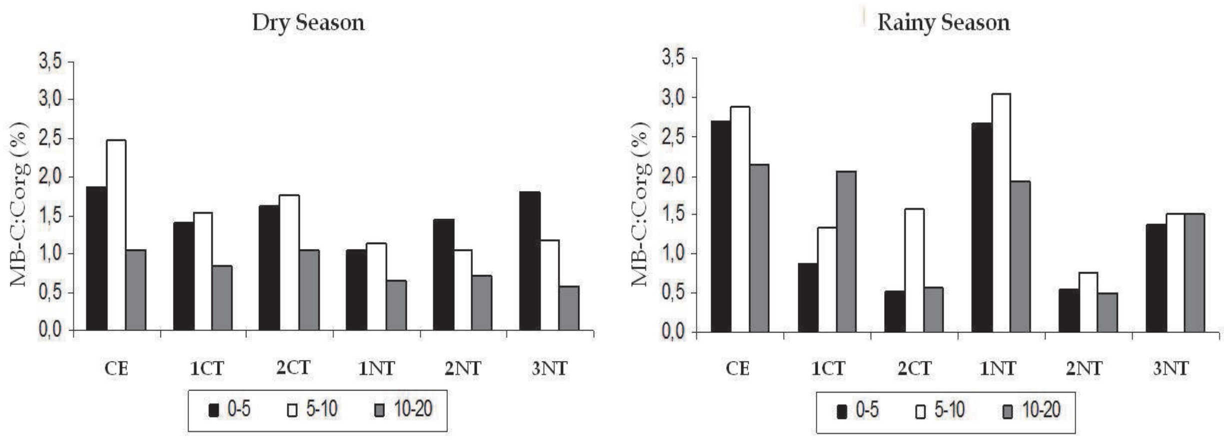

Fig. 7. MB-C:Corg ratio (%) in dry and rainy season. Situations evaluated: Cerrado (CE), Conventional tillage (1CTand 2CT) and No-tillage (1NT, 2NT and 3NT).

In the rainy season the MB-C:Corg ratio in the 0-20 cm soil layer was 2.5, 1.6, 0.8, 2.4, 0.6 and 1.5% for CE, 1CT, 2CT, 2CT, 1NT, 2NT and 3NT, respectively (figure 7). Anderson & Domsh (1989) evaluated the MB-C:Corg ratio in 134 plots of agricultural experiments to long term in temperate regions and obtained values between 0.27 and 7.0%.This variation was attributed to type and management of soil, type of vegetation, sampling season and analytical conditions. The authors suggested equilibrium values of 2.3% for monoculture and 2.9% for crop rotation.

However, according to Sá (2001), the MB-C:Corg ratio are lower in tropical regions than in temperate regions. Cerri et al. (1985) studying an Oxisol in the Amazon region found values of 0.73% under natural conditions and 0.04% in an area recently cleared and burned. Other study carried out in Parana State in soil cultivated with soybean under NT system found values ranging between 0.79 and 1.59% (Colozzi-Filho et al., 2005).

The MB-N:Ntotal ratio represents the mineralizable N fraction, i.e., it expresses the potential of inorganic N available in the soil for the next crop (McGill et al., 1988).

The ratio between microbial biomass nitrogen and total nitrogen (MB-N:Ntotal) varied in both seasons (Figure 8). The highest ratios were observed in 3NT, CE and 2NT. In NT situations (3NT and 2NT) the highest MB-N content were observed at 0-5 cm deep. Considering the MB-N:Norg ratio (%) at 0-20 cm, the values were 1.5 for CE area, 1.1 for 1CT and 2CT area, 0.6 for 1NT, 1.2 for 2NT and 1.3 for 3NT (Figure 8). In all situations were observed a reduction in the MB-N:Norg ratio at 10-20 cm compared to the superficial layers.

In the rainy season were founded the highest contribution of theMB-N into the N stocks. However, there was oscillation between the situations in the dry season. The MB-N:Ntotal ratio (%) at 0-20 cm in depth was 2.5 for CE, 2.0 for 1CT, 1.1 for 2CT, 2.9 for 1NT, 0.8 for 2NT and 1,5 for 3NT. The higher MB-N:Ntotal values in the CE and 1PD areas represent higher inorganic N cycling efficiency and availability in the medium term (Xavier et al., 2007).

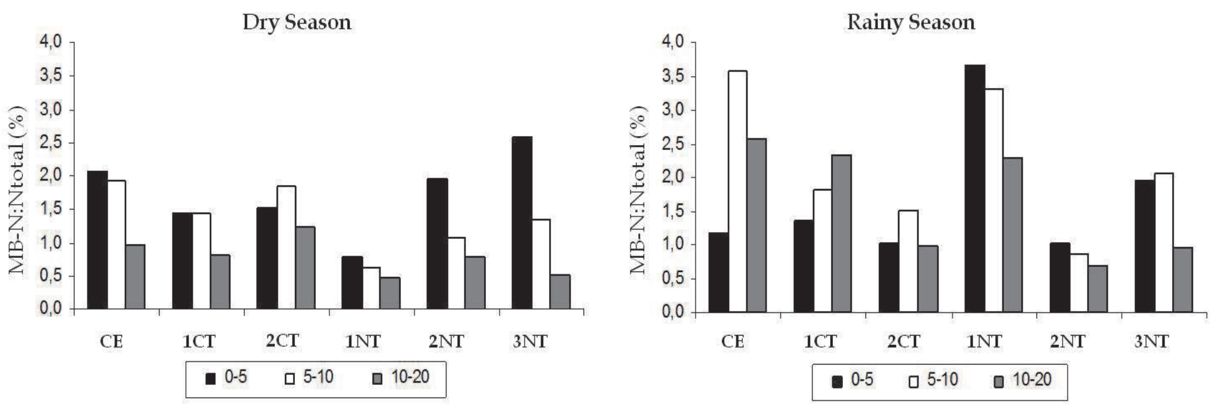

Fig. 8. MB-N:Ntotal ratio (%) in dry and rainy season. Situations evaluated: Cerrado (CE), Conventional tillage (1CTand 2CT) and No-tillage (1NT, 2NT and 3NT).

3. Conclusions

The land-use-change in the Cerrado region for pasture and agricultural purposes using different soil management systems promote alterations in the microbial components of soil.

The highest values of MB-C and MB-N were found in the Cerrado and pasture areas. The permanent soil cover and the lack of soil disturbance with the absence of agricultural practices produced more favorable conditions for microbial development in those systems.

The largest variations in the agricultural systems can be attributed more to climatic seasonality than to differences in the management systems.

In general, it is possible to conclude that soils under native system and agriculture with minimal disturbance of soil contribute to development and maintenance of soil microbial attributes.

4. Acknowledgments

The authors thank FAPESP - Fundação de Apoio a Pesquisa do Estado de São Paulo (The State of São Paulo Research Foundation) and CNPq - Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (National Council for Scientific and Technological Development) for concession of scholarships and financial resources.

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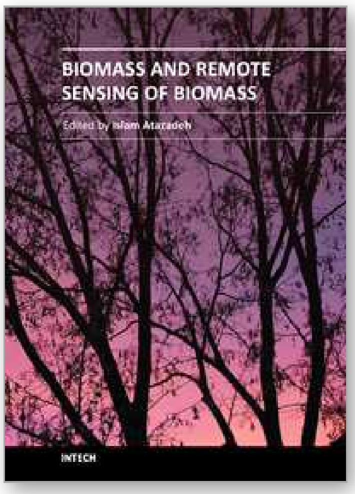

Biomass and Remote Sensing of Biomass
Edited by Dr. Islam Atazadeh

ISBN 978-953-307-490-0
Hard cover, 262 pages
Publisher InTech
Published online 06, September, 2011
Published in print edition September, 2011