Impacts of integrated crop-livestock-forest on microbiological indicators of soil

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ABSTRACT

The integrated systems are management practices that maximize the area use through the integration of crops with forest species. Impacts on the microbial biomass in an integrated crop-livestock-forest (iCLF) system are evaluated. The latter consists of simple, double and triple rows of eucalyptus and soil with soybean/pasture alternation with a native forest for comparison. Soil was collected at a depth of 0-0.05 m during the rainy and dry periods (March and October/2010). Evaluated microbiological indexes were biomass carbon, basal respiration, metabolic and microbial quotients, activity of acid and alkaline phosphatase enzymes and β-glucosidase. General means of biomass microbial carbon and microbial quotients were higher during the dry period, whereas variables basal respiration and metabolic quotients were higher during the rainy period, and in both cases activities in iCLF soil with single eucalyptus rows. Enzyme activities were more stable in integrated crop-livestock-forest soil between the two periods and slightly higher than the other systems. As a rule, iCLF systems had a positive impact on the microbiology of soil.

Key words: enzyme activity, integrated systems, microbial biomass carbon, soil microorganisms

Impactos da integração lavoura-pecuária-floresta sobre indicadores microbiológicos do solo

RESUMO

Os sistemas de integração são práticas de manejo que buscam maximizar a utilização da área, integrando grandes culturas com espécies florestais. O objetivo deste trabalho foi avaliar os impactos na biomassa microbiana em um sistema de integração Lavoura-Pecuária-Floresta (iLPF) com linhas simples, duplas e triplas de eucalipto e, para comparação, um solo sob rotação soja/pastagem e sob mata nativa. O solo foi coletado na profundidade de 0-0,05 m, nos períodos de chuva e seca (março e outubro/2010). Os indicadores microbiológicos avaliados foram o carbono da biomassa, respiração basal, quociente metabólico e microbiano, atividade das enzimas fosfatase ácida e alcalina e β-glicosidase. A média geral do carbono da biomassa microbiana e o quociente microbiano foram superiores no período de seca em relação à chuva enquanto as variáveis respiração basal e quociente metabólico foram superiores no período de chuva; em ambos os casos as atividades sob solo de iLPF linha simples de eucalipto. No solo sob iLPF as atividades enzimáticas se mantiveram mais estáveis entre os períodos e ligeiramente superiores aos demais sistemas; no geral, os sistemas de iLPF apresentaram impactos positivos na microbiota do solo.

Palavras-chave: atividade enzimática, sistemas integrados, carbono da biomassa microbiana, microrganismos do solo

Introduction

During the 1980s and 1990s the annual grain crops expanded the Brazilian savanna's agricultural frontier and intensified several production factors, especially soil resources, investments in mechanization and techniques based on high energy consumption with fertilizers and insecticides. At the same time, monocultures, which raised production costs and degraded the environment, destabilized most of the agricultural exploitations (Kluthcouski et al., 2003).

Differential soil use and management systems have been proposed and implemented in the Brazilian savanna thus degraded areas could be recovered and productivity maintained. Integrated crop-livestock-forest (iCLF) system, or rather, the agriculture-forest practice of soil use coupled with agricultural and livestock production, should be highlighted. The system provided environmental benefits in addition to economic and social advantages for producers. Management conservationist systems of soil, such as no-tillage and integration systems, are alternatives for the economic and environmental sustainability of the agroecosystem (Conte et al., 2003; Matsuoka et al., 2003; Carneiro et al., 2008).

Pastures in the iCLF system leaves great quantities of straw and roots in the soil with an increase in organic matter. The latter is highly important for the improvement of the physical and chemical structure and a relevant source of nutrients for soil organisms (Gonçalves et al., 1999).

Soil is a dynamic system where physical, chemical and biological factors continually interact. Enzyme-mediated microbial transformations and the different chemical reactions of soil may be altered according to the types of management adopted (Tabatabai, 1994; Paul & Clark, 1996; Makoi & Ndakidemi, 2008).

The iCLF system may be an efficient alternative for the improvement of the chemical properties of soil since it favors soil stability with the addition of organic matter and provides favorable conditions for microbial activities (Vinhal-Freitas et al., 2010).

Current assay evaluates the alterations in the soil microbial biomass by microbial biomass carbon, basal respiration, metabolic and microbial quotients, acid and alkaline phosphatase, and β-glucosidase in an Oxisol under an integrated crop-livestock-forest system with three designs: simple, double and triple rows of eucalyptus; soil with soybean/pasture rotation; and soil with native forest.

Material and Methods

The assay was performed with soil samples collected at the Technological Reference Unit (TRU) of Embrapa Arroz e Feijão (S 10º33'29"and W 55º57'11"), in Nova Canaã do Norte, northern Mato Grosso, Brazil. Soil was classified as Oxisol, according to Seplan-MT (2012). Soils with soybean/pasture rotation and from native forests were also collected. Table 1 shows a detailed description of the collection sites.

All areas under analysis were contiguous and the iCLF system was established in a degraded pastureland in 2009, whereas the soybean/pasture rotation area was introduced in

Table 1. Description of the sampled areas in Nova Canaã do Norte city, MT, Brazil

Areas	Description
iCLF 1 = iCLF simple row of eucalyptus	Enclosure with five hectares (200 x 250 m) and simple rows of eucalyptus (*Eucalyptus urograndis*), 250 m, spacing of 20 m between the eucalyptus rows and a 2 m distance between the plants. Soybean (*Glycine max*) and maize (*Zea mays*) are cultivated in the summer; *Brachiaria ruziziensis* is cultivated in the winter.
iCLF 2 = iCLF double row of eucalyptus	Enclosure with five hectares (200 x 250 m) and double rows of eucalyptus (*Eucalyptus urograndis*), 250 m, spacing of 20 m between the eucalyptus rows, with a 3 m distance between the rows and a 2 m distance between the plants. Soybean (*Glycine max*) and maize (*Zea mays*) are cultivated in the summer; *Brachiaria ruziziensis* is cultivated in the winter.
iCLF 3 = iCLF triple row of eucalyptus	Enclosure with five hectares (200 x 250 m) and triple rows of eucalyptus (*Eucalyptus urograndis*), 250 m, spacing of 20 m between the eucalyptus rows, with a 3 m distance between the rows and 2 m distance between the plants; 50 % of the enclosure has forest and grass in the sub-forest and 50 % with soybean (*Glycine max*) and maize (*Zea mays*) cultivated in the summer; *Brachiaria ruziziensis* cultivated in the winter.
Rotation soybean/pasture	Five hectare with soybean (*Glycine max*) in the harvest; maize (*Zea mays*) in the intermediate harvest and *Brachiaria ruziziensis* between the harvests, in non-tillage system.
Native forest	Five hectares with native forest character of the savanna region.

2006. The native forest belongs to the savanna preservation area.

According to Köppen's classification, the climate of region is Aw, with mean annual minimum and maximum temperatures 4 and 40 ºC, respectively, and mean annual rainfall of 2.500 mm.

Deformed samples of soil were collected at a depth of 0-0.05 m, with eight samples comprising two sub-samples in each area. Collections were undertaken by an earth auger, during the rainy and dry periods, in March and October/2010, respectively. Collected soil was stored in previously identified plastic bags and placed in Styrofoam boxes filled with ice. Sampled material was sent to the Soil Microbiology Laboratory, sieved in 2 mm mesh and stored at ± 4 °C prior to analysis.

The fumigation-incubation method by Jenkinson & Powlson (1976) was used to determine microbial biomass carbon (MBC). Each sample was divided into four sub-samples. MBC rates were obtained from the difference between titer rates of CO_2 amount released from fumigated and non-fumigated samples. Calculations were carried out according to Alef & Nannipieri (1995), by correction factor 0.45.

Microbial activity determined by basal respiration (BR) was obtained by CO_2 evolution caught by NaOH from non-fumigated soil samples, incubated for 5 days in sealed flasks (Alef & Nannipieri, 1995). The metabolic quotient (qCO_2) was obtained from the relation between basal respiration and the microbial biomass carbon. The microbial quotient (qMic)

was obtained from the relation between the microbial biomass carbon and the organic matter of soil (Anderson & Domsch, 1993).

Enzyme activities, acid and alkaline phosphatase, and β-glucosidase were evaluated according the methodology by Tabatabai (1994).

Data underwent Kruskal-Wallis's non-parametric multiple comparison test since the experiment comprised areas with laterally disposed treatments with no randomization. Statistic package Assistat 7.6 beta 2012 (Silva & Azevedo, 2002) was employed for calculation.

Results and Discussion

Rates of microbial biomass carbon (MBC) varied among the areas and evaluation periods. Areas with soybean/pasture rotation and native forest had the highest MBC rates during the rainy season, whereas native forest had high rates during the dry period. In the rainy season, iCLF system areas did not differ statistically but differed from the other evaluated areas, even though by lower rates (Table 2).

The native forest evaluated is compounded for Brazilian savanna vegetation with high diversity of vegetal species. This implies, as mentioned by D'Andréa et al. (2002), in a favorable factor for the survival and growth of the different groups of soil microorganisms. Due the absence of perturbations coming from anthropogenic actions, the rates of microbial biomass in these soils are higher, indicating a greater equilibrium on soil microbiota in the ecosystem.

It is important to observe that during the rainy period, the area under rotation of soybean/pasture did not differ statistically from native forest related to MBC and metabolic quotient (qCO_2). The treatment showed the smallest tax of qCO_2 and highest of MBC, representing in this way a more stable microbial biomass (Anderson & Domsch, 1985). This suggests that the no-tillage system in the area favors the microbial biomass, leading it to an equilibrium, as already mentioned in this paper.

The no-tillage condition leads to a stability in soil microbial population, which indicates that the iCLF over the years can have this stable condition, like the rotation treatment shows, once this one was established longer on the site. Besides the benefits that the variety of crop residues will bring to the soil microbiota, which tend to be bigger and more diverse.

No statistical differences were found with regard to basal respiration (BR) in the different areas and periods. However, BR rates during the rainy season were always higher than those during the dry periods. This fact may be perceived from the periods general means (Table 2). Although without any statistical difference, absolute rates indicate that microorganisms in the iCLF system areas had a higher respiratory activity but were not proportional to MBC rates.

When compared to native forest, BR increase in iCLF systems was due to a greater deposit of different crop waste by iCLF. Yan et al. (2009) showed that BR represented the metabolization capacity of organic matter of the soil by the microbiology and its behavior in decomposition. The latter may have been caused either by the decomposition of organic matter from the soil of a great substrate reserve or by a small reserve caused by soil mobilization, for instance.

According to Anderson & Domsch (1985) the BR on soil reflects the general activity or the energy expenditure of the microbial biomass, which explains why the iCLF systems obtained the bigger tax. Once these areas are freshly implanted and the microbial biomass did not establish its equilibrium on soil yet.

Lowest absolute rates for BR were found in native forest in both periods. According to Yan et al. (2009), the microbial respiration per microbial biomass unit decreased in more stable systems. However, alterations occurred according to the succession gradient of forest, especially throughout its natural succession.

The present study does not build the comparison with an area of conventional farming system, however the study realized by D'Andréa et al. (2002) should be cited, because it corroborated to the presuppose already cited here.

These authors evaluated the microbiological soil attributes (MBC, BR and qCO_2) comparing three no-tillage systems of annual crops to a conventional farming system. They observed that the results of the areas under no-tillage, even though recently implanted, by the time the study was made, had the most stable microbial biomass than in the conventional farming system. According to authors it can be attributed to the fact that in the conventional farming system occurs at high soil tillage and low diversification of crop residues (only soybean/ maize over 15 years), contributing to reduce the quantity and the heterogeneity of soil microorganisms.

As well found by Carneiro et al. (2008), the results reached in this study show that the qCO_2 was bigger in

Table 2. Microbial biomass carbon (MBC), basal respiration (BR), metabolic quotient (qCO_2) and microbial quotient (qMic) of an Oxisol for five areas in the 0 - 0.05 m[1]

Areas	MBC		BR		qCO_2		qMic	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
	(μg C g soil[-1])		(μg CO_2 g soil[-1])		(μg CO_2 μg Cmic day[-1])		(%)	
iCLF 1	183.53 bB	1381.92 bA	1263.16 aA	595.14 aB	7.23 aA	0.56 aB	0.18 bB	1.38 bA
iCLF 2	294.20 bB	623.48 cA	1280.16 aA	473.68 aB	5.90 aA	0.62 aB	0.29 bB	0.62 cA
iCLF 3	261.81 bA	221.32 eA	1202.43 aA	792.71 aB	5.66 aA	4.32 aA	0.26 bA	0.22 eA
Rotation soybean/pasture	788.12 aA	516.42 dB	1190.28 aA	680.16 aB	1.58 bA	0.15 aB	0.79 aA	0.51 dB
Native forest	723.35 aB	2215.92 aA	1180.57 aA	389.88 aB	1.66 bA	0.18 aB	0.72 aB	2.21 aA
Averages	450.20	991.81	1223.32	586.31	4.41	1.17	0.45	0.99
Standard deviation	141.74	239.40	76.95	239.40	1.74	0.67	0.14	0.26
VC %	55.39		35.95		70.65		55.39	

[1]Means followed by the same lower case and upper case letters in the column and in the line do not differ by Kruskal-Wallis test (p <0.05)

stressful environmental conditions, which is the case of the areas under iCLF system. Due the short time of system implantation the soil still does not have a stable condition, like the native forest does. Thus the microbial biomass spends more carbon to its maintenance, reflecting to increase this parameter.

Further, qMic had the highest percentages in soybean/ pasture rotation areas and native forest during the rainy period and in native forest areas during the dry period. This fact shows a highly active organic matter subject to transformations. In fact, the relationship qMic:TOC (total organic carbon) causing qMic obtained rates, indicates the availability of organic matter for microorganisms (Sampaio et al., 2008).

Averages of qMic were 0.45% and 0.99%, respectively during the rainy and dry seasons. These results were lower than those by Matias et al. (2009), with qMic rates equal to 8.02% and 1.0%, very much higher than those in current analysis. Difference in rates may be due to the adoption time in the management system in which the above mentioned researchers evaluated areas with higher adoption time in the management system. In other words, for the time period, the greater is the organic matter available.

Moreover, the ratio qMic:TOC is affected by several factors, such as the stabilizing degree of organic carbon and the history of soil management. The author indicated that to find out whether the ratio qMic:TOC was in equilibrium or in the degradation/recuperation phase, the basic rate for each situation had to be established, with seemingly scanty surpassing for different conditions (Moreira & Malavolta, 2004). Great evaluation time was required to state correctly the sustainability of the systems when compared to native forest.

For enzymatic activities, no behavior standards existed between the areas and the periods evaluated. Mean rates were always higher during the rainy season than those in the dry period (Table 3).

Table 3 shows the variation in the different conditions and periods under analysis. Significant differences between managements were reported for all systems during the rainy and dry seasons. The iCLF 2 had higher rates of β-glucosidase activity in the two periods. They were significantly higher than rates for the other systems.

Current assay reported high alkaline phosphatase rates to the detriment of acid phosphatase ones (Table 3). Soils analyzed featured pH 5.3. They were classified as acid, characteristic of the savanna soil. Several authors reported that phosphatase

activities were directly related to the pH of soil where it was expected that acid phosphatase would be higher in acid soils and alkaline in soils with a high pH (Matsuoka et al., 2003). Current research failed to corroborate the above.

Few research works are extant on savanna soils that quantify alkaline phosphatase. Most soils of the region are acid and it is believed that alkaline enzymatic activity is not relevant. However, this does not always take place and it may be stated that a kind of alkaline activity occurs in the soil. Matsuoka et al. (2003) corroborated the above information. In fact, they tested several types of soil under different managements in the savanna and found acid phosphatase activity. Absolute rates in the iCLF2 system were always higher for all enzymes and in all periods, except low acid phosphatase rates during the dry period.

Chaer & Tótola (2007) assessed the impact of organic residues management during the eucalyptus planting reform on the activity of the same enzymes evaluated in their research. They used a native forest area for comparison and perceived that it comprised a higher enzyme activity.

Vinhal-Freitas et al. (2010) found fewer activities for alkaline phosphatase when compared to acid phosphatase in soils with pH 4 in which organic matter had been added. This fact may have caused a decrease in activity.

Several studies showed that the activity of acid phosphatase enzymes and β-glucosidase were influenced by soil management. In fact, high increase in the conventional management system occurred to the detriment of conservationist systems such as no-tillage (Vinhal-Freitas et al., 2010). Current research did not corroborate with above results.

In their investigations on managed and non-managed soil in fields in southern Brazil, Conte et al. (2003) reported that soils with forests had a higher acid phosphatase activity, 1.504 µg p-nitrophenol h^{-1} g^{-1} of soil. This paper demonstrated the importance of the enzyme in P supply to plants in natural systems. Consequently, the native forest system and all iCLF systems in current assay should have higher rates than those actually found.

β-glucosidase activity was directly related to C rates in the soil, or rather, rates varied according to the amount and composition of the organic matter added to the soil. This was due to the fact that the enzyme acted in the final stage of the cellulose decomposition process (Tabatabai, 1994; Paul & Clark, 1996).

Table 3. Alkaline phosphatase, acid phosphatase and β-glucosidase activity of an oxisol, in different areas, depth of 0-0.05m [1]

Areas	Alkaline phosphatase		Acid phosphatase		β-glucosidase	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
	(µg p-nitrofenol h^{-1} g dry soil^{-1})					
iCLF 1	576.37 cB	665.58 aA	440.50 cB	473.08 aA	411.57 eB	572.49 cA
iCLF 2	1253.99 aA	813.57 aA	902.14 aA	382.77 bcB	780.70 aA	634.40 aB
iCLF 3	628.92 bA	746.12 aB	462.52 cA	393.87 bB	579. 83 cA	610.02 bA
Rotation	579.97 cA	728.36 aB	670.65 bA	499.13 aB	572.20 dA	499.53 dB
Native forest	663.31 bA	633.69 aA	632.32 bA	370.55 cB	743.80 bA	417.09 eB
Averages	740.51	717.46	621.63	423.88	627.70	546.71
Standard deviation	45.07	46.80	40.81	23.61	28.22	32.66
VC %	9.61		9.01		8.43	

[1]Means followed by the same lower case and upper case letter in the column and in the line do not differ by Kruskal-Wallis test (p <0.05)

Chaer & Tótola (2007) evaluated the enzyme activity in soil with eucalyptus trees and reported lower β-glucosidase rates than those found in current research. Highest concentrations could be found in areas with natural vegetation, which was not corroborated by results in current assay for most systems (Table 3). However, soil with iCLF2 had higher rates than others in both periods.

Moreover, β-glucosidase is a quality index and thus highly sensitive to changes in soil use, addition of organic matter, soil stability and pH changes. The above mentioned factor may be employed as an index of ecological modifications since, in the wake of modification conditions, the enzyme's activity might acidify the soil (Makoi & Ndakidemi, 2008).

Moreover, enzymes are important for soils since most biochemical transformations in this environment are dependent on or related to the enzymes. The evaluation of their activities may be useful to indicate whether a type of soil adequately performs the processes closely linked to its quality (Reis Junior & Mendes, 2009).

Permanent vegetal covering in natural ecosystems provided continuous protection to soil and added high amounts of nutrients mainly through wastes. Its effects on the microbial community may interact with the effects caused by water and heat fluctuations throughout the year and thus influence these populations in a higher or lesser degree (Tsai et al., 1992).

The iCLF system reported the same soil-plant relationship provided by natural ecosystems since integration with forest species was constant and permanent in the area. This fact is of great benefit to the microbial activity of soil.

Habitats, in fact, presented great differences in their biological characteristics within the different areas evaluated. They also depend on the abiotic factor of the site, such as the nutrient content in soil, rainfall, type of management and mainly the period when the system was introduced.

Conclusion

The iCLF system had been established for only one year at the time of collection and in an area with a history of degradation. Many years have to pass so that a system might stabilize and increase its organic matter and nutrient rates.

iCLF systems generally produce a positive impact for all microbiological features.

Soybean/maize rotation and native forest fail to provide any positive impacts on the microbiological features of soil.

Collection period (rainy or dry) affect the microbiological qualities of soil and the adoption time of system was required to evaluate the microbiological evaluations in the iCLF system.

Acknowledgements

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