UNIVERSIDADE ESTADUAL DE MATO GROSSO DO SUL
UNIDADE UNIVERSITÁRIA DE DOURADOS
PÓS-GRADUAÇÃO EM RECURSOS NATURAIS

SOIL QUALITY INDICATORS IN TROPICAL AGRICULTURAL PRODUCTION SYSTEMS

Irzo Isaac Rosa Portilho

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PÁGINA DE APROVAÇÃO

“A rich human being seeks out gold in society, a wise human being pours gold into the soles of his being”.

Augusto Cury

I dedicate this work to God and my family.

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To God for everything He has given me.

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SUMMARY

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ABSTRACT

The intensive use of tropical soils for food production has as consequence the modification of the soil environment from management schemes which may improve or reduce its quality. The adoption of more diversified management systems such as crop-livestock (CL) integration with crop rotation and the use of mechanized harvesting of sugarcane (SC) necessitates increased knowledge about the functional diversity within the soil in these agricultural and livestock production systems. Biological processes are related to physico-chemical characteristics, management practices and soil type, texture, temperature, humidity and plant residue diversity. The present work had as general objective to evaluate the influence of different production systems and their soil management practices, established in two agricultural and livestock farms, on the invertebrate fauna of the soil and microbial structure. This thesis is composed of four chapters with the following objectives: (i) general considerations; (ii) evaluate the terrestrial invertebrate community under management systems involving agricultural and livestock activities and the relationships of environmental variables of these systems with the soil invertebrate faunal communities; (iii) evaluate the effect of different systems of agricultural and livestock management on the microbial community abundance and the composition of denitrifier *nirK* communities; and (iv) final considerations. The invertebrate fauna was evaluated through the diversity of functional groups, density and richness of organisms, and environmental variables of the soil. Soil functional biology was evaluated using molecular approaches (DGGE and real time-qPCR) and soil physical-chemical properties. The crop-livestock integration system and the mechanized harvesting of sugarcane can fit into a planned biodiversity program, where the plant residue cover enriches the soil and assists in the development of soybean, corn and sugarcane intercrop systems. In addition, as an indirect function, these management systems provide greater diversity of functional groups and establishment of invertebrate fauna considered rare or soil specialists, as well as colonies of ants characterizing the forest fragments. Richness and diversity of soil *nirK* community is reduced with the transition from a forest to the agricultural and livestock production in tropical soil. However, the management systems under integrated crop-livestock farming and cultivation of sugarcane with mechanized harvesting maintain a relatively diverse community, possibly with conditions promoting balance in the N cycle. Maintaining fertility associated with better management practices stimulates the soil *nirK* community composition. In addition, management systems with the second year of crop rotation after pasture (CL-c), the third year of crop rotation after pasture (CL-d), mechanized harvesting of sugarcane (SC) (farm A) and (B farm) the second year of the crop-livestock integration system (CL-2) and the third year of the crop-livestock integration system (CL-3) deserve special attention because they have maintained microbial communities and greater fertility from soil. On the other hand, fields under pasture (CL-a) and continuous pasture (CP) reduced the diversity of the *nirK* community.

KEYWORDS: Soil biology, crop-livestock integration, *nirK* gene, soil invertebrate biota.

RESUMO

O uso intensivo de solos tropicais para a produção de alimentos tem como consequência a modificação do ambiente do solo em sistemas de manejos que podem melhorar ou reduzir sua qualidade. A partir da adoção de sistemas de manejo mais diversificados, como a integração lavoura-pecuária (CL), com a rotação de culturas e o uso de colheita mecanizada de cana-de-açúcar (SC), altera a dinâmica da diversidade funcional do solo, requerendo maior conhecimento sobre alguns processos que ocorrem nesses sistemas agropecuários. Os processos biológicos estão relacionados às características físico-químicas, práticas de manejo e tipo de solo, textura, temperatura, umidade e diversidade de resíduos vegetais. O presente trabalho teve como objetivo geral avaliar a influência de diferentes sistemas de produção e suas práticas de manejo de solo, estabelecido em duas fazendas, na fauna invertebrada de solo e estrutura microbiana. Esta tese é composta por quatro capítulos com os seguintes objetivos: (i) considerações gerais; (ii) avaliar a comunidade de invertebrados do solo sob sistemas de manejo envolvendo atividades agropecuárias e a relevância das variáveis ambientais desses sistemas na estrutura de invertebrados do solo; (iii) avaliar o efeito de diferentes sistemas de manejo agropecuário sobre a abundância de comunidades microbianas e a composição das comunidades desnitrificadoras *nirK*; (iv) considerações finais. Para a fauna invertebrada do solo foi observado a diversidade de grupos funcionais, densidade e riqueza dos organismos, além das variações ambientais. A biologia molecular do solo foi avaliada por meio de comunidades microbianas funcionais (DGGE e qPCR em tempo real) e das propriedades físico-químicas do solo. O sistema de integração lavoura-pecuária e a colheita mecanizada de cana-de-açúcar podem enquadrar-se em um programa de biodiversidade planejado, onde o sistema de cobertura enriquece o solo e auxilia no desenvolvimento das culturas de soja, milho e cana-de-açúcar. Além disso, com uma função indireta, esta cobertura proporciona maior diversidade de grupos funcionais e estabelecimento de fauna invertebrada considerada rara ou especialistas em solo, bem como colônias de formigas originárias dos ambientes ecossistêmicos em fragmentos florestais. A riqueza e a diversidade da comunidade *nirk* é reduzida com a transição de floresta para a produção agrícola e pecuária em solo tropical. No entanto, o sistema de gestão sob cultivo integrado-pecuária e cultivo de cana-de-açúcar com colheita mecanizada mantêm uma comunidade relativamente diversa, possivelmente com condições que promovem o equilíbrio do ciclo do nitrogênio (N). A manutenção da fertilidade associada a melhores práticas de manejo estimula a composição da comunidade *nirK* do solo. Além disso, os sistemas de manejo com o segundo ano de rotação de culturas após a pastagem (CL-c), o terceiro ano de rotação de culturas após a pastagem (CL-d), colheita mecanizada da cultura de cana-de-açúcar (SC) (fazenda A) e o (fazenda B) segundo ano do sistema integração lavoura-pecuária (CL-2) e o terceiro ano do sistema d integração lavoura-pecuária (CL-3) merecem atenção especial, pois mantiveram comunidades microbianas e maior fertilidade do solo. Por outro lado, os campos sob pastagem (CL-a) e pastagem contínua (CP) reduziu a diversidade da comunidade *nirK*.

PALAVRAS-CHAVE: biologia do solo, integração lavoura-pecuária, gene *nirK*, biota invertebrada do solo.

CHAPTER 1 - GENERAL CONSIDERATIONS

1.1 General Introduction

The constant challenge of agriculture is to achieve high levels of productivity within a context of sustainability of natural resources (soil and water). Within this dimension of production the use of conservationist systems such as crop-livestock integration (CL) and the mechanized harvest of sugarcane (SC) are inserted. The rotation of fields with *Brachiaria* pastures, cultivated with soybean or corn, in CL and the largest volume of vegetal residues in the fields in SC, have presented significant results regarding productivity increase and stability of production (LEAL et al., 2013, FRANZLUEBBERS et al., 2014). These management scenarios have aroused the interests of researchers in the search for functional understanding of the soil complex, with the goal of maximizing sustainability and crop productivity (MORALES et al., 2010; BARTZ et al., 2014).

In general, the soil complex is described as an interacting system, where the flow of matter and energy is controlled by its internal processes and, above all, by its relations with the external environment (KANTER et al., 2016). In this environment, the biological dynamics are related to the source of energy and matter, together with residues of plants, animals and root exudates (DORAN & PARKIN, 1994; BROWN et al., 2007). The different residues deposited in the soil are gradually transformed into organic matter (OM) mainly by the action of the soil organisms, and OM can interact with the mineral fraction in the process of soil aggregation (ROSCOE et al., 2006; RESENDE et al., 2013).

Soil management systems with permanent or rotational pasture with no-tillage systems favor the formation of larger stable aggregates in relation to systems only with crops or with rotating crops with pastures in cycles greater than three years (SALTON et al., 2008). The necessary energy for the formation of these larger aggregates comes mainly from the growth of fungi and roots and from the mechanical action of soil invertebrate fauna such as termites, ants and earthworms, through feeding, excavation, formation of tunnels, and the presence of organic matter (LAVELLE & SPAIN, 2001).

In addition, microorganisms (denitrifying bacteria) may also contribute to the nitrogen cycle process in the soil, through a diverse subset of facultative anaerobic bacteria that participate in various reductions in the nitrate (NO^-_3) pathway for nitrite (NO^-_2) and possibly for molecular nitrogen (N_2) (KOCH et al., 2015). Nitrogen retention in soil and cycling are important for plant growth and development (PHILIPPOT & HALLIN, 2006).

This dynamic system results in the degree of soil management and the complexity of its relationships, which results in a level of soil quality, a capacity for the system to withstand disturbances, infiltration and storage of water, aeration, and level of soil structure or compaction. Soils facilitating root growth with an environment of chemical, physical and biological balance consequently tend to have better conditions for the development of plants (BAYER, 2004; CHAER et al., 2009).

In order to better understand soil processes, the use of soil biological indicators, such as functional evaluation of microorganisms or the invertebrate fauna community, has been proposed (ROUSSEAU et al., 2013; LONG et al., 2014). We note the importance of greater efforts in research on the processes involved for the conservation of production systems and conserving the natural resources, such as the maintenance of soil quality (TILMAN et al., 2011). Soil sustainability in production systems depends on the dynamics between soil-plant systems (VEZZANI & MIELNICZUK, 2009), which may reflect greater economic viability (productivity and quality of the generated products).

In order to understand the richness, density, diversity and community composition of functional bacteria populations and invertebrate fauna in tropical soils, two agricultural and livestock farms with different management systems in Brazil were studied. The objective of CHAPTER 2 was to evaluate the community of terrestrial invertebrates under management systems involving agricultural activities and the relevance of the environmental variables of these systems on the invertebrate community composition. CHAPTER 3 has the objective to evaluate the effect of different agricultural management systems on the abundance of bacterial communities and the composition of the denitrifier communities.

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CHAPTER 2 - SOIL INVERTEBRATE BIOTA INDICATE THE BENEFITS OF INCORPORATING CROP-LIVESTOCK SYSTEM INTO AGRICULTURAL MANAGEMENT

SOIL INVERTEBRATE BIOTA INDICATE THE BENEFITS OF INCORPORATING CROP-LIVESTOCK SYSTEM INTO AGRICULTURAL MANAGEMENT

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ABSTRACT

Soil invertebrate fauna contribute to decomposition and nutrient cycling and are useful ecological indicators of soil quality. The objective of this study was to evaluate the terrestrial invertebrate community under management systems involving agricultural and livestock activities and the relationship of environmental variables in the structure of soil invertebrate fauna. The study was conducted in the southern region of Mato Grosso do Sul State, Brazil, in January 2014 in Hapludox soil. Two farms utilizing agricultural and livestock activities were evaluated. Farm A had fields with the following: third year pasture (CL-a), first year of crop rotation after grazing (CL-b), second year of crop rotation after grazing (CL-c), third year of crop rotation after grazing (CL-d) and sugarcane with mechanized harvesting (SC). Farm B had fields in the second year of crop-livestock integration cycle (CL-2), third year of crop-livestock integration cycle (CL-3), no-tillage (NT), sugarcane with mechanical harvesting (SC-b), and continuous pasture (CP). In both farms (A and B), a forest fragment (F and F-b, respectively) was included in the study as a reference soil of the region. The crop-livestock integration system and mechanized harvesting of sugarcane provide greater diversity of functional groups and establishment of invertebrate fauna considered rare or soil specialists. The effect of soil compaction, decreased nutrient content and less volume and diversity of plant residues may be factors related to the decline of soil functional diversity.

KEYWORDS: Ants, agroecosystems, bio-indicators, crop rotation, functional groups.

BIOTA INVERTEBRADA DO SOLO INDICA BENEFÍCIOS DA INCORPORAÇÃO DO SISTEMA INTEGRAÇÃO LAVOURA-PECUÁRIA EM MANEJOS AGRÍCOLAS

RESUMO

A fauna de invertebrados do solo contribui para a decomposição e ciclagem de nutrientes, como também é indicador ecológico útil da qualidade do solo. O objetivo deste estudo foi avaliar a comunidade de invertebrados do solo sob sistemas de manejo envolvendo atividades agropecuárias e a relevância de variáveis ambientais desses sistemas na estrutura de fauna invertebrada do solo. O estudo foi realizado na região sul do Estado de Mato Grosso do Sul, Brasil, em janeiro de 2014, em Latossolo Vermelho distroférrico de textura muito argilosa. Foram avaliados dois cenários envolvendo atividades agropecuárias. Fazenda A: pastagem sob terceiro ano do ciclo integração lavoura-pecuária (CL-a), primeiro ano de rotação de culturas após a pastagem (CL-b), segundo ano de rotação de culturas após a pastagem (CL-c), terceiro ano de rotação de culturas após a pastagem (CL-d) e cana-de-açúcar com colheita mecanizada (SC). A fazenda B: campo com segundo ano do sistema integração lavoura-pecuária (CL-2), terceiro ano do sistema integração lavoura-pecuária (CL-3), plantio direto (NT), cana-de-açúcar com colheita mecanizada (SC) e pastagem contínua (CP). Em ambas os cenários agrícolas e pecuários (A e B), um fragmento florestal (F e F-b, respectivamente) foi incluído no estudo como um solo de referência da região. O sistema de integração lavoura-pecuária e a colheita mecanizada de cana-de-açúcar proporcionam maior diversidade de grupos funcionais e estabelecimento de fauna invertebrada considerada rara ou especialistas em solo. A compactação do solo, menor teor de nutrientes e menor volume e diversidade de resíduos vegetais podem ser fatores relacionados ao declínio da diversidade funcional do solo.

PALAVRAS-CHAVE: formigas, bioindicador, agroecossistemas, rotação de culturas, grupos funcionais.

2.1 Introduction

The development of the agricultural and livestock sector for food production has reduced production costs by increasing crop yields (FRANZLUEBBERS et al., 2014). However, land use for the production of grains and meat has fragmented natural ecosystems and changed the vegetative composition from native species, which are important in the framework for conservation of flora and fauna (ZIMMERER, 2010). In

Brazil, as in other countries, intensification of the production process has negatively affected the biodiversity of organisms (ROGER-ESTRADE et al., 2010). Furthermore, these changes have also been shown in soil featuring an artificial and unstable environment that requires the use of fertilizers, correctives, pesticides and growth regulators (KASSAM et al., 2015).

The type of management system adopted is important because different agroecosystems have presented different richness and diversity of organisms (ROUSSEAU et al., 2010; BARTZ et al., 2014). Considering conservation management, we can highlight the integrated crop-livestock (CL) system of crop rotation into *Brachiaria* pastures cultivated with soybean or corn (SALTON et al., 2014). The sundry residues deposited on the soil surface gradually increase soil organic matter (SOM), facilitated through the action of soil organisms (RESENDE et al., 2013), and provide nitrogen, improve nutrient availability, alleviate compaction, and conserve soil moisture. Moreover, the formation of aggregates may result from crop rotation (SALTON et al., 2008), and the mechanical action of soil invertebrates such as termites, ants and earthworms (BROWN et al., 2007). Furthermore, these systems can contribute to soil quality and increase the capacity of the soil system to withstand disturbances (TURMEL et al., 2015).

For a better understanding of soil processes in systems involving agricultural and livestock activities, the use of indicators of soil quality has been proposed (DORAN & PARKIN,1994; PAOLETTI, 2012; SANABRIA et al., 2014) as a strategy to monitor the environment and assess the quality of processes and products for potential use in agriculture (SPIEGEL et al., 2015). This model allows the researcher to infer the environmental quality or effect of an agent on parameters (KANTER et al. 2016). Among potential indicators, invertebrate soil fauna feature a wide variety of forms, behavior, size, and foraging strategies (food and excavation) (ROUSSEAU et al., 2013).

These organisms play a key role in the functioning of the ecosystem because they occupy different trophic levels in the soil food chain and can modify their environment by participating in biogeochemical cycles and contribute to the soil structural development (COLEMAN, 2004). Changes in invertebrate soil fauna can be evaluated for quantitative aspects (density and richness) and diversity of functional groups of soil organisms; both have been used as potential bio-indicators of soil quality, providing a sense of their current status and changes induced by biotic and abiotic factors over time (GERLACH et al., 2013; ROUSSEAU et al., 2013). Thus, diversity and density of soil invertebrate fauna can serve

as an indicator of soil quality in determining the biological conditions of the soil production systems (VAN LEEUWEN et al., 2015). The interactions that these biological communities have with the chemical and physical processes in soils are essential to ensure the maintenance of soil quality for agricultural and livestock activities (LAVELLE et al., 2006).

However, there is little knowledge of invertebrate soil fauna as related to organic matter content and physical and chemical parameters with changes in the environment from the management activity conducted across different tropical agricultural production systems. While there is more known about individual properties under different management systems, there is a need to assess the interaction of indicators (DECAËNS, 2010), especially in soils occupied with agricultural and livestock activities. A better understanding of soil processes, including the flow of energy and recycling of nutrients, can help support the paradigm for sustainable management practices and therefore the maintenance of soil quality (BRIONES, 2014).

Thus, the hypotheses for this study were that: i) soil invertebrate diversity will decline with loss of invertebrate functionality under different agricultural management systems; and ii) changes in the faunal community could be explained by changes in physical and chemical soil properties. This study aimed to assess whether soil biological parameters are promoted by different agriculture systems and to investigate the relationships of soil physical and chemical properties in explaining the loss of soil biodiversity in agriculture management systems.

2.2 Materials and methods

2.2.1 Field sites

Fields from two farms in the southern region of Mato Grosso do Sul State, Brazil were investigated. Soil at both farms is classified as Hapludox according to the Brazilian System of Soil Classification - SiBCS (EMBRAPA, 2013). The climate of the region is classified as Cwa, humid mesothermal with warm summer and dry winter (FIETZ &

FISCH, 2008). To support the discussion and understanding of the results, precipitation and temperatures were recorded throughout the experimental period (Figure 1).

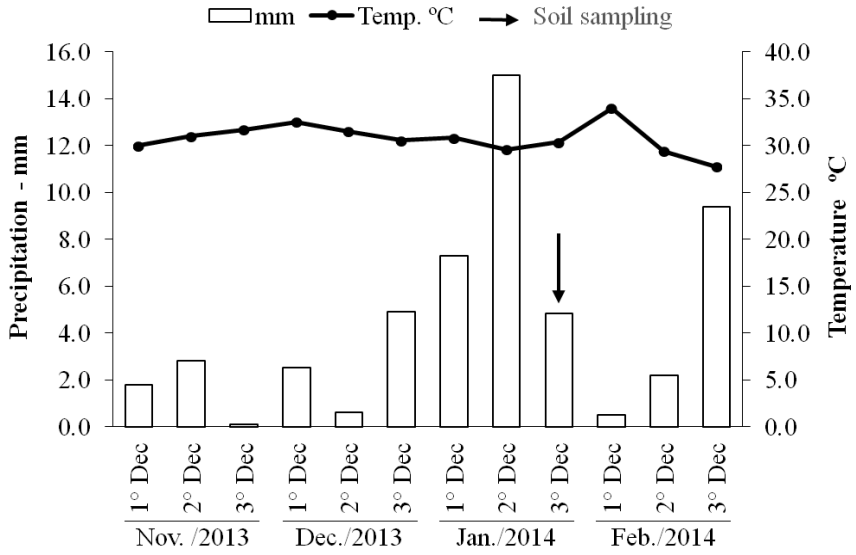

Figure 1. Precipitation and temperatures recorded during soil sampling in the region of Maracaju, Mato Grosso do Sul, Brazil. Centro de Previsão de Tempo e Estudos Climáticos - CEPTEC. Nov./2013: 1° Dec (1 to 10 days), 2° Dec (10 to 20 days), 3° Dec (20 to 30 days); Dec./2013: 1° Dec (1 to 10 days), 2° Dec (11 to 21 days), 3° Dec (21 to 31 days); Jan./2014: 1° Dec (1 to 10 days), 2° Dec (11 to 21 days), 3° Dec (21 to 31 days); Feb./2014: 1° Dec (1 to 10 days), 2° Dec (10 to 18 days), 3° Dec (18 to 28 days). Averages of precipitation and temperature evaluations at approximately every ten days in the month.

In farm A (Figure 2, Table 1), the main management is the integrated crop-livestock (CL), managed in succession with no-tillage between pasture (*Brachiaria brizantha* cv.) and row crops. Two or three years of pasture are followed by three years of soybean in the summer and corn with *Brachiaria ruziziensis* cv. in the winter. At the time of sampling, the field that was in its third year of pasture is denoted CL-a, while the field occupied by the first year of soybean after two years of grazing is named CL-b. The field that was in its second year of soybean is CL-c and the field that was in its third year of soybean is CL-d. Samples from the sugarcane fields continuously cropped for 5 years to variety SP-81-3250 with green harvest are labeled SC. Soil was also collected from a forest fragment (F) as a reference for native soil conditions.

In farm B (Figure 2, Table 1), two additional management strategies were sampled: no-till (NT) production has been utilized since 2009 and there is also continuous pasture (CP) of *Brachiaria brizantha stapf* cv. with cattle grazing in rotation according to the amount of dry matter in pastures (15-20 cm grass height).

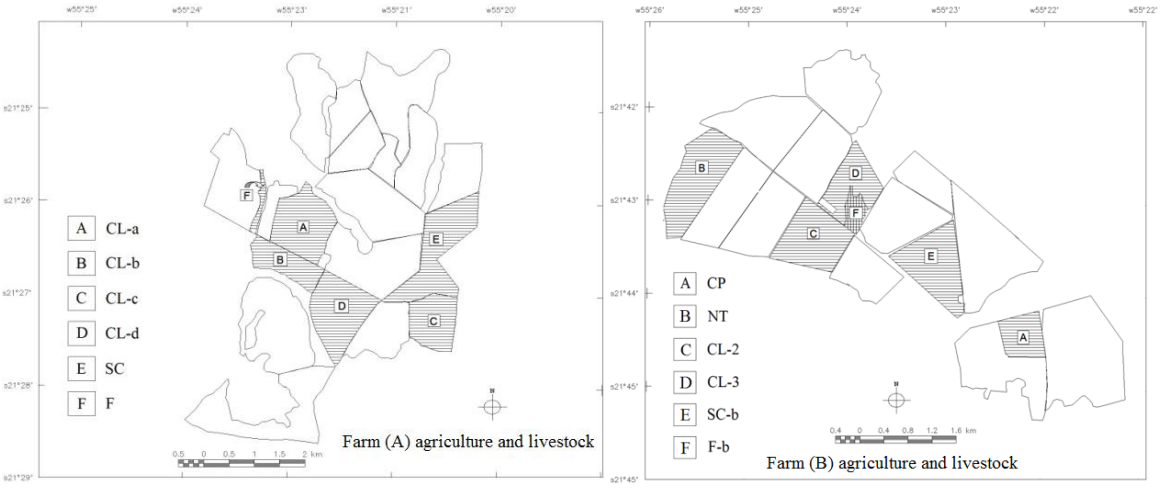

Figure 2. Maps of agriculture and livestock management at two farms (A) and (B) in the region of Maracaju, Mato Grosso do Sul, Brazil. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanized harvesting; CP, continuous pasture; F-b, forest fragment.

Table 1. Rotation succession for each field in farms A and B from winter 2009/2010 through 2013/2014.

Fields (ha)		*DM kg/m^2	09/10	2010	10/11	2011	11/12	2012	12/13	2013	**13/14
			Farm (A) agriculture and livestock								
CL-a	130.5	0.4c	Soyb.	C+*B.*	Soyb.	C+*B.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*
CL-b	70.1	0.7bc	Soyb.	C+*B.*	Soyb.	C+*B.*	*B.ruz.*	*B.ruz.*	*B.ruzi*	*B.ruz.*	Soyb.
CL-c	96.4	0.9ab	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	Soyb.	C+*B.*	Soyb.
CL-d	237.0	1.0ab	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.
SC	26.4	1.3ab	.....Sugarcane, SP 81 3250.....								
F	17.3	1.4a	.....Forest fragment…..….....								
			Farm (B) agriculture and livestock								
CL-2	103.5	0.8bc	Soyb.	Corn	Soyb.	Corn	Soyb.	Corn	Soyb.	*B.ruz.*	Soyb.
CL-3	59.1	1.0b	Soyb.	Corn	Soyb.	Corn	Soyb.	*B.ruz.*	Soyb.	*B.ruz.*	Soyb.
NT	122.4	0.8bc	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.
SC-b	101.2	1.3ab	Soyb.	Corn	.....Sugarcane, SP 80 1842.....						
CP	91.5	0.3c	.....…..*Brachiaria brizantha Stapf* cv.....……								
F-b	25.8	1.7a	..... Forest fragment …..….....								

Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment. Soyb., Soybean; B.ruz., *Brachiaria ruziziensis* cv. Common; C+B., Corn grown in consortium with *Brachiaria ruziziensis* cv. Common. *DM = Crop dry matter, (n=5). **Time of soil sampling. Values with different letters in the column differ significantly by Duncan test ($p<0.05$) (n=5).

The NT system has been in place with the rotation of soybean during the summer and the winter cultivated in corn grown in consortium with *Brachiaria ruziziensis* cv. Since the 2010/2011 season (i.e. fourth year), there has been green harvesting of sugarcane (SP-80 1842) (SC-b), and in 2011/2012 the crop-livestock integration (CL) began (Table 1).

Before the CL system deployment, fields were planted with soybean under no-tillage in summer and corn during the winter. Two fields were evaluated in CL: a field that was in its second year of soybean cycle after the first winter pasture (CL-2), and a field that was in the third year of soybean cycle in summer with pasture in winter (CL-3). Soil of a forest fragment (F-b) was included as a reference of the original soil condition.

In both farms (A and B) systems with pasture Bovine (CL-a and CP) were conducted under of grazing adjusted to 7% (7 kg of material mass dry forage to 100 kg live weight per day).

2.2.2 Sampling of soil invertebrate fauna

The invertebrate fauna was collected in different cropping systems (January 2014) in five equidistant points along the 300 m transect, for a total of 60 sampling points. Invertebrate fauna from the top of the soil were captured in four pitfall traps at each collection point for seven days, totaling 20 traps in each management system (MOLDENKE, 1994). In addition, a square meter of litter fall was collected at the same points of collection for the evaluation of invertebrate fauna from the top of the soil. After the removal of the litter layer, the invertebrate fauna was evaluated in the soil profile, with the collection of soil monoliths (25 x 25 cm wide and 40 cm deep) divided into 0-10, 10-20 and 20-40 cm, method adapted from "Tropical Soil Biology and Fertility" (ANDERSON & INGRAM, 1993). Different collection techniques were adopted in order to obtain more accurate data about the composition of the invertebrate fauna community (DELABIE et al., 2000).

The invertebrate fauna of the soil was extracted manually (traps, leaf litter and soil monoliths), identified and counted at the level of family, groups and immature larvae (TRIPLEHORN & JONNSON, 2010), with individuals separated by developmental stage into adult or immature (larvae) with the help of binocular loupe. The organisms collected were stored in 70% ethanol solution. Within the community of invertebrate soil fauna, the Formicidae family is indicated as a potential indicator because it presents great structural complexity and sensitivity to environmental changes (ANDERSEN, 1997). In this context, the organisms of the Formicidae family were separated and identified at the morphospecies

level in order to contribute to the discussion and understanding of the results of the soil invertebrate communities. For identification, the ants were mounted with entomological pin, placed in plastic triangles and labeled in vegetal paper, and identified following available guides (BOLTON, 1994; FERNANDEZ, 2003; WARD, 2012).

In the same collection locations as invertebrate soil fauna, physical and chemical parameters of the soil were evaluated, in order to evaluate possible correlations with the soil faunal community (Figure 2, Table A1 in appendices). For physical samples steel rings were used (Kopecky) with sharp edges and an internal volume of 100 cm^3 to collect soil to determine soil bulk density (Ds), macroporosity (Macro), microporosity (Micro), penetration resistance (Rp), and total porosity (TP) (EMBRAPA, 1997). The relative density (Dr), an important tool for measuring compaction, was obtained from Proctor test (EMBRAPA, 1997).

Soil extractable phosphorus, potassium, sodium, and micronutrients were measured by ion exchange with 0.0125 *M* H_2SO_4 + 0.05 *M* HCl solution followed by determination of phosphorus by colorimetry, potassium and sodium by flame photometry, and micronutrients (copper, iron, magnesium and zinc) by atomic absorption spectrophotometry (MACHADO, 2005). The determination of aluminum, calcium and magnesium were made after extraction with 1 *M* KCl where aluminum was determined by titration with 0.025 *M* NaOH, and calcium and magnesium were measured by atomic absorption (MACHADO, 2005). The organic carbon and total nitrogen was determined via thermal conductivity detector (TCD - CHNS) (MACHADO, 2005).

2.2.3 Statistical analyses

The characterization of the soil invertebrate fauna was based on the density (number of individuals per pitfall and per square meter), richness (number of species) and diversity (Shannon-Wiener index) (KREBS, 1999). The Shannon-Wiener index was calculated as

$$H' = -\sum pilogpi$$

Σ = summation, *pi* = proportion of total sample represented by species i divided by the total number of individuals, S = number of species or species richness.

Due to their heterogeneity, the invertebrate soil fauna data obtained (x) for density were converted into $(x + 0.5)^{0.5}$ and compared by Duncan test at the at the 5% level ($p < 0.05$). The data obtained (x) for richness were not transformed, and the means were compared by Duncan test at the at the 5% level ($p < 0.05$). Statistical analyzes were processed with the use of Assistat program (SILVA & AZEVEDO, 2009). For the ants, the characterization of the morphospecies was based on the frequency of registers (pitfall and litter) and richness (number of morphospecies) according Romero & Jaffe (1989).

The soil invertebrate community, ants and environmental variables were submitted to. principal component analysis (PCA). This method was adopted because it reduces the multidimensionality of datasets and generates interpretable axes (PCA axes), finding linear combinations of the variables in order to describe the most important sources of variation (LEGENDRE & LEGENDRE, 1998). Principal component analysis was performed through the vegan package on the R platform (R DEVELOPMENT CORE TEAM, 2012).

The determination of functional groups is represented in Tables A10 and A11 in the Appendices. The functional diversity was based on the Shannon-Wiener index, according to Mendes et al. (2015). In addition, the functional groups were also subjected to cluster analysis, performed using the method closest to the Euclidean distance to describe the similarity between management fields. The cluster analyses were processed through the Statistica program (HILL & LEWICKI, 2007).

2.3 Results

The community of soil invertebrate fauna in the farm A (Table A2 in appendices) was represented in 1,446 captured individuals, distributed across 14 orders, 40 families and a group of larvae on top of the soil. Most of the adults and larvae were identified as Coleoptera (11 families). The order Diptera was represented by seven families, followed by five families in Hemiptera, four families in the order Hymenoptera, three families in the order Orthoptera, two families in the orders Lepdoptera and Blattodea, and one family each in the Arachnida, Dermaptera, Chilopoda, Collembola, Gastropoda and Psocoptera.

For the soil profile (0-10, 10-20 and 20-40 cm depths) the number of individuals captured was smaller in comparison to the organisms found on the top of the soil, with 406 individuals, distributed across 11 orders, 23 families and two groups of immatures (larvae) (Table A3 in appendices). Families in Scarabaeidae, Cydnidae, Formicidae, Lumbricidae and a group of immature (larvae), and Coleoptera were the only ones represented in all depths of the soil.

In the agriculture and livestock farm B (Table A6 in appendices), 1,432 individuals were captured on the top of the soil, distributed across 14 orders, 47 families and two groups of larvae. The most numerous of the families, considering adults and larvae, in the farm B were identified in the order Coleoptera (12). The orders Hymenoptera, Diptera and Hemiptera were represented by seven families, followed by three families in the order Orthoptera, two families in the orders Lepdoptera and Blattodea, and one family in each of the orders Arachnida, Dermaptera, Millipede, Chilopoda, Collembola, Haplotaxida and Gastropoda.

A number of individuals captured in the soil profile (0-10, 10-20 and 20-40 cm depths) was also smaller (229 individuals) compared to the organisms captured at the soil surface (Table A7 in appendices), distributed across 11 orders, 23 families and a group of larvae. The orders Coleoptera and Hemiptera presented the majority of families collected (6), considering adults and larvae. The order Hymenoptera was represented by three families, and each of the orders Diptera, Haplotaxida, Orthoptera, Arachnida, Millipede, Dermaptera, Chilopoda and Gastropoda by one.

The results of the ecological parameters (Table 2) at the soil surface (pitfall and litter fall) of farm A showed that density and richness of invertebrate fauna did not differ ($p<0.05$) for the different management practices. However, in the litter fall, F was significantly ($p < 0.05$) greater, only not differing ($p < 0.05$) from the CL-d field for density or richness or from SC for richness. For the soil profile (depth 0-10, 10-20, 20-40 cm) the density and richness followed the same trend observed for the organisms collected on the soil surface; they did not differ ($p < 0.05$) among evaluated fields, except of the third-year grazing system (CL-a) that had significantly ($p<0.05$) less richness compared to F in the 0-10 cm layer. In the 10-20 cm layer the richness in CL-b field had smaller values ($p < 0.05$) than F (Table 2).

The Shannon-Wiener index (Table 2) indicated that F had the greatest diversity index values for the soil surface invertebrate communities (pitfall and litter fall) and within the soil

profile (0-10, 10-20, 20-40 cm depths), followed by the CL-d and SC fields. The exception was in the 20-40 cm depth of soil, where the SC field showed the lowest diversity index value in comparison to the other evaluated systems.

Table 2. Ecological parameters for invertebrate biota, under the farm A agriculture and livestock management in the region of Maracaju, Mato Grosso do Sul, Brazil.

Ecological parameters	.....Farm A agriculture and livestock.....					
	CL-a	CL-b	CL-c	CL-d	SC	F
			Pitfall			
Density	*4.4±1.1 a	4.0±2.0 a	5.9±0.7 a	7.8±0.8 a	4.5±1.3 a	7.9±1.3 a
Richness	2.8±0.8 a	3.2±1.5 a	5.2±1.0 a	4.8±1.6 a	5.4±2.0 a	6.8±2.7 a
Index (H')	1.7	2.2	2.4	2.7	2.6	3.0
			Litter fall			
Density	1.3±0.2 b	1.3±0.2 b	1.4±0.1 b	2.5±0.4 ab	2.2±0.4 b	3.9±0.4 a
Richness	1.4±0.5 b	1.4±0.5 b	1.6±0.4 b	4.4±0.8 a	2.0±0.4 ab	4.4±0.8 a
Index (H')	1.4	1.7	1.7	2.3	1.9	2.4
			Depth 0-10 cm			
Density	1.7±0.1 a	2.6±0.4 a	2.4±0.4 a	2.6±0.4 a	2.1±0.5 a	3.2±0.3 a
Richness	1.8±0.3 b	3.8±1.2 ab	2.8±0.8 ab	5.2±1.2 ab	3.0±1.2 ab	7.4±1.6 a
Index (H')	1.7	1.9	2.2	2.7	2.3	2.9
			Depth 10-20 cm			
Density	1.3±0.2 a	1.0±0.2 a	1.9±0.4 a	2.8±0.9 a	2.3±0.4 a	2.1±0.3 a
Richness	1.2±0.3 ab	0.8±0.5 b	1.2±0.3 ab	1.4±0.5 ab	1.8±0.5 ab	3.2±0.7 a
Index (H')	1.0	1.0	1.2	1.3	1.5	2.2
			Depth 20-40 cm			
Density	1.3±0.2 a	1.2±0.1 a	1.1±0.1 a	1.8±0.5 a	1.2±0.2 a	1.8±0.6 a
Richness	1.2±0.4 a	1.0±0.3 a	0.8±0.3 a	1.0±0.5 a	1.2±0.4 a	1.0±0.4 a
Index (H')	1.0	1.1	1.0	1.1	0.7	1.3

CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. (n = 5). *Averages ± standard error. Values within a row followed by a similar letter are not significantly different by the Duncan test ($p < 0.05$) (n = 5).

In farm B, the results of the ecological parameters (Table 3) on the soil surface indicate F-b was significantly ($p < 0.05$) greater than the other evaluated fields, except for no difference ($p < 0.05$) from the CL-3 field in density and from CL-3 and SC-b for richness. The litter fall community followed the same trend observed for pitfall, except for the density where F-b was significantly greater ($p < 0.05$) than the other evaluated fields, except for no difference ($p < 0.05$) from the SC-b field in richness or diversity and CL-3 in richness. For the soil profile (0-10 depth), the SC-b and CP fields had significantly lower density ($p < 0.05$) than the F-b. For richness, F-b was significantly ($p < 0.05$) greater than the other evaluated fields, except for CL-3 for which was significantly similar. In the 10-20 and 20-40 cm depths, there were no significant differences ($p < 0.05$) among the different management systems (Table 3).

The Shannon-Wiener index (Table 3) indicated that F-b had greatest diversity values of the invertebrate community at the soil surface (pitfall and litter fall), followed by the CL-3, CL-2 and SC-b fields. The diversity in the soil profile showed the same tendency observed at the soil surface, except in the depth of 0-10 cm where the SC-b field under the sixth mechanized harvest of the sugarcane crop and the CP field presented the smallest diversity indices.

Table 3.Ecological parameters for invertebrate biota, under the farm B agriculture and livestock management, in the region of Maracaju, Mato Grosso do Sul, Brazil.

Ecological parameters	.....Farm B agriculture and livestock.....					
	CL-2	CL-3	NT	SC-b	CP	F-b
			Pitfall			
Density	4.4±0.7 bc	7.8±1.3 ab	2.4±0.3 c	5.4±0.8 bc	3.5±0.7 c	9.1±0.6 a
Richness	4.4±1.4 b	8.0±1.2 a	3.8±0.8 b	6.8±1.5 ab	3.6±0,5 b	8.6±0.9 a
Index (H')	2.4	2.7	2.1	2.5	1.9	2.8
			Litter fall			
Density	1.7±0.3 b	2.2±0.3 b	1.5±0.2 b	5.7±0.8 a	2.0±0.2 b	3.2±0.3 a
Richness	0.8±3.4 b	3.4±0.8 ab	2.0±0.6 b	5.8±0.9 a	2.4±0.5 b	6.4±1.1 a
Index (H')	1.9	2.2	1.5	2.5	1.6	2.7
			Depth 0-10 cm			
Density	2.3±0.2 ab	2.1±0.3 ab	2.3±0.2 ab	1.6±0.2 b	1.6±0.2 b	3.3±0.2 a
Richness	2.8±0.7 b	4.0±0.7 ab	2.0±0.4 b	1.2±0.37 b	1.2±0.3 b	6.8±1.4 a
Index (H')	1.9	2.3	1.4	1.2	1.2	2.5
			Depth 10-20 cm			
Density	1.4±0.1 a	1.2±0.1 a	1.3±0.1 a	1.4±0.3 a	1.1±0.2 a	1.7±0.1 a
Richness	1.6±0.2 a	0.8±0.2 a	1.4±0.5 a	1.2±0.4 a	0.6±0.2 a	1.6±0.2 a
Index (H')	1.6	1.6	1.1	1.3	0.6	1.7
			Depth 20-40 cm			
Density	1.3±0.3 a	1.4±0.5 a	1.1±0.2 a	1.1±0.1 a	1.2±0.1 a	1.2±0.2 a
Richness	0.8±0.3 a	1.0±0.6 a	0.6±0.2 a	1.0±0.3 a	1.0±0.1 a	1.0±0.4 a
Index (H')	1.0	1.1	1.1	1.1	0.7	1.3

Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanized harvesting; CP, continuous pasture; F-b, forest fragment. *Averages ± standard error. Values within a row followed by a different letter differ significantly by the Duncan test ($p < 0.05$) ($n = 5$).

The performance of the PCA with the fields under different management practices showed close or isolated communities among the sample groups (Figure 3 and 4). Using ordination in farms A and B it is possible to observe the physical and chemical soil properties that are related to the distribution of invertebrate faunal families and morphospecies of ants (Figure 3 and 4).

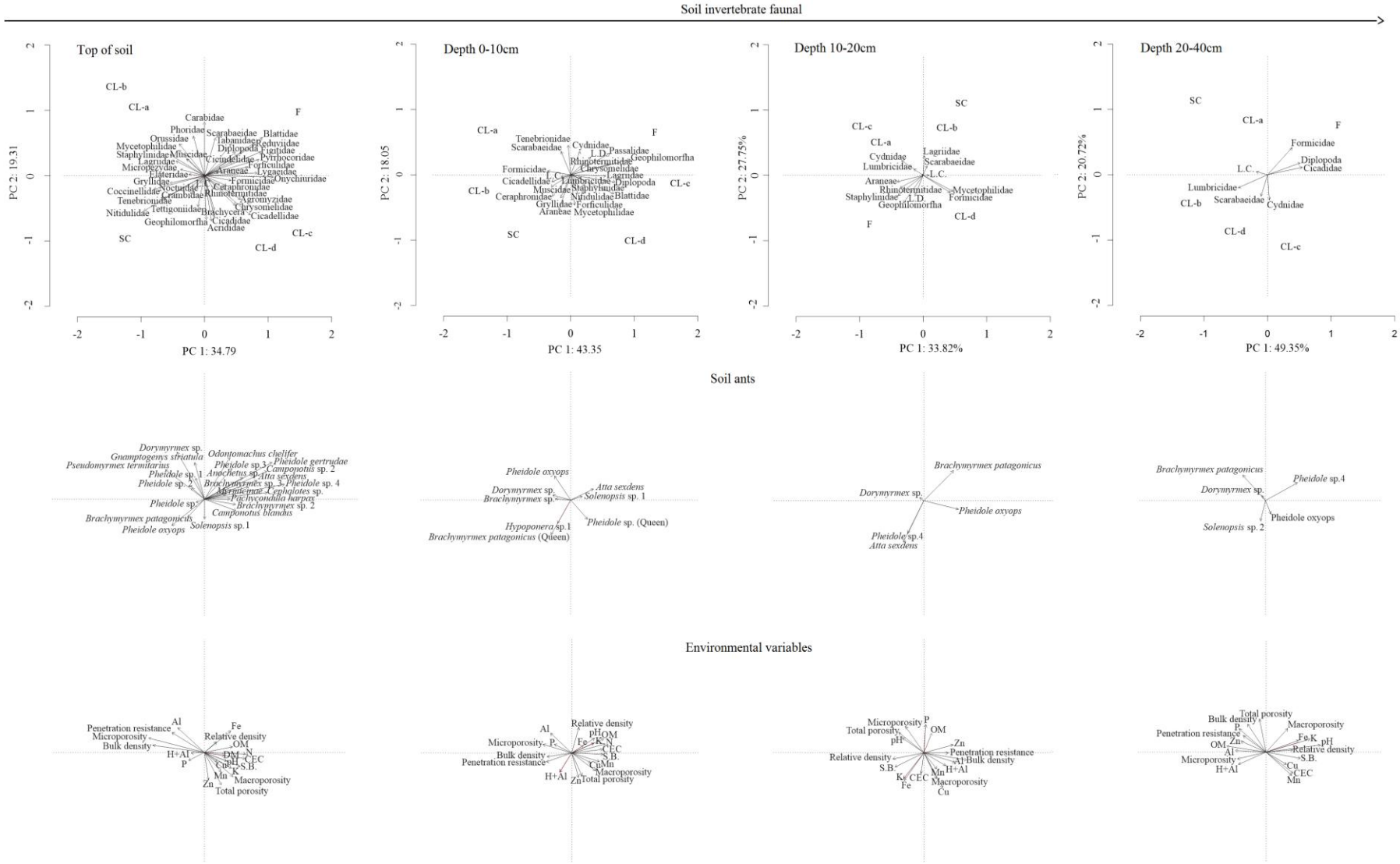

Figure 3. Principal Component Analysis (PCA) of community structure of soil invertebrates, ants and environmental variables in the farm (A) fields under different agricultural and livestock management. Independent biplots move to the right (from soil surface through depths of the soil).

Total nitrogen (N), sum of bases (SB), phosphorus (P), iron (Fe), aluminum (Al), and soil physical parameters were altered as the soil profile and management system changed (Figure 3 and 4). At the surface of the soil of farm A (Figure 3), PCA axes explained 34.79% and 19.31% of the variability of invertebrate fauna community composition, ants, and chemical and physical properties of the soil. The forest fragment (F) and sugar cane (SC) separated from fields under the crop-livestock integration system (CL-a, CL-b, CL-c and CL-d). The CL-a and CL-b fields were close, as were CL-c and CL-d. It is possible to observe a broad interaction among the biological, physical and chemical variables in the field under forest fragment (F) and the fields under crop-livestock integration CL-c and CL-d, involving for example the cycles of OM, N, SB, and total macroporosity with the greatest diversity of the fauna community and soil ants. On the other hand, there was a lower proximity of soil attributes in the SC, CL-a and CL-b fields, presenting larger concentrations of H+Al, soil density and less diversity of organisms.

For the soil profile in farm A (depth 0-10, 10-20 and 20-40 cm) the greater depth reduces the biological composition and modifies the distribution of the physical and chemical parameters of the soil (Figure 3). In the 0-10-cm depth, PCA axes explained 43.35% and 18.05% of the variability of invertebrate faunal community composition, ants, and chemical and physical properties of the soil. The different fields followed the same trend observed for the soil surface except the CL-b and SC fields grouped together and the CL-a was isolated. For the 10-20-cm depth, PCA axes explained 33.82% and 27.75% of the variability, showing the separation of F and CL-d fields and the similarity between CL-a and CL-c fields, as well as CL-b and SC. At the greatest depth of the soil (20-40 cm), PCA axes explained 49.35% and 20.72% of the variability, with the separation of F, and plotting of CL-c and the CL-b and CL-d fields in close proximity to each other, as well as CL-a and SC.

At the soil surface of farm B (Figure 4), PCA axes explained 28.75% and 25.27% of the variability of invertebrate faunal community composition, ants, and soil chemical and physical properties. The results show the fields under mechanized harvest of sugarcane (SC-b) and continuous pasture (CP) isolated in relationship to the other evaluated fields. The CL-2 and CL-3 fields are close plotted in close proximity to each other, as did the NT and F. It is possible to observe a broad interaction among the biological, physical and chemical variables in the fields under CL-2 and CL-3 integration and the fields under forest fragment (F-b) and no-tillage (NT) (Figure 4).

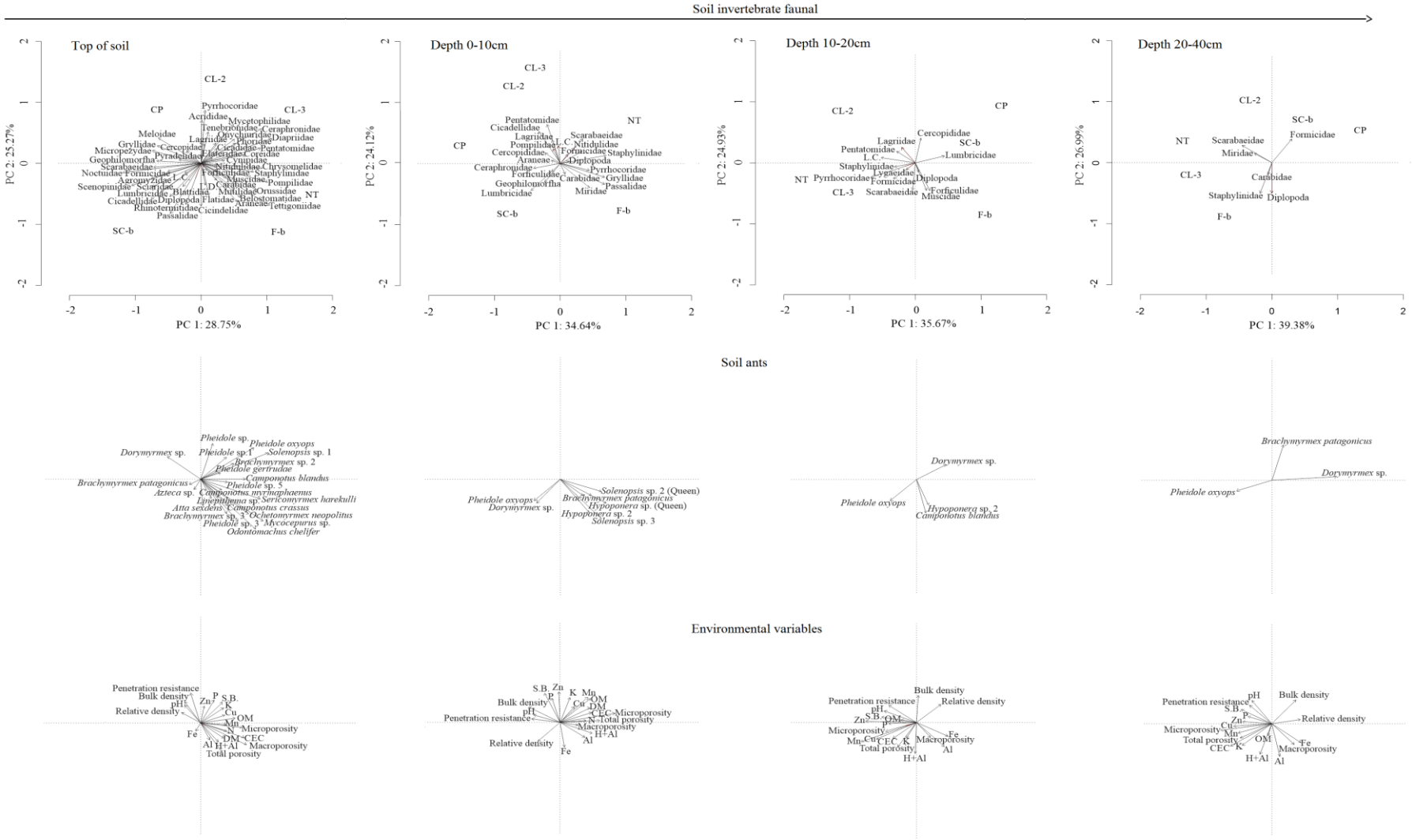

Figure 4. Principal Component Analysis (PCA) of community structure of soil invertebrates, ants and environmental variables in the farm (B) fields under different agricultural and livestock management. Independent biplots move to the right (from soil surface through depths of the soil).

These interactions involve physical (microporosity, macroporosity and total porosity) and chemical (OM, N, S, C, P, K, Cu, Zn) and biological processes with the greatest diversity of invertebrate fauna and soil ants (Figure 4). On the other hand, there was lower interaction of soil attributes in the SC and CP fields. The SC field had stronger interaction with the larger Fe contents. The CP field was grouped with the largest values of pH, penetration resistance, bulk density and relative density (Figure 4).

For the soil profile in farm B (0-10, 10-20 and 20-40 cm depth), the greater depth reduced the biological composition and modified the distribution of the physical and chemical parameters of the soil (Figure 4). In the 0-10 cm depth, PCA axes explained 34.64% and 24.12% of the variability of soil invertebrate faunal community composition, ants, and chemical and physical properties of the soil. The different fields F-b, NT, SC-b, CP are isolated and the CL-2 and CL-3 fields grouped together. For the 10-20 cm depth the PCA axes explained 35.67% and 24.93% of the variability, showing the separation of CL-2, CL-3 and F-b fields, and the grouping of CP and SC-b fields. In the greater depths of the soil (20-40 cm) the PCA axes explained 39.38% and 26.99% of the variability, with the CP and SC-b fields, the CL-2 and NT fields, and the CL-3 and F-b forming groupings.

In farm A (Figure 3), observe the antagonism between the presence of ants and the soil properties such as penetration resistance, bulk density, Zn, Al, H + Al, P and pH at the soil surface. In farm B (Figure 4), observe the opposite position between the presence of ants and the soil properties such as penetration resistance, bulk density, Zn, S.B., P and pH at 0-10, 10-20, and 20-40 cm depths.

The distribution of the functional groups in farm A and B varied across the different fields evaluated (Figures 5 and 6; Table A10, A11 in Appendices). In farm A, five functional groups were represented in CL-a, six in CL-b, CL-c, and CL-d, seven in SC and eight in F. Families with more than one ecological function in the environment (plastic species) were predominant in most fields, except for the field under mechanical harvesting of sugarcane (SC), where the highest percentage (27%) organisms were phytophagous. In Farm B, five functional groups were represented in the CL-2 and CP fields, six in NT and SC-b, seven in CL-3, and eight in F-b. The predominance of families with more than one ecological function in the environment (plastic species) can also be observed in farm B among the different fields. However, the CL-2 field revealed a large percentage of phytophagous organisms (35%). In the F-b field the greatest percentages were represented by phytophagous (25%) and omnivorous (27%) organisms.

Functional diversity values (Figure 5) were greatest under forest fragment (F) at farm A. Among the fields under agricultural production practices, functional diversity was greatest in SC and CL-d. In farm B, the field under forest fragment (F-b) followed the same trend observed in farm A, with the greatest values of functional diversity. Among the fields managed under agricultural production practices, the field under continuous pasture (CP) reduced the values of functional diversity.

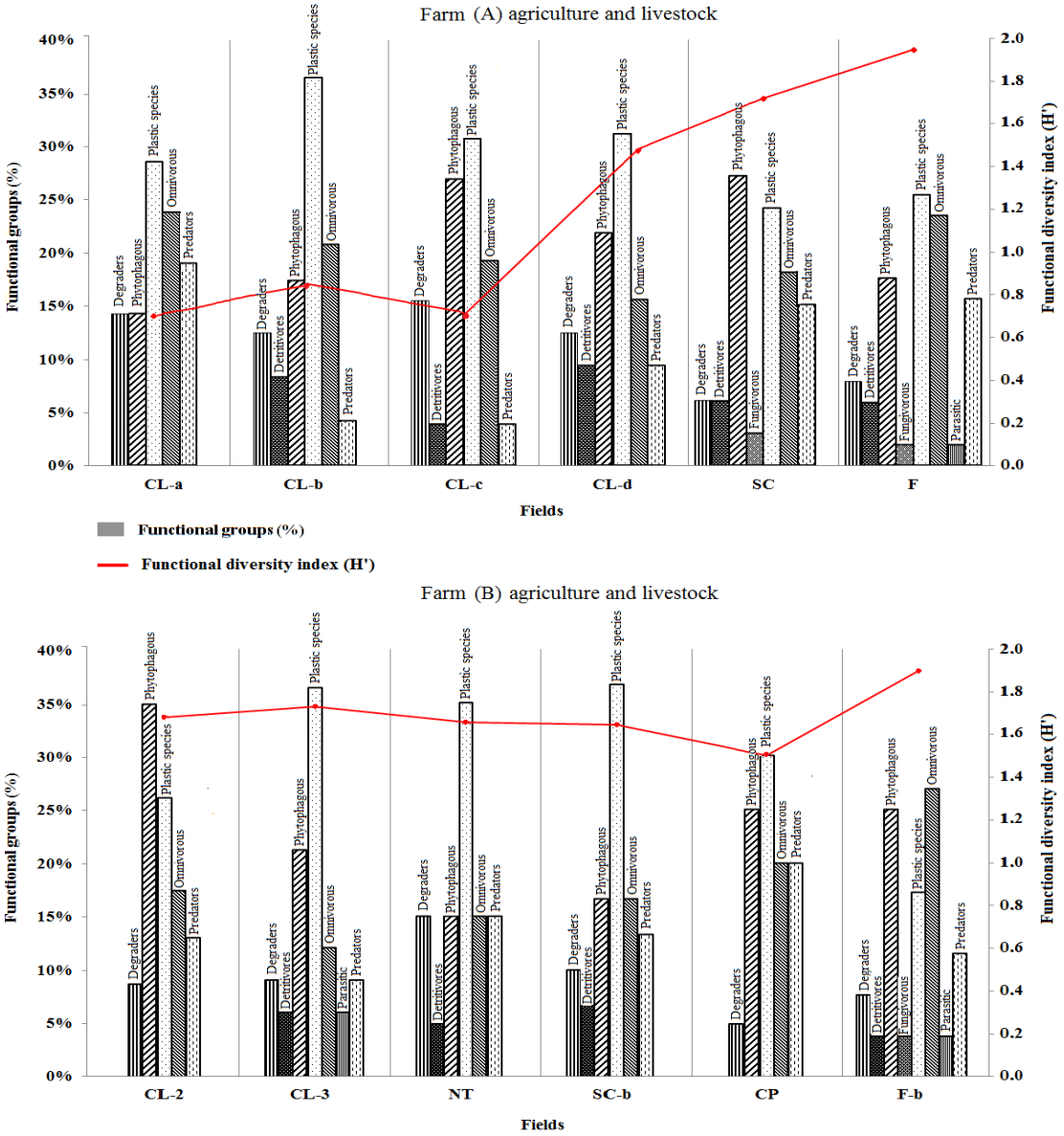

Figure 5. Percentage distribution and diversity index (H') of the functional groups. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third-year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanized harvesting; CP, continuous pasture; F-b, forest fragment. (n = 5).

Cluster analysis from the data of the functional groups and functional diversity index in farm A resulted in the formation of two interpretable groups (G1 and G2) (Figure 6). One group "G1" was comprised of CL-b and CL-c linked with distance less than 69%, which allows inference that the similarity between the two communities is 31%. The second group "G2" formed by CL-d, SC and F presented 28% similarity. The community in the CL-a field remained isolated from the others (Figure 6).

In farm B, the field under forest fragment (F-b) was 100% different in relation to the other evaluated fields (Figure 7). The functional community in CL-3 did not cluster with the other fields, showing distance less than 42% . However, two distinct groups were formed G1 (comprising CL-2 and SC-b) and G2 (comprising NT and CP) (Figure 7).

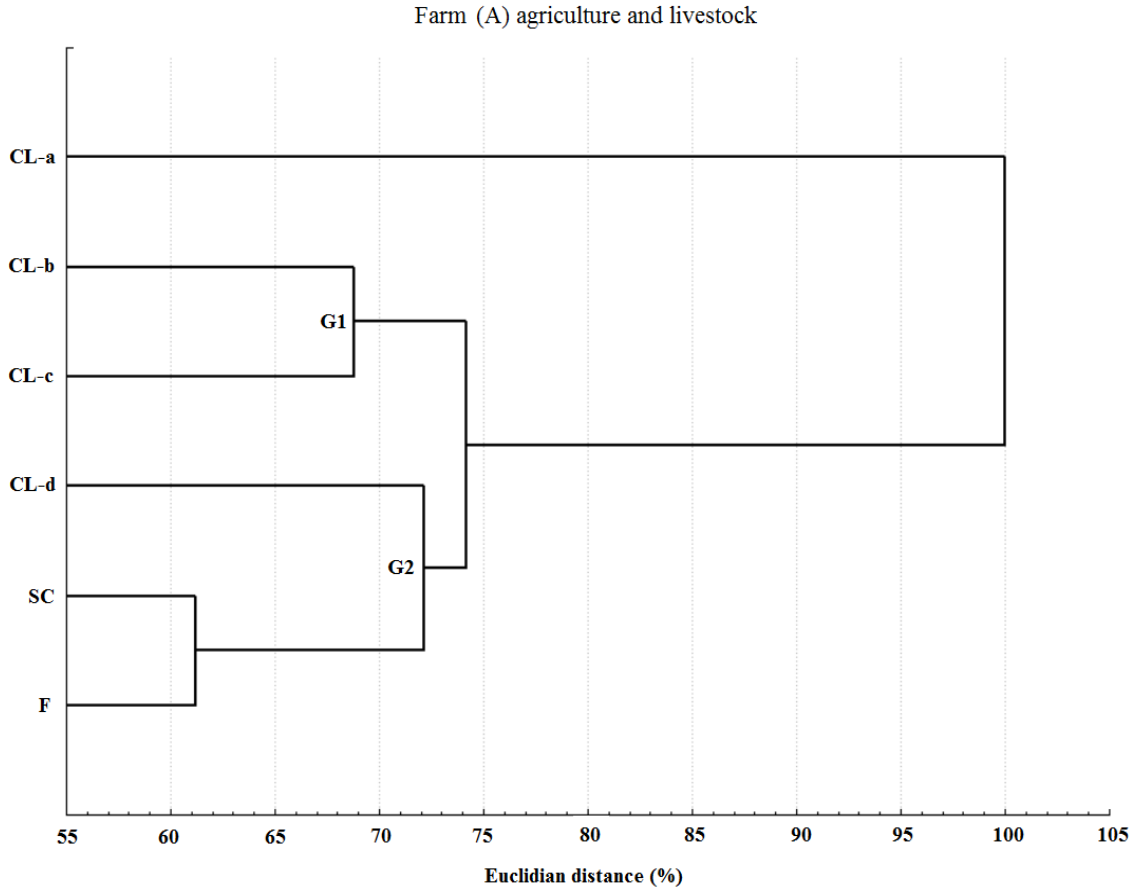

Figure 6. Dendrogram of similarity of functional groups. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. (n=5). G1 and G2, groups formed from Euclidean distance.

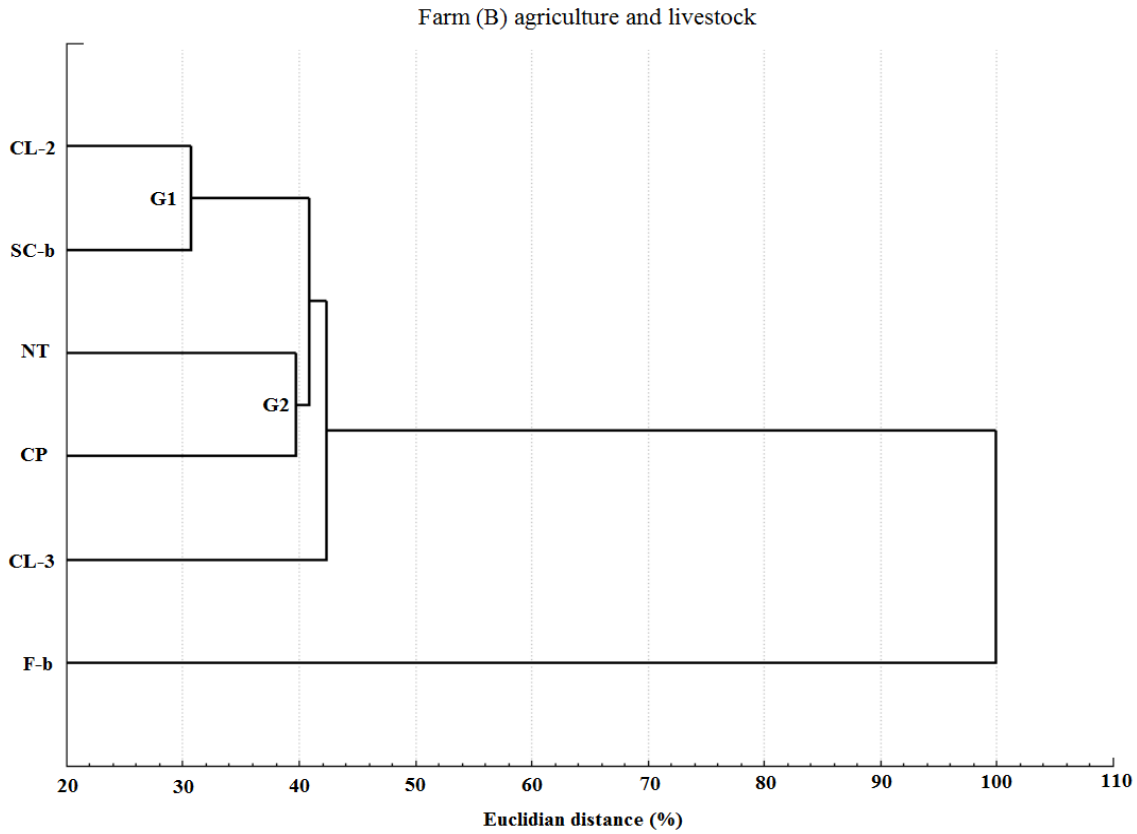

Figure 7. Dendrogram of similarity of functional groups. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanized harvesting; CP, continuous pasture; F-b, forest fragment. (n = 5). G1 and G2, groups formed from Euclidean distance.

The evaluation of organisms considered rare or specialists showed that in the soil surface of farm A, fifteen families (37.5%) were represented by only one record in all evaluated fields, most of them represented in the field under forest fragment (F) (Table A2 in appendices). In addition, the families Agromyzidae, Lygaeidae, Onychiuridae and Cicadellidae were found only in the CL-d and F fields (Table A2 in appendices). For the soil profile (0-10, 10-20 and 20-40 cm depths) five families (21.7%) were represented by only one registry in all evaluated fields, most of them represented in the field under mechanical harvesting of sugarcane (SC) (Table A3 in appendices). In addition, some families presented in the forest fragment were observed in the CL-c, CL-d, SC, for example Lagriidae, Carabidae, Passalidae, Staphylinidae, Cydnidae, Mycetophilidae and Blattidae.

On the soil surface of farm B (Table A6 in appendices), twenty-one families (44.7%) were represented by only one record in all the evaluated fields, most of them present in the forest (F-b) and mechanized harvesting of sugarcane field (SC-b). Some families (Staphylinidae, Cynipidae, Mycetophilidae, Cercopidae, Cicadidae, Acrididae and

Gryllidae) found at the top of the soil on forest fragment (F-b) are represented only in CL-2, CL-3 and SC systems. For the soil profile (0-10, 10-20 and 20-40 cm depths) seven families (30.4%) were represented by only one record in all evaluated fields, most represented in the forest fragment (F-b) (Table A7 in appendices). The Nitidulidae and Pyrrhocoridae families present in the F-b were also found in the CL-3 field. The organisms Carabidae, Scarabaeidae, Staphylinidae, Pyrrhocoridae, Formicidae, Lumbricidae and Diplopoda were the only organisms represented at all depths of the soil.

For the ants *Pheidole* and *Solenopsis* were the most diverse genera in farms A and B. These taxa keep their nests inside the soil (Table A4, A5, A8 and A9 in appendices). On farm A, twenty-three morphospecies were collected at the soil surface, distributed across 13 genera among the different fields (Table A4 in appendices). Fourteen morphospecies (60.9%) were represented by only one record in all evaluated fields, most of them represented in the forest fragment (F). Some morphospecies present in the forest fragment were also observed in the CL-d field, for example *Atta sexdens* and *Pheidole gertrudae*. For the soil profile (0-10, 10-20 and 20-40 cm depths) the ants were represented by 12 morphospecies distributed among 7 genera (Table A5 in appendices). Eight morphospecies (66.7%) were represented by only one record in all the evaluated fields, following the same tendency of the fauna in the soil profile. The morphospecies *Brachymyrmex* sp., *Brachymyrmex patagonicus* Queen, *Hypoponera* sp.1, *Pheidole* sp. Queen, *Solenopsis* sp., *Solenopsis* sp. 1 were found only in the soil profile.

For ants in farm B, twenty-one morphospecies were collected at the soil surface, distributed across 12 genera among the different fields (Table A8 in appendices). Ten morphospecies (47.6%) were represented by only one record in all evaluated fields, most of them present in forest fragment (F-b). In addition, some families in the forest fragment (*Azteca* sp., *Brachymyrmex* sp. 2, *Brachymyrmex* sp. 3, *Pheidole gertrudae*) are also represented in the CL-2 and SC-b fields. For the soil profile (0-10, 10-20 and 20-40 cm depths) the ants were represented by eight morphospecies distributed among six genera (Table A9 in appendices). In all depths of the soil, ants were observed; however, no individuals were found in CL-2, CL-3, NT, and SC fields at the 10-20 cm depth; and CL-2, NT, and F-b fields at the 20-40 cm depth. Five morphospecies (62.5%) were represented by only one record in all evaluated fields, with most represented in the forest fragment (F-b). The *Brachymyrmex patagonicus* family present in the F-b were also found in the SC-b field.

2.4 Discussion

The use of soil invertebrates as bioindicators is an important tool to monitor the responses of ecosystem functions due to changes from environmental management practices (SILVA & SILVESTRE, 2000; COLEMAN, 2008). Recently, studies have shown that transitions from forest fragments to agricultural systems can restructure soil invertebrate communities (SANABRIA et al., 2014; LAVELLE et al., 2016). Recording the results from changes in management can contribute to the preservation of biological processes in natural and agricultural ecosystems (XAVIER et al., 2010).

The general diversity of different soil faunal groups indicates that the collection methodology was representative in relationship to the taxonomic diversity of the different groups of invertebrate fauna present in the soil (TRIPLEHORN & JONNSON, 2010). According to Brown et al. (2006), approximately 250,000 species of edaphic fauna are estimated in Brazil and little is known regarding their biology and ecology. Most of the collected invertebrates are active at the soil surface and some families move through the profile, perhaps related to greater availability of food and preferred habitat at the soil surface, as well as to the biology of these organisms (FRANCHINI et al., 2011). The large proportional representation of the order Coleoptera is characteristic in most tropical soils, for example, in soils with systems of agricultural and livestock production with maintenance of vegetal residues in the top soil (PORTILHO et al., 2011). According to Purvis & Fadl (2002), soil beetles are among the most active insects at the soil surface in agroecosystems. Families of this order may be beneficial to fertility and soil physics, especially in the larval phase; on the other hand, some families may be considered as pests to agriculture (NICHOLS et al., 2008).

Density, richness and diversity of soil organisms show the fields under forest fragment (F and F-b), integrated crop-livestock systems (CL-d, CL-2 and CL-3) and sugarcane crop with mechanized harvesting (SC and SC-b) with the best conditions for the maintenance of the community of organisms in the soil. Despite the fragmentation of the forests the greater diversity of soil invertebrates was maintained, showing the importance of the preservation of these fields under natural vegetation for the local biodiversity. In the tropical soils, this fact had already been evidenced in a study with ant communities in systems with crop rotations and natural vegetation. The authors suggest that this type of

stock adjacent to rotational systems is important for conservation and ecosystems replenishment in agricultural management (CREPALDI et al., 2014). Trees play a crucial role in the maintenance of healthy soil conditions through the action of their roots and litter (SCHROTH & SINCLAIR, 2003)

Among the agricultural and livestock production systems, the results, in general, may be related to the crop-livestock integration cycle in the two farms evaluated. In the farm A, the field in the third year of pasture (CL-a) reflected a reduction of invertebrate diversity, greater soil compaction in the superficial layers, and lower concentration of important chemical properties (OM, N, P, K and sum of bases). However, with the onset of crop rotation in CL-b, CL-c and CL-d, the soil physical, chemical and biological conditions are modified, improving soil quality. Plant residues together with a diversity among plant roots increase the energy flow in the soil system, reflecting a greater potential for biological actions (consumption and interactions) in the environment, and avoid declines within carbon, nitrogen and phosphorus biogeochemical cycles of the soil (BROWN et al., 1999; BENCKISER, 2010; CHAPUIS-LARDY et al., 2011; FILSER et al., 2016). The maintenance of these soil nutrient cycles is important for crop development (corn, soybean, sugarcane) in agricultural production systems (NOVAIS et al., 2007).

In farm B, the no-tillage (NT) field grouping with fields under integrated crop-livestock (CL-2 and CL3) may be explained by the fact that soil tillage is different at the moment of planting. In no-tillage, the seeders are equipped with stems which are used in the opening of the planting line to the depth of 0-10 cm. However, the preparation model adopted in the fields under crop-livestock integration (CL-2 and CL-3) was minimum cultivation, with opening of each planting line to a greater soil depth of 35 cm. This management was adopted in farm B for the integrated crop-livestock system because of the greater resistance to soil penetration in the superficial layers (0-10, 10-20 cm) reflecting the periods with pasture. The opening of the planting line at greater soil depth in the CL-2 and CL-3 fields may be favoring lower soil density and, consequently, the action of soil organisms in the deeper layers. On the other hand, the absence of plant diversity in crop rotation and the presence of livestock may have influenced soil biological, physical and chemical processes, and reduced soil quality in the CP field.

The fields under sugarcane harvest (SC and SC-b) maintain 20 Mg ha^{-1} of dry biomass (‘straw’) on the soil surface, which may explain the invertebrate community results in the superficial layers of the soil in these fields. However, due to the sugarcane crop and

fertilization processes, soil compaction occurred at the deepest depths (20-40 cm), reducing the soil invertebrate community. The decreasing of soil chemical contents in these fields can be explained by the collection period that occurred soon after the harvest of the sugarcane crop, as the crop may have used the maximum amount of nutrients available in the soil during its development. Therefore, it can be observed that changes in the invertebrate biota community can be explained by changes in the soil environment of natural and productive systems (e.g. physical and chemical properties of the soil).

Overall to facilitate the understanding of soil invertebrate communities and their importance in ecosystems, functional group concepts (e.g. predators, detritivores, degraders, hematophagous, parasitic and microphytophagous organisms) for soil fauna and ant guilds were adopted in the present study (LAVELLE et al., 2003). In both farms A and B, there was a large percentage of organisms with more than one ecological function (plastic species) across management practices. The interaction between different functional groups, climatic and microbiological factors in the soil, can accelerate the litter decomposition (PEREIRA et al., 2013, CORREIA et al., 2005) and, consequently, greater availability of nutrients to the soil (ROSOLEM et al., 2003). In some fields in both farms A and B, phytophagous organisms dominated. The phytophagous species may be comprised of organisms of different orders, for example Coleoptera, Hymenoptera, Hemiptera, Diptera and Arachnida (BROWN et al., 2015). Greater availability of food and reduction of natural enemies in agricultural production systems may have favored the development and multiplication of phytophagous organisms. The high density of these insects can cause damage to the aerial parts and the roots of plants, and thus these insects would be considered pests in agriculture (CRUZ et al., 2013).

In addition to quantifying functional groups, determining the functional diversity of organisms has emerged as a new way of measuring the ecological importance of species in a community and understanding how their activities can affect specific ecosystems (CADOTTE et al., 2011; LAURETO et al., 2015). The results of functional diversity in farms A and B showed that the crop-livestock integration system over time (CL-d, CL-2, CL-3), mechanized harvesting of sugarcane (SC, SC-b) and forest fragments (F, F-b) may favor the greater functionality of the system compared to pasture fields. The rotation of plant species and their processes directly influence the occurrence of communities of soil organisms (AQUINO et al., 2008) and consequently the diversity of organisms (LAOSSI et al., 2008). In this way, the greater diversity of functional groups can contribute to the

maintenance of the soil processes, considering that each functional group is important for the community and consequently for the dynamics of the system.

Soil invertebrates are involved in major soil biogeochemical cycles (C, N, and P), controlling almost all aspects involving organic matter interactions and nutrient recycling, regulating the activity and functional composition of soil microorganisms (LAVELLE et al., 2006; FILSER et al., 2016). The invertebrate biota can be found on the surface and in the soil profile, which also contributes to the active transport of organic matter from the top to the deeper layers, favoring organic matter dynamics and nutrient cycles within the soil profile (LAVELLE et al., 2006; VAN GROENIGEN et al., 2014; BOTTINELLI et al., 2015). In addition, organisms classified as ecosystem engineers are able to modify the soil physical environment by digging burrows and modifying soil aggregation (LAVELLE, 1997; BOTTINELLI et al., 2015).

Another important ecological indicator is the registration of organisms considered rare or specialists in the different fields evaluated. Nine taxa were recorded only in forest fragments, which may reflect potential soil quality indicators because of their rarity. Organisms considered rare in abundance have a relevant role in the composition of soil biodiversity, in addition to serving in the role as an indicator of environmental quality (LUTINSKI & GARCIA, 2005; LUTINSKIA et al., 2016). The contribution of rare organisms to the total community density is in agreement with the results of Portilho et al. (2011) and Crepaldi et al. (2014), who report densities of soil faunal families and ants represented by only one record between fields with a forest fragment and those under agricultural management. Specialist invertebrates are normally found in environments with a higher concentration of litter, roots, stems, trunks, tree limbs and remains of dead animals (TRIPLEHORN & JONNSON, 2010; WARD, 2012).

Through functional groups, functional diversity, and rarity it was possible to observe that the diversity of invertebrates of the soil will decrease with the loss of invertebrate functionality in different systems of agricultural management, characterizing these ecological parameters as potential tools to understand the invertebrate soil biota. These results can be incorporated into decision making in the conservation and restoration of environments, especially for those who attempt to rebuild or preserve natural and production fields (PETCHEY et al., 2004; CADOTTE et al., 2011).

The ants were sensitive to changes in the soil environment, confirming their potential to indicate ecosystem quality (ANDERSEN, 1997). The results in this study are in

full agreement with those described by Andersen, 1997 and Bieber et al. 2006. According to those authors *Pheidole* is always the best represented genera among litter ants collected in forested areas. Other genera of soil ants have also been reported in the literature, including *Camponotus, Solenopsis* and *Crematogaster* (BRAGA et al., 2010; CREPALDI et al., 2014). These ants have a large geographical distribution, species diversity and adaptations, and are therefore considered to be common on a global scale (WILSON, 1976; WARD, 2012).

The greater diversity of morphospecies and ant guilds may be related to diversity and volume of plant residues; physical conditions and availability of nutrients in the soil of the crop-livestock integration system and sugarcane production may have been important factors for the balance of the ant community. However, the correlations between chemical properties that presented different interactions with the diversity of ants in the two farms evaluated need further investigation. Determining the diversity of ants and the processes responsible for changes in the community contributes to the modeling of ecosystem conservation plans (ANDRE et al., 2002; DIAS et al., 2008).

Fields with the most compacted soils may have interfered with the diversity of ants; this may be related to the habitation of some of these organisms that live in inter-aggregate pore spaces of the soil and are not able to create their own galleries. Soil properties (soil density, porosity, grain size, stability of aggregates, moisture, Mg, Ca, carbon, and acidity potential); vegetation (basal area and dry litter mass), temperature, and humidity can affect the occurrence of ants in tropical soils (SILVA, 2014). With a diversified food metabolism, ants play an important role in the energy flow and cycling of matter in ecosystems; ants can contribute to seed dispersal, predation, herbivory, nutrient cycling, soil physical and chemical structuring, plant protection against herbivores, and their interaction (SILVA & SILVESTRE, 2000; BENCKISER, 2010).

2.5 Conclusions

The crop-livestock integration system and the mechanized harvesting of sugarcane can fit into a planned biodiversity program where the holistic agricultural production system enriches the soil and assists in the development of soybean, corn and sugarcane

intercropping systems. In addition, with an indirect function, integration systems provide greater diversity of invertebrate functional groups and establishment of invertebrate fauna considered rare or soil specialists, as well as colonies of ants originating from the forested environments. The fields with soil compaction, lower nutrient concentrations, and reduced bulk density, and plant residue diversity may be factors related to the decline in soil functional diversity. On the other hand, fields that provided greater protection of the soil and greater amount of plant residues in the long-term favored physical, chemical and biological soil conditions related to increased faunal diversity.

2.6 Acknowledgements

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2.7 In memoriam

On the 18th of July, 2016, Fabio Martins Mercante, an Embrapa Agropecuária Oeste Researcher passed away. He is sorely missed by his family, friends, and the wider scientific community who knew and respected him. Most recently, aware of his illness, he dedicated his efforts to transfer his knowledge about soil quality in Brazil. We appreciated your teachings, the opportunities to work with you, and your example. Rest in peace our friend.

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CHAPTER 3 - SOIL BACTERIAL DENITRIFIER DIVERSITY ACROSS TROPICAL MANAGEMENT SYSTEMS

SOIL BACTERIAL DENITRIFIER DIVERSITY ACROSS TROPICAL MANAGEMENT SYSTEMS

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ABSTRACT

The integration of soil physical, chemical and microbial diversity in agricultural management systems was investigated to determine which systems maintained denitrifier (*nirK*) diversity. This study was conducted in southern Mato Grosso do Sul, Brazil, in January 2014, in Hapludox soil. Two farms that incorporate integrated crop-livestock systems were assessed through evaluation of functional microbial communities (DGGE and real time qPCR), and soil physical and chemical properties. The *nirK* gene community showed forest fragments (F, F-b) had the highest range weighted richness (*Rr*) values. Among the fields with agricultural and livestock systems, the largest *Rr* and functional organization (*Fo*) were observed in third-year of crop rotation after grazing (CL-d), third year of crop-livestock integration cycle (CL-3) and mechanized harvesting of sugarcane (SC). Fields in pasture (CL-a, CP) and first year of crop rotation after pasture (CL-b) had reduced *Rr* and *Fo* values. Fertility and better soil physical conditions were positively correlated with *nirK* diversity. The AOB, 16S rRNA and *nosZ* gene density were positively related with the *nirK* diversity in fields with a longer time under the integrated crop-livestock. Richness and diversity of soil *nirK* community is reduced with the transition from a forest to the agricultural and livestock production in tropical soil. However, the management system under integrated crop-livestock farming and cultivation of sugarcane with mechanized harvesting maintain a relatively diverse community, possibly with conditions promoting balance in the N cycle.

KEYWORDS: agroecosystem, denitrification, crop-livestock integration, PCR-DGGE.

DIVERSIDADE DE BACTERIAS DESNITRIFICANTES EM SISTEMAS DE MANEJO TROPICAL

RESUMO

A integração da diversidade física, química e microbiana do solo em sistemas de manejo agrícola e pecuário foi investigada para determinar quais sistemas mantinham a diversidade denitrificante (*nirK*) do solo. Este estudo foi conduzido no sul de Mato Grosso do Sul, Brasil, em janeiro de 2014, em Latossolo Vermelho distroférrico de textura muito argilosa. Duas fazendas que incorporam o sistema integração lavoura-pecuária foram avaliadas através das comunidades microbianas funcionais (DGGE e qPCR em tempo real) e propriedades físico-químicas do solo. A comunidade de genes *nirK* mostrou que os fragmentos florestais (F, F-b) apresentaram os valores de riqueza ponderada mais elevada (*Rr*). Entre os campos com sistemas agrícolas e pecuários, observou-se o maior *Rr* e organização funcional (*Fo*) no terceiro ano de rotação de culturas após a pastagem (CL-d), terceiro ano do ciclo de integração lavoura-pecuária (CL-3) e colheita mecanizada de cana-de-açúcar (SC). Os campos sob pastagem (CL-a, CP) e o primeiro ano de rotação após a pastagem (CL-b) apresentaram valores de *Rr* e *Fo* reduzidos. A fertilidade e melhores condições físicas do solo foram positivamente correlacionadas com a diversidade *nirK*. A densidade dos genes AOB, 16S, rRNA e nosZ foi positivamente relacionada com a diversidade *nirK* em campos com maior tempo de cultivo integrado. A riqueza e a diversidade da comunidade *nirK* do solo é reduzida com a transição de uma floresta para a produção agrícola e pecuária em solo tropical. No entanto, o sistema de gestão sob cultivo integrado-pecuária e cultivo de cana-de-açúcar com colheita mecanizada mantêm uma comunidade relativamente diversa, possivelmente com condições que promovem o equilíbrio no ciclo N.

PALAVRAS-CHAVE: agroecossistema, desnitrificação, integração lavoura-pecuária, PCR-DGGE.

3.1 Introduction

The simultaneous goals to maximize productivity and soil conservation have been featured in integrated row crop agriculture and livestock production systems (PALM et al., 2014). Soil management is a critical element of sustainable agricultural production systems because agricultural management practices can modify soil structure to various degrees

which may vary in magnitude and direction and either improve or reduce soil quality (BONO et al., 2013). The maintenance of soil quality can be related to greater soil organic matter content and moisture and nutrient retention (KIBBLEWHITE et al., 2008; BHARDWAJ et al., 2011). Therefore, the ultimate challenge for agricultural and livestock production is to achieve the highest level of productivity within the context of natural resources (e.g. soil and water) conservation.

This approach to farming that integrates productivity with soil and water conservation has led to more diversified agricultural and livestock management systems, such as crop-livestock integration (CL) with soybean or corn into *Brachiaria* pastures (MACHADO et al., 2011). In a soil classified as very clayey texture Oxisol, the process of crop rotation in a crop-livestock integrated system enabled the maintenance of soil quality and soil carbon levels equal to the native forest, and greater capacity of the system to withstand disturbances in the 0 – 5 cm layer (TIRLONI et al., 2012). However, how biological communities respond to these integrated management systems remains unclear (CAETANO et al., 2013; PEZARICO et al., 2013). Furthermore, inherent soil properties, such as texture can modify the structure of microbial communities (PASTORELLI et al., 2010). More research about organisms and biological processes in soil will contribute to the understanding of how management systems impact soil productivity and nutrient conservation.

Here we propose that biological indicators, specifically the microbial structure of *nirK* communities, can be used as strategies to monitor the soil environment in agriculture (PEIXOTO et al., 2010) to allow researchers to infer environmental quality or the effects of an agent, process, or integration of decisions on parameters in the environment (CASALINHO et al., 2007). Biological attributes, particularly soil microorganisms, have shown great sensitivity to changes in agro-ecosystems (ROSA et al., 2014). Soil nitrogen retention and cycling are important to plant growth and development (PHILIPPOT & HALLIN, 2006). Denitrifiers comprise about 5% of the soil bacterial community, are a diverse subset of facultative anaerobic bacteria participating in various reductions in the pathway of nitrate (NO^-_3) to nitrite (NO^-_2) and eventually to molecular nitrogen (N_2) (KOCH et al., 2015).

The use of molecular biology allows analysis of soil microbial communities without the reliance and limitations of growing microorganisms in culture (MARZORATI et al., 2008). Studies of specific denitrifier genes in tropical agricultural production

systems are limited (HENRY et al., 2008; MORALES et al., 2010). However, the *nirK* gene has been used successfully as a molecular marker of denitrifying bacteria across multiple environments including agricultural soils and compost farm waste (CHEN et al. 2013; LONG et al., 2014). A wide distribution of taxonomically diverse organisms carry the *nirK* gene, contributing to the reduction of nitrate to nitrite in soil (THROBACK et al., 2004; ENWALL et al., 2010; ORLANDO et al., 2012).

Given the potential of production systems to impact so many variables in soil that influence denitrification, e.g. organic matter, pH, nutrients, moisture, and soil aggregate structure, it needs to be understood how crop-livestock integration systems in Brazilian agriculture will change microbial communities in general and denitrifier communities specifically (MEYER et al., 2013; MORAES et al., 2014). Therefore, to evaluate denitrifying microorganisms in different management systems, which have different soil physical and chemical conditions, the *nirK* gene was investigated to generate information on which agricultural and livestock management models promote maintenance of diverse microbial communities (PASTORELLI et al., 2013). We predicted that the establishment of livestock management would increase the soil microbial abundance as well as the diversity of denitrifier communities. The objective of this study was to evaluate the effect of different systems of agricultural and livestock management on microbial community abundance and the composition of denitrifier *nirK* communities. Specifically, we wanted: i) to compare *nirK* richness and community relationships across soil management systems; and ii) to explore relationships between *nirK* communities and soil biological and physico-chemical properties.

3.2 Materials and Methods

3.2.1 Field sites

Fields from two farm systems in the southern region of Mato Grosso do Sul State, Brazil were investigated. Soil at both farms is classified as Hapludox according to the

Brazilian System of Soil Classification - SiBCS (EMBRAPA, 2013). The climate of the region is classified as Cwa, humid mesothermal with warm summer and dry winter (FIETZ & FISCH, 2008). To support the discussion and understanding of the results, precipitation and temperature were recorded throughout the experimental period (Figure 1).

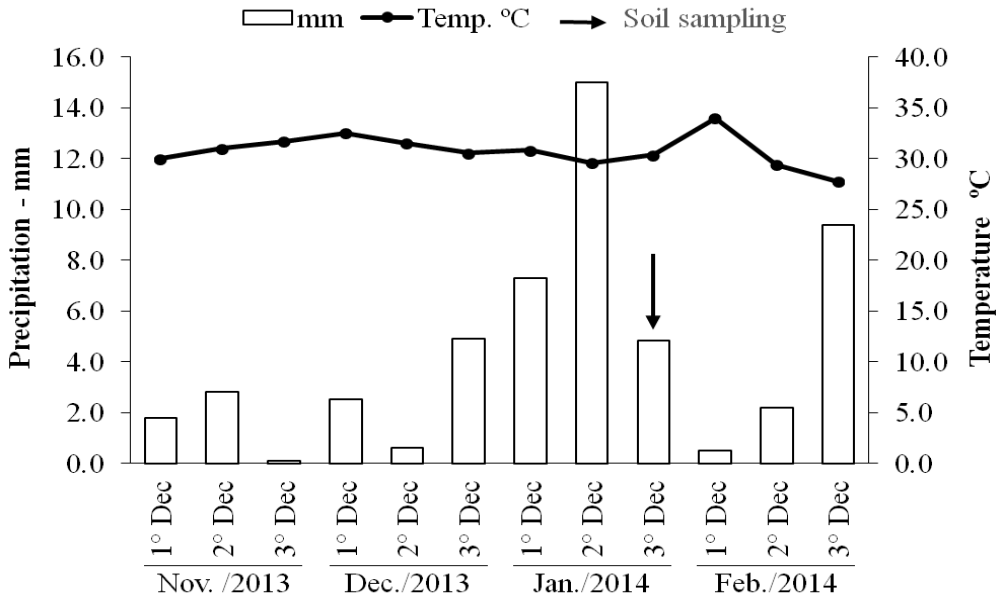

Figure 1. Precipitation and temperatures recorded during soil sampling in the region of Maracaju, Mato Grosso do Sul, Brazil. Centro de Previsão de Tempo e Estudos Climáticos - CEPTEC. Nov./2013: 1° Dec (1 to 10 days), 2° Dec (10 to 20 days), 3° Dec (20 to 30 days); Dec./2013: 1° Dec (1 to 10 days), 2° Dec (11 to 21 days), 3° Dec (21 to 31 days); Jan./2014: 1° Dec (1 to 10 days), 2° Dec (11 to 21 days), 3° Dec (21 to 31 days); Feb./2014: 1° Dec (1 to 10 days), 2° Dec (10 to 18 days), 3° Dec (18 to 28 days). Averages of precipitation and temperature evaluations approximately every ten days in the month.

At farm A (Figure 2, Table 1), the main management is the integrated crop-livestock (CL), managed in rotation with no-tillage between pasture (*Brachiaria brizantha* cv.) and row crops. Two or three years of pasture are followed by three years of soybean in the summer and corn with *Brachiaria ruziziensis* cv. in the winter. At the time of sampling, the field that was in its third year of pasture is denoted CL-a, while the area occupied by the first year of soybean after two years of grazing is named CL-b. The field that was in its second year of soybean is CL-c and the field that was in its third year of soybean is CL-d. Samples from the sugarcane fields continuously for 5 years cropped in variety SP-81-3250 with green harvest is denoted SC. Soil was also collected from a forest fragment (F) as a reference for native soil conditions.

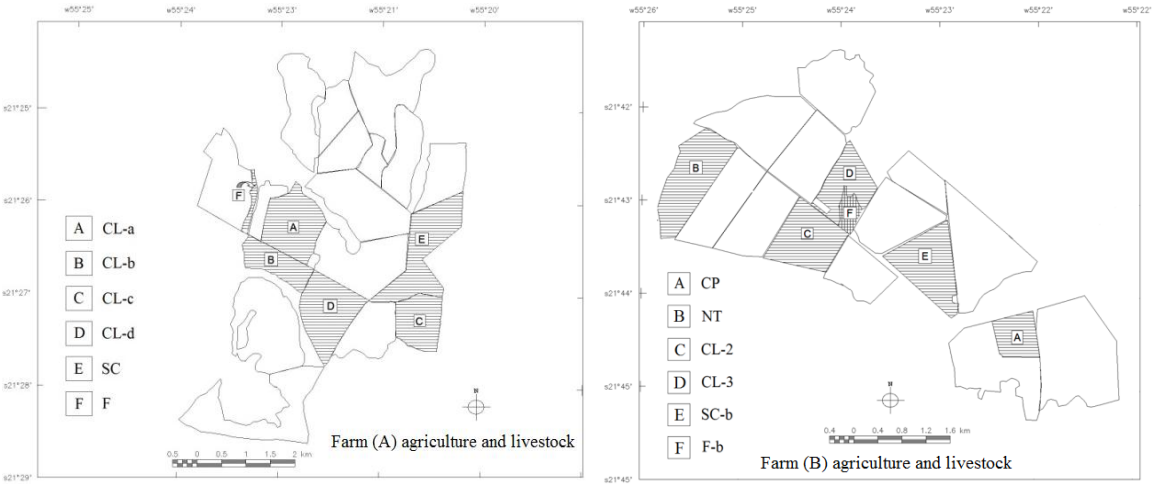

Figure 2. Maps of agriculture and livestock management at two farms (A) and (B) in the region of Maracaju, Mato Grosso do Sul, Brazil. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanized harvesting; CP, continuous pasture; F-b, forest fragment.

Table 1. Rotation succession for each field in farms A and B from winter 2009/2010 through 2013/2014.

Fields (ha)		*DM kg/m^2	09/10	2010	10/11	2011	11/12	2012	12/13	2013	**13/14
			Farm (A) agriculture and livestock								
CL-a	130.5	0.4c	Soyb.	C+*B.*	Soyb.	C+*B.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*
CL-b	70.1	0.7bc	Soyb.	C+*B.*	Soyb.	C+*B.*	*B.ruz.*	*B.ruz.*	*B.ruzi*	*B.ruz.*	Soyb.
CL-c	96.4	0.9ab	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	Soyb.	C+*B.*	Soyb.
CL-d	237.0	1.0ab	*B.ruz.*	*B.ruz.*	*B.ruz.*	*B.ruz.*	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.
SC	26.4	1.3ab	.....Sugarcane, SP 81 3250.....								
F	17.3	1.4a	.....Forest fragment…..….....								
			Farm (B) agriculture and livestock								
CL-2	103.5	0.8bc	Soyb.	Corn	Soyb.	Corn	Soyb.	Corn	Soyb.	*B.ruz.*	Soyb.
CL-3	59.1	1.0b	Soyb.	Corn	Soyb.	Corn	Soyb.	*B.ruz.*	Soyb.	*B.ruz.*	Soyb.
NT	122.4	0.8bc	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.	C+*B.*	Soyb.
SC-b	101.2	1.3ab	Soyb.	Corn	.....Sugarcane, SP 80 1842.....						
CP	91.5	0.3c	.....…..*Brachiaria brizantha Stapf* cv.....……								
F-b	25.8	1.7a	.....… Forest fragment …..…..……....								

Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment. Soyb., Soybean; B.ruz., *Brachiaria ruziziensis* cv. Common; C+B., Corn grown in consortium with *Brachiaria ruziziensis* cv. Common. *DM = Crop dry matter, (n=5). **Time of soil sampling. Values with different letters in the column differ significantly by Duncan test ($p<0.05$) (n=5).

In farm B, two additional management strategies were sampled: no-till (NT) production has been utilized since 2009 and there is also continuous pasture (CP). The NT system has been in place with the rotation of soybean during the summer and the winter cultivated in corn grown in consortium with *Brachiaria ruziziensis* cv. The CP utilizes

rotational cattle grazing of *Brachiaria brizantha Stapf* cv. according to the amount of dry matter in pastures (15-20 cm grass height). Since the 2010/2011 season, there has been green harvesting of sugarcane (SC-b) and in 2011/2012 crop-livestock integration (CL) began. Before the CL system deployment, fields were planted with soybean under no-tillage in summer and corn during the winter. Two fields were evaluated in CL: a field was in its second year of soybean cycle after the first winter pasture (CL-2), and another was in the third year of soybean cycle in summer with pasture during the winter (CL-3). The SC system consisted of sugarcane (SP-80 1842) in its fourth year of green harvest. Soil from a forest fragment (F-b) was included as a reference of the original soil condition.

3.2.2 Soil sampling

Samples were collected in January 2014 from five equidistant points along a 300-m transect for a total of 60 sampling points. After the removal of the litter layer, a 5-cm diameter soil standard core was used to collect the 0-to-10 cm depth, which was homogenized and chilled immediately (4°C), and shipped to the laboratory within hours. Soil samples were passed through a 2-mm mesh sieve and samples were stored at −20°C for molecular analyses or 4°C soil for soil physico-chemical analysis (RODRIGUES et al., 2013).

To determine soil bulk density (Ds), macroporosity (Macro), microporosity (Micro), penetration resistance (Rp), and total porosity (PT), soil was collected in steel rings (Kopecky) with sharp edges and an internal volume of 100 cm^3 (EMBRAPA, 1997). Relative density (Dr), an important tool for measuring the compaction, was obtained from the Proctor test (EMBRAPA, 1997).

Soil extractable phosphorus, potassium, sodium, and micronutrient concentrations were measured by ion exchange with 0.0125 *M* H_2SO_4 + 0.05 *M* HCl solution followed by determination of phosphorus by colorimetry, potassium and sodium by flame photometry, and micronutrients (copper, iron, manganese and zinc) by atomic absorption spectrophotometry (MACHADO, 2005). The determination of aluminum, calcium and magnesium were made after extraction with 1 *M* KCl where aluminum was determined by titration with 0.025 *M* NaOH, and calcium and magnesium were measured by atomic

absorption (MACHADO, 2005). The organic carbon and total nitrogen was determined via thermal conductivity detector (TCD - CHNS) (MACHADO, 2005).

Table 2. Soil chemical and physical properties (0-10 cm depth) in the two farms under different agriculture and livestock management in the region of Maracaju, Mato Grosso do Sul, Brazil.

Fields	pH	OM	P	N	H+Al	S.B.	CEC	Sand	Silt	Clay	Texture
	H_2O	g/dm^3	mg/dm^3	g/kg^{-1}	.....…$mmol_c/ dm^3$ …...			....….....(g/kg^{-1}).....			
						Farm A					
CL-a	5.7a	34.2b	11.7ab	0.14d	4.0a	6.4bc	10.4ab	248	160	620	Clay
CL-b	5.6a	34.2b	12.8a	0.13d	5.3a	5.3c	10.6ab	212	111	677	Clay
CL-c	6.0a	39.1a	14.7a	0.30c	4.1a	9.8a	13.8ab	212	144	644	Clay
CL-d	5.8a	43.8a	6.6b	0.37b	4.9a	7.9a	12.7ab	295	128	629	Clay
SC	5.6a	29.2b	7.6b	0.11d	4.4a	5.7c	10.0b	279	94	627	Clay
F	6.0a	56.4a	3.9c	0.57a	4.0a	11.1a	15.0a	298	127	622	Clay
						Farm B					
CL-2	5.9a	48.5ab	29.6ab	0.26c	4.4b	9.8ab	13.1bc	298	144	558	Clay
CL-3	6.1ab	50.4a	22.2a	0.35b	3.3b	10.1a	14.5ab	348	110	542	Clay
NT	5.3bc	39.7bc	28.6ab	0.17d	8.3ab	7.8bc	16.1ab	117	186	697	Clay
SC-b	5.8bc	24.6cd	15.5ab	0.12de	5.2b	6.7bc	12.0bc	300	103	597	Clay
CP	6.0a	15.5d	6.1b	0.06e	2.9b	4.2d	7.1c	600	87	313	Sandy Clay
F-b	5.3c	55.6a	7.5b	0.52a	14.2a	5.4cd	19.6a	317	86	614	Clay

Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment. pH, hydrogen potential; OM, organic matter; P, phosphorus; N, total nitrogen; H+Al, acidity potential; S.B., sum of bases; CEC, cation exchange capacity. Values for a property in a column at a farm followed by the same letter do not differ significantly by Duncan test ($p<0.05$) (n=5).

3.2.3 DNA extraction

DNA was extracted from 0.25 g soil in triplicate, using the PowerSoil PowerLyzer DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). The quantity of extracted DNA was measured with a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). For amplification of DNA during PCR, samples were diluted to a target concentration of 2.5 ng DNA μL^{-1} with ultra-pure water.

3.2.4 PCR amplification of *nirK* gene

PCR amplification was performed in a total volume of 25 µL containing 5 µL of 5x Taq Buffer (1x final conc.), 0.2 µL of 25 mM each dNTP (200 µM each dNTP final conc.), 1.5 µL of 1% BSA (600 ng / µL final conc.), 1.25 µL of 10 µM each of forward and reverse primers (0.5 µM final conc.), 0.25 µL of Taq Polymerase (5 U / µL) (1.25 U / rxn final conc.) and 14.55 µL of ultrapure water and 1 µL of 2.5 ng μL^{-1} DNA template. The conditions for PCR amplification in a PTC-200 DNA Engine (MJ Research Inc., Waltham, MA) were denaturation at 94°C for 2 min, touchdown 4 cycles for 30s denaturation at 94°C, 30s at 59°C for annealing with 0.5°C decrease in annealing temperature each cycle to 57°C, and 30s at 72°C for extension. Finally, 35 cycles were performed at a constant annealing temperature of 57°C, with a final extension step of 7 min at 72°C.

The following primers were used for PCR amplification of the *nirK* gene: R3Cu (5' GCC TCG ATC AGR TTG TGG TT -3') and FlaCu (5'ATC ATG GTC CTG CCG CG-3'), with a 33-bp GC-clamp (5' GGC GGC GCG CCG CCC GCC CCG CCC CCG TCG CCC 3') attached to the 5' of R3Cu primer (THROBÄCK et al., 2004). The resulting PCR products were checked by gel electrophoresis for 60 min at 80 V in agarose gels in 0.5x Tris-borate-EDTA (TBE) buffer stained with ethidium bromide (10 mg / mL) (EtBr). Gel images were captured on a gel-doc 290 system using Kodak EDAS 1D software package (Kodak, New Haven, CT).

3.2.5 Denaturant gradient gel electrophoresis (DGGE)

The PCR products were subjected to DGGE in a D-code system (Bio Rad Laboratories, Hercules, CA). The PCR samples were loaded onto 1.0-mm thick 7 % (w/v) polyacrylamide gels (acrylamide:bisacrylamide ratio of 37.5:1) with a denaturing gradient that ranged from 45 to 75 % and were electrophoresed in 1.5x TAE buffer (40 m*M* Tris-acetate and 1 m*M* EDTA, pH 8.0) at 90V and 60°C for 16 hrs. One hundred percent denaturant corresponded to 7 M urea and 40 % (v/v) deionized formamide. After electrophoresis, the gels were stained in SYBR Green for 20 min, and images were captured on a gel-doc 290 system using a Kodak EDAS 1D software package (Kodak, New Haven, CT).

3.2.6 qPCR assay

Genes were quantified by quantitative PCR method *(*qPCR) using a ViiA7 Real-Time PCR System (Applied Biosystems, Foster City, USA). The reactions contained 10 µg/µL, 1 µL BSA, 5.5 µM of each primer, 10 µL of qPCR SYBR master mix 2x (Fermentas) and 10 to 20 ng DNA, and 10 nM primers (16S rRNA gene, ammonium-oxidizing bacteria (AOB), and nitrous oxide reductase *nos*Z). The standard curve was performed with pre-amplification of the primer sets using known genes, with a serial dilution of 10^2 to 10^7 µL genes^{-1}. PCR products were cloned and 3-5 clones were sequenced to confirm specificity.

The primers were bac 968f (5'-AAC GCG AAG AAC CTT AC-3'), bac 1387r (5'-GGG CGG WGT GTA CAA GGC-3') (Marchesi et al., 1998), amoA-1F (GGG GTT TCT ACT GGT GGT), amoA-2R (5'-CCC CTC KGS AAA GCC TTC TTC-3') (Rotthauwe et al., 1997), and nosZ2F (5'-CGC RAC GGC AAS AAG GTS MSS GT-3'), nosZ2R (5'-CAK RTG CAK SGC RTG GCA GAA-3') (HENRY et al., 2006). Thermal cycling conditions were as follows for 16S rRNA: 5 min at 95 °C, followed by 40 cycles of 20s at 95 °C, 15s at 56 °C and 40s at 72 °C; amoA: 5 min at 95 °C, followed by 40 cycles of 20s at 95 °C, 15s at 53 °C and 40s at 72 °C; and *nos*Z used a touchdown with 5 min at 95 °C, followed by 6 cycles of 20s at 95 °C, 40s at 66 °C (-1 ºC by cycle) and 40s at 72 °C, 40 cycles of 20s at 95 °C, 40s at 61 °C and 40s at 72 °C. The specificity of the all marker genes was checked by observing a single melting peak melting (60°C to 95°C), which confirmed the purity of the amplified product, and displayed a single band in 1% agarose gel to confirm the size of the amplicons. Analysis was only accepted if the r^2 for the standard curve was above 0.995. The amplification efficiencies for quantitative PCR were 95% ± 4%.

3.2.7 Statistical analyses

The detected bands were analyzed with the Bionumerics program (Applied Mathematics, Kortrijk, Belgium), using the Ward algorithm (SNEATH & SOKAL, 1973) and the Neighbor Joining coefficient. The tolerance position was set at 2 % and

background subtraction was applied. Both band migration distance and intensity within a gel were included in the analysis. The band richness within a DDGE profile for a sample was considered an expression of the total number of detectable *nirK* gene amplicons from a soil sample. The analysis of similarities (ANOSIM) for *nirK* gene DGGE patterns was performed considering both band presence and absence. Bootstrap analysis was performed with the above definitions, 100 repetitions.

The characterization of the functional microbial structure *nirk* gene was based on the range-weighted richness (*Rr*) and functional organization (*Fo*) according to Marzorati et al. (2008). *Rr* is the total number of bands multiplied by the percentage of denaturing gradient needed to describe the total diversity of the sample analyzed, according to the following formula (1):

$$Rr = (N^2 \ X \ D_g)$$

where *N* represents the total number of bands in the pattern, and *Dg* the denaturing gradient comprised between the first and the last band of the pattern. Subsequently, the *Fo* is the cumulative normalized number of bands is used as x-axis, and their respective cumulative normalized intensities represented by the y-axis. The data (*Rr*) were compared by the Duncan test at 5% probability. Statistical analyses were processed with the use of Assistat program (SILVA & AZEVEDO, 2009).

The DGGE patterns were ordinated by cluster group, and the effect of environmental variables was assessed with distance-based redundancy analysis (db-RDA) using the Bray-Curtis distance estimation of microbial abundance matrices with permutation tests (9999 permutations) and Hellinger transformation to eliminate the effect of unit and distribution range differences (R statistical program, http://vegan.r-forge.r-project.org).

3.3 Results

The adjacent forest systems (F, F-b) evaluated on both farms A and B showed relatively great range-weighted richness (*Rr*) (Figure 3). On another hand, functional organization (*Fo*) showed that SC and F in farm A, and CL-2, CL-3, and F-b in farm B

showed different abundances and species accumulations than other management scenarios (Figure 4).

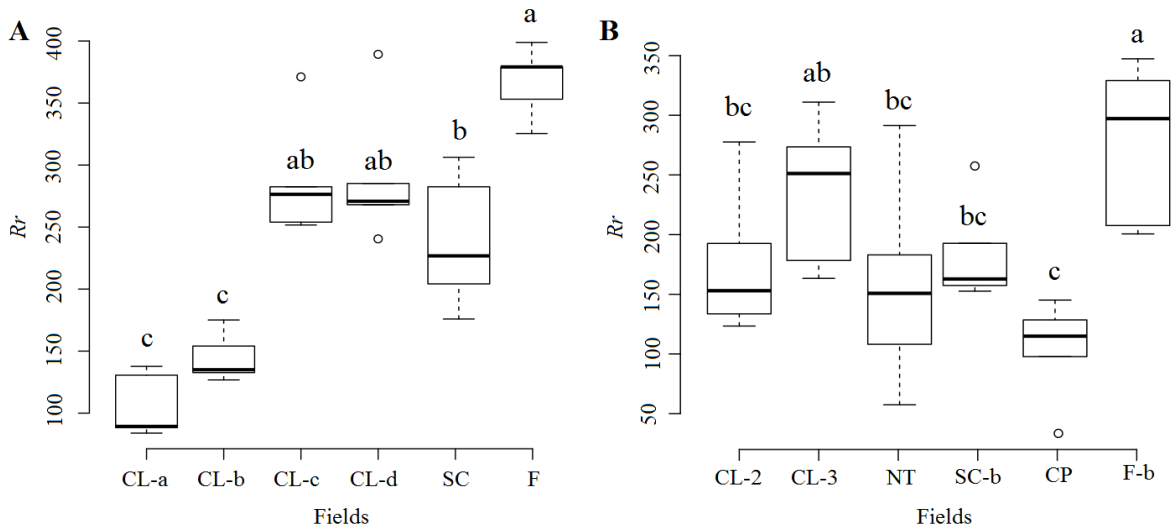

Figure 3. Range-weighted richness (*Rr*) of *nirK* community. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment. Treatments with similar letters are not significantly different by Duncan test ($p<0.05$) (n=5).

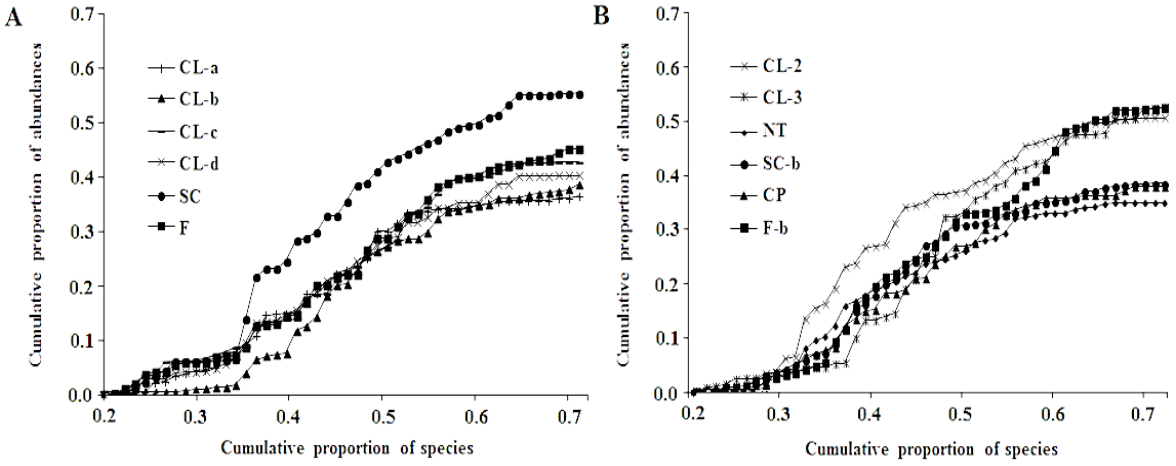

Figure 4. Functional organization (*Fo*) of *nirK* community. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment.

Among management systems at Farm A, the area under the third year of pasture (CL-a) and the first year of cultivation after grazing (CL-b) showed the smallest *Rr* values and *Fo* (Figures 3 and 4). Values in the CL-c fields and CL-d with the second and third year of cultivation after grazing are larger and *Rr* values are not different from F. In farm B, the CP field had the smallest *Rr* values, although values were not significantly different ($p < 0.05$) from NT, SC-b, and CL-2. On the other hand, the field with the third year of crop-farming (CL-3) maintained *Rr* that was not statistically lower ($p < 0.05$) than F-b. Functional organization showed somewhat similar tendency as *Rr*.

The *Rr* of *nirK* communities in SC and SC-b, although smaller than F and F-b, was larger than some other cultivation systems or not different from other systems at both farms (Figure 3). At farm A, *Fo* in SC was maintained large abundances with accumulating proportions of species, and in farm B, the SC-b field had a reduced *Fo* curve compared to CL-2 and CL-3 (Figure 4). It is possible to define the *Fo* as the ability of the community to organize into an adequate distribution of dominant microorganisms and resilient ones, a condition that should assure the potential of counteracting the effect of a sudden exposure to changes in the environment (stress) (MARZORATI et al., 2008).

The DGGE profiling in the soil management systems (Figure 5 and 6) revealed visible differences in the *nirK* microbial community composition among the samples. In the first farm (A), in the dendrogram generated by neighbor joining, the majority of forest fragment (F) samples had the greatest similarity to some replications from the *nirK* community in the CL-d field, but there is little consistent clustering by management systems among replicate samples. In farm B, replicate samples from a management system showed greater clustering of *nirK* microbial communities than in Farm A using neighbor joining and cluster dendrograms (Figures 6). The *nirK* microbial composition in CL-3 communities clustered separately from the other fields using clustering methods.

Redundancy analysis (RDA) was performed in an effort to elucidate the soil attributes involved in changing the *nirK* microbial community composition. In ordination of farms A and B it is possible to observe physical, chemical and biological properties related ($p < 0.01$) to the functional *nirK* community (Figures 7 and 8). Chemical soil conditions (e.g., total nitrogen (N), sum of bases (SB), phosphorus (P), iron (Fe), aluminum (Al) and soil physical parameters (e.g. total porosity (TP) and relative density (BD) were changed as the management system adopted (Table 2).

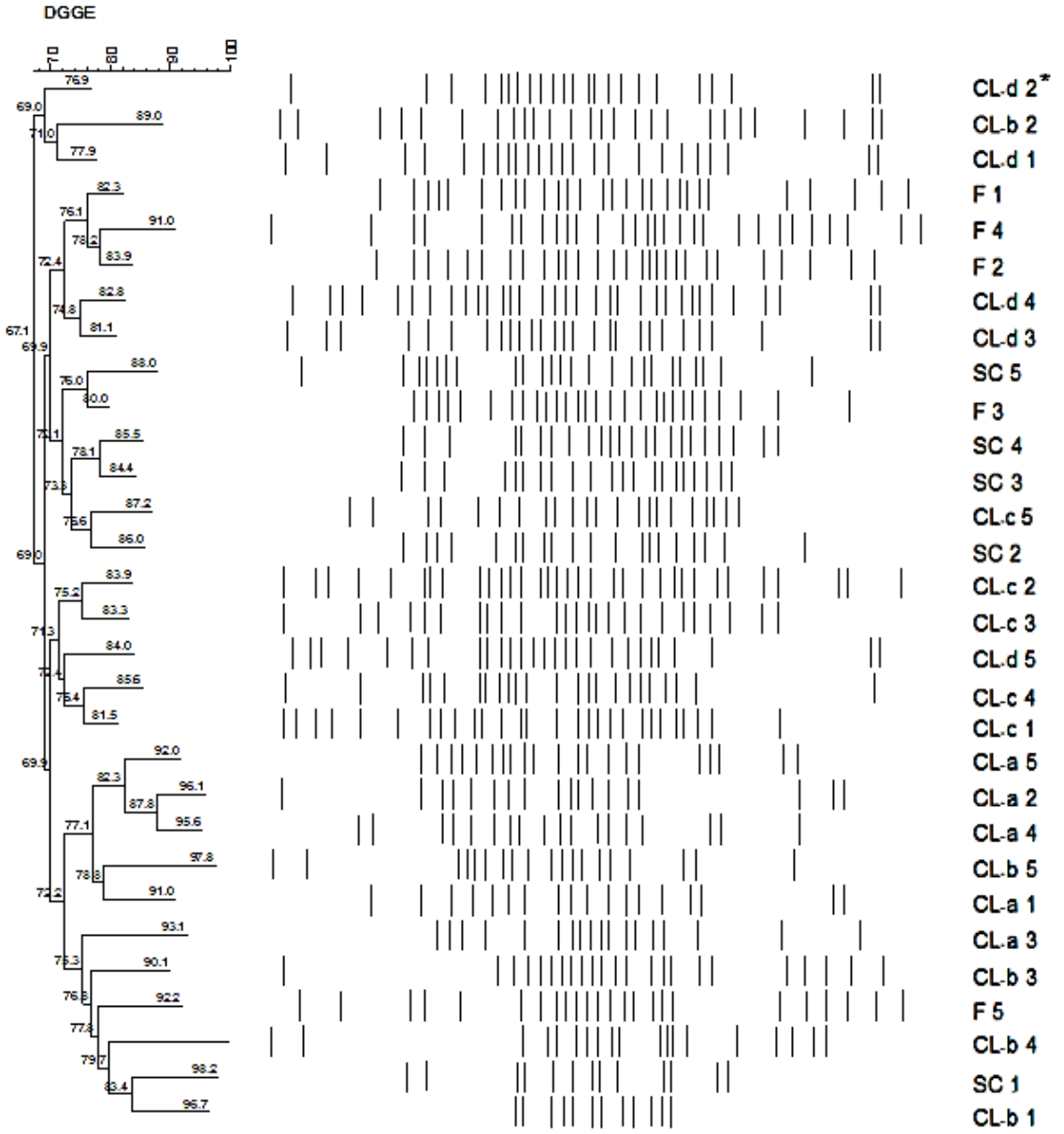

Figure 5. Neighbor-joining representation of DGGE band positions of *nirK* microbial community. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. *(n=5).

In farm A (Figure 7), the axes of the RDA explained 24% and 17% of the variability contained in *nirK* community and soil chemical properties. The *nirK* gene communities found in fields under forest fragment (F) and third year crop rotation after grazing (CL-d) showed a majority relationship ($p < 0.01$) with the contents of N, Cu, K, pH and S.B. In the SC, CL-a and CL-c fields, there was a positive relationship with the chemical elements P and soil Al. The H+Al was highlighted in the CL-b field.

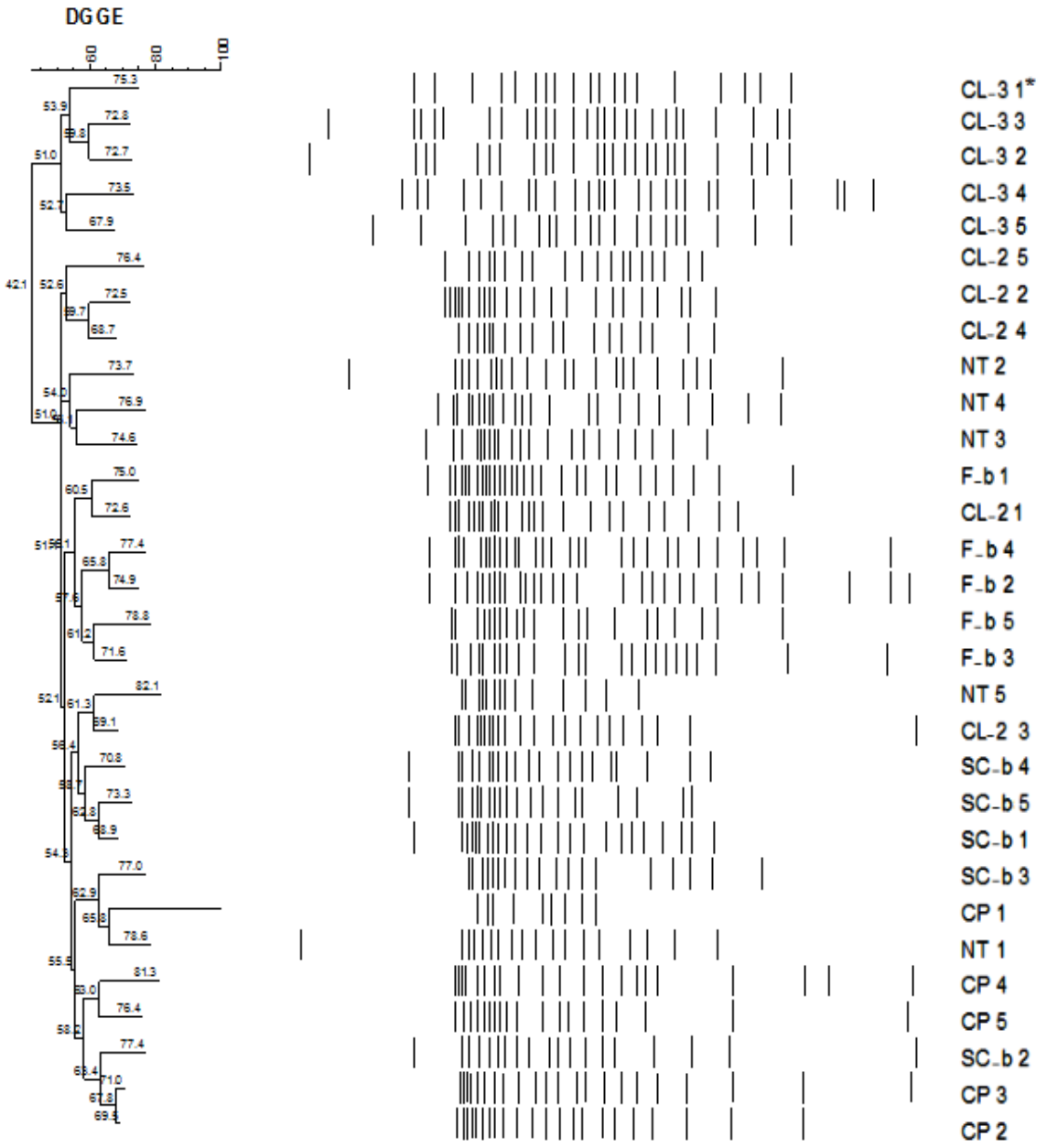

Figure 6. Neighbor-joining representation of DGGE band positions of *nirK* microbial community. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment. *(n=5).

Among the soil physical parameters and microbial *nirK* community composition (Figure 7), the axes of the RDA explained 16% and 13% of the variability. The total porosity, microporosity and macroporosity were significant ($p < 0.01$) with the microbial *nirK* community composition in CL-b and CL-c fields. In the CL-d and F fields, the *nirK* community was related to macroporosity, while in CL-a and SC fields, bulk density showed a positive relationship. The RDA abundance of 16S, AOB, and *nosZ* genes explained 47% and 33% of the total variability in the ordination (Figure 7). The AOB and

16S rDNA had strong positive correlation ($p < 0.01$) with microbial *nirK* community composition in CL-b, while *nosZ* and *nirK* community were related in CL-d and F.

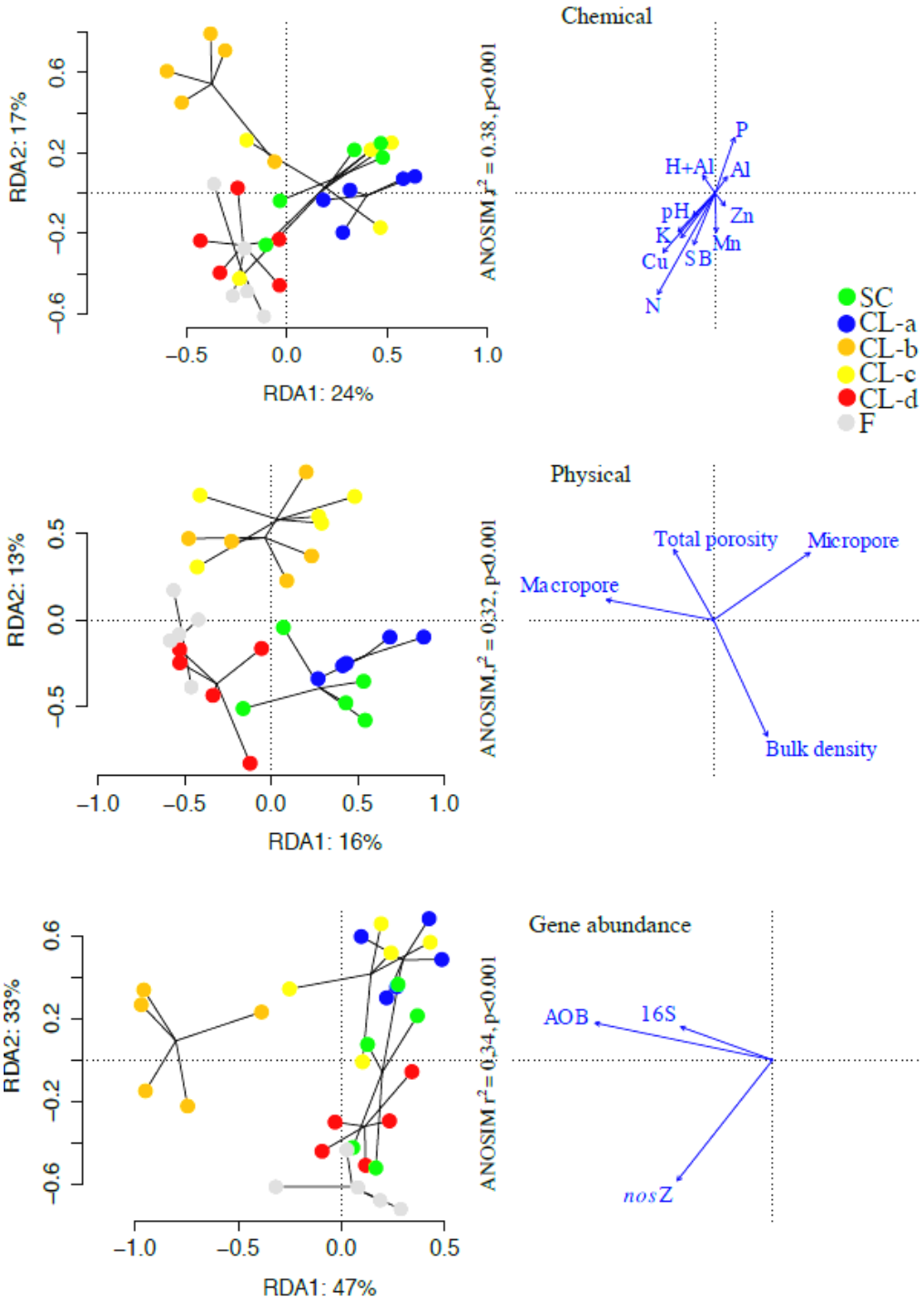

Figure 7. Redundancy analysis (RDA) of *nirK* community and soil properties. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Fe, iron; OM, organic matter; Mg, magnesium; Zn, zinc; P, phosphorus, K, potassium; Cu, copper; S.B., sum of bases.

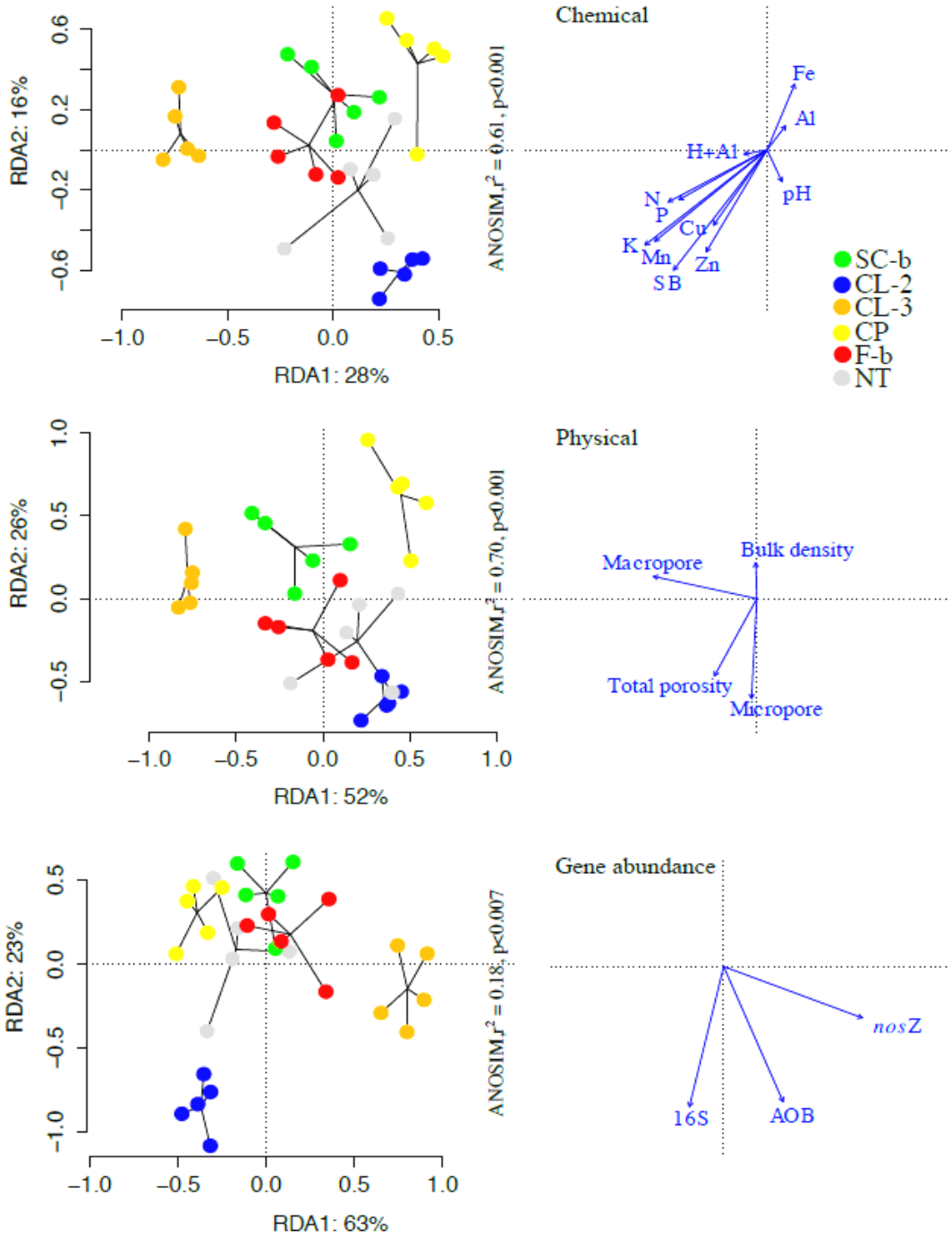

Figure 8. Redundancy analysis (RDA) of *nirK* community and soil properties. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment. Fe, iron; OM, organic matter; Mg, magnesium; Zn, zinc; P, phosphorus, K, potassium; Cu, copper; S.B., sum of bases.

In farm B (Figure 8), the axes of the RDA explained 28% and 16% of the variability between *nirK* community and soil chemical properties. The *nirK* community in CL-3 and F-b is associated with to the content of P, N, Cu, K, Mn, Zn, and S.B. In NT and CL-2, soil pH influenced the *nirK* community composition. The Fe and Al grouped with the *nirK* community in CP and SC-b.

The RDA axes explained 52% and 26% of total variability between the physical properties and microbial *nirK* community composition. The *nirK* community under F-b was related ($p < 0.01$) to total porosity and microporosity. In CL-3, the *nirK* community was related to macroporosity, while in SC-b and CP, the BD was positively related to *nirK*. No-tillage (NT) and CL-2 were not associated with soil physical parameters (Figure 8).

The RDA explained 63% and 23% of the variability between the *nirK* community and the abundance of 16S rDNA, AOB, and *nosZ* genes. The nosZ gene and AOB gene had greater significant relationships ($p < 0.01$) with *nirK* community in CL-3. The field CL-2 *nirK* community showed strong relationship ($p < 0.01$) with the 16S rRNA gene.

3.4 Discussion

The aim was to detect changes in the diversity of the *nirK* community across different agricultural and livestock management systems. The change from forest to agricultural systems was the strongest driver in shaping the functional microbial community in soil. Further changes in the functional microbial community may provide evidence that soil management can alter diversity, and may confer benefits to plants growing in those systems.

Soil in forest fragments (F, F-b) showed the greatest ability to maintain *nirK* gene richness and diversity. Functional organization (*Fo*) results from the action of soil microorganisms with better adaptation to environmental-microbial interactions (MARZORATI et al., 2008). Factors such as temperature, humidity and soil chemical properties have been related to conditions of denitrifying communities in forest fragments (SZUKICS et al., 2009 and 2010). The *nirK* gene has been detected in greater abundance in forest soil compared to agricultural and livestock management systems (DANDIE et al., 2011).

The results of this study suggest that despite potentially high aboveground primary productivity in agricultural soils, belowground primary productivity decreases with institution of agricultural management systems. Agriculture and livestock systems in Farms A and B show the importance of crop rotation in the integrated management of crop-livestock systems to maintain *nirK* community composition and diversity. The crop

rotation with soybean in the summer and corn with *Brachiaria* in winter after grazing (CL-c, CL-d) in farm A improved *nirK* diversity. This effect was also observed in farm B with the adoption of crop-livestock integration (CL-2 and CL-3) under management with pasture in winter and soybean in the summer. The nutrient turnover that occurs with the diversity of rotation of annual crops with pasture may be the most viable method for sustainable soil and water use management in tropical regions (VILELA et al., 2011). This may be related to greater quantity and diversity of crop residue retained on the soil surface and the excreta (urine and dung) promoted by integrated crop-livestock management (MACHADO et al., 2011). Furthermore, these management schemes may promote soil organic matter accumulation (Roscoe et al., 2006), increasing microbial activity (MERCANTE et al., 2008) and C and N concentrations in soil (KANDELER et al., 2006). These components are seen as important in maintaining denitrifier bacterial communities (BREMER et al., 2007; BOZ et al., 2013).

On the other hand, the *nirK* gene community diversity was reduced in systems with a long period of continuous pasture. This may result from fewer inputs into the soil by fertilization and, in this case the pastures are degraded, reducing *nirK* gene diversity (YANG et al. 2013). The reduced crop residue and fertilizers inputs affect the balance of soil organisms and reduce individual and bacterial species (BARETTA et al., 2006). A reduced *Fo* may feature a poorly defined microbial community structure dominated by a few specific species present in high concentrations (MARZORATI et al., 2008).

Overall, the results of this study suggest that the *nirK* community has been impacted significantly by management system. Changes in microbial diversity are generally associated with plant communities (LANGE et al., 2015; TIEMANN et al. 2015). Our findings are in full agree with this conclusion, suggesting that the communities of *nirK* gene are shaped by management system with same diversity of crops. The impact of soil management system on the microbial community has been demonstrated by others using DGGE profiles (SMITH et al, 2010; ZHOU et al, 2011; RACHID et al., 2012 and 2013). Here we show that plant diversity is more important to soil biodiversity composition.

The understanding of the concomitant changes in soil chemical and physical that also change biological properties is important to understanding sustainable systems management and soil fertility. Denitrification potential can be promoted from Sugarcane management which has a high input of N fertilization and with plant residue addition and turnover of high densities of straw (PITOMBO et al. 2016). Itakura et al. (2013) suggest

that soybean can increase N in soil through biological nitrogen fixation. Another factor to explain greater diversity of the *nirK* gene community in lixisol ferric soil is the greater N concentration in the soil which results in increased activity of nitrifying and denitrifying genes (HAI et al., 2009). However, anoxic conditions in Australian soils occur in sugarcane crop fields which inhibit the activity of nitrifying genes and stimulate soil denitrifying genes abundance under high N fertilization (YEOH et al., 2016). This is can be possible due the metabolic versatility of nitrite-oxidizing bacteria including the participation in different nitrogen cycling processes (KOCH et al., 2015).

In both farms (A and B), greater *nirK* richness was related to fertility and improved soil physical conditions, but richness reduced in areas with low pH and soil compaction. Bru et al. (2011) showed that 43-85% of the spatial distribution of soil microbial communities in pasture can be explained by chemical and physical soil properties. The relationship between soil attributes and microbes has been much discussed in many studies in different types of soil under agricultural and livestock production systems (LINDSAY et al., 2010; PASTORELLI et al., 2013; PESSOA-FILHO et al., 2015). Pastorelli et al. (2010, 2011 and 2013) and Samad et al. (2016) evaluated the influence of soil parameters on *nirK* microbial communities in systems under no-tillage with crop-rotation and in pastures and showed that high bulk density, elevated clay content, pH and organic matter can promote the *nirK* profile community. According to Enwall et al. (2010) the pH range and Cu concentrations selected the *nirK* community.

Crop rotation systems may be favoring a better balance of nitrification and denitrification processes in soil. The presence of straw and residues may increase the abundance of decomposable substrate and N species and thus AOB and denitrifying soil communities (*nirK*, nosZ) (HAI et al., 2009). In contrast to these results, organic compared to conventional systems on American silt loam and Rincon silt clay loam soil reduced AOB and nosZ communities (KONG et al., 2010). In soils in Canada with 29% sand, 52% silt, and 19% clay, higher densities and richness of *nirK* and nosZ genes coincided with reduction of straw on the soil surface (BENT et al., 2016). The contrasting results with this study may be explained by the different soil microbiome selected by plant composition of each system, or the decreased diversity of plant communities reducing microbiological abundance in the tropical soil. Our results agree with Hai et al. (2009) and Yeoh et al. (2016) who showed low diversity of microbes coincided with least plant diversity. Hai et al. (2016) and Yeoh et al. (2016) it also observed the reduction of nitrifying and soil

denitrifying communities with the incorporation of only the corn straw in the soil without crop rotation (BENT et al., 2016).

Previous research conducted in the same geographic region has shown mechanical harvesting of sugarcane and systems under crop rotation-livestock rotations are important for maintaining biological, physical and chemical balance in soil (SALTON et al., 2008, 2014; PORTILHO et al., 2011; PAREDES Jr. et al., 2015). Likely the interaction of other soil biological processes with physical and chemical parameters are also involved with the *nirK* gene functional microbial community in soil of agricultural and livestock production systems. Rainfall and temperature are important factors to consider; it is possible to observe greater precipitation and moderate temperatures during the month of soil collection. Greater availability of water and temperatures may favor the maintenance of nitrifying and denitrifying communities (HAI et al, 2009; SMITH et al, 2010).

3.5 Conclusions

Maintaining fertility associated with better management practices stimulates the soil *nirK* community composition. In farm A the systems Cl-c, Cl-d, SC and in farm B the systems CL-2 and CL-3 deserve special attention since they maintained microbial communities. While integrated crop-livestock systems did not increase microbial abundances, they maintained a relatively high functional diversity in soil. Maintenance of diverse microbial communities may be critical if the benefit for N cycling in sugarcane and integrated crop-livestock systems and mitigation of negative environmental impacts are to be fully realized. Conversely, grazing on continuous pasture reduced *nirK* community diversity.

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3.7 In memmoriam

On the 18th of July, 2016, Fabio Martins Mercante an Embrapa Agropecuária Oeste Researcher passed away. He is sorely missed by his family, friends and the wider scientific community who knew and respected him. Most recently, aware of his illness, he dedicated his efforts to knowledge transfer about soil quality in Brazil. We are thankful for his teachings and dedication. He was an inspiration for all of us. Rest in peace our friend.

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4.1 APPENDICES

Table A1. Chemical attributes and physical soil in the soil profile (0-10, 10-20 and 20-40 cm depths) in the two agriculture and livestock farms in the region of Maracaju, Mato Grosso do Sul, Brazil.

Fields	pH H_2O	OM g/dm^3	P mg/dm^3	N g/kg^{-1}	H+Al	S.B.	CEC	Sand	Silt	Clay	Texture
					.....…mmol$_c$/ dm^3 …...			...….....(g/kg^{-1}).....			
Depth 0-10cm											
Farm (A) agriculture and Livestock											
CL-a	5.7a	34.2b	11.7ab	0.14d	4.0a	6.4bc	10.4ab	248	160	620	Clay
CL-b	5.6a	34.2b	12.8a	0.13d	5.3a	5.3c	10.6ab	212	111	677	Clay
CL-c	6.0a	39.1a	14.7a	0.30c	4.1a	9.8a	13.8ab	212	144	644	Clay
CL-d	5.8a	43.8a	6.6b	0.37b	4.9a	7.9a	12.7ab	295	128	629	Clay
SC	5.6a	29.2b	7.6b	0.11d	4.4a	5.7c	10.0b	279	94	627	Clay
F	6.0a	56.4a	3.9c	0.57a	4.0a	11.1a	15.0a	298	127	622	Clay
Farm (B) agriculture and Livestock											
CL-2	5.9a	48.5ab	29.6ab	0.26c	4.4b	9.8ab	13.1bc	298	144	558	Clay
CL-3	6.1ab	50.4a	22.2a	0.35b	3.3b	10.1a	14.5ab	348	110	542	Clay
NT	5.3bc	39.7bc	28.6ab	0.17d	8.3ab	7.8bc	16.1ab	117	186	697	Clay
SC-b	5.8bc	24.6cd	15.5ab	0.12de	5.2b	6.7bc	12.0bc	300	103	597	Clay
CP	6.0a	15.5d	6.1b	0.06e	2.9b	4.2d	7.1c	600	87	313	Sandy Clay
F-b	5.3c	55.6a	7.5b	0.52a	14.2a	5.4cd	19.6a	317	86	614	Clay
Depth 10-20cm											
Farm (A) agriculture and Livestock											
CL-a	5.7a	25.1c	4.2cd	-	4.3b	4.9c	9.2c	248	160	620	Clay
CL-b	5.6a	32.5b	11.2b	-	5.2b	5.1b	10.3ab	212	111	677	Clay
CL-c	5.4a	36.7b	17.3a	-	7.0a	6.3a	13.4a	212	144	644	Clay
CL-d	5.1a	41.4a	6.7c	-	8.0a	6.0a	12.5a	295	128	629	Clay
SC	5.9a	30.7b	16.7a	-	4.0b	5.6b	9.7bc	279	94	627	Clay
F	5.7a	49.5a	1.1d	-	4.7ab	7.9a	12.7a	298	127	622	Clay
Farm (B) agriculture and Livestock											
CL-2	5.4a	42.1a	9.7b	-	6.8b	6.4a	13.2a	298	144	558	Clay
CL-3	5.2a	39.2ab	20.8a	-	7.1ab	5.1a	12.2ab	348	110	542	Clay
NT	5.3a	22.0b	23.6a	-	8.2a	7.2a	15.4a	117	186	697	Clay
SC-b	5.1a	21.3bc	13.5ab	-	7.5ab	2.9b	10.5b	300	103	597	Clay
CP	4.9b	19.2c	1.9c	-	5.7c	1.6c	7.3c	600	87	313	Sandy Clay
F-b	4.9b	47.4a	2.7c	-	10.9a	2.3b	13.2a	317	86	614	Clay
Depth 20-40cm											
Farm (A) agriculture and Livestock											
CL-a	5.3a	24.1d	3.9bc	-	5.7b	3.4bc	9.1ab	248	160	620	Clay
CL-b	5.3a	27.1cd	4.7b	-	6.8a	3.3bc	10.2a	212	111	677	Clay
CL-c	5.5a	34.2ab	2.0cd	-	5.5b	5.4a	10.9a	212	144	644	Clay
CL-d	5.1a	36.0ab	2.1c	-	6.1a	5.7a	9.9ab	295	128	629	Clay
SC	5.2a	29.9c	7.6a	-	5.5b	2.9c	8.4b	279	94	627	Clay
F	5.8a	41.2a	1.0d	-	4.2c	6.8a	11.1a	298	127	622	Clay
Farm (B) agriculture and Livestock											
CL-2	5.5a	22.6b	2.2ab	-	5.2c	5.2a	10.5b	298	144	558	Clay
CL-3	5.1a	37.9a	3.4ab	-	7.1b	3.2ab	10.3bc	348	110	542	Clay
NT	5.2a	16.0c	2.1ab	-	9.1ab	5.9a	15.1a	117	186	697	Clay
SC-b	5.0a	21.0b	7.2a	-	7.0b	2.2b	9.3c	300	103	597	Clay
CP	4.8a	16.5c	1.6b	-	6.2c	1.1c	7.4d	600	87	313	Sandy Clay
F-b	4.8a	38.0a	1.7b	-	12.4a	1.5c	13.9ab	317	86	614	Clay

Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanical harvesting; CP, continuous pasture; F-b, forest fragment. pH, hydrogen potential; OM, organic matter; P, phosphorus; N, total nitrogen; H+Al, acidity potential; S.B., sum of bases; CEC, Cation exchange capacity. Values at a specific depth for an individual farm followed by a similar letter in the column are not significantly different by Duncan test ($p<0.05$). (n=5).

Table A2. Density of individuals and richness of invertebrate fauna community on the top of the soil on farm (A) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Orders / Families	Individuals for Pitfall .....Fields.....						Individuals for Litter fall (m^2) ..... Fields.....					
	A	B	C	D	E	F	A	B	C	D	E	F
Coleoptera												
Carabidae	2	2	1	3	0	0	0	1	0	0	2	8
Chrysomelidae	0	0	0	0	0	1	0	0	3	0	0	0
Cicindelidae	0	0	0	0	0	0	0	0	0	0	0	9
Coccinellidae	0	0	0	0	0	0	0	0	0	0	1	0
Elateridae	0	0	0	0	0	0	0	4	0	0	10	0
Lagriidae	2	1	0	3	0	0	0	0	0	0	0	0
Nitidulidae	0	0	2	0	14	16	0	0	2	1	7	6
Scarabaeidae	3	1	2	3	0	8	2	0	1	1	2	2
Staphylinidae	0	1	1	3	0	0	0	5	0	2	0	3
Tenebrionidae	0	0	0	0	3	0	0	0	0	0	0	0
L. Coleoptera	0	0	0	0	1	2	0	0	0	2	1	0
Diptera												
Agromyzidae	0	0	0	0	0	0	0	0	0	12	0	1
Brachycera	0	0	0	4	0	0	2	0	3	5	0	0
Micropezydae	0	0	0	0	0	0	0	0	0	0	0	1
Muscidae	0	0	0	10	0	0	13	8	3	12	4	10
Mycetophilidae	0	0	0	4	1	0	6	31	0	31	2	16
Phoridae	0	0	0	0	0	0	0	13	0	6	1	11
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	2
Hemiptera												
Cicadellidae	0	0	1	0	0	2	0	0	0	3	0	0
Cicadidae	0	0	0	0	0	4	0	0	6	0	3	2
Lygaeidae	0	0	0	0	0	0	0	0	0	1	0	1
Pyrrhocoridae	0	0	0	0	0	0	0	0	0	0	0	1
Reduviidae	0	0	0	0	0	2	0	0	0	0	0	0
Hymenoptera												
Ceraphronidae	0	0	0	0	0	0	0	0	0	1	0	0
Figitidae	0	0	0	0	0	0	0	0	0	0	0	1
Formicidae	85	45	150	232	38	254	0	0	0	0	0	11
Orussidae	0	0	0	0	0	0	13	51	0	0	0	3
Orthoptera												
Acrididae	0	0	0	0	0	0	0	0	9	0	0	0
Gryllidae	0	0	0	0	0	0	0	3	0	3	59	1
Tettigoniidae	0	0	0	0	0	0	0	0	2	0	1	0
Lepidoptera												
Crambidae	0	0	0	4	2	0	0	0	0	0	0	0
Noctuidae	0	0	0	0	0	0	0	0	0	3	4	0
Blattodea												
Blattidae	0	0	0	0	0	1	0	0	0	0	0	2
Rhinotermitidae	0	0	0	0	0	0	0	0	2	0	0	0
Arachnida												
Araneae	1	0	1	3	2	23	0	0	3	2	4	9
Dermaptera												
Forficulidae	0	0	0	0	0	1	0	0	0	0	0	2
Myriapoda												
Diplopoda	0	2	0	0	0	0	0	0	1	1	0	4
Chilopoda												
Geophilomorfha	0	0	0	0	0	0	0	0	0	1	0	0
Collembola												
Onychiuridae	0	0	0	3	0	3	0	0	0	0	0	0
Gastropoda	0	1	0	0	0	0	0	0	0	0	0	0
Psocoptera	0	0	0	0	5	6	0	0	0	0	0	0
Density	93	53	158	272	66	323	36	116	35	87	101	106

Richness	5	7	7	11	8	13	5	8	11	17	14	22

A= CL-a, crop-livestock integration system: third year pasture; B= CL-b, first year of crop rotation after grazing; C= CL-c, second year of crop rotation after grazing; D= CL-d, third year of crop rotation after grazing; E= SC, sugarcane with mechanized harvesting. F= F, forest fragment. (n=5).

Table A3. Density of individuals and richness of invertebrate fauna of community in soil (0-10 cm, 10-20 cm, 20-40 cm depths) at farm (A) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Orders / Families	Depth 0-10cm ..... Fields.....						Depth 10-20cm ..... Fields.....						Depth 20-40cm ..... Fields.....					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Coleoptera																		
Carabidae	0	0	1	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae	1	2	1	5	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Lagriidae	0	0	1	2	0	3	0	0	0	0	1	0	0	0	0	0	0	0
Nitidulidae	0	2	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Passalidae	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Scarabaeidae	4	1	2	3	1	3	0	2	0	0	3	1	0	0	0	3	0	0
Staphylinidae	0	0	0	2	1	3	0	0	1	0	0	2	0	0	0	0	0	0
Tenebrionidae	3	0	2	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
L. Coleoptera	1	0	0	2	2	3	0	1	0	5	1	2	2	2	0	0	0	0
Hemiptera																		
Cicadidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cicadellidae	0	6	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Cydnidae	0	0	0	0	0	2	0	0	15	0	9	1	0	0	2	2	0	0
Hymenoptera																		
Ceraphronidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Formicidae	4	20	5	4	4	1	3	0	0	45	2	2	3	0	1	1	3	18
Diptera																		
Mycetophilidae	0	0	1	2	2	2	0	0	0	1	0	0	0	0	0	0	0	0
Muscidae	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
L. Diptera	0	0	0	0	0	4	0	0	0	0	0	3	0	0	0	0	0	0
Blattodea																		
Blattidae	0	0	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Rhinotermitidae	0	2	0	0	0	4	1	0	0	0	0	3	0	0	0	0	0	0
Haplotaxida																		
Lumbricidae	1	0	15	5	4	3	4	1	0	4	15	6	3	2	2	15	4	3
Orthoptera																		
Gryllidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Arachnida																		
Araneae	0	3	0	1	2	2	0	0	1	0	0	1	0	0	0	0	0	0
Myriapoda																		
Diplopoda	0	1	2	2	0	2	0	0	0	0	0	0	0	0	0	0	0	2
Dermaptera																		
Forficulidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilopoda																		
Geophilomorfha	0	0	0	1	0	3	0	0	0	0	0	2	0	0	0	0	0	0
Density	14	37	31	39	26	52	8	4	17	55	31	23	8	4	5	21	7	24
Richness	6	8	10	16	11	19	3	3	3	4	6	10	3	2	3	4	2	4

A= CL-a, crop-livestock integration system: third year pasture; B= CL-b, first year of crop rotation after grazing; C= CL-c, second year of crop rotation after grazing; D= CL-d, third year of crop rotation after grazing; E= SC, sugarcane with mechanized harvesting. F= F, forest fragment. (n=5).

Table A4. Frequency and richness of the ant community at the soil surface at farm (A) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Morfoespécies	Individuals for Pitfall ..... Fields.....						Individuals for Litter fall ..... Fields.....					
	A	B	C	D	E	F	A	B	C	D	E	F
Anochetus sp.	0	0	0	0	0	0	0	0	0	0	0	1
Atta sexdens	0	0	0	0	1	1	0	0	0	0	0	1
Brachymyrmex patagonicus	1	1	1	1	1	0	0	0	0	0	0	0
Brachymyrmex sp. 2	0	0	0	0	0	1	0	0	0	0	0	0
Brachymyrmex sp. 3	0	0	0	0	0	1	0	0	0	0	0	0
Camponotus blandos	0	0	0	0	0	1	0	0	0	0	0	0
Camponotus sp. 2	0	0	0	0	0	1	0	0	0	0	0	0
Cephalotes sp.	0	0	0	0	0	0	0	0	0	0	0	1
Dorymyrmex sp.	1	1	1	0	1	0	0	0	0	0	0	0
Gnamptogenys striatula	1	0	0	0	0	0	0	0	0	0	0	0
Myrmicinae sp.	0	0	0	0	0	1	0	0	0	0	0	0
Odontomachus sp.	0	0	0	0	0	0	0	0	0	0	0	1
Odontomachus chelífer	1	0	0	0	0	1	0	0	0	0	0	0
Pachycondula harpax	0	0	0	0	0	0	0	0	0	0	0	1
Pheidole gertrudae	0	0	0	0	1	1	0	0	0	0	0	0
Pheidole oxyops	1	1	1	1	1	1	0	0	0	0	0	0
Pheidole sp.	0	0	0	0	1	0	0	0	0	0	0	0
Pheidole sp. 1	1	1	1	0	0	1	0	0	0	0	0	1
Pheidole sp. 2	1	0	0	0	0	1	0	0	0	0	0	0
Pheidole sp. 3	0	0	0	0	0	1	0	0	0	0	0	0
Pheidole sp. 4	0	0	0	0	0	1	0	0	0	0	0	0
Pseudomyrmex termitarius	1	0	0	0	1	0	0	0	0	0	0	0
Solenopsis sp. 1	0	0	0	1	0	0	0	0	0	0	0	0
Richness	8	4	4	3	7	13	0	0	0	0	0	6

A= CL-a, crop-livestock integration system: third year pasture; B= CL-b, first year of crop rotation after grazing; C= CL-c, second year of crop rotation after grazing; D= CL-d, third year of crop rotation after grazing; E= SC, sugarcane with mechanized harvesting. F= F, forest fragment (n=5). Individuals for litter fall (m^2).

Table A5. Frequency and richness of the ant community in soil (0-10 cm, 10-20 cm, 20-40 cm depths) at farm (A) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Morfoespécies	Depth 0-10cm ..... Fields.....						Depth 10-20cm ..... Fields.....						Depth 20-40cm ..... Fields.....					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Anochetus sp.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Atta sexdens	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Brachymyrmex sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachymyrmex patagonicus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Brachymyrmex patagonicus Queen	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Dorymyrmex sp.	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Hypoponera sp.1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pheidole oxyops	1	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0
Pheidole sp. Queen	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pheidole sp.4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Solenopsis sp. 1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solenopsis sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Richness	2	2	1	1	3	1	2	0	0	1	1	2	2	0	1	1	1	1

A= CL-a, crop-livestock integration system: third year pasture; B= CL-b, first year of crop rotation after grazing; C= CL-c, second year of crop rotation after grazing; D= CL-d, third year of crop rotation after grazing; E= SC, sugarcane with mechanized harvesting. F= F, forest fragment (n=5).

Table A6. Density of individuals and richness of invertebrate fauna community on the top of the soil at farm (B) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Orders / Families	Individuals for Pitfall ..... Fields.....						Individuals for Litter fall (m^2) ..... Fields.....					
	G	H	I	J	L	M	G	H	I	J	L	M
Coleoptera												
Carabidae	1	0	3	0	0	2	1	1	4	0	0	1
Chrysomelidae	0	0	0	0	0	0	0	1	1	0	0	3
Cicindelidae	0	0	0	2	0	0	0	0	0	0	0	0
Coreidae	0	0	0	0	0	0	0	1	0	0	0	0
Elateridae	0	30	1	0	0	0	0	1	0	0	0	0
Lagriidae	0	6	0	0	0	0	0	10	0	0	0	1
Meloidae	0	0	0	0	3	0	2	3	0	0	0	2
Nitidulidae	0	54	2	1	0	114	0	0	0	0	0	0
Passalidae	0	0	0	0	0	0	0	0	0	2	0	0
Scarabaeidae	11	0	0	3	2	8	2	2	1	10	3	9
Staphylinidae	0	4	0	0	0	2	0	0	0	5	0	3
Tenebrionidae	0	0	0	0	0	0	0	1	0	0	0	0
L. Coleoptera	0	0	0	1	2	0	3	0	0	27	0	2
Hymenoptera												
Ceraphronidae	0	3	0	0	0	0	0	0	0	0	0	0
Cynipidae	0	2	0	0	0	2	0	0	0	0	0	0
Diapriidae	0	1	0	0	0	0	0	0	0	0	0	0
Formicidae	47	39	11	52	57	156	0	0	0	40	0	1
Mutilidae	0	0	0	0	0	13	0	0	0	0	0	0
Orussidae	0	0	0	0	0	1	0	0	0	0	0	0
Pompilidae	1	0	3	4	0	1	0	0	0	0	0	0
Diptera												
Agromyzidae	0	0	0	2	0	0	0	0	0	0	0	0
Micropezydae	1	0	0	0	0	0	0	0	0	0	3	0
Muscidae	30	2	2	18	0	47	0	0	0	0	0	0
Mycetophilidae	0	53	0	0	2	10	2	0	0	0	0	0
Phoridae	0	46	0	0	0	0	0	0	0	0	0	0
Scenopinidae	0	0	0	0	0	0	0	0	0	1	0	0
Sciaridae	0	0	0	0	0	0	0	0	0	2	0	0
L. Diptera	0	0	0	0	0	1	0	0	0	5	0	5
Hemiptera												
Belostomatidae	0	0	0	0	0	1	0	0	0	0	0	0
Cercopidae	5	1	0	2	0	1	0	0	0	0	0	5
Cicadellidae	0	0	0	0	0	24	0	0	0	0	0	2
Cicadidae	1	2	0	0	0	1	0	0	0	0	0	0
Flatidae	0	0	0	0	0	0	0	0	0	0	0	1
Pentatomidae	0	0	0	0	0	0	0	1	0	0	0	0
Pyrrhocoridae	1	0	0	0	0	0	0	0	0	0	0	0
Orthoptera												
Acrididae	5	0	0	2	0	1	0	0	0	0	0	0
Gryllidae	2	0	0	0	0	2	0	0	0	0	5	0
Tettigoniidae	0	0	0	0	0	17	0	0	0	0	0	0
Lepdoptera												
Noctuidae	1	0	1	15	0	1	0	0	0	0	4	0
Pyralidae	0	0	3	0	0	0	0	0	0	0	4	0
Blattodea												
Blattidae	0	0	0	0	0	0	0	0	0	7	0	2
Rhinotermitidae	0	0	0	0	0	0	0	0	0	13	0	4
Arachnida												
Araneae	2	3	3	18	2	17	3	4	4	2	0	6
Dermaptera												
Forficulidae	0	1	0	1	0	0	0	0	1	2	0	0
Myriapoda												

Diplopoda	0	0	0	38	0	0	0	0	0	0	0	0
Chilopoda												
Geophilomorfha	0	0	0	0	3	0	3	0	0	3	0	3
Collembola												
Onychiuridae	0	96	0	0	0	0	0	0	0	0	0	0
Haplotaxida												
Lumbricidae	0	0	0	0	0	0	0	0	0	59	0	0
Gastropoda	0	0	0	0	0	0	0	0	0	0	0	1
Density	108	343	29	159	71	422	16	25	11	178	19	51
Richness	13	16	9	14	7	21	7	10	5	14	5	17

G= CL-2, second year of crop-livestock integration cycle; H= CL-3, third year of crop-livestock integration cycle; I= NT, no-tillage; J= SC-b, sugarcane with mechanical harvesting; L= CP, continuous pasture; M= F-b, forest fragment (n=5).

Table A7. Density of individuals and richness of invertebrate fauna of community in soil (0-10cm, 10-20cm, 20-40cm depths) at farm (B) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Orders / Families	Depth 0-10cm ..... Fields.....						Depth 10-20cm ..... Fields.....						Depth 20-40cm ..... Fields.....					
	G	H	I	J	L	M	G	H	I	J	L	M	G	H	I	J	L	M
Coleoptera																		
Carabidae	0	2	0	0	1	4	0	0	0	0	0	0	1	5	0	0	0	1
Lagriidae	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Nitidulidae	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
Passalidae	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Scarabaeidae	4	1	0	0	0	4	0	2	0	0	0	3	1	0	1	0	0	0
Staphylinidae	0	1	2	0	0	3	0	1	0	0	0	0	0	0	0	0	0	2
L. Coleoptera	0	7	0	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0
Hemiptera																		
Cercopididae	4	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Cicadellidae	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Lygaeidae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Miridae	0	0	0	0	0	3	0	0	0	0	0	0	0	0	1	1	0	0
Pentatomidae	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Pyrrhocoridae	0	0	0	0	0	3	0	1	0	0	0	0	0	7	0	0	0	0
Hymenoptera																		
Ceraphronidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formicidae	0	0	0	0	2	7	0	1	0	0	2	2	0	2	0	2	3	0
Pompilidae	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera																		
Muscidae	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Haplotaxida																		
Lumbricidae	4	2	8	0	8	6	0	0	2	4	4	4	0	0	3	2	2	2
Orthoptera																		
Gryllidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Arachnida																		
Araneae	2	1	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Myriapoda																		
Diplopoda	0	0	8	0	0	2	0	1	0	2	0	1	0	0	0	0	0	1
Dermaptera																		
Forficulidae	0	0	1	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Chilopoda																		
Geophilomorfha	0	2	1	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0
Gastropoda	0	0	0	5	3	0	0	0	0	2	0	0	0	0	0	0	0	0
Density	18	22	20	10	16	44	7	5	5	8	7	13	2	16	5	5	5	6
Richness	6	11	5	4	5	13	4	4	2	3	3	6	2	4	3	3	2	4

G= CL-2, second year of crop-livestock integration cycle; H= CL-3, third year of crop-livestock integration cycle; I= NT, no-tillage; J= SC-b, sugarcane with mechanical harvesting; L= CP, continuous pasture; M= F-b, forest fragment (n=5).

Table A8. Frequency and richness of the ant community at the top of the soil at farm (B) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Morfoespécies	Individuals for Pitfall ..... Fields.....						Individuals for Litter fall ..... Fields.....					
	G	H	I	J	L	M	G	H	I	J	L	M
Azteca sp.	0	0	0	1	0	1	0	0	0	0	0	0
Atta sexdens	0	0	0	1	1	1	0	0	0	0	0	0
Brachymyrmex patagonicus	0	1	1	1	1	1	0	0	0	1	0	0
Brachymyrmex sp. 2	1	0	0	0	0	1	0	0	0	0	0	0
Brachymyrmex sp. 3	0	0	0	1	0	1	0	0	0	0	0	0
Camponotus blandos	0	0	0	0	0	1	0	0	0	0	0	1
Camponotus crassus	0	0	0	0	0	1	0	0	0	0	0	0
Camponotus myrmaphaenus	0	0	0	0	0	1	0	0	0	0	0	0
Dorymyrmex sp.	0	0	0	1	1	1	0	0	0	0	0	0
Linepithema sp.	0	0	0	0	0	1	0	0	0	0	0	0
Mycocepurus sp.	0	0	0	0	0	1	0	0	0	0	0	0
Ochetomyrmex neopolitus	0	0	0	0	0	1	0	0	0	0	0	0
Odontomachus chelífer	0	0	0	0	1	1	0	0	0	0	0	0
Pheidole gertrudae	1	0	0	0	0	1	0	0	0	0	0	0
Pheidole oxyops	1	1	1	1	1	1	0	0	0	1	0	0
Pheidole sp.	0	1	0	0	0	0	0	0	0	0	0	0
Pheidole sp. 1	0	1	1	0	0	1	0	0	0	0	0	0
Pheidole sp. 3	0	0	0	0	1	1	0	0	0	0	0	0
Pheidole sp. 5	0	0	0	0	0	1	0	0	0	0	0	0
Sericomyrmex harekulli	0	0	0	0	0	1	0	0	0	0	0	0
Solenopsis sp. 1	1	0	0	0	0	0	0	0	0	0	0	0
Richness	4	4	3	6	6	19	0	0	0	2	0	1

G= CL-2, second year of crop-livestock integration cycle; H= CL-3, third year of crop-livestock integration cycle; I= NT, no-tillage; J= SC-b, sugarcane with mechanical harvesting; L= CP, continuous pasture; M= F-b, forest fragment. (n=5).

Table A9. Frequency and richness of the ant community in soil (0-10cm, 10-20cm, 20-40cm depths) at farm (B) in the region of Maracaju, Mato Grosso do Sul, Brazil.

Morfoespécies	Depth 0-10cm ..... Fields.....						Depth 10-20cm ..... Fields.....						Depth 20-40cm ..... Fields.....					
	G	H	I	J	L	M	G	H	I	J	L	M	G	H	I	J	L	M
Brachymyrmex patagonicus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
Camponotus blandus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Dorymyrmex sp.	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
Hypoponera Queen	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Hypoponera sp. 2	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Pheidole oxyops	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0
Solenopsis sp. 2 Queen	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Solenopsis sp. 3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Richness	0	0	0	0	2	5	0	1	0	0	1	2	0	1	0	1	1	0

G= CL-2, second year of crop-livestock integration cycle; H= CL-3, third year of crop-livestock integration cycle; I= NT, no-tillage; J= SC-b, sugarcane with mechanical harvesting; L= CP, continuous pasture; M= F-b, forest fragment. (n=5).

Table A10. Invertebrate fauna captured at the top of the soil and in the soil profile (0-10, 10-20, 20-40 cm depths) and their functional groups. Farms A and B in the region of Maracaju, Mato Grosso do Sul, Brazil..

Families	Functional group
Carabidae	Predators
Chrysomelidae	Degraders
Cicindelidae	Predators
Coccinellidae	Predators
Coreidae	Phytophagous / Predators
Elateridae	Detritivores / Phytophagous / Predators
Lagriidae	Phytophagous
Nitidulidae	Detritivores
Meloidae	Phytophagous
Scarabaeidae	Degraders
Staphylinidae	Predators / Degraders
Tenebrionidae	Phytophagous
Passalidae	Detritivores
Agromyzidae	Phytophagous
Brachycera	Detritivores / Predators
Micropezydae	Detritivores / Predators
Muscidae	Degraders
Mycetophilidae	Phytophagous / Detritivores
Phoridae	Detritivores
Tabanidae	Predators / Hematophagous
Scenopinidae	Detritivores / Myxophagous
Sciaridae	Predators / Detritivores / Myxophagous
Belostomatidae	Predators
Cercopidae	Phytophagous
Cicadellidae	Phytophagous
Cicadidae	Phytophagous
Cydnidae	Phytophagous
Flatidae	Phytophagous
Lygaeidae	Predators
Miridae	Phytophagous
Pentatomidae	Phytophagous / Predators
Pyrrhocoridae	Phytophagous
Reduviidae	Predators / Hematophagous
Ceraphronidae	Parasitic/ Detritivores
Cynipidae	Parasitic
Diapriidae	Parasitic
Figitidae	Parasitic
Formicidae	Phytophagous / Predators
Mutilidae	Parasitic
Orussidae	Phytophagous
Pompilidae	Predators
Acrididae	Phytophagous
Gryllidae	Phytophagous
Tettigoniidae	Phytophagous
Crambidae	Phytophagous
Noctuidae	Phytophagous
Pyralidae	Phytophagous
Blattidae	Degraders
Rhinotermitidae	Phytophagous / Degraders
Araneae	Phytophagous / Predators
Forficulidae	Degraders / Predators
Diplopoda	Detritivores / Phytophagous
Geophilomorfha	Predators
Onychiuridae	Degraders / Microphytophagous
Lumbricidae	Detritivores / Phytophagous
Gastropoda	Detritivores / Phytophagous

Psocoptera	Detritivores / Phytophagous

Functional group and soil invertebrate fauna according to Borror & Delong (1969), Righi (1997), Triplehorn & Jonnson (2010), Lavelle & Kohlmann (1984) and Eisenbeis & Wichard (2012).

Table A11. Ants captured at the top of the soil and in the soil profile (0-10, 10-20, 20-40 cm depths) and their Guild. Farms A and B, Maracaju, Mato Grosso do Sul, Brazil.

Morfoespécies	Guild
Anochetus sp.	Predators
Azteca sp.	Omnivorous / Dolichoderineas aggressive
Atta sexdens	Leaf stripper / fungivorous
Brachymyrmex patagonicus	Omnivorous
Brachymyrmex sp.	Omnivorous / Opportunist soil and vegetation
Camponotus sp.	Generalist omnivorous
Camponotus blandus	Omnivorous
Camponotus crassus	Omnivorous
Camponotus myrmaphaenus	Omnivorous
Cephalotes sp.	Arboreal / Omnivorous
Dorymyrmex sp.	Generalist omnivorous
Gnamptogenys striatula	Generalist predator
Hypoponera sp.	Generalist predator epigaeic
Linepithema sp.	Omnivorous
Mycocepurus sp.	Leaf stripper / fungivorous
Myrmicinae sp.	Diverse feed habits
Ochetomyrmex neopolitus	-
Odontomachus chelífer	Large-sized predators
Pheidole sp.	Generalist omnivorous
Pheidole gertrudae	Generalist omnivorous
Pheidole oxyops	Generalist omnivorous
Pseudomyrmex termitarius	Predators / Visitor of extrafloral nectaries
Pachycondula harpax	Generalist predator epigaeic
Sericomyrmex harekulli	Fungivorous
Solenopsis sp.	Generalist omnivorous

Guild and soil ants according to Bolton, 1994; Silvestre & Silva, 2001, Fernandez, 2003; Ward, 2012, Antwik. org.

CHAPTER 4 - FINAL CONSIDERATIONS

The present work showed in the crop-livestock integration system (three-year cycle), that the third year of pasture reduces soil quality mainly in the more superficial layers. Following the cycle, the presence of the crop rotation in no-tillage regains the conditions of the soil profile during the three-year period. In farm B, the crop-livestock integration (one-year cycle) conducted in winter in a corn consortium with *Brachiaria* and summer pasture also favored soil quality in relationship to the other evaluated systems. It is noted that this effect on soil profile conditions is intensified throughout the time of the crop-livestock integration management.

Another important result was in the fields under mechanical harvesting of sugarcane that favored the diversity of microorganisms and invertebrates mainly in the superficial layers of the soil. After periods of five and six years of mechanical harvesting of sugarcane, large volumes of plant residues were accumulated in the soil, and these conditions may have favored the establishment of biological communities. On the other hand, the presence of machinery over time led to the physical modification of the soil, with lower porosity and, consequently, greater compaction.

The different methods of agricultural and livestock management may help in decision making by the agricultural sector in the search for more sustainable management of corn, soybean, sugarcane and meat. In addition, with the results obtained, one can add another "positive characteristic" of these systems, since they favor greater diversity of microorganisms and invertebrate fauna. These are important communities for soil processes such as nutrient cycling.

Therefore, the present work contributes to the evaluation of the sustainability of production systems and conservation of natural resources (soil and water) in tropical soils, considering the continuous use of the soils mainly for the production of soybean, corn, sugarcane, and meat. This work opens doors to other research that includes the evaluation of the soil profile and the characterization of the sustainability of production systems in Mato Grosso do Sul, Brazil.