



Environmental Microbiology

Comparing how land use change impacts soil microbial catabolic respiration in Southwestern Amazon



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ABSTRACT

Land use changes strongly impact soil functions, particularly microbial biomass diversity and activity. We hypothesized that the catabolic respiration response of the microbial biomass would differ depending on land use and that these differences would be consistent at the landscape scale. In the present study, we analyzed the catabolic response profile of the soil microbial biomass through substrate-induced respiration in different land uses over a wide geographical range in Mato Grosso and Rondônia state (Southwest Amazon region). We analyzed the differences among native areas, pastures and crop areas and within each land use and examined only native areas (Forest, Dense Cerrado and Cerrado), pastures (Nominal, Degraded and Improved) and crop areas (Perennial, No-Tillage, Conventional Tillage). The metabolic profile of the microbial biomass was accessed using substrate-induced respiration. Pasture soils showed significant responses to amino acids and carboxylic acids, whereas native areas showed higher responses to malonic acid, malic acid and succinic acid. Within each land use category, the catabolic responses showed similar patterns in both large general comparisons (native area, pasture and crop areas) and more specific comparisons (biomes, pastures and crop types). The results showed that the catabolic responses of the microbial biomass are highly correlated with land use, independent of soil type or climate. The substrate induced respiration approach is useful to discriminate microbial communities, even on a large scale.

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Introduction

The growing demand for food, fiber and biofuels has led to many environmental problems and primarily reflect the occupation of the world's greatest agricultural frontier: the border between the Amazon rainforest and the Cerrado (savanna) of central Brazil.¹ Generally, the flat topography eases the mechanization and incentivizes the occupation of this region. The southwestern Amazon, particularly the states of Rondônia (RO) and Mato Grosso (MT), still practice deforestation for agricultural land use.² The intensive land use invariably has negative effects on both the environment and agricultural productivity when conservation practices are not adopted.^{3,4} Interest in the maintenance of soil quality, consistent with Karlen et al.,⁵ is essential for the sustainability of these new agricultural areas.

Increasing evidence has shown that soil microbial attributes are potential early indicators of the changes in soil quality because these parameters are more sensitive than are the chemical and physical properties of soil.^{6,7} The microbial biomass has been characterized as a sensitive index for changes in the soil organic carbon that result from management and land use. Initially, microbial biomass undergoes fluctuations until it reaches a new equilibrium.⁸ One common method for measuring the metabolic function of soil microorganisms is the catabolic response profile.^{9,10} According to San Miguel et al.,¹¹ analyzing the functional and catabolic diversity is important because it is difficult to infer whether some soil functions have been lost solely based on changes in genetic diversity. Stevenson et al.¹² showed different patterns in the catabolic capacity of the microbial community under forests and pastures in New Zealand.

In most land uses under agricultural practices (plowing, fertilizing, liming, pesticide application and other inputs), the available soil niches might be affected. Each change represents a renewal of selection pressure, which favors some components of the microbial community while eliminating others, thereby relocating the equilibrium between populations. Microbial diversity reduction implies the loss of species that metabolize certain functional groups, which results in a decrease in the resilience of the system.¹³ The aim of the present study was to increase the current knowledge concerning the impact of land use and land use changes on the metabolic capacity of the soil microbial biomass. Therefore, the objective of the present study was to compare the functional diversity of the soil microbial biomass in the natural vegetation prior to human interference and after land use changes. To this end, we sampled native areas, pastures and agricultural areas in the Southwest Amazon Region.

Materials and methods

Study area

The study area focuses on the states of Rondônia and Mato Grosso, which form a transitional region between the Amazon Basin and the highlands of the Brazilian Central Plateau. The regional climate varies according to latitude and is

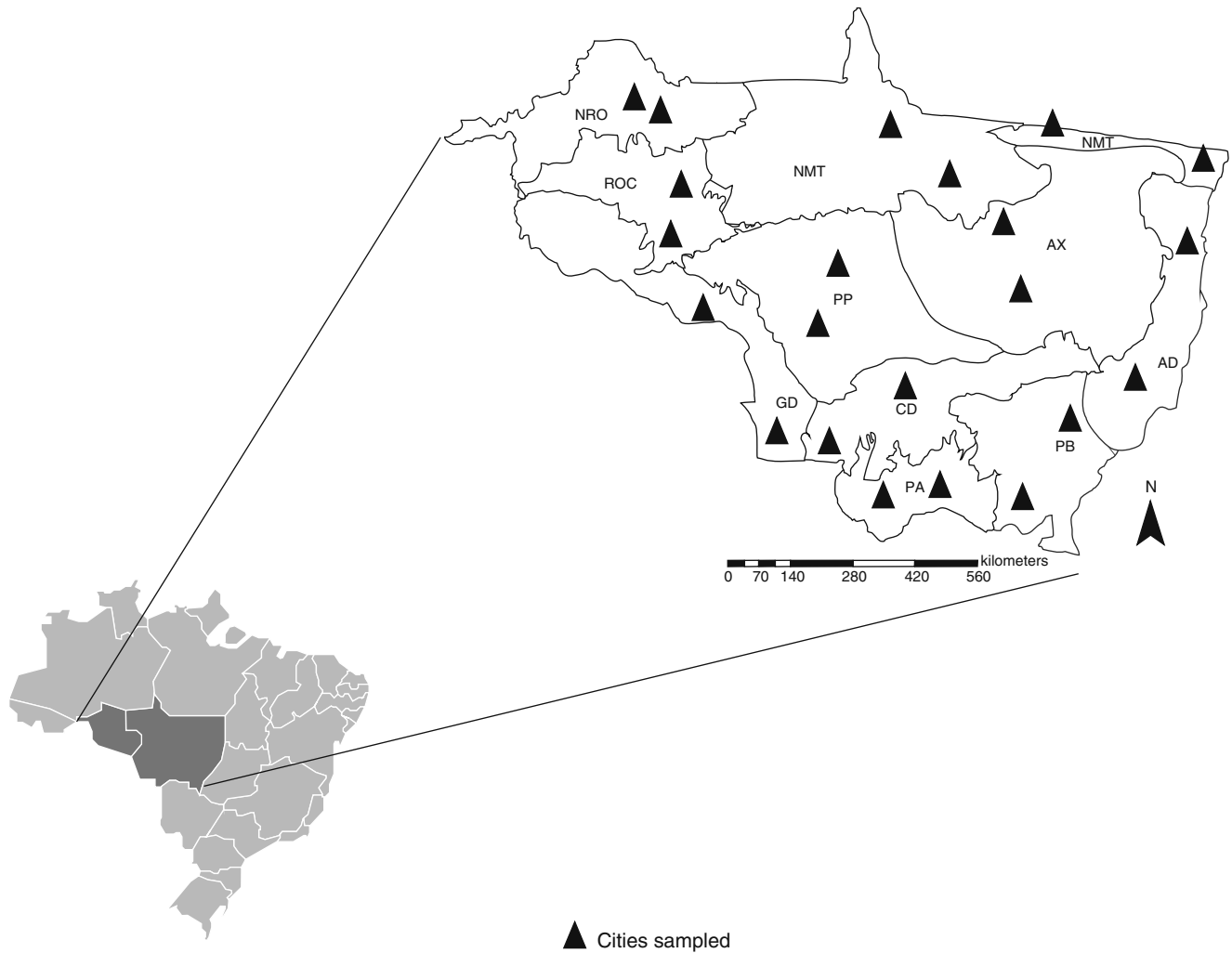
characterized as a humid tropical regime with short dry seasons. The sites covering the main bio and geo-climatic zones of the States of Rondônia and Mato Grosso (Fig. 1) were selected according to the Intergovernmental Panel for Climate Changes "Guidelines for National Greenhouse Gas Inventories".¹⁴ The delimitation of the zones was performed using the Geographic Information System ArcGis 9.0 with combined information on soils, native vegetation, geology, climate and relief. This methodology generated relatively homogeneous areas, thus facilitating the extrapolation of the microbiological parameters for the entire region. In each of the 11 zones, two sites were randomly selected, totaling 22 sampling points (Fig. 1). In all sites, we identified and sampled soil from one native area, one pasture and one agricultural area, totalizing 66 sampling points. In these locations, the soil of native systems was sampled to determine chemical, physical and microbiological attributes. The general characteristics of each ecoregion are provided in Table 1 (adapted from Maia et al.¹⁵) and in Online Appendix 1.

Soil samples collection

Soil cores were collected at 0–10 cm depths in five replicates from each area, for a total of 330 samples (110 samples in each management system). The soil samples were broken apart and sieved through a 2-mm mesh to remove rocks and plant fragments. Native areas were denominated Forest ($n=60$); Cerrado ($n=25$), which was defined as the tropical savanna, species-rich dense vegetation of shrubs and trees, 8–10 m high, with a grass undergrowth¹⁶; and Dense Cerrado or "Cerradão" ($n=25$), which was similar to a woodland savannah, with trees up to 20 m high. The pastures were described as Improved ($n=25$) when at least one improvement (fertilizer or lime application, irrigation) was received; Nominal ($n=25$) when reasonable productivity was maintained, despite no improvements occurring; and Degraded ($n=60$) when the typical management was received and a reduction in productivity due to weed infestation, bare soil and/or soil erosion.¹⁵ The agricultural sites were divided into Conventional-Tillage ($n=70$) when plant residue was incorporated into the soil and aggregates were routinely disrupted through tillage (physical release of protected organic matter); No-tillage ($n=20$); and perennial crops ($n=20$).

Substrate-induced respiration (SIR)

Estimates of the catabolic diversity in the soil microbial community were obtained based on the short-term respiratory responses of soil samples, consistent with Degens and Harris.⁹ Substrates (as 2 mL solution) were added to a 1-g equivalent dry weight of soil in MacCartney bottles sealed with vacutainer stoppers. The following substrates were included in this analysis: 2 amines (glutamine and glucosamine), 6 amino acids (arginine, glutamic acid, asparagine, histidine, lysine and serine), 2 carbohydrates (glucose and mannose) and 12 carboxylic acids (citric acid, ascorbic acid, gluconic acid, fumaric acid, malonic acid, malic acid, ketoglutaric acid, ketobutyric acid, pantothenic acid, quinic acid, succinic acid and tartaric acid). An analysis with water was also performed. The CO₂ flux from each sample was measured using an IRGA (LICOR-6262 Model) after incubation for 4 h at 25 °C.



▲ Cities sampled

Fig. 1 – Distribution of the ecoregions in the study area. AX, Alto Xingu; PB, Parana Basin; PP, Parecis Plateau; AD, Araguaia Depression; CD, Cuiabá Depression; DG, Guaporé Depression; NMT, North of Mato Grosso; NRO, North of Rondônia; PA, Pantanal; ROC, Central Rondônia.

Table 1 – Description of the ecoregions analyzed.

Ecoregion	Soil	Vegetation	Climate
Alto Xingu	Oxisols	Seasonal semi-deciduous forest to open Amazon forest	Climate: Ami – Rainfall: 1750–2250 mm year ⁻¹
Parana Basin	Oxisols and Quartzipsamments	Cerrado <i>sensu stricto</i>	Climate: Am and Cwa – Rainfall: 1250–1750 mm year ⁻¹
Parecis Plateau	Quartzipsamments and Oxisols	Cerrado <i>sensu stricto</i> and Seasonal semi-deciduous forest	Climate: Ami – Rainfall: 1500–2250 mm year ⁻¹
Araguaia Depression	Entisols and Aquent Entisols	Open Cerrado (dominated by grasses) and Cerrado <i>sensu stricto</i>	Climate: Ami – Rainfall: 1250–2000 mm year ⁻¹
Cuaiba Depression	Inceptisols and Entisols	Cerrado <i>sensu stricto</i>	Climate: Am – Rainfall: 1500–1750 mm year ⁻¹
Guapore Depression	Oxisols, Ultisols and Inceptisols	Open Amazon forest (north) and Seasonal semi-deciduous forest to Cerrado (south)	Climate: Ami – Rainfall: 1750–2250 mm year ⁻¹
Northeast of Mato Grosso	Ultisols	Cerrado to a Seasonal semi-deciduous forest	Climate: Ami – Rainfall: 2000–2250 mm year ⁻¹
North of Rondônia	Oxisols	Open Amazon Forest	Climate: Awi – Rainfall: 2000–2500 mm year ⁻¹
North of Mato Grosso	Ultisols, Oxisols and Inceptisols	Open Amazon Forest to Seasonal semi-deciduous forest	Climate: Ami and Awi – Rainfall: 2000–2700 mm year ⁻¹
Pantanal	Entisols and Alfisols	Open Cerrado and Seasonal semi-deciduous forest	Climate: Am and Cwa – Rainfall: 15,000–1750 mm year ⁻¹
Central Rondônia	Ultisols and Oxisols	Open Amazon Forest	Climate: Ami and Awi – Rainfall: 1750–2250 mm year ⁻¹

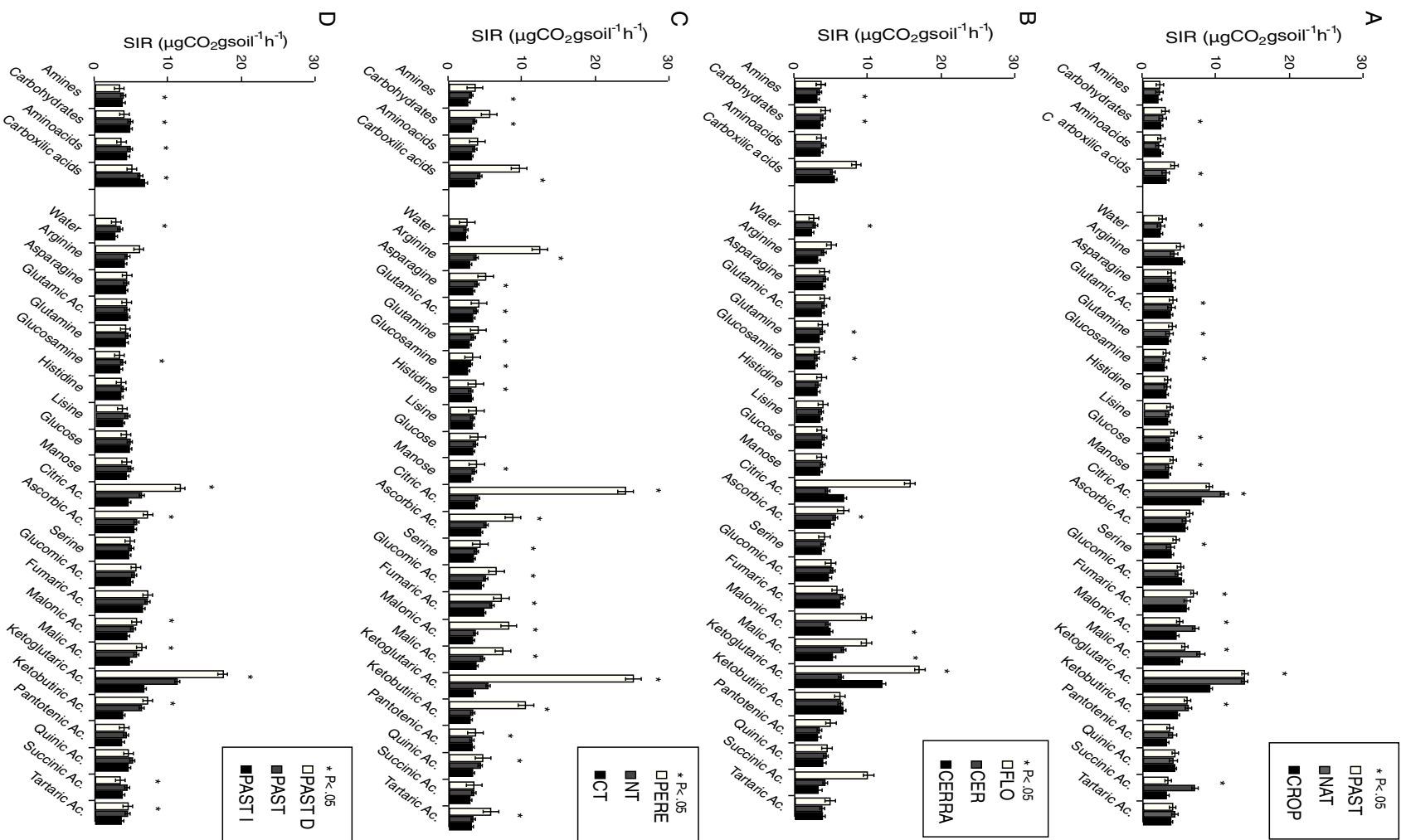


Fig. 2 – Community level physiological profile of the land uses analyzed. (A) Differences between the land uses: PAST I, pasture; NAT, native areas; CROP, areas under crops. (B) Differences within Native Areas: FLO, forest areas; CER, Cerrado areas; CERRA, Dense-Cerrado areas. (C) Differences within agricultural areas: PERE, perennial crops; CT, conventional-tillage; NT, no-tillage. (D) Differences within pasture areas: PAST I, nominal pasture; PAST D, degraded pasture; PAST I, improved pasture.

Chemical analysis

Total carbon was measured through dry combustion on an LECO CN elemental analyzer (furnace at 1350 °C in pure oxygen). The bulk density (ρ) was measured from each pit and layer using a volumetric (100 cm³) steel ring. For each soil layer, the carbon stocks were calculated after multiplying the concentration of the C (g g⁻¹) by ρ (kg m⁻³) and the layer thickness (m). The soil pH was assessed in both the water and the KCl solution.¹⁷

Statistical analysis

One-way analysis of variance (ANOVA) was applied to determine significant differences between the land uses analyzed. Tukey's test was applied at 5% significance. The Canonical Variate Analysis (CVA) was applied to determine whether the SIR can distinguish the land uses.

Results

Comparison between land uses

Pasture soils showed higher responses to the amino acid and carboxylic acid groups and to the individual substrates glucose, mannose, serine, fumaric acid, ketoglutaric acid, and ketobutyric acid (Fig. 2). Native areas had higher responses to malonic acid, malic acid and succinic acid. CVA showed good separation of the areas using SIR (Fig. 3). The substrates that contributed the observed separation in the first canonical axis (CV1) were glucosamine and glutamic acid (positive value), and asparagine (negative value) contributed to the observed separation of the pasture areas from the crop and native areas. Serine (negative value) was highlighted in the

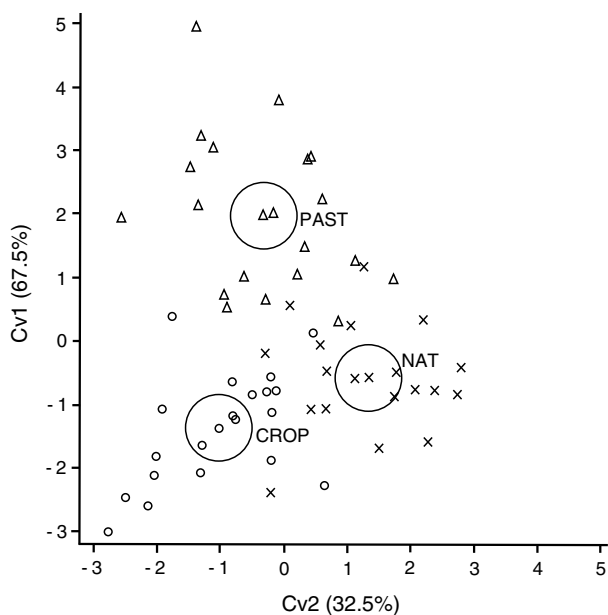


Fig. 3 – Canonical variate analysis of the catabolic profile of microorganisms in the studied areas. ○ (CROP) crop areas; × (NAT) native areas; Δ (PAST) pasture areas. The circle represents the 95% confidence area.

Table 2 – Canonical variate vectors and means for the comparison between land uses.

Native areas		
% Variation		
CV1	67.50	
CV2	32.50	
Total	100	
Vectors	CV1	CV2
Water	-2.46	-0.24
Arginine	0.37	-0.16
Asparagine	-3.29	0.59
Glutamic Ac.	3.08	-0.83
Glutamine	-0.88	0.83
Glucosamine	4.12	-0.88
Histidine	-2.30	-0.16
Lysine	0.64	-0.12
Glucose	0.23	0.81
Mannose	1.27	0.79
Citric Ac	0.10	-0.36
Ascorbic Ac	-0.09	0.33
Serine	1.66	-1.61
Gluconic Ac	-0.78	0.24
Fumaric Ac	0.98	-0.50
Malonic Ac	0.33	-0.17
Malic Ac	0.32	-0.06
Ketoglutaric Ac	-0.02	0.05
Ketobutyric Ac	-0.19	0.52
Pantothenic Ac	0.83	0.88
Quinic Ac	-2.07	-1.73
Succinic Ac	-0.55	0.37
Tartaric Ac	-0.98	1.75
C.M.	CV1	CV2
CROP	-1.38	-1.02
NAT	-0.58	1.34
PAST	1.96	-0.32

C.M., canonical mean; CROP, crop areas; NAT, native areas; PAST, pasture areas.

second axis (CV2), which separates native areas from pasture and crop areas (Table 2). The soil pH in native areas was different from that in other land uses. Soil pH was positively correlated ($r^2=0.72$) with histidine, whereas other chemical attributes did not show a significant correlation in all land uses. Pasture showed the highest density and C stock (Table 3).

Comparison within land uses

Forest areas had higher responses to amines and amino acid groups and the individual substrates arginine, glucosamine, histidine, ascorbic acid and ketoglutaric acid (Fig. 2). SIR was used to show separation among the native areas (Fig. 4). The substrates that contributed to the observed separation in CV1 were glucosamine and glucose (positive value), and glutamine (negative value) contributed to the observed separation of the forests from the Cerrado and Dense Cerrado areas. Glucosamine (positive value) was highlighted in the CV2 as asparagine, which separates the Dense Cerrado from the forest and Cerrado (Table 4). The soil chemical characteristics were similar within land uses. Significant differences were

Table 3 – Chemical characteristics of all studied land uses.

	C (%)			C stock (Mg ha ⁻¹)			Density (g cm ⁻³)			pH H ₂ O			pH KCL		
	Average	SD	CV	Average	SD	CV	Average	SD	CV	Average	SD	CV	Average	SD	CV
<i>Land uses</i>															
Native areas															
Native areas															
Pasture															
Agricultural areas															
<i>Native areas</i>															
Forest															
Cerrado															
Cerradão															
<i>Pasture</i>															
Nominal pasture															
Degraded pasture															
Improved pasture															
<i>Agricultural areas</i>															
No-tillage															
Tillage															
Perennial															

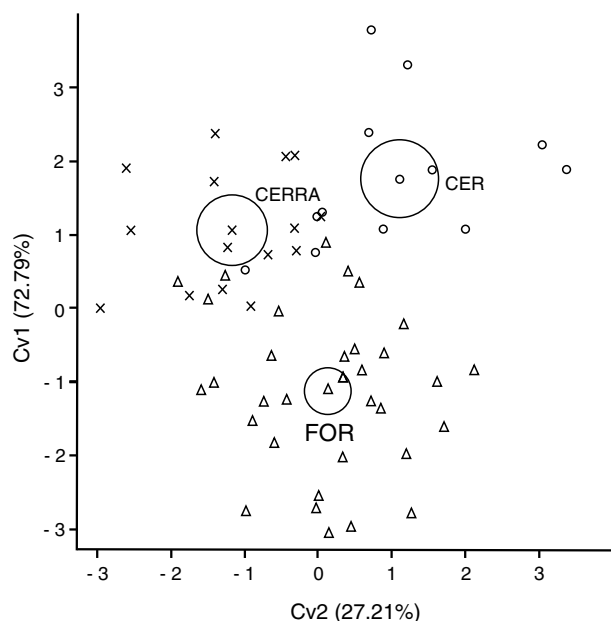


Fig. 4 – Canonical variate analysis of the catabolic profile of the microorganisms in native areas. ○ (CER) Cerrado; × (CERRA) Cerradão; Δ (FOR) forest. The circle represents the 95% confidence area.

only observed in soil pH H₂O and KCl (Table 3). In Cerrado, pH was highly correlated with arginine, whereas the C% was correlated with asparagine. In sites where the native area was the Cerradão, pH was highly correlated ($r^2 > 0.70$) with 16 substrates, including all groups (amines, carbohydrates, amino acids and carboxylic acids).

Nominal pastures showed higher responses to amines and carbohydrate groups and the individual substrates glucosamine, lysine and succinic acid. Improved pastures showed significantly responses to amino acid and carboxylic acid groups, whereas degraded pastures showed higher responses to the individual substrates citric acid, ascorbic acid, malonic acid, malic acid, ketoglutaric acid, ketobutyric acid, and tartaric acid (Fig. 2). SIR was used to showed the separation of the pasture areas (Fig. 5). The substrates that contributed to the observed separation in CV1 were quinic acid and mannose (positive value) and ascorbic acid, glucose and glutamic acid (negative value), thus separating the improved pastures from the degraded and nominal pastures. Glucosamine (positive value) was as highlighted in CV2, as asparagine, which separates degraded pastures from improved and nominal pastures (Table 4). Improved pastures showed higher C% and C stock, whereas nominal pasture showed less acidic pH (Table 3). The soil pH and soil bulk density of nominal pastures were highly correlated ($r^2 > 0.7$) with ketoglutaric acid, whereas the C% and C stock were highly correlated with 9 substrates, particularly carbohydrates. The soil pH in improved pastures was highly correlated with carboxylic acid and the C stock was correlated with tartaric acid.

Perennial crops showed a higher response to amines, amino acid and carboxylic acid groups and all of the individual substrates, except lysine, glucose, quinic acid and succinic acid (Fig. 2). Among the crop areas, CVA showed good separation using SIR (Fig. 6). The substrates that contributed to the observed separation in CV1 were serine, glucosamine and mannose, (positive value) and glucose, pantothenic acid and quinic acid (negative value), thus separating conventional-tillage areas from no-tillage and perennial areas. Histidine

Table 4 – Canonical variate vectors and means for the comparison within land uses.

	Native areas		Pasture		Agricultural areas			
% Variation								
CV1	72.79		63.43		95.55			
CV2	27.21		36.57		4.45			
Total	100		100		100			
	Vectors							
	CV1	CV2	CV1	CV2	CV1	CV2		
Water	–0.37	0.73	CV1	CV2	–14.22	15.82		
Arginine	–0.17	0.07	0.04	1.25	–0.01	0.04		
Asparagine	0.52	1.10	0.12	0.25	0.32	–15.55		
Glutamic Ac.	–0.41	–0.02	–0.94	–0.67	0.02	0.41		
Glutamine	–2.47	–0.03	–10.65	–0.82	0.23	–0.54		
Glucosamine	1.73	1.19	0.85	0.09	15.90	–10.53		
Histidine	–0.86	–1.31	0.98	–0.68	–0.29	30.47		
Lysine	–0.33	0.25	–0.06	0.73	0.34	–0.92		
Glucose	2.00	0.57	–0.68	0.33	–22.98	0.84		
Mannose	–0.84	–0.58	–10.66	–0.82	13.68	0.19		
Citric Ac	–0.07	0.27	10.61	0.47	–0.01	0.01		
Ascorbic Ac	–0.28	0.29	0.08	–0.03	0.06	–0.54		
Serine	–0.51	0.13	–12.51	–0.21	18.42	–14.14		
Gluconic Ac	0.19	0.40	0.02	–0.83	–0.29	0.02		
Fumaric Ac	0.21	–0.12	0.02	0.65	0.22	0.48		
Malonic Ac	0.06	0.35	–0.01	–0.39	–0.73	0.91		
Malic Ac	0.16	0.27	0.23	0.30	–0.73	–0.35		
Ketoglutaric Ac	–0.07	0.02	–0.11	0.33	0.61	–0.12		
Ketobutyric Ac	0.06	0.36	–0.10	0.01	0.43	0.12		
Pantothenic Ac	–0.20	0.41	–0.01	0.36	–12.94	16.18		
Quinic Ac	0.93	0.04	0.20	0.03	–10.45	–0.82		
Succinic Ac	–0.02	0.10	19.32	0.99	0.60	–0.84		
Tartaric Ac	0.09	0.61	0.62	0.28	0.21	0.10		
C.M.	CV1	CV2	C.M.	CV1	CV2	C.M.	CV1	CV2
CER	1.76	1.11	PAST N	2.93	0.98	PERE	8.51	0.14
CERRA	1.07	–1.17	PAST D	–0.90	0.42	NT	–1.88	–0.41
FOR	–1.12	0.13	PAST I	0.51	–2.00	T	–2.96	2.60

C.M., canonical mean; CER, Cerrado; CERRA, Cerradão; FOR, forest; PAST N, nominal pasture; PAST D, degraded pasture; PAST I, improved pasture; PERE, perennial crop; NT, no-tillage; T, conventional tillage.

and pantothenic acid (positive value) were as highlighted in the CV2 as serine, glucosamine and asparagine, which separate perennial areas from conventional-tillage and no-tillage (Table 4). The chemical attributes, except C stock and pH, were similar within agricultural areas (Table 4). The soil pH and soil bulk density in tillage areas were highly correlated ($r^2 > 0.70$) with amino acids and carboxylic acids.

Discussion

Several authors^{18–22} have demonstrated that plants significantly influence soil microbial biomass diversity. Microorganisms depend on external carbon sources. Therefore, litter quantity/quality and floristic diversity can be important factors in determining microbial biomass diversity. Zhang et al.²³ showed that litter quantity influenced soil microbial community structure and functional stability. According to the results of the present study, the most important substrates used to differentiate the land uses and/or managements (asparagine,

serine, glucose) were associated with root exudates²⁴ or components of plant tissue (quinic acid).²⁵ When the catabolic diversity of microorganisms partially depends on the varied quality and production of litter, it is expected that different land uses would result in different microbial communities.

For thousands of years, natural fire, during the wet season, and anthropogenic fire, during the dry season, coexisted in the Cerrado region, particularly in the more open physiognomic form. According to Arocena and Opio,²⁶ fire majorly impacts soil-aggregate stability and soil pH, significantly influencing the microbial biomass. According to Zimmerman and Frey,²⁷ the increase in soil pH in response to ashes contributes to the development of the microbial community. Roscoe et al.²⁸ demonstrated in Cerrado soils that the soil organic matter origin changes when fires are frequent in the system, with 34.6% of the original C derived from C3 plants substituted with C derived from C4 plants. According to Hart,²⁹ fire can be considered a selection factor of areas exposed to periodic fire events, as these events change the structure of the

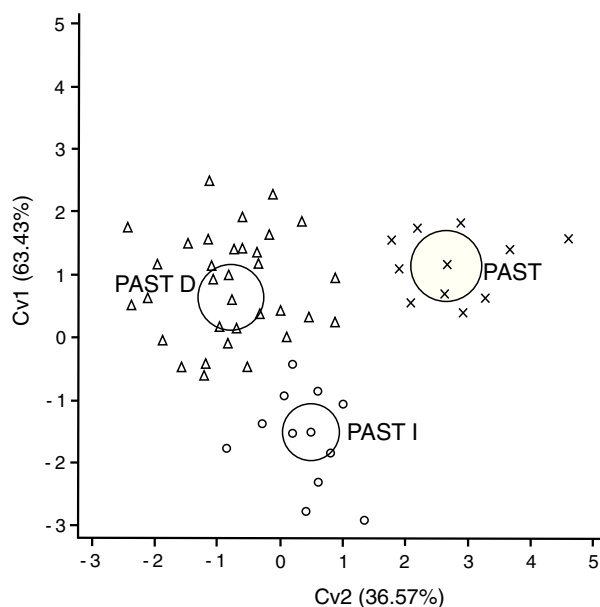


Fig. 5 – Canonical variate analysis of the catabolic profile of the microorganisms in pasture areas. Δ (PASTD) degraded pasture; \times (PAST) nominal pasture; \circ (PASTI) improved pasture. The circle represents the 95% confidence area.

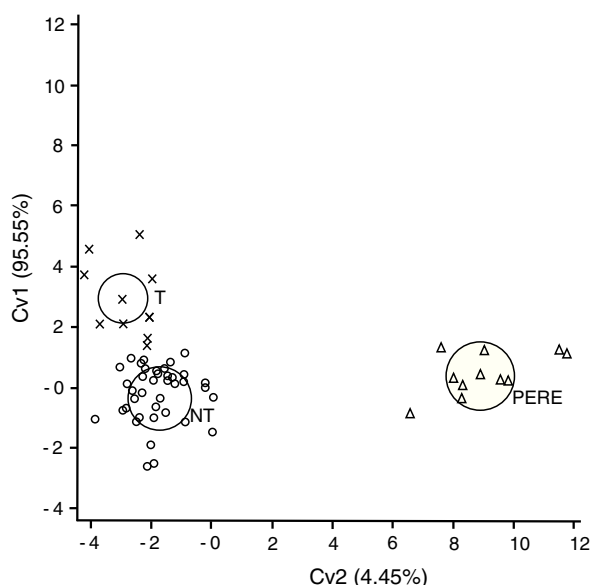


Fig. 6 – Canonical variate analysis of the catabolic profile of the microorganisms in crop areas. Δ (PERE) perennial crop; \times (T) tillage; \circ (NT) no-tillage. The circle represents the 95% confidence area.

microbial biomass. The results of the present study showed that pH was correlated with all substrate groups in Dense Cerrado areas. Campbell et al.³⁰ demonstrated that the utilization of carbonate substrates decreases with burning, suggesting the decreased resistance/resilience of the microbial community.

The separation observed in pasture areas might be associated with the total C stock in soil. Land uses or managements that induce the accumulation of organic matter increased

the catabolic diversity of the microbial community, consistent with previous studies.³¹⁻³³ The results of the present study showed that C stock was an important factor differentiating pastures (Table 3). In nominal pasture areas, soil C% and C stock were highly correlated with carbohydrates, whereas the soil pH was significantly correlated with carboxylic acids in improved pastures.

Lupwayi et al.³⁴ also observed differences in the microbial diversity in areas under conventional and no-tillage management. Conventional-tillage induces changes in the availability of substrates,³⁵ which modifies the pattern of use and the microbial community in these areas.³⁶ The different patterns suggest the presence of different microbial communities in each land use. No-tilled surface soils (0-5 cm) have more lightweight material.³⁷ These conditions stimulate the growth and activity of soil microorganisms. Furthermore, these characteristics indicate that SOM has chemical and structural differences in the two systems.^{37,38} Another factor that might contribute to the observed difference is the increased organic carbon storage in the soil due to the use of no-tillage systems. Maia et al.³⁹ reported the potential of areas under no-tillage to increase the organic carbon content in the same soils. The application of herbicides, which is a common practice in conventional tillage, also decreases soil microbial biomass and activity.⁴⁰

According to Brady and Weil,⁴¹ pH affects the physical, chemical and biological properties of soil. Zimmerman and Frey²⁷ demonstrated that pH plays a key role in the microbial community dynamics of forest soils. Other studies at the continental scale also identified pH as the main factor determining the microbial community structure.^{42,43} Campbell et al.³⁰ reported the same conclusion from studies of Australian forests.

Many studies have supported the idea that free-living microorganisms exhibit biogeographic patterns.⁴⁴ The idea that “every thing is in everywhere, but the environment selects”⁴⁵ is consistent with the results of the present study. The separation observed in the CRP analysis (Figs. 3-6) indicated the importance of the specific characteristics of each land use in microbial biomass diversity, even in areas with different climates and soils, such as the evaluated eco-regions. Differences in the C content and the nature of substrates induce changes in the utilization patterns of organic substrates.^{31,46} The different patterns suggest the presence of different microbial communities in each vegetation cover. Despite the large extension of the area evaluated, the microbial community was strongly correlated with the soil-plant component in the present study. Consistent with Stevenson et al.,¹² we also demonstrated that the functional microbial community changed in response to different land uses.

Conclusion

The different responses to the addition of these substrates suggest the presence of different microbial communities in each land use or management. Despite the different soil type, climate and topography observed in each ecoregion, the microbial communities showed similarities in each land

use. Plant composition (and consequently root exudates) and soil pH were the main factors determining the similarities between and within land uses. In the present study, differences were observed in large comparisons (Land uses – 3.1) to biomes and more specific managements (3.2), regardless of climate or soil type.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bjm.2015.11.025](https://doi.org/10.1016/j.bjm.2015.11.025).

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