Microbial biomass activity in neotropical savanna soils managed during six years with conservationist cereal-cattle systems

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Abstract
In well-drained savanna soils of Venezuelan Central Plains, the changes of microbial activities produced by different agricultural managements for maize were evaluated. The perennial cover crops; *Brachiaria dyctioneura* (grass) and *Centrosema macrocarpum* (legume) were established in 2002. Two years later, they were associated with maize cultivated under no-tillage and different phosphorus sources: phosphate rock, diammonium phosphate, native mycorrhizas used as maize inoculum and the phosphorus produced by organic residues mineralization of the cover crops. Temporal variations of the microbial activity were found, showing a significant increase ($P<0.05$) with the cover crop introduction. Their dynamic patterns during six years of the essay also were different ($P<0.05$), depending of the phosphorus source-cover crop interaction. Phosphate rock and biofertilizer induced a higher mineralization activity at beginning of maize development, just when there is a major demand. The dynamic variations of metabolic coefficient (qCO_2) suggested changes in the efficiency of use of C by the soil microorganisms.

Key Words
Microbial biomass, metabolic coefficient, well-drained savannas, cover crops, maize.

Introduction
The microbial biomass is a fundamental factor of the soil fertility because it transforms all the organic residues that enter into the soil. It has an important role in nutrient availability for plants and at the same time, in nutrient conservation in organic forms, during a short period of time. When the soils have a low fertility as the well-drained savanna soils have; the role of the microorganisms and their activities increase their relative importance on the agroecosystem sustainability. On the other hand, acid soils dominate in these well-drained savanna ecosystems. These edaphic conditions; acidity and gross textures, joined to the strong climatic seasonality, produce one of the principal problems for plant production; P and N deficiency. Mineral fertilization has been a palliative solution to mitigate these deficiencies, however, the risk of N loss by lixiviation and the chemical adsorption of P in these ecosystems, places fertilization and the nutrient availability at a crucial point to consider in the agriculture management of these savanna soils.

Knowledge of the microbial biomass sensitivity to changes that are produced in the soil by the agriculture management, its activity could be used to manipulate the mineralization or immobilization of important elements in the soil. In this sense, it is reasonable to combine organic residues with distinct C/N ratios with fertilizers that promote different P availabilities for the crops. This interaction can affect the mineralization activity of the microorganisms and to create key conditions affecting the availability of nutrients in mineral form for maize.
The objective of this study was to evaluate microbial activity changes in the well-drained savanna soils with different types of agricultural management; that included the use of cover crops and type of phosphate fertilization of maize cultivated under no-tillage.

Methods
The study was conducted at La Iguana Experimental Station, which is in the Orinoco River watershed. La Iguana Experimental Station presents representative savannas of the Venezuelan Central Plains and is located in the southeast of Guarico state, Venezuela (8° 25' N and 65° 24' W). The region has a marked seasonality with a dry season from November to May and a rainy season from June to October. The total annual precipitation is 1369 mm and the mean annual temperature of 27ºC. The soil at the experimental site is a sandy Ultisol, acid with low fertility.

Experimental design
The experimental design corresponded to big plots without repetition, one big plot for each treatment (cover crop-fertilization). A soil spatial variability study (Lozano *et al.* 2004) was conducted in the whole sampling area to find out whether it was similarly heterogeneous, and consequently to determine the size and shape of the sampling surface, which finally corresponded to 18 x 450 m plots. From that geo-statistical study, it was also established that in each plot, the minimum number of samples per treatment of cover crops-phosphorus fertilizers should be twelve. Perennial cover crops, one grass *Brachiaria dictyoneura* (BD) and one legume *Centrosema macrocarpum* (CM) were sown in experimental plots in 2002. The preparation of the soil to sow the cover crops implicated use of tillage (four ploughs) and fertilizers added in a typical dose for these soils. Two years after starting the experiment, the aerial cover crops biomass was mechanically mown and their residues were left on the soil surface. Then, maize was sown under no-tillage management. Three type of fertilizer combination were used: 1.-Phosphate rock (RF); N-P-K with 100% phosphate rock as phosphorus source. 2.-Mycorrizal inoculation (FB); N-P-K with 25% phosphate rock + 75% P got by native mycorrhizas. 3.- Reduced inorganic fertilization (IR); N-P-K with 50% phosphorus from diammonium phosphate and 50% from phosphate rock 4.- Without fertilization (Io), the control treatment, where the phosphorus only depended of residues decomposition. Once the maize was harvested, four animals (cows) per treatment were brought in for a three to four-months period to feed on the covers and the remaining maize residues. The same management system for the maize and the cattle herd was used for three consecutive years. Annually, the perennial cover crops were mechanically mown just before the maize was sown. The cover crops naturally re-grew slower than maize. The soil was ploughed only when the cover crops were sowed; the rest of the time only no-tillage management was used.

Sampling procedure
Twelve soil samples were collected randomly (0-5 cm depth) at each sampling plot and at three different stages of the agro-ecosystem: i) before cutting the covers (AC), which corresponds to the period between the end of the dry season and the beginning of the rainy season, ii) at the flowering peak of maize (F), that is in the rainy season, and finally iii) after taking the animals out of the plots (3 or 4 months after grazing) (DP), in the dry season. The sampling dates corresponded to 0, 671, 1044, 1120, 1408, 1506, 1742, 1783, 1884 and 2094 days after the cover crops were established (ddsc) in 2002. These dates also correspond to AC, F and DP, during the three maize-cattle cycles. Soil samples were stored at field humidity in polyethylene bags at 4ºC, until analysis was performed. At the beginning of the experiment, in the rainy season just before the agronomical management was established, an initial savanna soil characterization was made, that sampling was done in the same sites where each plot treatments would be established, it also corresponded to a randomized sampling (twelve samples) at native savanna soil (SN).

Analytical methods
Twenty-gram triplicates of each sample of soil, at field humidity, were used to determine microbial biomass carbon (CBM) using the fumigation-extraction method (Sparling and West 1988). Basal respiration (CO_2) was estimated using the Alef (1995) trap method. Soil samples (50 g each, in triplicate), at field humidity, were placed in 250-ml plastic bottles and incubated at 28°C and constant humidity content. The metabolic quotient, qCO_2, defined as the specific soil respiration of the microbial biomass (mg CO_2 / (mg CBM x h)$^{-1}$), was calculated using the formula:

$$qCO_2 = ([CO_2/CBM]/24) \quad (1)$$

Where CO_2 is the C mineralized by the soil basal respiration, MB-C is the C of microbial biomass and 24 are the hours of the measurement of both processes.

Results
The microbial biomass was affected by; i.- the cover crops development and the organic nutrients supply due to the cover crop's necromass decomposition (evaluated in AC between dry-rainy season), ii.- the maize development with the different types of phosphorus fertilizers (evaluated in F in the rainy season) and, iii.- the changes produced in the soil by the cattle effect (evaluated in DP in the dry season). It was evident the spatial variability of this parameter in the study area (Figure 1). At the beginning, before the essay was introduced, existed significant differences ($P<0.05$) between *Brachiaria´*s plot and *Centrosema´*s plot with native savanna´s plot (SN). At that time, higher values of CBM were observed in the plots (BD and CM), where phosphate rock would be applied (RF). Two years later, 671 ddsc, the CBM was similar among all treatments compared to native savanna (SN). After that the maize was sown and the different types of fertilizers were applied, CBM was changing among treatments (cover crops-fertilization interaction). It was

found that the same phosphorus source produced a different effect on the CBM depending which cover crops was associated to maize. An example of this occurred when 50% of phosphorus was used like diamonium phosphate (IR). Both, phosphate rock (RF) and diamonium phosphate (IR) promoted a higher microbial biomass when they were used with the legume cover crop associated to maize (CM) (Figure 1). In case of FB, the CBM has lower values in the rainy season, when the maize was flowered. On the contrary, at that same stage, the CBM was higher in native savanna (SN). The RF treatment produced a similar behavior to the observed in the SN soil. In the dry season, coinciding with the after grazing stage (DP), the CBM was higher in RF and IR with the grass, *Brachiaria*, but with the legume, *Centrosema*, the pattern was contrary and the RF soil shows the lowest value.

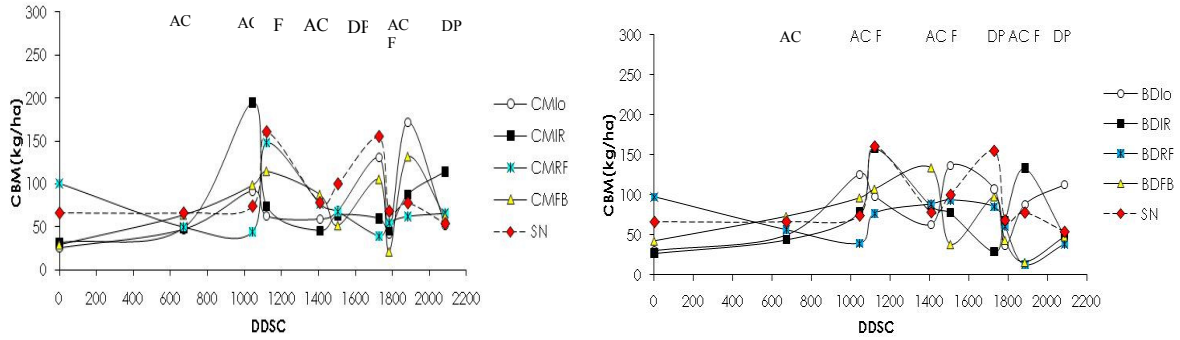

Figure 1. Microbial biomass content (CBM) during the development of the cover crops-maize-cattle system under no-tillage and fertilization types. BD: *Brachiaria dyctioneura,* CM: *Centrosema macrocarpum*, IR: diammonium phosphate, RF: phosphate rock, FB: mycorrhiza inoculation, Io: Without fertilization, SN: Native savanna. DDSC indicates the days after the cover crops were established.

The microbial respiration dynamic (Figure 2) showed fewer differences between cover crop treatments during the essay evolution. At the first four years, the CO_2 production was lower in the SN soil, and then, a significant increase ($P<0.05$) was produced in the rainy season, when the maize was flowered. In change, in the cover crops soils, the CO_2 production was significantly increasing since they were sown until the flowering of maize (F) in the rainy season. This diminution occurred in general in BD and CM, except in BDIR, where the C mineralization did not decrease. The CO_2 pattern changed at the second and third maize cycles because this parameter increased in F, especially when BD was associated to maize.

Metabolic changes of the microorganisms could occur during the six years of the production system, being the microbial biomass more efficient in the use of C per microbial biomass unity produced in some periods (Table 1). When the cover crops were introduced (671 ddsc), the metabolic efficiency decreased. It was evidenced that RF could promote minor efficient of the microorganisms in BD than CM, where there was minor constrains by N.

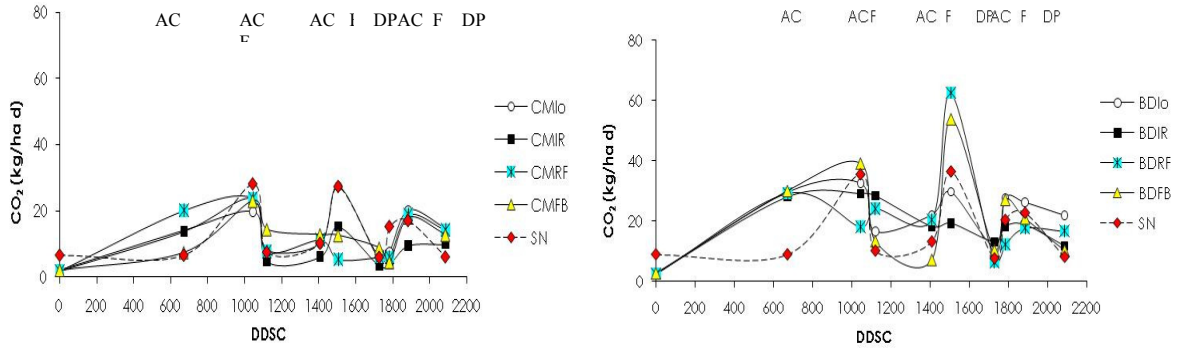

Figure 2. C mineralized by microbial biomass (CO_2) during the cover crops-maize-cattle system development under no-tillage and fertilization types. BD: *Brachiaria dyctioneura,* CM: *Centrosema macrocarpum*, IR: diammonium phosphate, RF: phosphate rock, FB: mycorrhiza inoculation, Io: Without fertilization, SN: Native savanna. DDSC indicates the days after the cover crops were established.

Table 1. Changes of metabolic efficiency (qCO_2) during the cover crops-maize-cattle system development under no-tillage and fertilization types.

Treat	0	671 (AC)	1044 (AC)	1120 (F)	1408 (AC)	1506 (F)	1742 (DP)	1783 (AC)	1884 (F)	2094 (DP)
BDIR	0,07a	0,45c	0,25bc	0,22bc	0,15b	0,17b	0,27c	0,19b	0,10a	0,17ab
BDIo	0,06a	0,44c	0,19b	0,18b	0,26c	0,13ab	0,07a	0,54d	0,22b	0,14a
BDFB	0,04a	0,29b	0,29bc	0,11ab	0,04a	0,88e	0,07a	0,46d	0,99d	0,15a
BDRF	0,02a	0,37bc	0,33c	0,22bc	0,15b	0,48d	0,04a	0,15a	1,02d	0,29b
CMIR	0,06a	0,28b	0,11ab	0,09a	0,14b	0,25c	0,05a	0,08a	0,11a	0,09a
CMIo	0,07a	0,29b	0,20b	0,18b	0,17b	0,40d	0,03a	0,18b	0,12a	0,28b
CMFB	0,06a	0,12a	0,23b	0,14ab	0,14b	0,25c	0,08a	0,21b	0,14a	0,20b
CMRF	0,02a	0,40c	0,50d	0,06a	0,15b	0,06a	0,10b	0,11a	0,30c	0,22b
SN	0,10b	0,10a	0,04a	0,05a	0,12b	0,25c	0,04a	0,24c	0,22b	0,11a

Different lowercases at the same column indicate significant differences among treatment $p<0.05$, Tukey. BD: *Brachiaria dyctioneura,* CM: *Centrosema macrocarpum*, IR: diammonium phosphate, RF: phosphate rock, FB: mycorrhiza inoculation, Io: Without fertilization, SN: Native savanna.

Conclusion

Microbial biomass was a good indicator of the agricultural management effect and seasonality of the well-drained savanna ecosystem. The cover crops with different C/N ratio could be modeling the microbial variation at the same fertilization treatment, as occurred with IR and RF treatments.

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