Microbial biomass-c, evolved CO_2-C, mycorrhizal colonization, soil fertility and corn yield under different soil management and liming

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ABSTRACT. The objective of this research was to quantify the microbial biomass carbon (MBC) and evolved CO_2-carbon (CO_2–C), mycorrhizal colonization, soil fertility and corn production under different soil managements and surface-limestone application. For that, an experiment was carried out consisting of four treatments, as follows: two soil managements - no-tillage (NT) and minimum tillage (MT) and two limestone treatments (0 and 2 t ha^{-1}), with four replications. Soil managements affected the soil chemical attributes in the 0-0.10 m depth layer: the highest P values were observed in MT and the lowest Al levels in the NT-treatments. Once no significant differences were found among treatments for the evaluated microbial variables and corn yield, it allowed affirming that it is possible to avoid disruption of the no-till sequence by applying lime on the soil surface, once the incorporation of organic matter and limestone caused little changes to the system stability.

Key words: no-tillage, minimum-tillage, arbuscular mycorrhizal fungi, Brazilian savannah.

RESUMO. Carbono da biomassa microbiana e do CO_2 liberado, micorrização, fertilidade e produtividade do milho sob diferentes manejos do solo e calagem. O objetivo deste trabalho foi quantificar o carbono da biomassa microbiana (CBM) e do CO_2 (C-CO_2) liberado, a micorrização, fertilidade e a produtividade do milho em razão do manejo do solo e da calagem aplicada à superfície. Para tanto, instalou-se um experimento com quatro tratamentos constituídos de dois manejos do solo, plantio direto (PD) e cultivo mínimo (CM), com e sem calcário (0 e 2 t ha^{-1}), com quatro repetições. Os manejos afetaram os atributos químicos do solo na camada 0-0,10 m: o maior valor de P foi observado no CM e o menor de Al no PD. Uma vez que não foram observadas diferenças significativas entre tratamentos para as variáveis microbiológicas e produtividade de milho, isto permite afirmar que é possível evitar a interrupção do PD com aplicação de calcário na superfície, visto que a incorporação do material de cobertura e a calagem pouco alteraram a estabilidade do sistema.

Palavras-chave: plantio direto, cultivo mínimo, fungos micorrízicos arbusculares, cerrado.

Introduction

There is a worldwide increasing concern about the preservation of natural soil attributes and properties. Activities related to the so-called conventional agriculture, associated with intensive and/or inadequate soil management, might exhaust the soil over the years. An uncovered soil surface, overheated by the sun, loses rapidly water content, which hinders conditions for organic matter maintenance (important in soil erosion protection) and is detrimental for development of crops and yield, while requiring more fertilizers, correctives and pesticides which are responsible for the physical, chemical and biological degradation of the soil (Sá, 1997; Silva e Resck, 1997).

In Brazil, several soil conservationist management systems have been studied looking toward a minimum soil disturbing. In the cerrado (Brazilian savannah), the no-tillage system has been extensively used. It consists of soil mobilization only in the planting row and the sowing is done over a partially or totally plant residue-covered soil (Furlani, 2000). The layers of plant residues accumulated on the soil surface minimize the erosion effect of intensive rainfalls, which usually occurs in tropical regions. They also reduce soil temperature oscillations, keeping it mild, and maintain the humidity during the hot and dry seasons (Colozzi-Filho, 2000). Moreover, it contributes to improve soil structure and preserve nutrients for the following crops, increasing yield (Sá,

1997).

The amount of organic matter that remains in the soil managed in a no-till and conventional systems are similar; however, in no-till it remains on the surface while in conventional it is incorporated into the soil. Incorporation favors aeration that, parallel to the addition of vegetal residues, speeds up microbial activity and provides fast decomposition. In no-till systems the organic matter remains on the surface, reducing contact with soil microorganisms, leading to a slower decomposition rate (Almeida, 1985).

Although the benefits of no-tillage on the agro-ecosystem, this system requires specific know-how, due to the alterations that occur in the soil. As a necessary practice, liming is effective correcting soil acidity, increasing pH, improving base saturation and providing calcium and magnesium. These are important attributes for the cerrado soils which usually have low pH, low exchangeable bases and phosphorus (P), and high H+Al (Silveira *et al.*, 2000).

The ideal soil management is supposed to integrate technologies with the purpose of reducing production costs and improving environmental quality, enhancing biological interactions and natural processes that benefit the soil (Freitas and Bernardi, 2003). Several biochemical processes occur in the soil due to the microbial activities, which affects the physical and chemical soil properties, and therefore, the plant development, agricultural productivity and environment quality (Balota, 1997).

The microbial biomass is the main constituent of soil carbon cycle, and according to the local soil, climate and residue composition remaining on the soil surface, this biomass might work as a nutrient reservoir (readily available nutrients) or as a catalyst in the organic matter decomposition (Mercante, 2001). The agronomic implications of nutrient cycling, particularly mineralization and immobilization, are of great interest, because they might result in gains or losses in productivity, thus affecting the economic threshold of the agronomic system. No-till planting, compared to conventional tillage, has showed to be more efficient in the maintenance of soil microorganism equilibrium, resulting in a better balance among soil conditions (Balota, 1997).

Mutualistic associations between roots and certain soil fungi, named mycorrhiza, have helped to improve plant nutritional status and survivability in the agro-ecosystem, besides improving the productivity in poor soils and the tolerance to abiotic and biotic stresses. Once gramineous species have a well-developed rooting system, which is efficient in the absorption of water and soil nutrients, this association is of great importance during their growth under stress conditions (Siqueira e Franco, 1988).

The objective of this research was to quantify the microbial biomass carbon (MBC) and evolved CO_2-carbon (CO_2–C), mycorrhizal colonization, soil fertility and corn production under different soil managements and surface-limestone application.

Material and methods

A cornfield experiment was conducted during 2002/2003 (summer growing season) at the Teaching and Research Farm of the Faculdade de Engenharia (Engineering College), Unesp (Universidade Estadual Paulista)/Campus de Ilha Solteira, located in Selvíria, Estado de Mato Grosso do Sul, Brazil. The temperature and rainfall annual averages are 24.4°C and 1232.2 mm, respectively. The experimental area was on an Oxysol soil (Dematte, 1980).

The area was originally covered with cerrado vegetation, but has been cropped with corn or soybeans during the summer growing season and sprinkler-irrigated common beans during the winter. Since 1982, no-tillage has been used in the area. In August 2002, the area was divided in plots for liming treatment: half plots was the control (0 t ha^{-1}) and half received 2 t ha^{-1} of dolomite limestone on soil surface (85% TNRP). In November 2002, these plots were subdivided for the tillage treatment: no-tillage (NT) and minimum tillage (MT) systems.

Fertilizers were applied, at the rate of 250 kg ha^{-1}, using a 08-28-16 formula (20 kg ha^{-1} N + 70 kg ha^{-1} P_2O_5 + 40 kg ha^{-1} k_2O) and 25 days after seedling emergence, 40 kg ha^{-1} N (ammonium sulfate) was side dressed.

Corn hybrid (DASB 420) appropriate for the region was seeded and spaced 0.90 m between rows. The experiment was a complete randomized block design, in split-plots, with four replications. The main plots were the tillage treatments and the subplots the liming. Each subplot consisted of eight 40-m long cornrows.

The soil sampling was done in February/March 2003 during the grain filling stage. For the chemical soil analyses, composite-samples, each consisting of 20-subsamples/subplot, were collected at 0-10 cm depth. The soil samples were air-dried, sieved and analyzed, according to the method of Raij and Quaggio (1983).

For the analyses of the microbial biomass-C (MBC) and evolved CO_2-C, two soil subsamples were collected from each subplot. Each replication value

was the average of two analyses. For the number of spores, eight soil subsamples from each treatment replication were evaluated.

Soil sample was composed by 4-subsamples/subplot, colleted at 0-10 cm depth, on the row, close to the plant root system. Samples were air-dried and sieved (2-mm mash), and the roots were separated, rinsed under tap water and kept in 50% alcohol until staining.

The MBC was evaluated by the extraction-fumigation method (Vance *et al.*, 1987) that uses chloroform to eliminate the soil microflora. To quantify the amount of evolved CO_2-C, 100 g of soil was placed in glass jars with screw lids, and a flask containing 10 mL of 0.1 mol L^{-1} NaOH was placed in the center of the jar. These jars were hermetically sealed and maintained in an incubator at 27ºC during 48h. The free sodium hydroxide (NaOH) was treated with 0.1 mol L^{-1} HCl for the estimation of the quantity of evolved CO_2 (Anderson and Domsch, 1982).

For the native arbuscular mycorrhizal fungi (AMF) colonization evaluation, the roots were bleached in 10% KOH, acidified in 1% HCl, stained with 0.05% Trypan blue, and preserved in lactoglycerol (Phillips and Hayman, 1970). The colonization evaluation was done in forty 1-cm size root segments, per replication, using an optical microscope. The number of spores was determined in 100 g soil samples, which were processed according to the combination of the decanting and wet sieving methods (Gerdemann and Nicolson, 1963), followed by centrifugation in a sucrose solution (Jenkins, 1964). The spore number was determined in Petri dishes with concentric rings, under a stereoscopic microscope (40x).

Plant shoots from three 4-m rows/subplots were harvested for dry matter determination (DM) and threshed to separate the straw residues from the grains. The grain production was determined and corrected for 13% humidity. Samples of straw residues (200 g) were collected and kept at 65ºC for 5 days for the estimation of total dry matter mass.

The data obtained were submitted to analysis of variance and Tukey test was used for mean comparisons. Correlation analyses were done for all parameters.

Results and discussion

Significant differences in soil chemical attributes due to management were found only for phosphorus and aluminum with higher P values in the MT treatment whilst higher Al was found in the NT treatment (Table 1).

Liming did not affect any soil chemical attribute; however, the interaction management x liming was significant. The OM was higher in the absence of lime, and K in the MT and NT treatments, respectively. Higher values for pH and Ca were found in NT with lime application (Table 2). The OM content showed a positive correlation with pH (0.97[**]), Mg (0,75[**]) and H+AL (0,91[**]).

Table 1. Soil chemical attributes in the 0-10 cm depth layer, and analysis of variance results as affected by soil managements (NT and MT) and liming in Selvíria, Estado de Mato Grosso do Sul, Brazil.

Treatments		P	OM	pH	K	Ca	Mg	H+Al	Al	CTC	V
		mg dm^{-3}	g dm^{-3}	$CaCl_2$	--------------------------- mmol$_c$ dm^{-3} ---------------------------						%
Management	NT	28.43b	29.56	5.27	4.95	33.81	26.75	32.12	1.37[a]	97.77	63.50
(M)	MT	44.56a	30.81	5.29	4.23	31.25	23.06	28.81	0.31b	87.43	65.75
Liming	0 t	36.50	30.43	5.21	4.56	29.92	22.62	32.00	0.94	89.11	62.31
(L)	2 t	36.50	29.93	5.35	4.61	35.12	27.18	28.93	0.75	96.09	66.93
M		0.048[*]	0.208[ns]	0.839[ns]	0.140[ns]	0.500[ns]	0.260[ns]	0.112[ns]	0.010[*]	0.117[ns]	0.527[ns]
L		1.000[ns]	0.607[ns]	0.193[ns]	0.898[ns]	0.180[ns]	0.170[ns]	0.138[ns]	0.587[ns]	0.275[ns]	0.201[ns]
M x L		0.604[ns]	0.005[**]	0.042[*]	0.044[*]	0.037[*]	0.063[ns]	0.278[ns]	0.630[ns]	0.078[ns]	0.160[ns]
CV (%)		39.06	6.14	3.54	19.48	22.07	24.71	12.45	77.93	13.00	10.44

(*) and (**) F test significant at P ≤ 0.05 and 0.01, respectively; (ns) non-significant. Means followed by the same letter in the column and within each parameter are not different by the Tukey test (P ≤ 0.05).

Table 2. Soil chemical attributes and analysis of variance results as affected by soil management (NT and MT) and liming in Selvíria, Estado de Mato Grosso do Sul, Brazil.

Management	OM		pH ($CaCl_2$)		K		Ca	
Liming	NT	MT	NT	MT	NT	MT	NT	MTM
	------------g dm^{-3}---------		---------------------mmol$_c$ dm^{-3}------------------					
0 t	28.13aB	32.75aA	5.10bA	5.34aA	5.44aA	3.69bB	26.88bA	33.00aA
2 t	31.00aA	28.88bA	5.45aA	5.25aA	4.46aA	4.77aA	40.75aA	29.50aA

Means followed by the same letter, small in the columns and capital in the lines, do not differ by Tukey test (P ≤ 0.05).

The highest value for P found in MT (Table 1), could be possibly explained by the greater nutrient accumulation on the soil surface provided by conservationist management systems. As the results

found on this study, Souza and Alves (2003) reported that, on the cerrado soil, the highest value for P were also found in MT, possible due to the element dispersion through the soil profile. Valpassos *et al.* (2001) and Caires *et al.* (2001) also reported results that corroborate those found on the present study. The highest Ca values in the surface layer of NT was due to liming, due to Ca cycling via plant residue decomposition and due to the increase of cation exchange capacity (CEC), which was able to retain more cations in this layer. Caires *et al.* (2001; 2003) and Souza and Alves (2003) reported results similar to those found on this study.

A higher pH value was observed in the NT treatment with lime (Table 2). Caires *et al.* (2001) observed different pH between soil management, and justify those values by the correction of the soil acidity. Increases of pH in the NT treatment, according to Souza and Alves (2003), were due to the application of lime on the soil surface as well as to the presence of plant residues. The plant residue potential to correct soil pH is due to the nutrient release from decomposition. Therefore, the effect of liming on soil pH is probably enhanced when plant residues are present on the soil. In the MT treatment the lowest Al values observed were probably due to the soil tillage, causing partial lime incorporation and soil revolving and thus, increasing the contact of lime and soil particles (Furlani, 2000).

According to Caires *et al.* (2001; 2003) and Souza and Alves (2003) the tendency toward soil attributes homogenization might be explained by the non-soil revolving, which causes a downward nutrient movement through soil profile in channels formed by decomposed roots, soil microorganisms action as well as by the formation of organic complexes.

Microbiological indices, based on more than one variable, are able to determine the effect of different soil management systems (Waker and Reuter, 1996). The no-till has as a characteristic soil plowing only at the seedling line, keeping residues on the surface, which reduces the soil temperature and moisture oscillations, providing conditions for a higher microbial activity (Colozzi-Filho, 2000). In the present study, only after 20 years with no-till system that the soil was revolved (MT), there was a trend of this soil to stabilize over time (in this case, after one year), which was also verified for microbial activity and for corn yield.

The variables MBC, evolved CO_2-C, mycorrhizal colonization, number of native arbuscular mycorrhizal fungi spores, shoot straw dry matter and grain yield (Table 3), did not differ significantly due

to soil management, liming or the interaction between them, showing that they were not dependent on the treatment used.

Table 3. Microbial biomass carbon (MBC - μg C dry soil^{-1} day^{-1}), CO_2 carbon (CO_2-C - μg C g^{-1} dry soil), mycorrhizal colonization (COL %), native arbuscular mycorrhizal fungi spores (n^o), shoot straw dry matter (SDM), corn yield and analysis of variance results as affected by soil management (NT and MT) and liming in Selvíria, Estado de Mato Grosso do Sul, Brazil.

Treatments		MBC	CO_2-C	COL	Spores	SDM	Yield
		μg C day^{-1}	μg C g^{-1}	%	N^o.	t ha^{-1}	kg ha^{-1}
Management	NT	115.31	12.01	94.95	119.63	10.43	9293.75
(M)	MT	115.93	10.92	99.58	116.50	9.30	7177.25
Liming	0 t	110.13	11.44	96.20	113.50	8.85	8450.75
(L)	2 t	121.13	11.50	98.33	122.63	10.89	8020.25
M		0.914ns	0.052ns	0.053ns	0.674ns	0.571ns	0.165ns
L		0.092ns	0.913ns	0.339ns	0.232ns	0.306ns	0.763ns
M x L		0.214ns	0.633ns	0.190ns	0.198ns	0.523ns	0.921ns
CV (%)		10.25	8.56	4.32	12.09	38.15	34.22

(ns) non-significant.

Different from the results observed in the present study, it is possible to find higher values of MBC on NT in literature, even under tropical conditions. In the NT system, the mineralization of the plant residues maintained on the soil surface occurs in a gradual manner, thus causing an increase in the MBC content (Vargas and Scholles, 2000; Valpassos *et al.*, 2001). The MBC content showed a positive correlation with evolved C-CO_2 (0,72*), OM (0,83**), pH (0.80*), Mg (0,73*) and H+AL (0,92**), and these results could be explained by the soil chemical characteristics, i.e., by the pH elevation, which has a direct effect on exchangeable Al sedimentation. In addition, its concentration is reduced with the addition of vegetal residues in the soil, as shown by Sá (1997) and Silva and Resck (1997).

The great soil conditions promoted by the plant residue incorporated into soil stimulate microbial activity (Salinas-Garcia *et al.*, 1997). However, in the present case, MT or NT and liming did not alter the respiratory activity, once results have shown that the rate of evolved CO_2 – C in the NT treatment was not significantly different from MT treatment. These results are not the same from the ones observed by Vargas and Scholles (2000). They found higher CO_2-C in NT than in MT.

A lower respiratory rate can be considered an indicative of higher soil organic matter maturity and might be related to soil improvement (Insam and Domsch, 1988). On the other hand, a greater microbial activity is commonly related with higher mineralization rate and faster nutrient cycling (Priha and Smolander, 1994). Thus, it is possible to infer that

the crop residue incorporation through tillage, into this area that had been no tilled for 20 years, showed little stability alteration. This might be confirmed by the lack of significant differences among the evaluated parameters.

The quantification of evolved CO_2-C is an important procedure and a precise determination of soil microorganism activity (Colozzi-Filho *et al.*, 1999). The microbial community monitoring has been utilized as an indicator of soil quality under different management systems and crop rotations (Mercante, 2001).

Mycorrhizal colonization is a beneficial biological system for plants, mainly under stress edaphic conditions usually found in the tropics. Soil management is the most drastic stress that may affect mycorrhiza formation. Soil management practices might alter not only the mycorrhizal colonization but also the number of spores (Colozzi-Filho *et al.*, 1999).

Even though mycorrhizal colonization was high (over 94.0%) in this study, no significant differences were observed between management or lime application for this variable as well as for sporulation (Table 3). It should also be noticed that mycorrhizal colonization and number of spores had a positive correlation only with MS. Different from the results found on this study, Colozzi-Filho *et al.* (1999) reported that management and crop rotation have been shown to increase the natural inoculum potential of these organisms into soil. Miranda *et al.* (2001) and Alvarenga *et al.* (1999) reported lower mycorrhizal colonization values for corn in cerrado soils under NT (79% in Goiás and 17-57% in Minas Gerais, respectively) than the ones found in this study (95-99%).

Martins *et al.* (1999) showed that lime application at low rates does not significantly alter mycorrhizal populations. However, at higher lime rates (4 t ha^{-1}), they observed a decrease in spore density and suggested that mycorrhiza had different degrees of sensibility to liming.

Plants symbiotically associated with arbuscular mycorrhizal fungi showed higher dry matter content when compared to plants without mycorrhiza (Martins *et al.*, 1999). Siqueira and Franco (1988) found an increase of 32% (801 kg ha^{-1}) in the production of common beans when plants were associated with mycorrhiza in limed soil. On the other hand, they showed that plants grown in no-liming soil, inoculated with mycorrhiza, had the percentage of infection varying from 30 to 60% and an increase on yield. In the present study no significant differences in dry matter production of shoot straw and grain yield for any of the treatments were found

(Table 3), and there was a positive and significant correlation between shoot dry matter and grain yield (0.908**), when plant residues were added to the soil due to chisel plowing, which accelerates residue decomposition, as stated by Sá (1997) and Silva and Resck (1997).

The soil pH raises due to lime application increasing the availability of nutrients such as Ca, Mg, P and K, which can be taken up by plants and thus, possibly increasing dry matter and grain production. Caires *et al.* (2001) found that high rates of lime caused an increase in soybean and corn grain yields.

Conclusion

The incorporation of the crop residues through minimum tillage caused small changes on the system stabilities evidenced.

Differences among treatments were not found for the evaluated microbial variables and corn yield.

It is possible to avoid the disruption of NT sequence by soil surface lime application.

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